Solitary Bees: conservation, rearing and management for pollination

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2 authors:

Breno Magalhães Freitas
Universidade Federal do Ceará

Júlio Otávio Portela Pereira
Instituto Federal de Educação, Ciência e Tecnologia do Ceará

Solitary Bees

Conservation, Rearing and Management for Pollination

FEDERAL UNIVERSITY OF CEARA

SOLITARY BEES

CONSERVATION, REARING AND MANAGEMENT FOR POLLINATION

A contribution to the International Workshop on Solitary Bees and Their Role in Pollination,
held in Beberibe, Ceará, Brazil, in April 2004

Edited by

Breno M. Freitas and Júlio Otávio P. Pereira

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Universidade Federal do Ceará
Departamento de Zootecnia – CCA
C. P. 12168, Campus do Pici – CEP: 60.021-970
Fortaleza – CE, Brazil – Phone: +55 (85) 288.9697
www.abelhas.ufc.br – E-mail: freitas@ufc.br
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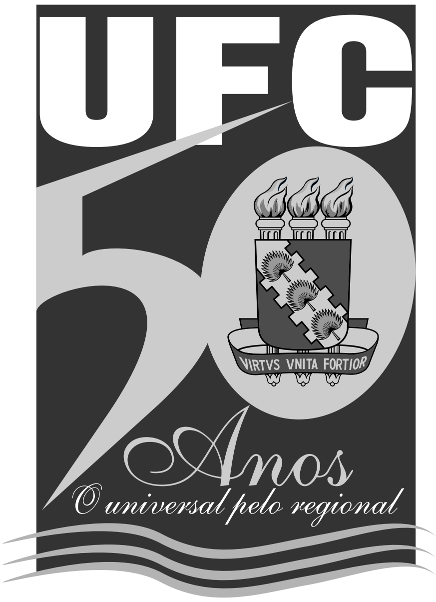
DEDICATION

This book is dedicated to the fiftieth anniversary of foundation of the Federal University of Ceara (1954 - 2004)



PREFACE

This book was prepared as a contribution of scientists from all around the world, experts on solitary bees, to the **International Workshop on Solitary Bees and their Role in Pollination**, which was held in Beberibe, state of Ceara, Brazil, from 26 to 29 April, 2004. The book and the workshop are initiatives of the Brazilian Pollinator Initiative, Ministry of Environment and celebrates the fiftieth anniversary of foundation of the Federal University of Ceara.

The main objective of this book is to bring together updated knowledge on solitary bees, especially their use for crop pollination. Subjects such as rearing, building-up population techniques, standardized methodologies, losses of species diversity, population decline & management practices, assessment of the economic value of their pollination services and the economic impact of the decline of pollination services are covered in the six sessions and 25 chapters in which the book is arranged.

Session I "The Pollinator Initiatives" comprises 6 chapters and covers the International Pollinator Initiative (**Chapter 1**) coordinated by FAO, as well as the five regional initiatives underway in Brazil (**Chapter 2**), EUA (**Chapter 3**), Europe (**Chapter 4**), SE Asia (**Chapter 5**) and Africa (**Chapter 6**). These initiatives deal with pollinators in general, but bees constitute the most important group of pollinators and 85 % of the 20,000 known species of bees are solitary.

Despite the great number of solitary bees, little is known about most species and their population status. **Session II "Monitoring and Population Dynamics of Solitary Bees"** discusses the need of monitoring natural populations and standardize methodologies (**Chapter 7**), revises trap-nest techniques for monitoring solitary bees and lists species caught in trap nests in Brazil (**Chapter 8**). This session also discusses population dynamics and genetics of solitary bees through two elegant studies carried out in temperate (**Chapter 9**) and tropical climates (**Chapter 10**).

Although reckon as important pollinators, many species of solitary bees are endangered and their economic value to natural and agricultural ecosystems is still little known. **Session III "Conservation and Economic Valuation of Solitary Bee Pollination Services"** presents experiences for restoring native bee pollinators (**Chapter 11**), discusses the dependence of pollination services on community composition (**Chapter 12**), the economic valuation of bee pollination services and its implications for farm managing and policy (**Chapter 13**) and uses the bumblebee rearing and commercializing industry to discuss the potential of managing other bee species for pollination (**Chapter 14**).

A few species of solitary bees have been used for large-scale crop pollination. **Session IV "Rearing and Managing Solitary Bees: *Osmia* and *Megachile*"** shows how *Osmia* bees have been studied in their life cycle (**Chapter 15**) and reared for crop pollination (**Chapter 16**), and discusses the difficulties in identifying *Megachile* species (**Chapter 17**) and the valuable use of pollen analysis to monitor pollen sources of solitary bees (**Chapter 18**).

Other promising solitary bee species for crop pollination are covered in **Session V “Solitary Bees in Agriculture Systems: *Centris* and *Xylocopa* “**. These two bee genus are being proposed for pollination of a variety of crops, such as cashew (*Anacardium occidentale*) and West Indian cherry (*Malpighia emarginata*) (**Chapter 19**), wild plant species just coming into agriculture like nance (*Byrsonima crassifolia*) and Peanut Butter Fruit (*Bunchosia argentea*) (**Chapter 20**), and greenhouse crops like tomatoes (*Lycopersicum esculentum*) (**Chapter 21**).

Finally, the importance of solitary bees to the pollination of wild plants is exemplified in **Session VI “Wild Pollination Systems Involving Solitary Bees”** with a beautiful preliminary communication of ricochet pollination in the Cassiinae (**Chapter 22**), the discussion whether oligolectic bees are always the most effective pollinators (**Chapter 23**), their role on the reproductive biology of timber trees (**Chapter 24**) and a review on oil-collecting bees and their related plant species (**Chapter 25**).

Breno M. Freitas and Júlio Otávio P. Pereira

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The editors thanks the contributors to this book for their time and knowledge put into each chapter. We are also thankful to sponsors which supported the *International Workshop on Solitary Bees and Their Role in Pollination* that motivated the conception of this book and for funding to allow its publication.

We are grateful to the Bee Research Group of the Federal University of Ceara for the work and dedication its members put into the preparation of this book and we are particularly indebted to Mr. Marcelo Casimiro Cavalcante for his expertise in formatting the book.

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CONSERVATION AND MANAGEMENT OF POLLINATORS FOR SUSTAINABLE AGRICULTURE - THE INTERNATIONAL RESPONSE

Food and Agriculture Organization of the United Nations

Pollinators – an essential component for ecosystem functioning

Crop-associated biodiversity (CAB) refers to biodiversity that supports the functioning of ecosystem services necessary for agriculture, as well as contributing to the maintenance of ecosystem health and resilience. CAB is an intrinsic and important part of agricultural ecosystems, and includes components such as pollinators. Pollinators contribute to the maintenance of biodiversity, and ensure the survival of plant species including plants that provide food security to innumerable rural households. Pollination is an essential ecosystem service that enables plant reproduction and food production for humans and animals (fruits and seeds – also impacting on the quality and yield) that depend, to a large extent, on the symbiosis between species, i.e., the pollinated and the pollinator. The reduction and/or loss of either will affect the survival of both.

Pollinator diversity is directly dependent on plant diversity and vice-versa - no other natural phenomenon illustrates more vividly the principle that conservation measures must be directed at ecological processes, and not just individual species. Indeed, pollination, a fundamental step for plant reproduction, is an ecological service that cannot be taken for granted. Plants are the primary producers in terrestrial ecosystems and direct providers of many ecosystem services such as carbon sequestration, prevention of soil erosion, nitrogen fixation, maintenance of water tables, greenhouse gas absorption, and food and habitat providers for most other terrestrial and many aquatic life forms. Pollinators, through facilitating plant reproduction, thus play a crucial role in the maintenance of ecosystem services. Pollination requires pollinating agents which themselves require resources for nesting, feeding and reproduction in the form of vegetation, prey, and certain habitat conditions, as well as the application of “pollinator-friendly” land-use management practices to ensuring their survival.

The pollinators

Over 75% of the major world crops and 80% of all flowering plant species rely on animal pollinators (Nabhan and Buchmann, 1997; Kevan and Imperatriz-Fonseca, 2002). Of the hundred or so animal-pollinated crops which make up most of the world’s food supply, 15% are pollinated by domestic bees, while at least 80% are pollinated by wild bee species and other wildlife (Prescott-Allen and Prescott-Allen, 1990; Ingram *et al.* 1996). Diversity among species, including agricultural crops, depends on animal pollination. Thus, pollinators are essential for “diet diversity”, biodiversity, and the maintenance of natural resources.

The principle pollinators are bees. Approximately 73% of the world’s cultivated crops, such as cashews, squash, mangoes, cocoa, cranberries and blueberries, are pollinated by some variety of bees, 19% by flies, 6.5% by bats, 5% by wasps, 5% by beetles, 4% by birds, and

4% by butterflies and moths (Fact Sheet: Pollinator Diversity, 2004). Of the hundred principal crops that make up most of the world's food supply, only 15% are pollinated by domestic bees (mostly honey bees, bumble bees and alfalfa leafcutter bees), while at least 80% are pollinated by wild bees and other wildlife (as there are an estimated 25,000 bee species, the total number of pollinators probably exceeds 40,000 species).

The 25,000 different species of bees differ significantly in size and habit requirements, and diverge accordingly in the plants they visit and pollinate. Though bees form the most important group of pollinators, other animals, such as bats, birds, butterflies, moths, flies and beetles also play key roles in pollination. Both the diversity of wild plants and the variability of food crops depend on this diversity.

Pollination is a complicated process with some pollinators being "generalists" and others being species-specific. Likewise, many different pollinators visit some plants, while other plants have species-specific pollinator requirements. Given this complexity, managing pollination as an ecosystem service requires a comprehensive understanding of the pollination process and the application of that knowledge in the design and implementation of intricate management practices. In most cases, there is limited knowledge about the exact relations between individual plant species and their pollinators.

The issues: the impact of declining pollinator populations on agriculture

It is recognized that agricultural production, agro-ecosystem diversity and biodiversity are threatened by declining populations of pollinators. Many pollinator population densities are being reduced below levels at which they can sustain pollination services in agroecosystems, natural ecosystems, and for the maintenance of wild plant reproductive capacity. The major contributors to this decline in pollinator populations are, *inter alia*, habitat loss and fragmentation, land management practices, agricultural and industrial chemicals, parasites and diseases, and the introduction of alien species.

Ecological dangers of pollinator decline include the loss of essential ecosystem services (particularly agro-ecosystem services) and functions that pollinators provide. Ecosystem services in their turn have their own value – biophysical, but also economic. For example, for the entire biosphere, the value of ecological services (most of which outside the market) was estimated to be in the range of US$16-54 trillion per year, with an average of US$33 trillion per year (Costanza *et al.*, 1987). Services that are provided by native pollinators (non-honeybee species) are estimated to be worth US$ 4.1 billion a year to United States agriculture alone (Prescott-Allen and Prescott-Allen, 1990). The value of the annual global contribution of pollinators to the major pollinator-dependant crops is estimated to exceed US$ 54 billion (Kenmore and Krell, 1998). In the Canadian prairies, the value of pollinators to the alfalfa seed industry has been placed at about CAD 6 million per year (Kevan and Phillips, 2001).

Examples from Asia (e.g. northern Pakistan, parts of China) show linkages between declining natural insect populations and decreasing crop yields - as a result, people have begun to *manage the crop-associated biodiversity* (i.e. pollinators) in order to maintain their crop yields and quality. For example, farmers in Himachal Pradesh (in northwestern India) are using honeybees to pollinate their apples (Partap, 2003). Due to declining pollinator populations and changing cultivation practices, an increasing number of farmers around the world are now paying for pollination services and are importing and raising non-native pollinators to ensure crop production. In many developing countries, however, external pollination services are not available and rural communities have to live with reduced quantity, quality, and diversity of foods. In fruit orchards in Western China, the decline of useful insect populations has led farmers to pollinating by hand, acting as “human bees” (Ibid).

Despite a general recognition of the impact of declining pollinator populations on ecosystem functioning, and despite the examples of the ecological and economic impacts as well as examples of where this is occurring, bottlenecks and constraints hinder the conservation and management of pollinators in sustainable agriculture. An example of a globally recognised bottleneck is the lack of taxonomic information, which hampers progress that could be made in identifying and analysing firstly pollinator populations important to agriculture, and their behaviour patterns, but also best management practices. Best management practices are not readily available or known in all areas of the world, and especially not to all peoples. Indeed, a lack of awareness of pollinator issues –from the farmer to the extension worker to the policy maker – is also a set-back for the promotion of issues related to the conservation and management of pollinators within the context of sustainable agriculture. Recognition of these bottlenecks and constraints as well as a need for action contributed to the international arena’s response to the conservation and management of pollinators, in agricultural systems (and non).

The international response

Although agricultural international instruments that address sustainable agriculture, and implicitly pollinators, exist, such as the Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture and the International Treaty on Plant Genetic Resources for Food and Agriculture, this section focuses mainly on the Convention on Biological Diversity (CBD) and its programme of work on agricultural biological diversity.

The third meeting of the Conference of the Parties (COP) of the CBD (decision III/11) recognized the importance of agricultural biodiversity and decided to establish a multi-year programme of activities on agricultural biological diversity. This also involved calling for priority attention to components of biological diversity responsible for the maintenance of ecosystem services important for the sustainability of agriculture, including pollinators.

COP Decision III/11 also encouraged interested parties and international organizations to conduct case studies on pollinators, including:

- consideration of the monitoring of the loss of pollinators worldwide; the identification of the specific causes of pollinator decline;
- the estimation of the economic cost associated with reduced pollination of crops;
- the identification and promotion of best practices and technologies for more sustainable agriculture; and
- the identification and encouragement of the adoption of conservation practices to maintain pollinators or to promote their re-establishment.

In October 1998, a Workshop on the Conservation and Sustainable Use of Pollinators in Agriculture, with an Emphasis on Bees, was held in São Paulo, Brazil. The outcome of this workshop was the São Paulo Declaration on Pollinators, which was submitted by the Government of Brazil to the fifth meeting of the Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA).

At its fifth meeting in Nairobi, Kenya, in 2000, COP endorsed the Programme of Work on Agricultural Biodiversity (decision COP V/5). The objectives of the Programme of Wok are:

- To promote the positive effects and mitigate the negative impacts of agricultural systems and practices on biological diversity in agro-ecosystems and their interface with other ecosystems;
- To promote the conservation and sustainable use of genetic resources of actual and potential value for food and agriculture;
- To promote the fair and equitable sharing of benefits arising out of the use of genetic resources.

Considering the recommendations of the São Paulo Declaration on Pollinators, and its subsequent submission to SBSTTA 5, COP also decided to establish an International Initiative for the Conservation and Sustainable Use of Pollinators (also referred to as the International Pollinators Initiative, or “IPI”), as a cross-cutting initiative within the programme of work on agricultural biodiversity to promote coordinated action worldwide, and requested the development of a Plan of Action for the IPI (Decision COP V/5). In addition, as per decision COP V/5, the CBD Executive Secretary was also requested to “invite the Food and Agriculture Organization of the United Nations to facilitate and co-ordinate the Initiative in close co-operation with other relevant organizations...”

A Plan of Action of the IPI was subsequently developed by FAO in collaboration with the Secretariat of the CBD, and adopted at the sixth meeting of the COP (decision VI/5).

The objectives of the International Initiative for the Conservation and Sustainable Use of Pollinators (IPI) are to promote co-ordinated action world-wide to:

- Monitor pollinator decline, its causes and its impact on pollination services;
- Address the lack of taxonomic information on pollinators;
- Assess the economic value of pollination and the economic impact of the decline of pollination services; and
- Promote the conservation and the restoration and sustainable use of pollinator diversity in agriculture and related ecosystems.

The Plan of Action of the International Pollinators Initiative is structured into four main Elements:

1. Assessment:

The Operational Objective is to provide comprehensive analysis of the status and trends of the world’s pollinator diversity and of their underlying causes of its decline (including a focus on the goods and services provided by pollinator diversity), as well as of local knowledge of its management.

2. Adaptive Management:

The Operational Objective is to identify management practices, technologies and policies that promote the positive and mitigate the negative impacts of agriculture on pollinator diversity and activity, in order to enhance productivity and the capacity to sustain livelihoods, by expanding knowledge, understanding and awareness of the multiple goods and services provided by pollinators.

3. **Capacity Building**:

The Operational Objective is to strengthen the capacities of farmers, indigenous and local communities, and their organizations and other stakeholders, to manage pollinator diversity so as to increase its benefits, and to promote awareness and responsible action.

4. **Mainstreaming**:

The Operational Objective is to support the development of national plans or strategies for the conservation and sustainable use of pollinator diversity and to promote their mainstreaming and integration in sectoral and cross-sectoral plans and programmes.

The IPI is to be implemented as a cross-cutting initiative within the programme of work on agricultural biodiversity, with appropriate links to other thematic programmes of work, particularly those on forest biological diversity and on the biodiversity of dry and sub-humid lands.

Some cross-cutting issues addressed at the level of the Convention on Biological Diversity are also associated with the various issues and considerations highlighted in the different elements of the International Pollinators Initiative. One such example is the cross-cutting issue of taxonomy, through the Global Taxonomy Initiative - more specifically. It has linkages to Element 1 of the Plan of Action of the IPI (i.e. “assessment”) which includes addressing the deficit in taxonomic knowledge on pollinators. Another example is the cross-cutting theme “alien species”, which has an impact on the issue of the conservation and sustainable use of pollinators, given that alien invasive species are recognized as a contributor to the decline in pollinator populations.

Another cross-cutting theme that applies to the conservation and sustainable use of pollinators is the “ecosystem approach”, which is defined by the CBD as “...a strategy for the integrated management of land, water and living resources that promotes conservation and sustainable use in an equitable way. Application of the ecosystem approach will help to reach a balance of the three objectives of the Convention. It is based on the application of appropriate scientific methodologies focused on levels of biological organization which encompass the essential processes, functions and interactions among organisms and their environment. It recognizes that humans, with their cultural diversity, are an integral component of ecosystems.” The ecosystem approach, for example, is promoted and used in the GEF-funded PDF-B activities, for a global project on pollinators, that will commence in the first part of 2004 (for a period of two years), entitled “Conservation and Management of Pollinators for Sustainable Agriculture, Through an Ecosystem Approach” - which will be a contribution towards the implementation of the IPI Plan of Action.

The main technical components of the PDF-B are stocktaking of current status of pollinators, demonstration sites and replication strategies as well as capacity building and awareness raising. The final objective of this PDF-B phase is to produce a Full-size GEF project proposal for a global project on Pollinators. The three principal objectives of the global project are to:

- Develop and implement tools, methodologies, strategies and best management practices for pollinator conservation and sustainable use.
- Build local/national/regional/global capacities to enable design, planning and implementation of interventions to mitigate pollinator population declines, and establish sustainable pollinator management practices – including raising awareness and strengthening existing networks dedicated to conservation of pollinators.
- Promote co-ordination and integration of activities related to the conservation and sustainable use of pollinators at the international level to enhance global synergies.

These aims would be achieved through the implementation of the following main components of the Full-size Global project on pollinators:

- Development of a Knowledge Base
- Extension and Promotion of Pollinator-friendly Best Management Practices
- Capacity Building and Awareness Raising
- Sharing of Experiences and Dissemination of Results

A number of other activities and initiatives have been developed, and are being implemented, to respond to the issues related to the conservation and sustainable use of pollinators. Just a few examples of such initiatives include the African Pollinator Initiative, whose Plan of Action was developed and published in 2003. The Brazilian Pollinator Initiative, the European Pollinator Initiative and the North American Pollinator Protection Campaign are other examples. The International Centre for Integrated Mountain Development (ICIMOD) is also undertaking extensive work in the area of pollination in the Hindu-Kush Himalaya region.

Synergies and partnerships

A concerted effort, from the global community that deals with issues related to environment and agriculture, to undertake its activities by taking into account pollinator considerations would assist in the implementation of the IPI. In this regard, COP decision V/5 invites relevant leading organizations to collaborate in supporting actions in Parties and countries subject to pollinator decline. In addition, increasing awareness and understanding of the role and value of pollinator conservation and sustainable use should lead to the development and implementation of local, national, regional and international policies, programmes and projects that integrate pollinator's considerations, hence contributing to sustainable agriculture.

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BRAZILIAN POLLINATORS INITIATIVE

Vera Lucia Imperatriz-Fonseca
Braulio Ferreira Souza Dias

The CDB and the world Pollinators decline

In October 1998 the Brazilian Ministry of Environment (MMA) held an international workshop of experts, the "Workshop on the Conservation and Sustainable use of Pollinators in Agriculture, with Emphasis on Bees", to propose a framework for an International Initiative on Pollinators as a key element in the Convention on Biological Diversity (CBD) thematic program of work on Agricultural Biological Diversity. A total of 61 scientists attended it from 15 countries and 5 International organizations.

As a consequence of this meeting a document was produced, entitled "The Sao Paulo Declaration on Pollinators" (1) that was endorsed in May 2000 by the fifth Conference of Parties on Convention on Biological Diversity (COP5), held in Nairobi (section II of the decision V/5, that reviewed the implementation of decisions III/11 and IV/6 on the program of work on Agricultural Biological Diversity). COP5 established an "International Initiative for the Conservation and Sustainable Use of Pollinators", hereafter referred to as the "International Pollinators Initiative" (2).

An Action Plan was then prepared by FAO and the CBD Secretariat, based on the "Sao Paulo declaration on Pollinators" document, was endorsed by SBSTTA7, and recommended for adoption by CBD COP6. The Plan of Action of the IPI was accepted by member countries and adopted at COP 6 (decision VI/5)(3).

Since then, most regions of the world have established or are in process of establishing wide-ranging pollinators initiatives. The Brazilian Pollinators Initiative also keeps the core objectives of IPI. They are:

- Monitor pollinator decline, its causes and its impact on pollinator services
- Address the lack of taxonomic information on pollinators
- Assess the economic value of pollination and the economic impact of the decline of pollination services
- Promote the conservation, the restoration and sustainable use of pollinator diversity in agriculture as well as in related ecosystems

The Brazilian Pollinators Initiative

The Brazilian Pollinators Initiative (BPI) was officially established during the biannual meeting of Bees in Ribeirão Preto, organized by the University of Sao Paulo, in September 2000. Leading this Initiative were the Brazilian Ministry of Environment, the University of Sao Paulo and the Brazilian Corporation of Agricultural Research. An initial steering committee was formed, and began to work in a national agenda.

To follow its agenda, the Brazilian Pollinator Initiative (BPI), under the facilitation of FAO, participated in the preparation of project proposal submitted to the Global Environmental Facility (GEF) for funding, called "Adaptive Management for the Sustainable Use of Pollinators through an Ecosystem Approach", together with the African Pollinators Initiative and the ICIMOD, in Southeast Asia.

Parallel to the project development to GEF, several other activities characterized this initial phase of BPI. Among them, should be mentioned:

The Pollinators Symposium at the XXI International Congress of Entomology, Iguassu Falls, (Parana State, Brazil) July 2000, funded by MMA; the BPI sessions at the Biennial Bee Meetings, Ribeirao Preto (Sao Paulo State, Brazil) in September 2000 & September 2002; the publication of the book "Pollinating Bees: the Conservation link between Agriculture and Nature" (4), funded by MMA, 2002; Publication of the book "Brazilian Bees, Systematics and Identification", funded by MMA and Fundação Araucaria, 2002 (5); World Bees Checklist Workshop, Indaiatuba (S. Paulo State, Brazil) October 2002 (6); the inclusion of BPI in the Federal Government Multi-Year Work Program for 2004-7, within the program of work of MMA; the public call for projects on pollinators sustainable use, MMA September 2003 and January 2004 (see annex 1); a MOU between MMA and EMBRAPA on Biodiversity Research, October 2003; the EMBRAPA survey of activities and researchers on pollinators, 2003; the Sao Paulo Declaration on Pollinators plus 5 Forum (7), 2003, funded by FAO, MMA and USP, with two workshops, *Standard Methodologies Workshop* and *Pollinators Initiatives and the role of Information Technology: building synergism and cooperation*. The mentioned workshops were organized inside broader related forums (for instance, *World Bee Checklist* in the *Trends and Developments in Biodiversity Informatics Forum*) or in special meetings for them, in order to have data mining, to construct global databases and to develop standardized methods for evaluation of pollinator's abundance and management in crops.

The S. Paulo Declaration Forum plus 5 put together the regional pollinators initiatives (the European Pollinators Initiative; the African Pollinators Initiative; the International Centre for Integrated Mountain Development Initiative, from South Asia; North American Pollinators Campaign; and the Brazilian Pollinators Initiative) and promoted the first discussion on the role of Information Technology in the Pollinators Initiatives. A total of 77 participants from 12 countries (Brazil, Canada, Colombia, Germany, Ghana, Italy, Kenya, Panama, Nepal, South Africa, United States of America, United Kingdom) joined these meetings. The standard methodology workshop addressed the methods related to pollinator-mediated gene flow; to bee surveys and monitoring of bees as pollinators in natural landscapes; bee management for pollination purposes. The talks presented also focused the state of the art of each regional Initiative, as well as methods used to evaluate the pollinators decline and status, their efficiency and number in some crops. The main challenges for next years were pointed out, among them the meta-analyzes of existing data and modeling in order to estimate next activities and make predictions. The IT workshop addressed the importance of the global facilities (as GBIF and other current services, for example ITIS), as tools for supporting and to join the local knowledge on bee names, checklists and regional catalogs, providing knowledge for policy makers.

Other participations of BPI were in workshops realized in 2003 in Africa: the Mabula workshop, for the development of "A guide-line for the development of a Legal and Institutional Framework for Pollinator Conservation", was a very important meeting. The next one was realized in Kenya, in the workshop on Managing Agricultural Biodiversity for Sustainable Development, organized by the CGIAR in the week before its annual meeting at Nairobi. In this workshop, BPI presented a talk on The role and maintenance of pollinator diversity in agricultural production, and had important role in the intensive discussion on this subject**.**

In February 2004, BPI took part of the Apimondia *Tropical Beekeeping: Research and Development for Pollination and Conservation* in *Costa Rica* discussing pollination and the pollinator initiatives. The *International Workshop on Solitary bees and Their role in Pollination* (8), held in state of Ceara, April 2004, and organized by UFC, was the second achievement of BPI for this year. The purpose of this meeting was to update knowledge on solitary bees, especially their use for crop pollination. Subjects such as rearing, building-up population techniques, standardized methodologies, losses of species diversity, population decline & management practices, assessment of the economic value of their pollination services and the economic impact of the decline of pollination services were addressed and discussed.

Brazil has many challenges to fill for the improvement of the activities scheduled for BPI. The design of the full project for five years is the goal of next two years for the GEF project. The structure of the development plan for this project is listed below. Some points are already under development, although the GEF project, already approved, is not signed yet.

Brazilian Pollinators Initiative Projects

BPI PROPOSED SUB-PROJECT 1: BEE SURVEYS & MONITORING

PROPOSED PDF-B ACTIVITIES:
-Workshop to define standard survey methods & sites
-Publish survey methods manual
-Assess existing surveys
-Pilot test of proposed assessment & monitoring
-Visit potential sites for surveys and monitoring methods
-Consolidate & publish pollen catalogs
-Develop and detail full project proposal

PROPOSED FULL PROJECT PRODUCTS:
-Assess bee diversity in x sites in major biomes & crop systems
-Monitor bee diversity in x sites in major biomes & crop systems
-Publish a synthesis report on pollinators' assessment
-Publish a National List of Endangered Pollinators
-Train experts, students and technicians on methods

BPI PROPOSED SUB-PROJECT 2: CROP POLLINATION ASSESSMENTS

PROPOSED PDF-B ACTIVITIES:
-Consolidate existing data on pollination dependant crops
-Collect complementary *in loco* data on crop pollination
-Workshop to consolidate list of pollination dependent crops
-Publish a list of pollination dependent crops (database)
-Publish updated edition of economic valuation methods
-Develop and detail full project proposal

PROPOSED FULL PROJECT PRODUCTS:
-Identify pollinators of pollination dependent crops
-Assess pollination deficit in crops
-Assess economic value of pollination to crops
-Publish assessment of economic importance of pollination to crops
-Train experts, students and technicians on methods

BPI PROPOSED SUB-PROJECT 3: STINGLESS BEES MANAGEMENT

PROPOSED PDF-B ACTIVITIES:
-Workshop on preliminary assessment of conservation status of Meliponini bees
-Select target species and sites
-Identify and visit potential partners and sites
-Select appropriate methods
-Consolidate case studies of best practices
-Develop and detail full project proposal

PROPOSED FULL PROJECT PRODUCTS:
-Assessment report on conservation & use status
-Increase by x folds the number of farmers with Meliponiculture
-Increase by x folds the availability of nesting substracts
-Increase by x folds pollination of selected crops
-Increase by x % the income of poor farming families with byproducts of Meliponiculture
-Manual of stingless bees management
-Train experts, students and technicians on methods

BPI PROPOSED SUB-PROJECT 4: HONEY BEES MANAGEMENT

PROPOSED PDF-B ACTIVITIES:
-Select protected areas
-Identify and visit potential partners and sites
-Identify priority crops to reduce pollination deficits
-Consolidate case studies of best practices
-Develop and detail full project proposal

PROPOSED FULL PROJECT PRODUCTS:
-Assessment report on abundance of honey bees in natural ecosystems
-Reduce abundance of honeybees in x protected areas with hive trapping
-Increase by x folds the use of honeybees in crop pollination
-Manual of honeybees' management
-Train experts, students and technicians on methods

BPI PROPOSED SUB-PROJECT 5: SOLITARY BEES MANAGEMENT

PROPOSED PDF-B ACTIVITIES:

-Identify candidate bees to work
-Consolidate information on selected bee species
-Identify and visit potential partners and sites

-Identify priority crops to reduce pollination deficits
-Select appropriate methods
-Consolidate case studies of best practices
-Develop and detail full project proposal

PROPOSED FULL PROJECT PRODUCTS:

-Assessment report on conservation & use status
-Increase by x folds the availability of nesting substracts
-Increase by x folds pollination of selected crops
-Manual of solitary bees management
-Train experts, students and technicians on methods

BPI PROPOSED SUB-PROJECT 6:TAXONOMIC SUPPORT

PROPOSED PDF-B ACTIVITIES:

-Assessment of bee collections
-Publish “Bees of Brazil” book
-Preliminary Checklist of Brazilian bees
-Provide supplies for Bee Taxonomy Centers
-Develop and detail Full Project proposal

PROPOSED FULL PROJECT PRODUCTS:

-7 Bee Taxonomy Centers equipped
-Data basis of bees in collections
-Checklist of Brazilian bees
-Bee taxa revisions & keys
-Regional Identification guides
-Training on identification of bees
-Bee identification services
-Train parataxonomists

BPI PROPOSED SUB-PROJECT 7: VIRTUAL INSTITUTE

PROPOSED PDF-B ACTIVITIES:

-Fully develop planning and program of training courses (themes, lecturers, materials, demands, selection process)
-Develop Information System structure and contents
-Develop and detail Full Project proposal

PROPOSED FULL PROJECT PRODUCTS:

-5 Annual International Training Courses
-10 National Training Courses
-Information System fully operational, uploaded and updated through the Internet

BPI PROPOSED SUB-PROJECT 8: POLICY & PUBLIC AWARENESS

PROPOSED PDF-B ACTIVITIES:
-Assess impacts of existing legislation and policies
-Assess availability of incentive measures and credit

-Assess public opinion in major regions and stakeholder groups
-Identify potential partners
-Regional workshops with potential partners
-Develop and detail Full Project proposal

PROPOSED FULL PROJECT PRODUCTS:
-National campaign reaching all stakeholders launched
-Public policies developed and applied
-Incentive measures developed and applied
-Inter-sectored partnerships established

Acronyms

API – African Pollinators Initiative
BPI – Brazilian Pollinators Initiative
CBD – Convention on Biological Diversity
CGIAR – Consultative Group on International Agricultural Research
COP – Conference of the Parties
EMBRAPA – Brazilian Agricultural Research Corporation
EPI – European Pollinators Initiative
FAO – Food and Agriculture Organization of the United Nations
GEF – Global Environmental Facility
GBIF– Global Biodiversity Information Facility
ICIMOD – International Centre for Integrated Mountain Development
IPI – International Pollinators Initiative
IT – Information Technology
ITIS – International Taxonomy Information Service
MMA – Brazilian Ministry of the Environment
MOU– Memorandum of Understanding
PDF B – Project Development Facility phase B
SBSTTA – Subsidiary Body on Scientific, Technical and Technological Advice
UFC – Federal University of Ceara, Brazil
USP – University of Sao Paulo, Brazil

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The Sao Paulo Declaration on Pollinators Plus 5 Forum. Assess: March 28th 2004
http://graviola.pcs.usp.br/%7Ewebbee/ibp/php/workshop/index.html

International Workshop on solitary bees and their role in Pollination. Assess: March 28th 2004
http://eco.ib.usp.br/beelab/ibp/pdfs/programa_abelhas_solitarias.pdf

ANNEX 1

Brazilian Pollinators Initiative Public Call MMA 2003-4

Public Call made by the Brazilian Ministry of the Environment - MMA, through the National Biodiversity Project – PROBIO, to support projects to develop management plans for native pollinators of plants of economic value (cultivated or explored through extractivism).

The Public Call was issued in two stages in September 2003 and January 2004. A total of 53 proposals were received and 13 projects were selected and are being contracted, with a total sum of R$ 1,543,702.80 (equivalent to approximately US$ 500,000.00) of financing from MMA plus counterpart funding from the implementing organizations. These projects will be implemented till the first half of 2005 in the following regions and states of Brazil (plants indicated in parenthesis):

Amazon Region (States of Amazonas (cupuassu) and Pará (assaí palm));

Northeast Region (States of Maranhão (murici), Pernambuco (cotton, soursop, acerola, mangaba, mango and passion fruit), Bahia (guava, mango, umbu and passion fruit);

Central Region (State of Mato Grosso (araticum));

Southeast Region (state of Minas Gerais (passion fruit and tomato), Rio de Janeiro (passion fruit) and São Paulo (tomato));

Southern Region (State of Paraná (passion fruit))

The target plants and pollinators of these 13 projects are:

TARGET PLANTS	TARGET POLLINATORS	STATES
Annona muricata – “graviola” or soursop (Annonaceae)	*Cotalus* spp (Nitidulidae, Coleoptera)	Pernambuco
Annona crassifolia – “araticum” or marolo (Annonaceae)	*Cyclocephala* spp (Scarabaeidae, Coleoptera)	Mato Grosso
Hancornia speciosa – “mangaba” (Apocynaceae)	Sphingidae and Hesperidae (Lepidoptera)	Pernambuco
Spondias tuberosa – “umbu” or imbu (Anacardiaceae)	*Frieseomelitta* spp and *Trigona* spp (Meliponinae, Apidae, Hymenoptera)	Bahia
Mangifera indica – “manga” or mango (Anacardiaceae)	Diptera & Lepidoptera	Pernambuco and Bahia
Gossypium hirsutum – “algodão” or cotton (Malvaceae)	*Bombus* spp and *Xylocopa* spp (Apidae, Hymenoptera)	Pernambuco
Byrsonima crassifolia – “murici” or nance (Malpighiaceae)	*Centris* spp (Apidae, Hymenoptera)	Maranhão
Malpighia emarginata – “acerola” or west indian cherry (Malpighiaceae)	*Centris* spp and other Centridini (Apidae, Hymenoptera)	Pernambuco
Theobroma grandiflorum – “cupuaçu” or cupuassu (Sterculiaceae)	*Plebeia* spp, *Paratrigona* spp and *Frieseomelitta* spp (Meliponinae, Apidae, Hymenoptera)	Amazonas
Psidium guajava – “goiaba” or guava (Myrtaceae)	*Frieseomelitta* spp (Meliponinae, Apidae, Hymenoptera)	Bahia
Passiflora edulis – “maracujá” or passion fruit (Passifloraceae)	*Xylocopa* spp, *Centris* spp, *Epicharis* spp & *Eulaema* (Apidae, Hymenoptera)	Pernambuco, Bahia, Minas Gerais, Rio de Janeiro and Paraná
Passiflora alata & *Passiflora cincinnata* – “maracujá” or passion fruit (Passifloraceae)	*Xylocopa* spp (Apidae, Hymenoptera)	Pernambuco
Lycopersicon esculentum – “tomate” or tomato (Solanaceae)	*Melipona* spp (Meliponinae, Apidae, Hym.) and Halictidae (Hymenoptera)	Minas Gerais and São Paulo
Euterpe oleraceae – “açaí” or assai palm (Palmae)	*Melipona* spp (Meliponinae, Apidae, Hymenoptera)	Pará

THE NORTH AMERICAN POLLINATOR INITIATIVE

Michael Ruggiero
Stephen Buchmann
Laurie Adams

More than 218,000 of the world's quarter million flowering plants, including 80% of the world's crop plants, rely on animal pollinators for their reproduction and survival (Emblidge and Schuster, 1999; Buchmann and Nabhan, 1996). For more than a decade, biologists and conservationists have been concerned about apparent global declines in pollinators, especially those that pollinate crops, or those that migrate between regions. This concern about plant-pollinator interactions has contributed to a paradigm shift from protecting individual species to protecting inter-specific relationships, landscape-level ecological processes and threatened habitats.

While an awareness of these relationships and ecosystem services is not new to conservation biology, the recent attention given to these ideas by resource managers, policy-makers, environmental scholars, and the media has been unprecedented. Government officials have urged conservationists to embrace pollinator conservation, which benefits many species including humans. The U.S. Departments of Agriculture and Interior have defined activities and cooperative research and management agendas to address declines in pollinating animals (Tepedino and Ginsberg, 2000; Ruggiero and Healy, 2002). Many other organizations, institutions and individuals also have added plant-pollinator interactions to their conservation mandates and programs (Allen-Wardell *et al.*, 1998; Kremen and Ricketts, 2000).

The North American Pollinator Initiative is actually a portfolio of programs, projects, and activities from the public and private sector, connected by a spirit of cooperation. From 1996 to the present there have been several important milestones in North American cooperation: the Forgotten Pollinators Campaign (1996), the Sao Paulo Declaration on Pollinator Conservation (1998), Pollinator Roundtable and Seminar (1999), the Migratory Pollinators Workshop (1999), the Joint Interior - Agriculture Research Workshop (1999), the North American Pollinator Protection Campaign NAPPC (1999), and activities surrounding the International Pollinator Initiative (2002). Many of these activities are described or linked at <http://www.nappc.org> and <http://pollinators.nbii.gov>.

During 1994, the idea for a tri-national (U.S.A., Canada, Mexico) pollinator and plant conservation grassroots campaign was conceived by Stephen Buchmann, at the time a research entomologist with the federal Carl Hayden Bee Research Center in Tucson, AZ. With his colleague, Gary Nabhan, the two approached the administration of the Arizona-Sonora Desert Museum, also in Tucson, with their shared ideas for a pollinator conservation campaign based at the museum.

In early 1995, the Arizona-Sonora Desert Museum (ASDM) officially launched the Forgotten Pollinators Campaign (FPC) along with partners Bat Conservation International, The Bee Works, Centro de Conservacion para el Aprovechamiento de los Recursos Naturales

(CECARENA), Centro Ecologico de Sonora, Centro Intercultural para de Estudios de Desiertos y Oceanos (CEDO), Instituto del Medio Ambiente y el Desarrolio Sustentable del Estado de Sonora (IMADES), Instituto Technologico y de Estudios Superiores de Monterey (ITESM)/Guaymas, Pronatura Sonora, Programa de Conservacion de Murciealagos Migratorios (PCMM), the Seri Tribe, Universidad Nacional Autonoma de Mexico, Universidad de Sonora, the University of Arizona, the University of Miami, the University of New Mexico, and the University of Wyoming. The FPC had funding support from the Wallace Genetic and Wallace Global Foundations, the CS Fund, the National Fish and Wildlife Foundation, the Turner Foundation, and the Turner Endangered Species Fund.

As the centerpiece of the Forgotten Pollinators Campaign, Buchmann and Nabhan published a “call-to-arms” award-winning book, *The Forgotten Pollinators,* from Island Press, as a hardcover edition (1996) and paperback format (1997). A television documentary on endangered pollinators was also produced and released on the TBS television channel in 2000. The documentary was hosted and narrated by actor Peter Fonda, and was made possible with funding from the National Wildlife Federation and Turner Original Productions. A suite of 13 pollinator gardens by pollinator type was created as a permanent ASDM exhibit. Over 300,000 visitors view these gardens each year. The Forgotten Pollinators Campaign continued during the years 1995 until 1998, co-directed by Drs. Nabhan and Buchmann. After 1998, most of the educational materials and outreach efforts were handled personally by Dr. Buchmann of The Bee Works (www.thebeeworks.com). The FPC also hosted various symposia and workshops, which brought together scientists and policy makers in Tucson and other locations to work on the shared goals of pollinator and plant conservation. The FPC was highly successful and gave rise to other national, tri-national (including NAPPC) and now other international pollinator initiatives and legislation to protect plants and their pollinators.

In 1998, the Forgotten Pollinators Campaign was restructured as a multi-institutional, bi-national Pollinator Conservation Consortium (PCC). The PCC was formed to strengthen the ASDM collaboration with Bat Conservation International, the University of Arizona, the University of Miami, and several Mexican universities and research organizations (UNAM, UNISON, IMADES). PCC partners had the complementary skills needed to mount a large-scale bi-national effort. This approach of “Conservation across Borders” recognized that these problems could not be resolved by one sovereign nation alone, nor by a few scientists working in isolation. The migratory pollinators effort was led by ASDM creating bi-national tracking and research efforts that focused on four target species (Lesser long-nosed bats, Rufous hummingbirds, White-winged doves and Monarch butterflies) that travel north and south along “nectar corridors” between the United States and Mexico. The migratory pollinators effort received a majority of its funding from the Turner Foundation and ended in 2001.

The Sao Paulo Declaration on Pollinators raised international interest, provided strategic direction for pollinator conservation planning, and provided impetus for national and regional programs. The Pollinator Roundtable, hosted by the United States Secretary of the Interior gathered high level government and environmental leaders to hear experts describe the current state of pollinators in the United States and to suggest strategies for improvement. Suggestions included using existing wetland, waterfowl, and agriculture conservation programs; identifying nectar and migratory corridors for pollinators; engaging the golf industry in pollinator-friendly management practices; and incorporating pollinators into other Federal monitoring programs; among others.

In 1999, in Logan, Utah, scientists from the U.S. Departments of Agriculture and the Interior held a workshop to jointly develop a pollinator research agenda. The major areas identified for needed research included pollination and ecosystem health, landscape scale and patterns of pollinators and pollination, multiple factors affecting pollination, bioprospecting for and biodiversity of pollinators, and restoration and management practices for pollinator conservation.

Pollination and ecosystem health topics included monitoring long term changes in pollinator guilds; assessing the effects on ecosystem processes resulting from changes in pollinator services; examining the value of pollinator redundancy for stability of natural systems; assessing whether plants are pollinator-limited and assessing pollination deficits in natural systems, agro-ecosystems, and rare plant species; identifying model ecosystems and appropriate sites for research; delineating ecosystem services provided by pollinators; identifying vulnerabilities in plants or ecosystems to pollinator declines; examining the question of what is a healthy ecosystem for pollinators; compiling additional information on host associations of pollinators; and assessing spatial-temporal variability in pollinator assemblages and its effect on plant reproduction.

Research topics for landscape scale and patterns of pollinators and pollination included studying pollination dynamics at the agriculture and wild land interface; identifying and mapping nectar corridors and assessing their role; studying the required configuration of corridors for migratory species; studying the effects of landscape fragmentation; studying how pollinator movements link landscape units; predicting the effects of pollinator declines on landscape patterns; studying how change in pollinator guilds within a plant's range effects its productivity; assessing the effects of size and distribution of plant populations on pollinator communities; examining the effect of invasive species on pollinator movement in landscapes; and evaluating the potential contribution of invasive species to increases in pollinator populations.

Research on multiple drivers affecting pollination includes such topics as studying the effect of habitat fragmentation on pollinator dynamics and plant reproduction; evaluating the effects of interaction of multiple stresses on pollinators; developing IPM on managed pollinators; examining interactions of invasive species, native plants, and pollinator guilds; studying pesticide effects on native pollinators; developing decision support systems for screening potential invasive species; assessing the effects of genetically modified organisms (GMO's) on pollinators; and assessing the potential effects of climate change and extreme events on pollinators.

Bioprospecting and Biodiversity research topics include completing the "Bees of North America"; compiling the "Pollinators of North America"; examining the effects of bioprospecting; coordinating and accelerating candidates for pollination; enhancing technology transfer to industry; assessing and demonstrating value of set-aside lands for pollinators; determining relative pollination efficiency by various taxa; enhancing bioprospecting and screening of surrogate pollinators, including foreign prospecting; developing a gap analysis program for pollinators; developing and integrating museum databases; evaluating the use of pollinators for biomonitoring for agricultural and wild lands; developing new identification tools for bees; and enhancing taxonomic capabilities in Departments of Agriculture and Interior.

Research topics in restoration and management practices include assessing the influence of public attitudes and behaviors on restoration; developing methods to reintroduce pollinators to restored lands and mitigated habitats; developing propagation techniques for pollinators and host plants; studying impacts of disturbance (fire, grazing, etc.) on pollinators; evaluating conflicts of management practices on pollinators; developing methods for control of invasive species; developing bee garden methodologies and methods for habitat enhancement for pollinators (roadsides, hedgerows, golf courses, etc.); studying interactions of multiply managed species (pollinators); developing criteria to evaluate restorations (relative to pollination); monitoring restoration programs; assessing impacts of introduced pollinators on genetic diversity of native pollinators; and evaluating critical habitat size for pollinators and host plants.

The North American Pollinator Protection Campaign (NAPPC) was launched in 1999 by the Coevolution Institute. The campaign's stated goals were to raise the profile of the importance of pollinators *before* there is a crisis, encourage collaborative dialogue and public/private partnerships, support and distribute research and information, and determine and encourage best practices. NAPPC is a science-based voluntary collaboration that provides opportunities for individuals and organizations to affect knowledge, awareness, and behaviors. Its members come from the United States, Canada, and Mexico and include private industry, academia, government agencies, non-government organizations, and environmental groups. NAPPC currently has more than 70 partner organizations.

Major accomplishments of NAPPC to date include the following:

Affecting the public and decision makers:

1. A briefing and reception for U.S. Congressional and federal agency representatives, Canadian and Mexican Embassy staff, NAPPC partners, and industry guests at the U.S. Botanic Garden held on October 8, 2003. The reception featured pollinator foods served with identification of the responsible pollinator.
2. U.S. Botanic Garden/NAPPC Pollinator Gardens Exhibit and Photography Exhibits ("The Great Pollination Partnership") scheduled to open May 26, 2004. Predicted attendance will be 250,000 during the 4-month run of the exhibit.

Increasing on the ground conservation:

1. NAPPC - Wildlife Habitat Council Pollinator Friendly Practices Award. This award was given to PPG Industries in 2003 in front of 150 major international corporations. The award will be offered in 2004 as well. Interest in protecting pollinators by corporations continues to increase.
2. Comments to the USDA Natural Resources Conservation Service about its Conservation Security Program in order to provide incentives for farmers to protect pollinators.

Advancing research on pollinators:

1. NAPPC has supported a proposed National Academy of Sciences study to survey the status of pollinators in North America. Chaired jointly by the Board of Life Sciences and the Board of Agriculture and Natural Resources, this survey has received more than 40 letters of support from institutions and agencies as diverse as the Environmental Defense Fund, the U.S. Farm Bureau, the U.S. Florists Association, and the U.S. Apple Growers Association.
2. Support for standardized protocol in bee monitoring. This cooperative effort among North American scientists will be widely distributed for use in research around the world.

"Cross-pollinated" information and ideas:

1. Three international NAPPC Meetings have been held 2000, 2001, and 2003.
2. A subscription-based NAPPC- LISTSERV has been established for more than two years. This administered service delivers news that is pertinent to pollination issues to all interested stakeholders. Individuals and institutions can join the LISTSERV by contacting NAPPC@coevolution.org.
3. NAPPC national meetings have been planned for Mexico and Canada.
4. The Turner Foundation and NAPPC collaborated to produce an independent report called "Funding Opportunities for Foundations." The report is available online at <http://www.ceaconsulting.com/clients/case_studies.html> or by contacting NAPPC at nappc@coevolution.org. It gives an overview of the pollinator issue and recommendations for immediate measures to attract funding to solve the problem.

Actions Affecting Policy:

1. NAPPC partners supported the jointly sponsored South Africa/United States meeting on international pollinator conservation policies. Several NAPPC partners are working with other international experts to complete the handbook begun at the meeting. NAPPC will help facilitate distribution of the final product.
2. The NAPPC Conservation Committee has provided comments to the U.S. Department of Agriculture to ensure that pollinators are given consideration in programs administered through the Natural Resources Conservation Service.
3. NAPPC has joined the U.S. Environmental Protection Agency's Pesticide Environmental Stewardship Program to help influence members to include pollinator protection in their work.

Ongoing and planned NAPPC activities include the following:

1. Revising the booklet entitled "How to Reduce Bee Poisoning From Pesticides." The booklet will be updated to eliminate pesticides that are no longer registered and to add any new pesticides. The booklet will also be redesigned and reoriented to become a resource sought by agricultural concerns.
2. Designing and funding a collaborative research proposal to study effects of native pollinators on specific crops in a variety of geographic areas.
3. Planning and convening the 4th NAPPC Conference at the Smithsonian Institution in Washington, D.C. on 20 and 21 September, 2004.
4. Including a session at the 4th NAPPC Conference on *Bombus terrestris* and inadvertent movement of this pollinator into the habitat of native *Bombus* species.
5. NAPPC will begin working with State Conservation Departments on a state-by-state basis. Montana and Indiana will be the first two states.
6. NAPPC will create a list of questions about pollinators for state pesticide applicator examinations (distributed through the federal EPA and administered by the States).
7. NAPPC will continue to participate in the Pesticide Environmental Stewardship Program (PESP) through the U.S. Environmental Protection Agency.
8. NAPPC will administer the second year of the NAPPC-Wildlife Habitat Council Pollinator Protection Award.
9. NAPPC will sponsor the "traveling" U.S. Botanic Garden-NAPPC Pollinator Photo Exhibit.
10. NAPPC will establish a funded NAPPC Coordinator position in 2004 in Washington, D.C. to serve as a resource for NAPPC partners and to increase the visibility of pollinator issues.
11. NAPPC will continue to follow its Implementation Plan as developed by the ongoing work of NAPPC standing committees (Research, Conservation, Education, Policy, and Partnerships).

The International Pollinator Initiative (Convention on Biological Diversity, 2002) has influenced the sponsorship of two key projects supported by the U.S. government: 1) a collaboration with Brazil to sponsor the development of a Checklist of Bees of the World and 2) a collaboration with South Africa to develop a Handbook on Pollinator Conservation Policy and Practices. Both projects have recruited and involved experts from around the world.

The World Bee Checklist Project (October 2002), begun at a workshop in Indaiatuba, Brazil, has produced a checklist of Colletinae of the World, a preliminary database of bee collections of the world, a preliminary database of bee taxonomists of the world and a commitment to complete and integrate existing bee checklists from Africa, North America, Australia, Brazil, Colombia, Mexico, and Central America into a global database.

The Handbook of Pollinator Conservation Policies and Practices begun in May 2003, at a workshop in Warmbaths, South Africa will provide an international compilation of best practices and policies in pollinator conservation that is targeted to resource managers and policy-makers.

In an era when human activities have reshaped the world and placed increasing demands on both natural and rural landscapes, we cannot ignore the vital services played by pollinating animals. We need to work to bring international cooperation, policies and best practices that stabilize and protect one of our important, underlying ecological support systems: the often misunderstood, little appreciated, and undervalued world of flowering plants and their pollinators. To successfully confront an impending pollination crisis, we must work together globally and as dedicated nations and citizens. Foresters, extension agents, farmers, entomologists, conservationists, gardeners and policy makers must devise workable plans for flowering plants that include the needs of pollinators. Educators must emphasize the importance of pollinators and pollination services in wild and agricultural lands and their interconnectedness.

The North American Pollinator Initiative provides a good model for regional cooperation in that it takes full advantage of public and private institutions and the strengths of each. The public institutions provide policy direction, scientific, and management support at all levels of government, while the private institutions provide actions and consciousness-raising from the "grass roots" to the international arenas.

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EUROPEAN POLLINATOR INITIATIVE (EPI): ASSESSING THE RISKS OF POLLINATOR LOSS

Edited by Simon G. Potts

A) Primary Objective

To integrate pan-European expertise relating to pollination into a cohesive network, in accordance with the aims of the International Pollinator Initiative, to overcome the currently fragmented activities of scientists, end-users and stakeholders.

B) Background

The International Convention on Biological Biodiversity (CBD) specifically cites pollination as a key ecosystem function that is threatened globally. This ecosystem service not only ensures production value in crops but is critical to the survival and maintenance of the diversity of plant populations. The São Paulo Declaration on Pollinators (1999), based on the available global evidence at the time, reported that 'the numbers of honeybee colonies have decreased dramatically' and 'the numbers of native bees are dwindling, some species seriously so'. The stated plan of action of the CBD International Pollinator Initiative is to:

1. Monitor pollinator decline, its causes and its impact on pollination services
2. Assess the economic value of pollination and the economic impact of the decline of pollination services
3. Address the lack of taxonomic information on pollinators
4. Promote the conservation and the restoration and sustainable use of pollinator diversity in agricultural and related ecosystems.

These global objectives are equally relevant at the European level and reflect EU concerns about pollinator loss and associated risks. The question of whether the perceived pollinator declines are real has received a great deal of attention recently. For Europe, regional comparisons between historical and actual distribution data revealed a significant loss for Non-*Apis* bee diversity in at least six European countries (e.g. Corbet *et al.*, 1991; Day, 1991). However these data sets are restricted to particular regions and did not attempt to relate observed changes in species distributions to possible driving forces.

C) Organisation

The European Pollinator Initiative (EPI) has adopted the same overall framework as the International Pollinator Initiative. The four key components being: assessment, adaptive management, capacity building and mainstreaming (Figure 1). Given that the rate and extent of pollinator loss throughout Europe still remains to be fully quantified, the primary focus of the EPI is on the assessment of the problem. This is provided by the European Union Framework 6 project **ALARM** (**A**ssessing of **LA**rge-scale environmental **R**isks with tested **M**ethods). The ALARM consortium combines the expertise of 54 partners from 26 countries and has a centrally funded work programme for an initial period of 5 years (starting 1st February 2004). Research will focus on assessment and forecast of changes in biodiversity and in structure, function, and dynamics of ecosystems. This relates to ecosystem services and includes the relationship between society, economy and biodiversity. In particular, risks arising from pollinator loss, climate change, environmental chemicals and biological invasions in the context of current and future European land use patterns will be assessed.

SUPER (**S**ustainable **U**se of **P**ollinators as a **E**uropean **R**esource) will build directly upon ALARM to address identified declines in European pollinator resources in a socially and economically viable way. SUPER will focus upon adaptive management, capacity building and mainstreaming issues to overcome the economic and biodiversity costs associated with pollinator loss (Figure 1). To achieve this, there is an urgent need for an innovative and multi-disciplinary approach to understand the function and value of pollination in natural and agricultural systems and develop sustainable management of this key resource. Only by developing and integrating ground breaking trans –disciplinary research, and directly linking this to the expertise of the relevant end-users and stake holders, can pollination be sustained and improved throughout Europe. SUPER ims to achieve this through the:

- Development of a state-of-the-art understanding of the complex ecological, behavioural and evolutionary driving forces of plant-pollinator interactions;
- Building of European taxonomic capacity for pollinators;
- Elucidation, promotion and facilitation of best land-use and conservation
- practices to restore and conserve pollinator communities;
- Promotion of the long-term sustainable management of pollinators as a standard
- practice in all major agricultural and natural systems within the EU;
- Maximisation of the socio-economic benefits from effective pollination services.

This will be realised through complementary trans -disciplinary research programmes, enhanced training and mobility of relevant parties, and direct linking of scientists with policy makers, end-users and the commercial sector. The EPI aims to implement SUPER once ALARM has clearly quantified the rate and extent of pollinator loss across Europe.

D) ALARM: Assessing large-scale environmental risks with tested methods

1. Organisation and objectives

The ALARM consortium comprises four independent modules assessing risks consequent on: Pollinator loss, Climate change, Invasive species and Environmental chemicals (Figure 2). These four modules are integrated into a cohesive research programme through a series of ‘cross-cutting’ links which include socio-economics and land use patterns.

With respect to pollinators, ALARM will identify indicator groups to develop thresholds for the quantification of: (1) pressure (probability of pollinator loss) linked with (2) impact (consequences of loss of pollination function). This ecological basis for risk assessment will use a package of standardized protocols developed to allow comparable assessments to be undertaken in different ecosystems and in different EU regions.

Specific scientific and technological innovations will include:

- Development of rigorously standardised protocols for pollinator abundance, diversity and pollination services to agricultural crops and wild plants;
- Establishment of long-term monitoring schemes across Europe;
- Quantification of pollination requirements of key European crops and wild plants;
- Building a definitive catalogue of pollinator taxa and functional groups with regionally relevant risk probability evaluations;
- Production of a European (and first continental scale) pressure and impact risk assessment map;
- Identification of vulnerable ecosystem types, risk zones and pollinator groups;
- Development of predictive models for pollinator risk assessment;
- Identification of the drivers of pollinator loss at the local, national, and continental level and an understanding of the synergism between drivers at different scales ;
- Construction of a knowledge base to underpin future research programmes for the conservation, restoration and sustainable management of pollinators in agriculture and related ecosystems.

2. Work Programme

The work programme is divided into two broad and complementary blocks. The first aims to assess the risk probability of losing pollinators across Europe. The second focuses on the impact of this loss on ecosystems services i.e. pollination.

2.1 Assessing the risk probability of pollinator decline

To determine the risk of losing pollinators and make future predictions it is necessary to quantify the distribution shifts in key pollinator groups across Europe. ALARM will realize this through three approaches: (1) Data Mining; (2) Repeating Historical Observations; and (3) Developing Standardised Methods .

2.1.1 Data mining

A substantial amount of data relating to European pollinator distributions is contained within museum and private collections; electronic databases; published literature; and unpublished records. These resources differ markedly in their taxonomic and geographical coverage and also their accessibility to researchers. The majority of governmental statistics on pollinators refer exclusively to managed honeybees. This highlights the necessity to bring EU taxonomic resources into a cohesive centralized structure and underpin ongoing field collection and taxonomic work. Analysis of the data will generate a substantial set of point estimates of shifts in pollinator communities across Europe and allow us to map regional and continental-wide shifts in distributions and pollinator diversity.

2.1.2 Repeating Historical Observations

A substantial literature of pollinator observations was amassed in the late 19th and early 20th centuries, much of which consisted of lists of species found visiting a focal plant species at a focal site. A subset of the most useful will be selected on the basis of geographic coverage, taxonomic composition and other criteria. The historical surveys will then be repeated, keeping as close to the original protocols as possible. In tandem, with this contemporary standardised techniques will be used to calibrate the historical methods and allow comparison across sites. This approach will deliver a series of point estimates for changes in pollinator distributions which can be combined in a higher order analysis.

2.1.3 Developing Standardised Methods

Given the variety of insect orders European pollinators include, and the range of plant forms and habitats they are found in, it is hardly surprising that approaches to assessing the abundance and diversity of pollinators are equally as varied. The large variety of methods, spatial scales, time periods, taxonomic groups and sample units employed make it very difficult to reliably compare and up-scale the results of existing surveys.

A wide range of potential methods will be tested in parallel in several European regions using replicated sets of (semi-)natural and agricultural habitats. Methods being tested include: standardised transect walks; non-standardised transect walks; trap nests (Figure 3); water filled pantraps; intercept traps and focal observation plots. Species accumulation curves, rarefaction methods and species estimator techniques will be used to assess the optimal sampling effort for different methods. The intensive sampling effort on the focal study sites will result in a more or less complete survey of all pollinator species. This provides a the basis to test the indicator value of a variety of sampling methods, samplings efforts or different species groups by relating them to the known total species richness.

In tandem with the assessment of species diversity, there will be an assessment of genetic diversity for key groups of pollinators. The method development will include the screening of microsatellite DNA loci and testing for optimal sample size for measuring the genetic diversity of bee populations.

2.1.4 The Impact of Landscape Complexity

Using standardised methods will allow an analysis of the effects of habitat fragmentation and land use on pollinator communities in different European regions and ecosystems.

a) Impact of landscape complexity

Case studies, along regional gradients of increasing habitat fragmentation and land use intensity in selected European regions, will allow quantification of the impact of intensification on pollinator communities (Figure 4). The effects of habitat area, habitat isolation and landscape context on pollinator communities, the most threatened regional habitat types will examined. In each region replicated study sites will be selected covering a gradient of habitat patches from very small to large, and isolated to well connected. The impact of the surrounding landscape matrix will be assessed by comparing species area relationships on (1) real islands, (2) terrestrial habitat islands in a structurally complex landscape matrix, and (3) habitat islands in an intensively managed agricultural landscape matrix.

b) Impact of disturbance Intensity

A second focus will be on the effects of different management and disturbance regimes of threatened semi-natural habitat types on habitat quality and consequent effects on pollinator communities. Replicated pair-wise comparisons of intensive vs. extensive disturbance regimes will be tested in different European regions. The regional variation in dominant management regimes and socio-economic drivers will also be considered.

c) Impact of organic vs. conventional farming
The impact of organic farming on pollinator communities will be studied in different European regions so as to cover Mediterranean and temperate areas. In each region paired farms with organic and conventional management practices will be selected with increasing proportions of semi-natural or natural habitats.

d) Impact of agricultural policy
Pollinator diversity in agricultural landscapes that have been subject to long-term EU agricultural policy will be compared with traditional agricultural landscapes of an accession country to get an insight into the future impact of agricultural policy in accession countries.

2.2 Impact on Pollination Services

In addition to understanding distribution changes in pollinators it is essential to quantify changes in the function of pollinators in providing ecosystem services. Changes in the pollination services to both crops and wild plant systems have important socio-economic and biodiversity implications. Three tasks are being undertaken to assess the consequences of pollinator loss on pollination services: (1) development of standardised methods to measure pollination limitation; (2) quantification of pollination failure in natural, and (3) agricultural ecosystems.

2.2.1 Testing Methods to Assess Pollination Limitation

There is very extensive literature dealing with methods for quantitatively determining pollination limitation and the efficiency of floral visitors in providing pollination services to crops and wild plants (Dafni, 1992; Kearns and Inouye, 1993). Pollen-limited systems are those in which plant reproductive output increases with the addition of compatible pollen on stigmas. The variety of methodologies and protocols that have been developed reflect the wide range of plant functional groups that have been studied and the variety of ecological, evolutionary and economic questions asked about plant-pollinator interactions (Figure 5). Currently, no single protocol is applicable across different plant groups or allows easy comparability across regions. However, the number of techniques currently used to assess pollinator abundance, diversity, and pollination services, provide a clear basis for the development of integrated methodologies allowing rapid, reliable and repeatable assessments across plant functional types and in different geographic regions.

We will review extensive existing literature on methodologies and select the most promising subset of methods to assess pollination limitation based on their applicability to a range of flowering plant types across regions, ease of use, and repeatability. In replicated field experiments we will then calibrate the selected methods against the recognised state of the art methods to identify those which can most reliably adopted as part of the standardised protocol.

2.2.2 Assessing the Impact of Pollinator Shifts on Wild Plants

More than 80% of all wild plant species depend on insect pollination and an estimated 62% of plants studied show actual pollination limitation (Burd, 1994). Yet for wild plants, there are only few studies that have examined their pollination needs and the consequences of pollen limitation on their survival (Wilcock and Neiland, 2002). Plant ecologists have found clear evidence that small and isolated plant populations have reduced seed set (Matthies *et al.*, 1995), suffer from genetic erosion (Oostermeijer *et al.*, 1994) and face a higher extinction risk (Fischer and Stöcklin, 1997). However, it is still unclear as to what extent shifts of pollinator communities threaten the survival of rare plant species.

The survival and maintenance of genetic diversity of many wild plant populations depends largely upon pollinators other than honeybees (Kearns *et al.*, 1998). It has been recognised that many plant and pollinator traits are associated with the risk of disruption of their interaction (e.g. Bond, 1995). For instance, plant-pollinator interactions vary in their levels of specificity and consequently plants with more obligate and specialist pollinators will be more vulnerable to extinction.

We will assess the effects of habitat fragmentation and disturbance regimes on pollination limitation for rare plant species occurring across Europe, using standardized methods developed. Different representative species will be used as model plants occurring on comparable habitat types. We will monitor insect species composition and flower visitation rates in target plant populations on semi-natural grassland habitats covering a fragmentation gradient and relate these observation to fruit or seed set.

Additional experiments will test for effects of genetic fitness loss and gene flow between isolated plant populations in situations with reduced pollinator availability. Standardised protocols in all studies provide the opportunity for between-species comparisons as well as cross-comparisons between regions.

A second focus will be on the effects of the community succession (e.g. after fire) on plant-pollinator interactions that may lead to elimination of native plant species (Figure 6). This will be done in the Mediterranean area and compared to successional changes in semi-natural grassland habitats in across Europe. Distinct post-fire community age classes will be considered in each region. The variation of plant-pollinator relationships and the resulting fruit and seed-set of open-pollinated flowers will be evaluated in terms of quantity and quality.

2.2.3 Assessing the Impact of Pollinator Shifts on Crops

It is estimated that 84% of EU crops are directly dependent upon biotic pollination (Williams, 1994) as are the majority of wild plants. Pollination limitation results in reduced economic yields due to lower fruit set and seed set, reduced fruit or seed quality or a lower germination ability (review in Free, 1993). Crop pollination by honeybees alone has been estimated to be 5-14 billion dollars per year in the US (Kremen *et al.*, 2002) and 4.25 billion ecus for the EU (Borneck and Merle, 1989). Honeybees have been estimated to carry out 85% of the pollination for EU crops requiring insect-mediated pollen transfer (Borneck and Merle, 1989). This highlights the high level of dependency of European agriculture on honeybees, and associated high risks of relying on a single pollinator species. The value of non-*Apis* pollination services to individual crops is unknown in general, but the few studies to date demonstrate that diverse native pollinator communities make a significant contribution to crop production (e.g. Kremen *et al.*, 2002), and that pollination services are lost due to inappropriate use of pesticides (Kevan, 1975).

To improve the understanding of pollination services in European agro-ecosystems, case studies set in the Mediterranean and Central Europe will focus on pollinator shifts in agricultural landscapes and consequent impact on crop pollination. A focal crop species with regional model characteristics will be chosen. In each region replicates of paired study sites with low (organic farming) and high (intensive farming) local environmental risk pressure will be selected embedded in a gradient ranging from simple to complex landscape matrix. This experimental design makes it possible to test independently for both, local environmental risks from pesticide use and regional risks from habitat loss of natural and semi-natural habitats.

2.3 Assessing Multiple Environmental Risks to Pollinators

Our understanding of the environmental drivers of pollinator loss comes from a series of case studies with limited value for large-scale risk assessment. Even though most of continental Europe has not been assessed for pollinator loss, declines are likely to be widespread as the known drivers of loss are also widespread. ALARM will quantify the relative importance and combined effects of the main drivers of pollinator shifts and their consequences on ecosystem functions. This will be approached in two ways: establishing a pan-European focal site network and by examining the interactions between pollinator loss and potential drivers both on their own and in combination.

2.3.1 Focal Site network

To provide the necessary testing ground for aspects of ALARM, a focal site network is being established with a comprehensive biogeographical and environmental coverage of Europe. The aim for the pollinator module is to provide a longer-term monitoring scheme for pollinators, and allow cross-cutting experiments between pollinator and other ALARM modules to be set within a pan-European framework. The site network will utilise a subset of the existing locations of ALARM partners where appropriate (see Figure 7).

2.3.2 Multiple Drivers of Pollinator Loss

Several drivers of pollinator loss have been identified for Europe: changing la nd use practices including agricultural intensification (Banaszak, 1995), habitat fragmentation and isolation (Steffan-Dewenter and Tscharntke, 2002); agrochemicals (O'Toole, 1993); fire (Potts *et al.*, 2003); disease and parasite spread (Watanabe, 1994); climate change (Warren *et al.*, 2001); introduction of non-native plants (Chittka and Schürkens, 2001) and competition with managed pollinators (Butz-Huryn, 1997). The pollinator module within ALARM will interact with the climate change, environmental chemicals, invasive species and socio-economics modules to identify the large-scale drivers of pollinator loss across Europe:

a) Climate Change
Pollinator communities will be monitored on a network of European field sites using standardised methods. Monitoring of pollinator communities will include methods to assess genetic diversity of selected pollinator species as well as species richness, density and community structure. Multivariate statistical analyses will be used to relate the distribution of single pollinator species and pollinator guilds to climatic parameters. These results can then be used to predict potential shifts of pollinator communities in climate change scenarios.

b) Environmental Chemicals
Comparing agricultural practices between focal sites at which pollinators are being studied, and, knowing the proximity to non-agricultural pollution sources we will be able to estimate risks to pollinators from the chemical and land-use pressures they were under. Further we will

develop a risk assessment tool using pollinators and their forage products to integrate multiple pathways by which chemicals enter the environment. Bees and their forage products will be sampled from the focal network of sites and the concentration of chemicals measured using body burdens and extractions from pollen loads, pollen stores and honey. The utility of these newly developed methods will be tested by comparing results against known levels of various environmental chemicals at the same sites.

c) Invasive Species

We will test for the potential effect of pollination failure in slowing the movement of self-incompatible invasive plant populations. This will allow us to estimate the degree to which reproduction at advancing edges of distributions is influenced in self-incompatible species, relative to patterns displayed in self-compatible counterparts. In addition we will, quantify the impact of escaped domestic bumblebees on the genetic makeup of wild populations of the same species across Europe. A combination of large-scale field sampling, laboratory work, and regional experiments will be used to quantify the genetic impact of invasive bees.

d) Socio-Economics

There are two approaches: (1) Pressure and trend analysis; and (2) Policy driver analysis. The first aims to link the drivers of biodiversity pressures to their underlying socio-economic driving forces as a precondition to derive conclusions regarding policy formulation on the European and the national level. This will identify the most relevant socioeconomic driving forces, be they global trends, or the results of European or national politics. The second approach aims to identify the crucial elements for biodiversity protection beyond broad trends and to develop easy-to-communicate but directionally secure indicators as tools to highlight immediate action priorities, and as instruments for policy monitoring and assessment.

e) Multi-level Interactions

ALARM will integrate outcomes from the modules on pollinators, biological invasions, climate change and environmental chemicals and produce matrices in which the pressures can be ranked and weighted for their severity, extent and duration so that risk indices can be produced. Outputs from this work will be relevant to socio-economic studies as a matrix of this form, whose contributory parameters were understood and extractable as indicators, could be used to help decide whether what should/needs to be protected and can in fact be protected without great disruption to the economy and society of the EU and its neighbours. We will use these rules to create an overall model of the interactions - initially at a qualitative and conceptual level and then adding more quantitative material. These rule based models will provide information on overall risk and hazard hot-spots across several scales and will develop an information system permitting the analysis of the relative importance of threats and pressures. Especially, it will help to identify those factors which contribute most to risk, hazard or the resulting peril, in a specific region. A product will be specific maps of risk, hazard or peril including most if not all aspects regarded within ALARM on different scales.

2.3.3 Testing Predictions for Pollinator and Ecosystem Function Loss

ALARM will produce high quality data sets relating to European-scale pollinator distributions and provision of pollination services. A key requirement for the testing of large-scale risk assessment predictions is the identification of pollinator, floral and habitat traits associated with increased risk. This is necessary to underpin the construction of prediction maps indicating European areas most likely to experience loss of pollinators and consequent ecosystem function. Risk criteria are likely to be related to pollinator species traits (e.g. taxa, body size, degree of specialisation, range size or local abundance etc.); flower species traits (e.g. taxa, breeding system, flowering period, rarity, degree of specialisation etc.) and also site traits (e.g. latitude, longitude, elevation, habitat, landscape structure, degree of disturbance, isolation and dominant local land use). Site and species risk criteria may interact; for instance, certain pollinator groups may be at risk in particular habitats, whereas other species may thrive under the same conditions Such criteria may allow conclusions to be drawn as to which European pollinators and pollination systems are most vulnerable and therefore need of protecting and conserving.

3. Output and deliverables

ALARM will deliver a number of specific outputs, including:

- An integrated European pollinator database
- Risk assessment toolkit for pollinators and pollination
- Continental assessment of the economic and biodiversity risks
- Quantification of the drivers of pollinator loss
- Predictive models for pollinator loss and consequent risks
- Knowledge base to underpin future development of the European Pollinator Initiative (e.g. SUPER).

4. Dissemination and Training

The objectives of dissemination are (1) to interact with stakeholders to define research needs and ensure applicable products (2) to establish an advisory board of users for regular consultations, (3) to communicate and disseminate results to the scientific community (scientific articles, conferences), (4) to communicate and disseminate results to the general public (project website, brochures, articles in popular journals and newspapers), and (5) to disseminate major project results to other partners. ALARM will provide advanced training to researchers, key staff and research managers, to provide training for industrial executives and potential users and to organise exchange programs for PhD students at partner organisations to facilitate the exchange of know-how.

5. Partners for the Pollination Module of ALARM (Total number of partners is 54).

Göttingen University (Germany): Department of Agroecology, Dr. Ingolf Steffan-Dewenter, Dr Catrin Westphal and Birgit Meyer

Aegean University (Greece): Department of Geography, Prof. Theodora Petanidou, Dr Ellen Lamborn and Olivia Messenger.

Reading University (UK): Centre for Agri-Environmental Research, Dr. Simon G. Potts and Mr. Stuart Roberts

Institut National de la Recherche Agronomique (France): Laboratoire de Pollinisation Entomophile, Dr. Bernard Vaissière

Leeds University (UK): Centre for Biodiversity and Conservation, Dr. Bill Kunin and Prof Chris Thomas
Natural History Museum (UK): Department of Entomology, Dr Andy Polaszek, Mr. George Else and Dr Paul Williams

Haifa University (Israel): Laboratory of Pollination Ecology, Prof. Amots Dafni and Dr Gidi Ne'eman

Jagiellonian University (Poland): Prof. Michal Woyciechowski

Swedish University of Agricultural Science (Sweden): Prof. Jan Bengtsson and Dr Riccardo Bommarco

Halle-Wittenberg University (Germany): Prof Robin Moritz and Dr Bernd Kraus

Oxford University Museum (UK): Mr. Chris O'Toole and Dr Mark O'Neill

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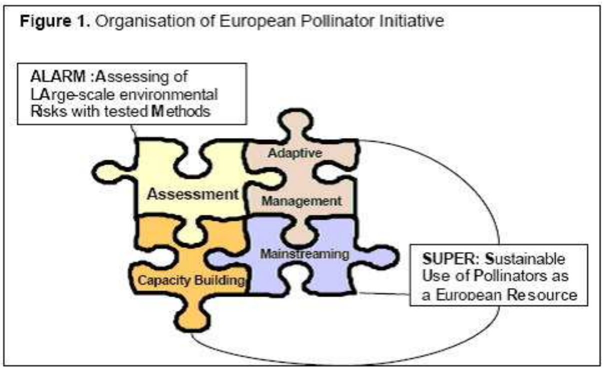

Figure 1. Organisation of the European Pollinator Initiative.

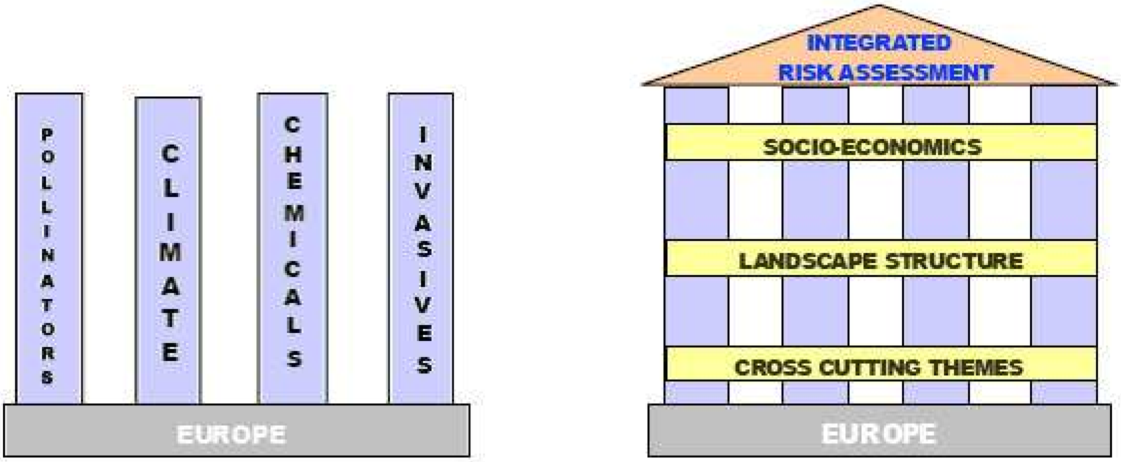

Figure 2. Organisation of the ALARM project.

Figure 3. Several methods, including trap nests, will be tested to develop a toolkit for pollinator assessment.

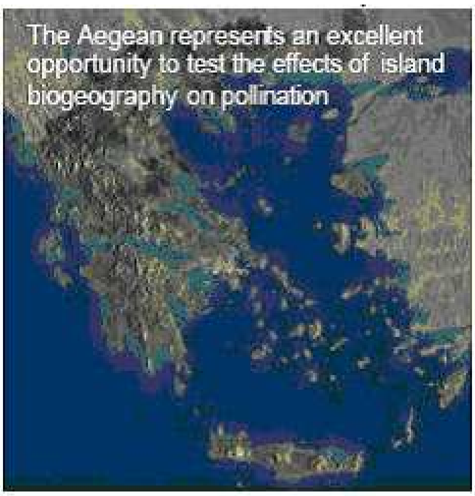

Figure 4. ALARM will include many case studies across Europe, including an assessment of the impacts of fragmentation and isolation on plant-pollinator communities.

Figure 5. ALARM will develop a toolkit of methods to assess pollination limitation in both crops and wild plants.

Figure 6. Multiple drivers of pollinator loss will be studied across Europe.

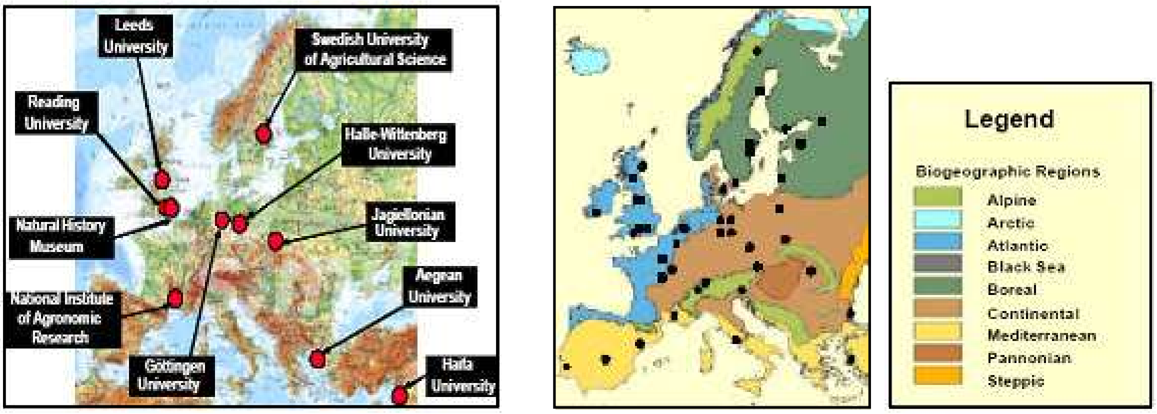

Figure 7. Location of Pollinator module partners (large red dots) and all ALARM partners (small black dots) with respect to the major biogeograhic regions across Europe.

AN OVERVIEW OF POLLINATORS RESEARCH AND DEVELOPMENT IN THE HINDU KUSH-HIMALAYAN REGION

Uma Partap

Abstract

Pollinators play an important role in poverty alleviation and food security by pollinating crops and other plants. But, in recent years the nature is experiencing a serious decline in pollinator populations and diversity, the impact of which is clearly visible in several areas as the agricultural productivity is seriously declining as a result of inadequate pollination due to the lack of sufficient numbers of pollinators in these agricultural landscapes. This decline in pollinators is attributed to several factors such as habitat loss, land-use changes, monoculture-dominated agriculture, and excessive and indiscriminate use of agricultural chemicals and pesticides. The decline in pollinators, both number and diversity and its impact on agricultural productivity and biodiversity has become a major concern worldwide. In developing world this issue is relatively new and needs due attention at this stage. There is need to study the factors and causes of pollinator decline and suggest an integrated approach to conserve them. This can be done by developing best management such as integrated pest management, judicious use of pesticides and agrochemicals, promoting habitat conservation and managing food sources for pollinators. In addition, raising awareness about the importance of pollinators and educating and training communities in conservation practices may also be required. At the same time bringing conservation and development GOs, NGOs, and CBOs on a common platform and formulate strategies and actions plans will also be needed. A balance between policy formulation and conservation actions would yield better results. There is need to promote integration of pollinator conservation concerns with agricultural development. In some intensively cultivated areas there is need to suggest some readily available solutions such as promoting rearing and use of manageable pollinators such as honeybees, stingless bees etc. The international Centre for Integrated Mountain Development (ICIMOD) is trying to address the issues related to the declining pollinator populations through conducting research, raising awareness at all levels about the value of conserving pollinators and promoting use of honeybees for pollination, improving institutional capabilities and human resource development. This paper highlights the efforts being carried out by ICIMOD's efforts in enhancing agricultural productivity and maintaining biodiversity through promoting conservation of pollinators and managed pollination.

Introduction

Pollinators play an indispensable role in improving agricultural productivity and maintaining biodiversity through providing essential ecoservice – 'pollination', which is a prerequisite to fertilization and is, therefore, crucial for seed/ fruit set. A large majority of people in the Hindu Kush-Himalayan (HKH) region depends primarily on subsistence agriculture and renewable natural resources for their livelihood. Mountain agriculture is largely based upon seasonal variations and is also dependent on crucial support from sustained ecological services.

ICIMOD's past work experiences, spanning more than a decade, showed that pollinators in general and honey bees in particular provide highly needed ecological services of pollination in mountain environments and can contribute effectively towards cash income from honey and other bee products.

Keeping in view a steady decline in pollinator populations, the agriculture sector has suffered from low productivity due to the lack of pollination services. ICIMOD studies revealed that the productivity of crops, for example apple is declining throughout the HKH region and this decline is a result of inadequate crop pollination (Partap, 2001, 2002; Partap and Partap, 1997; Partap *et al.*, 2001). ICIMOD studies have also revealed that due to the absence of pollinators a few apple farmers in India and China are trying to manage it using different approaches to manage pollination of their crops. While farmers in Himachal Pradesh, India are using honeybees for this purpose, those in China are pollinating their apple orchards by hand. Unless productivity of subsistence agriculture, pastures, and forests are increased it is difficult to achieve the goals of poverty reduction especially when renewable natural resources provide the basis for livelihoods.

ICIMOD understood the importance of conserving natural pollinators and its role in sustaining agricultural productivity and biodiversity maintenance and is trying to address this issue of declining pollinator populations for the past one decade through promoting conservation of indigenous pollinators on one hand and managed pollination on the other.

Review of Pollination R&D Work in Asia

Pollination is an essential ecosystem service, which is crucial for the production of fruits and seeds in plants and therefore contributes in enhancing agricultural productivity and conserving biodiversity. It has been estimated that over 75% of the world's crops and over 80% of all flowering plants depend on biotic pollinators. Globally, the annual contribution of pollinators to agricultural crops has been estimated at about US$ 54 billion (Kenmore and Krell, 1998). In the USA, native pollinators are estimated to provide annual pollination services worth US$ 4.1 billion to agriculture (Prescott-Allen and Prescott-Allen, 1990).

A great deal of research has been carried out in the HKH region to show the impact of pollination especially by honeybees - *Apis cerana* and *Apis mellifera* on various crops. They are reported to play a vital role in enhancing the productivity levels of different crops such as fruit and nuts, vegetables, pulses, oilseeds, spices, fiber and forage crops. A number of studies have been done to show the impact of honeybee pollination on different cash crops e.g., apple (Dulta and Verma, 1987; Gupta *et al.*, 1993), peach and plum (Partap *et al.*, 2000), citrus (Partap, 2000a), kiwi (Gupta *et al.*, 2000) and strawberry (Partap, 2000b). Bee pollination did not only increase the fruit set but also reduced fruit drop in apple (Dulta and Verma, 1987), peach and plum (Partap *et al.*, 2000) and citrus (Partap, 2000a). Reports have also indicated an increase in the fruit juice and sugar content in citrus fruits (Partap, 2000a). Honeybee pollination reportedly reduces the percentage of misshapen fruits in strawberry (Partap, 2000b). Research has also been conducted to show that honeybee pollination enhanced seed production and quality of seeds in various vegetable crops such as cabbage, cauliflower, radish, broad leaf mustard, and lettuce (Partap and Verma, 1992, 1994; Verma and Partap, 1993, 1994), asparagus, carrots, onion, turnips and several other crops (Deodikar and Suryanarayana, 1977). Recent experiments carried out in different parts of the Himalayan region show that honeybee pollination does not only increase fruit set in rapeseed and sunflower but also increases the oil contents in these oilseed crops (Singh *et al.*, 2000).

Pollinators/ Pollination Programme at ICIMOD

Scientists and researchers in the universities and agricultural research institutions across the Hindu Kush-Himalayan region are engaged in pollinators/ pollination research primarily of academic interest. As explained, the emphasis has been to study the impact of honeybees as pollinators of crops. There is only a little focus on applied research and development issues related to pollinators in the HKH region. However, in recent years a lot of interest is being shown in pollinators and focus is given to the assessment of the role of pollinators in plant biodiversity globally due to the realization of the fact that there is worldwide decline in pollinators, which needs to be addressed. The Convention on Biological Diversity (CBD) has, therefore established the International Pollinator Initiative (IPI) to address this issue at global level, and invited FAO to facilitate and coordinate this in collaboration with other relevant organisations.

The International Centre for Integrated Mountain Development (ICIMOD) initiated its pollinator / pollination programme in 1991 to address applied research, development and policies related issues of pollinators and pollination. The overall goal of ICIMOD's pollinator / pollination programme is to improve livelihoods of mountain people through enhancing agricultural productivity and biodiversity conservation through conservation of indigenous pollinator species including honeybees and beekeeping to ensure sustainable pollination of crops and other indigenous plant species of the HKH Region. The specific objectives are: to study and assess crop pollination problems in mountain areas, assess the status of pollinators and factors affecting them, to enhance understanding about the role of beekeeping in mountain crops' pollination and promote beekeeping for pollination of mountain crops, to create a network of pollination experts at regional and international level and to build capacities of individuals (human resources development) and institutions in the field of apiculture development and facilitate networks.

Development of Pollinator Programme at ICIMOD

- Phase I (May 1991-July 1993): Research on the exploration of genetic diversity of the indigenous *Apis cerana* and on farm experiments on the impact of bee pollination on various crops.
- Phase II (July 1993-December 1998): Genetic improvement of *Apis cerana* through selection, and mass queen rearing; research of honey plant resources and field studies on pollination.
- Phase III (1999- to date): Expanded to include all species of indigenous honeybees, action research on *Apis cerana* selection and multiplication, field studies on the issues related to pollination and pollinators.
- 2002 Joined hands with FAO to implement International Pollinators Initiative (IPI).

Activities Being Undertaken Under ICIMOD's Pollinators Programme

- Conduct studies to assess pollination problem of mountain crops.
- Conduct studies on natural pollinators and factors influencing natural pollination in mountain areas.
- Set up on-farm bee pollination research and demonstration facilities for selected crops (farmer-participatory action research).
- Training and capacity building of local institutions and individuals.
- Prepare publicity material to raise awareness at different levels – farmers, development workers, and professionals at policy and planning level.
- Conduct research to suggest appropriate policy options for promoting managed pollination and conservation of pollinators.
- Networking of institutions involved in research and development of pollinators/pollination.

ICIMOD's Achievements

ICIMOD has been carrying out a region-wide programme on community-based research and development on pollinator/ pollination related issues in mountain crops productivity for the past one decade in collaboration with partner institutions in the Hindu Kush-Himalayan region countries, which include Bangladesh, Bhutan, China, India, Myanmar, Nepal and Pakistan. This is one of the biggest projects on the conservation and development of indigenous honeybees in the Hindu Kush-Himalayan region focused on promoting conservation-based apiculture. ICIMOD's achievements are in the areas of review of pollination status, field studies, farmer participatory action research, demonstrations and awareness raising, training and policy analysis. The outputs include a variety of materials such as videos, policy-discussion papers, policy-issue papers, awareness handbooks, books, trainers' manuals and articles.

Some of the major achievements are:

- Studies on the identification of pollination related productivity issues in mountain cash crops have been carried out in different countries of the HKH region including Bhutan, China, India, Nepal and Pakistan. The causes of inadequate pollination including the causes of pollinator decline have been identified. Strategic research on critical andless understood issues related to pollination to fill in the knowledge gaps in understanding the factors and processes affecting pollination of mountain crops is underway. The results, so far, revealed the lack of pollinator numbers as well as diversity, lack of appropriate polliniser proportions especially in case of fruit crops, and global climate change as causes of inadequate pollination in the HKH region. In addition on farm action researchon pollination methodology has been carried out with farmers / stakeholders participation.
- Lack of adequate numbers of pollinators has been identified as one of the important factors causing crop pollination failure in fruit growing areas of the HKH region. The fact that the populations of natural insect pollinators are declining in intensively cash crops cultivated areas of the region has been confirmed through field studies and farmers interviews. This decline is very much related to the factors like excessive and indiscriminate use of pesticides, increase in area under cash crops, clearance of forests and grassland areas for cultivation (land use changes) resulting the loss of nesting habitat

and diversity of food sources of pollinators and climatic factors. Further research to understand which factor contribute what / how much to affect insect pollinator diversity and pollination problem is underway.

- A lot of work on identifying the causes of pollinator decline in mountain areas has been carried out. The main reasons identified include loss of habitat due to extension of farming into forests and grassland areas resulting in decline in food sources and nesting sites of the pollinators (also including non- honeybee pollinators), monoculture-dominated agriculture and modern agricultural technologies and inputs such as use of pesticides and other agrochemicals as causes of pollinator decline.
- Conducted case studies on indigenous honeybee pollinators in the Himalayan region. Case study on *Apis laboriosa* – the Himalayan cliff bee in Kaski district of Nepal has been completed and published in the form of a book, while on other species such as *Apis cerana, Apis dorsata* and *Apis florea* are underway. The case studies document information on the number of nesting sites and nests in a particular area, their status whether the numbers are increasing or decreasing, the causes of decline and recommendations on how to conserve them. A big programme focusing on conservation and development of *Apis cerana* through promoting its selection and multiplication is being carried out in the countries of the HKH region.
- Case studies on pollination problems in mountain cash crops' particularly apple, citrus, pear, and apricot, and vegetable crops and mountain farmers and institution management efforts / practices. So far studies on apple pollination issues have been completed and published in the form of a book and a video. Studies on other crops are underway.
- Capacity building in areas of human resources development and institutional strengthening activities included development of training extension material and training of farmers and development workers. ICIMOD has developed training and extension material on pollination such as manuals, posters, handouts and videos etc. Trainers; resource book on management of mountain crops through beekeeping prepared and published in English as well as in regional languages such as Hindi, Nepali and Urdu. The Centre has also trained farmers and development workers through regional partners in India, Nepal and Pakistan.
- ICIMOD has successfully raised the pollination related issues and the potential of honeybees for enhancing crop production and farm income of the small and marginal mountain farmers at policy and planning level. It has generated knowledge and information that has been (will be in some countries) used as an input to formulate policies for including beekeeping for crop pollination as one of the essential components of mountain agricultural diversification package. The knowledge generated has been published as awareness raising materials such as policy briefing papers, policy issue papers, and policy discussion papers, and a video depicting pollination issues in the HKH region and need to address it.
- ICIMOD has also conducted research on honey plant resources of the Hindu Kush-Himalayan region. Created a reference pollen data bank consisting of more than 1,000 plants species in ICIMOD, which has potentials for monitoring plant biodiversity in mountain areas. Published a book entitled "Bee Flora of the Hindu Kush-Himalayas: Inventory and Management". This book is being widely used by NGOs in training and university researchers and field workers to identifying plants in the field. It describes 237 plant species, which provide pollen and nectar to honeybees and other pollinators. In addition, plant sources producing nectar and pollen toxic to bees/ pollinators and humans are also described.

Major R&D Areas Needing Attention and ICIMOD's Efforts in Addressing Them

Awareness Raising about the Value of Pollinators

Lack of awareness at all levels - be it farmers, extension workers, and professionals at policy and planning level is one of the main problems in promoting conservation and sustainable use of pollinators. With few exceptions in those areas where there is large-scale pollination problem, farmers and general public are not even aware of the value of honeybees and other pollinators for agricultural production. This is because cash crops' farming is a new activity in many developing countries, and there is no indigenous knowledge on the importance and need for managing crop pollination for enhancing cash crops' production. Raising awareness at all levels about the importance of pollinators to agriculture and biodiversity is, therefore, the first step. ICIMOD is trying to do this through organising awareness workshops for different media and decision makers, developing and distributing awareness material such as briefing papers, issues papers, policy papers and discussions papers and video films, organising a number of one-day pollination awareness camps for farmers and extension workers and producing relevant literature and translating it into regional languages.

Promoting Integration of Pollinator Considerations into Policies

The inputs of pollinators in agriculture husbandry and biodiversity conservation have not been recognized by policy makers, planners, development workers and farmers. There is no conceptual clarity and recognition of the value of pollinators. The role of pollinators and pollination has been overlooked in agricultural development strategies and is not included as a technological input in agricultural development packages. High value agriculture is being promoted in several areas and official institutions offer packages of practices for each type of crop, but the importance of managing pollination to achieve a sustainable yield has been overlooked. Thus farmers have no way of knowing how essential it can be. This weakness in the agricultural extension system needs to be addressed. Since pollination is essential for the production of fruits and seeds, crop pollination management should be included in agricultural development packages. Changes in research and development investment policies may be needed to encourage this. It is necessary to evolve strategies to promote investment in research and development that will enhance the conservation and use of pollinators. This means developing area-based approaches, and making full use of the existing diversity of pollinators including honeybees. ICIMOD has done a lot of effort in this direction. It has carried out field research and conducted case studies to show the importance of pollinators for agriculture and biodiversity, produced material to brief those at the level of decision-making and suggested strategies to include pollinators and pollination as part of agriculture development strategies.

Mainstreaming Pollinator Concerns in R&D System

Economic and ecological importance of pollinators and the issue of their decline have not been brought in the mainstream of research and development efforts. Therefore, many people do not know about the service pollinators provide to agriculture and natural ecosystems. To effectively address this issue, it is necessary to bring pollinator concerns to the R&D mainstream. This can be achieved by promoting its integration into agricultural research policies and programmes, extension and outreach programmes etc. ICIMOD for the past few years has been working on bringing pollinator concerns into R&D mainstream through conducting studies to highlight the importance of pollinators and pollination in enhancing agricultural productivity and conserving biodiversity, raising awareness and producing material – both technical and non technical nature to brief research and development workers and professionals at policy and planning level.

Capacity Building: Human Resources Development and Institutional Strengthening

Examples from developing countries of Asia e.g. Nepal and Myanmar reveal that farmers think that honeybees damage their crops by extracting all the nectar and pollen. This is because of the lack of awareness, knowledge and public understanding about the significance of pollinators. Thus, the issue of declining pollinators, its impacts on agricultural production and natural ecosystems, and therefore the need to protect them, is not very well understood. The research and extension system is weak and is unable to translate the results of research into development. Moreover, the issues relating to the decline in pollinators, need for their conservation, rearing, and managing them for pollination are relatively new areas to institutions in the developing countries. There are only a few institutions in the region with explicit mandates or expertise with ongoing research and extension in this area at the national and international levels. Most institutions are working only with beekeeping and promoting it as a cottage industry to increase family income through the sale of honey. Promoting honeybees as reliable pollinators and conservation of other pollinators will require special efforts to strengthen research and extension systems. This is necessary in order to underline applied research in key areas of managed crop pollination. Thus, there is need for strengthening R&D institutions and building capacities of farmers and farmer-led organisations, development workers, and professionals at policy and planning level through raising awareness about the value of pollinators and the services they provide to agriculture, biodiversity and enhancing livelihoods and to conserve the pollinator diversity for the benefit of agricultural and natural ecosystems.

ICIMOD’s efforts in human resources development and institutional capacity building include setting up pollination research and demonstration sites and using farmers as partners in action research in different villages in the HKH region, developing training materials and organising training courses for farmers and extension workers, organising study tours and facilitating exchange visits, and providing advisory services on pollinators / pollination research. In addition, ICIMOD has created and informal network of individual researchers and institutions working on pollination in the region.

Facilitating Exchanges of Knowledge and Information in Pollinator Conservation

Developed countries of the world have a lot of knowledge about pollinators and pollination. The countries like USA, Japan, and Canada and Europe have developed knowledge and experience in rearing and using different species of bees for pollination of their crops and are benefiting from the pollinator services. These countries have developed technologies for rearing various pollinators and there are established companies in these countries that rear and sell these pollinators. However, the developing countries lag far behind in using even honeybees for pollination. The knowledge and technologies developed in the developed countries can be taken down to developing countries. For this there is need to build information on such success stories of promoting pollinators’ conservation and use in the developed countries and use it for training and human resources development and replicating them in developing countries. The pollinator programme at ICIMOD is playing an active role in facilitating the exchange of knowledge and information at regional as well as international level through participating in and organising workshops, meetings and conferences, developing and distributing relevant publications, and through organising trainings and awareness workshops. Recently ICIMOD has joined hands with FAO to address this issue through facilitating and coordinating the International Pollinator Initiative (IPI) established by CBD in close cooperation with other relevant organizations.

ICIMOD's Future Programme on Pollinators

ICIMOD plans to continue to work in the field of promoting conservation and sustainable use of pollinators at farmers, extension workers, researchers, development professionals, planners and policy makers' levels. Recently, it has embarked upon a much wider programme on conservation and sustainable use of pollinators in collaboration with the Food and Agricultural Organisation of the United Nations (FAO). The new programme (already identified by CBD and FAO) would focus on promotion of conservation and use of pollinators for sustainable agriculture through ecosystem approach. The four project components as identified by the CBD/ FAO include assessment, adaptive management, capacity building and mainstreaming.

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THE AFRICAN POLLINATOR INITIATIVE

Connal Eardley
Barbara Gemmill
Peter Kwapong
Wanja Kinuthia

Informed by increasing recognition worldwide that pollinators play a key role in ecosystem health, both in farmers' fields and in wild landscapes, an Africa-wide group of people formulated the African Pollinator Initiative. This group is interested in and committed to protecting, understanding and promoting the essential process of pollination for sustainable livelihoods. We realize that pollination is a service nature provides that we have tended to take for granted, and that we often do little to encourage it until we start to lose it. An estimated two-thirds of all flowering plants depend on animals, largely insects, for pollination. For these plants, the pollinator may be as critical as light and water. Pollination is a vital link in natural communities, connecting plants and animals in key and essential ways. Pollination is a service that is key to agriculture as well. Insect pollinators are essential for many fruit and vegetable crops, and the demand for pollinators grows as the need for agricultural productivity increases. Pollinators have real commercial value, although this is not always appreciated, as is evidenced by the US$150 million plus per year service that West African beetles provide to oil palm plantations in Southeast Asia. The contribution of pollinators to food security in Africa may have both tangible and intangible values in reducing wide disparities in production levels and dependence on imported foods.

As wild ecosystems are increasingly converted to more human-dominated uses to meet the compelling demands of food security, it is critical to understand how we can preserve the basic ecosystem functions that fostered tremendous diversity in the first place, such as pollination. Pollination precedes fertilization in plants, and fertilization results directly in seed and fruit production. As a part of reproduction, seeds comprise the dormancy and dispersal phase of many plants. Seeds and fruit are also food for many people and animals. The loss in biodiversity and the adverse ecological effect that would follow a broad-spectrum loss of pollinators is inconceivable.

Yet remarkably little is known about pollinators in Africa. Virtually nothing is known about the effectiveness of pollinators of wild plant species. The bulk of research on both crop plants and wild ecosystems resides in South African studies, while the rest of the continent has been unevenly covered in the scientific literature. We risk losing not just particular pollinators or plants with fragile pollination systems but also critical ecosystem interactions and long-evolved links that underpin African ecosystems.

The African Pollinator Initiative (API) was established in 1999, as the African network of the International Pollinator Initiative (IPI). Since then it has been involved in the development of IPI.

As a network, API strives to improve communication channels between all people and organizations interested in pollinators and pollination biology, including biodiversity conservation, agriculture and general awareness, and facilitate collective achievement. A large number of people, in numerous different countries, have shown an interest in API. But participation in API is voluntary and the involvement of individuals, and the amounts of their correspondence with the Secretariat, is ever changing. Therefore it is impossible to record all the activities in pollinator conservation and pollination in Africa. Relying on published works negates communication during project activities, and API needs to find ways to bridge this gap. There are, however, a lot more people interested in pollinators and pollination than immediately apparent, and it is not realistically possible to report on all the activities in Africa.

API operated as an informal network until 2002 when its first Secretariat was formed. Since then the major achievements initiated by the Secretariat include:

- The publication of the API - Plan of Action (API-POA).
- The publication of a dedicated issue of the journal *Insect Science and its Application* for pollinator conservation cases studies.
- A survey of pollinators.
- Pollinator identification training course.
- Involvement in a Project co-ordinated by the Food and Agriculture Organization of the United Nations (FAO) to be financed by the Global Environment Facility (GEF).
- A Workshop to facilitate the implication of IPI within countries.

The API-POA advocates a balanced approach to pollinator conservation, and it includes:

- Public education and awareness.
- Placing pollination in the mainstream.
- Conservation and restoration.
- Capacity building.

Repeatedly it is emphasized that it cannot assumed that African pollination systems are the same as those on other continents; the contrary having been demonstrated on many occasions, and that local research and customized conservation and sustainable-use programmes are needed.

African activities need to be better publicized and a dedicated journal issue comprising a suite of selected works was considered to be a mechanism for focusing attention on pollinator conservation in Africa.

A major need that exists is to know what the pollinators are. This initially requires rapid assessments and a suitably designed recording mechanism. To begin this FAO supported an estimation of the pollinators of selected crops in Ghana, Kenya and South Africa. This needs to be an on going project and the information needs to be recorded in a way that will allow continual expansion and improvement. Web-based catalogues can achieve this. Because bees are the most important group of pollinators a catalogue of the African bees, with host plants and countries of origin has been developed.

Assessment requires identification. Therefore a first African bee course was given, presented in both Kenya and Ghana. Better keys are needed, and an electronic interactive key is being developed.

IPI, through the expert co-ordination of FAO, applied for finance through GEF. Project development funds have been approved and the project will be developed during 2004-2005. The African countries involved in this project are Ghana, Kenya and South Africa. Because API includes all African countries and GEF priorities do not encompass all API goals, further activities and finance raising are on the API agenda. A particular need in Africa is capacity building, and the capacity developed in the countries participating in the "GEF project" should be expanded within those countries and in other countries.

Pollinator conservation is often overlooked; like most essential ecosystem services its effects are only appreciated after the service fails. To facilitate the implementation of IPI within countries a document, designed at a Workshop in South Africa, is being developed.

IPI is a young, yet vibrant network. It is inclusive and aims to include as many people and organization in Africa as wish to be involved. Its structure and mechanisms have been, and will continue to be determined by those involved in pollinator conservation and pollination studies and application. Thus it will change through time to meet the demands for better pollination in Africa.

SESSION II

MONITORING AND POPULATION DYNAMICS OF SOLITARY BEES

1. Fernando A. Silveira. "Monitoring Pollinating Wild Bees"

2. Carlos Alberto Garófalo, Celso Feitosa Martins and Isabel Alves-dos-Santos. "The Brazilian Solitary Bee Species Caught in Trap Nests"

3. Claudia Mohra, Martin Fellendorf and Robert J. Paxton. "The Population Dynamics and Genetics of Solitary Bees: A European Case Study, *Andrena vaga* (Hymenoptera, Andrenidae)"

4. David W. Roubik. "Long-term Studies of Solitary Bees: What the Orchid Bees are Telling Us"

MONITORING POLLINATING WILD BEES

Fernando A. Silveira

In recent years, the concern is increasing on the maintenance of stable populations of wild bees on which wild and cultivated plants can depend for their pollination needs. Factors affecting bee abundance and diversity in any given place may include natural phenomena, like severe rainy seasons or droughts, as well as human generated hazards such as deforestation and insecticide applications. At this moment we have only a pale idea of what are the dynamics of population and diversity variation in bee communities and we are not even able to distinguish between naturally and human induced effects on wild bees. Thus, monitoring programs are important for us to gain the predictive power we need to intervene on wild and cultivated landscapes to preserve wild bee populations and the continuity of the pollination service they offer. The establishment of efficient monitoring programs, however, depends on the solution of several methodological and analytical problems. Here we discuss some of the situations in which we may want to monitor wild bees and what are the problems we are going to face, then. Some alternative solutions for such problems are presented.

The natural landscapes

Conserving bees at natural landscapes is good for itself. Even from an agricultural perspective, however, natural landscapes are very important since they are the sources of colonizing bees for the crop fields. In croplands, bees are always at risk. In such conditions, their populations are always prone to disappear or, at least, to be drastically reduced at times. At these instances, surrounding natural landscapes is where new colonizer bees will come from to reestablish new populations able to keep up with the pollination service.

However, populations at natural landscapes are not themselves free from drastic fluctuations, as Roubik (2001) has shown for euglossine bees in Panamá. Under natural conditions, bee abundance (both of whole taxocenes or single species) varies according to the fluctuation of a series of frequently inter-related factors such as climate, food availability, predator and/or parasite abundance etc. Moreover, since natural landscapes in the surrounding of agricultural environments are generally mere fragments of pristine vegetation, their bee populations are also standing “unnatural” forces tending to push them to extinction. Invasion of exogenous predators, parasites and competitors, reduction of food and nesting substrate availability, and isolation of small, genetically impoverished populations are some of those forces.

So, it is important that we know how healthy bee populations are at natural patches, so that we can take action in helping them to keep themselves alive.

The agricultural landscapes

Agricultural landscapes are simplified ecosystems. As such, they tend to favor a few species that will adapt very well to the dominant, relatively homogeneous conditions and to eradicate most of everything else. The croplands are especially harsh environments due to agricultural practices that are hazardous to bees. Thus, they suffer from soil plowing and grading, which may destroy their nesting sites; they suffer from food shortage, when alternative flowering plants are not available while the crop is not in bloom and, most of all, they suffer from insecticide application.

Under such conditions, quite frequently, the bee species favored in or around crop fields are not necessarily what the crop plants need for their pollination. Thus, we must find ways to ameliorate the conditions for these target species to survive there. This will generally demand constant intervention, with actions such as habitat enrichment (planting nectar and/or pollen sources or supplying nesting substrate, for example) and bee translocation for population recovering and/or improvement of genetic variability. The knowledge of population tendencies is crucial to successfully deal with all details of such management programs.

What kind of methods should we use?

At different moments or situations, we may be interested in different information about bee taxocenes or species. We may want to know, for example, how the abundance of bees, in general, and/or species richness and/or taxocene composition is/are fluctuating through time or in response to any given factor. On the other hand, in specific crops, we may want to know how the population of a given pollinator species is varying along the years. We may want to be even more specific and monitor, not only population size at the fields, but its genetic variability or parasite infestation, for example.

For each situation, we will have one or more among a series of sampling methods available for a monitoring program. One main concern in choosing one such method, then, is the obtainment of unbiased, reliable data (Cane *et al.,* 2001). Sakagami *et al.* (1967), Silveira and Godinez (1996) and Williams *et al.* (2001) have called attention to problems in devising standard procedures for sampling whole bee taxocenes. Among the problems raised by those authors is the different ability of different people in finding and capturing bee species. Data obtained by Silveira and Azevedo (unpublished) and Cane *et al.* (unpublished) seem to indicate that this problem may be largely overcome by properly training collectors before they start sampling.

In any case, the less the data depend on individual ability of different data collectors, the best. Thus, use of trap-nests is a good choice when we are interested in specific species which nest in pre-existing borings in wood *(e.g.* Krombein, 1967). Unfortunately, however, collector-independent methods are not always efficient for bee sampling. Thus, trap-nests collect only a minor fraction of the bee species of any single local fauna; aromatic lures are very effective for orchid-bee monitoring (Apidae: Euglossina) but do not efficiently attract any other group of bees; light-traps may attract a few species of crepuscular bees or social species which nests happen to be close to the traps, but are ineffective for most bee species *(e.g.* Wolda and Roubik, 1986; Roubik and Wolda, 2001); flight interception traps (as the Malaise trap) are poor choices for collecting bees (personal observation); pan traps have shown to be effective in some instances *(e.g.* Laroca, 1980) but not in others (Cane *et al.,* 2001; pers. obs.).

Thus, except for a few special cases, sampling at flowers will be the best option to monitor pollinator bees. If the target species is/are easily spotted and identified on flowers, counting will be enough and monitoring will not impose an extra mortality factor on the species we are interested in preserving. However, if there is any doubt whether or not the target species will be confounded with other similar bees, than collecting becomes a necessity.

A few special problems in planning monitoring programs

Whatever the aims of a monitoring program, there will always be the need for comparisons such as of population abundance or taxocene composition between or along years. This poses a series of problems. We need sound, reliable data that will stand robust statistical testing so that we can trust on our results and confidently plan our actions upon them. Recipes for data analyses are beyond the scope of this talk but I want to emphasize some points, which must be taken in account when monitoring protocols are devised.

1. Most local bee faunas include many rare species that indeed may comprise the majority of the species. Thus, the smaller the sample sizes, the larger the number of missing species in specific year and/or local samples. These randomly derived absences in data matrices increase the differences among samples and may obscure true patterns. They also may be an impediment to the proper employment of given statistical tests *(e.g.* Cane, 2001). Thus, when monitoring whole taxocenes, the sampling effort should be carefully dimensioned so that sample sizes are adequate for analyses.

2. Distribution of species across the environment is not homogeneous, varying according to several factors, such as microclimate, and food and/or nesting site distribution. It is important, thus, that this spatial heterogeneity is accounted for through a convenient series of replications. Variations may be great enough that large standard deviations will make it impossible for true differences to show up if few replications are employed. This problem is especially important when comparing species composition of faunas containing many rare species (see above).

3. Frequently, it will be of interest to assess the impact of specific human activities on bee populations. In this case, proper controls should be set in the monitoring program. Not only patterns apparent before, during and after impact should be compared. It will be necessary that plots in nearby, similar environments not affected by the target activity are also monitored so that effects of other uncontrolled variables can be detected and taken in account. For example, Roubik (2001) found that strong El-Niño Southern Oscillation (ENSO) events were associated to high euglossine bee abundances in Panamanian forests. The coincidence of such an event with a given agricultural practice, for instance, may lead to the wrong interpretation that such practice might be benefic to bee populations if replications unaffected by this practice are not included in the monitoring design.

Final remarks

The establishment of monitoring programs of pollinating bees is important if we intend to act on their conservation and/or sustainable use. Different methods for sampling and analyzing data will be necessary for different aims and in different situations. However, definition of standard protocols for similar conditions would allow us to compare data generated by different researchers/monitoring programs, giving us better understanding of patterns and their causes and, thus, would give us conditions to make broader generalizations and better predictions. We would be, then, in better shape to propose and execute management practices both for conservation and use of wild bees as pollinators.

The proposal of a set of general monitoring protocols, including experimental design and analytical methods for different situations would be especially helpful for conservation and agricultural agents to step forward in starting useful monitoring programs for wild pollinators worldwide. This should be a priority task for the World and regional pollinators initiatives.

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THE BRAZILIAN SOLITARY BEE SPECIES CAUGHT IN TRAP NESTS

Carlos Alberto Garófalo
Celso Feitosa Martins
Isabel Alves-dos-Santos

Studying solitary species

It is not easy to locate nests of natural solitary bees, since most of the time they remain unperceived to our eyes. In the 60ths Karl Krombein published an extensive work about bee and wasps caught in trap nests (Krombein, 1967). After this work the use of traps to get solitary Apoidea spread to many parts of the world. In Brazil the first study using the technique was performed late in 70ths in Ribeirão Preto (southeastern Brazil) by C. A. Garófalo, which were responsible for the initiation of such studies in the country. Now a day there are people studying the trap nesting species everywhere in Brazil. The technique has been used to survey the species and to get biological data, most about nest structure and associated parasites.

The most common methods are the use of the following types of traps: 1. bamboo cane with variable diameters and lengths, which are cut so that the nodal septum closes one end of the cane. Some units of the canes are bundled together. 2. two rectangular pieces of wood firmly taped together, in which a round hole had been drilled through the contacting surfaces of the two pieces. 3. circular holes of different diameters, drilled some centimeters deep into a piece of wood. Straws of black card paper are rolled and inserted in each cavity, so that it could be later extracted and replaced by new paper. 4. small wooden boxes covered with glass plate with a circular entrance hole on one side. This last type is used for the Euglossini. In the field the traps are placed along shelves or suspended in trees, and examined periodically.

In this paper we summarize the results of studies performed in Brazil using the technique of trap nests for solitary bees. We discuss the potential of the bee species for the use in crop pollination.

The following works were consulted to obtain the names of the trapnests species: Serrano and Garófalo (1978), Garófalo *et al.* (1989), Garófalo *et al.* (1993), Camillo *et al.* (1995), Garófalo (2000), Morato and Campos (2000), Morato (2001), Viana *et al.* (2001), Aguiar (2002), Aguiar and Martins (2002), Martins *et al.* (2002), Alves dos Santos (2003), Gazola (2003).

Bees that use trap nests and the potential species for pollination

Members of the families Apidae e Megachilidae have been frequent in wood traps and were caught in all the studies performed in Brazil, while the Colletidae seldom occupied the traps. Around 60 species have been caught in trap nests (Table 1) and this number will be more exact when the morph species in each study will be compared and identified. On the table 1 we generalized this information using the genus following by spp. There were some species very common found in the traps and with wide distribution like: *Centris analis*, *C. tarsata*, *Tetrapedia diversipes* (= *dentipes*), *Euglossa cordata,* and *E. townsendi*. They do not show preference for the type of traps.

Besides the ranking of species on table 1 there were also the parasite bees of the genera *Coelioxys, Coelioxoides, Exaerete, Mesocheria, Stelis*, that occupied the nest of their hosts. But their role as pollinator will be not considered.

There are some species on the list (Table 1) that could be used for the pollination of crops. For example *Centris* spp. will be good pollinating agents for fields of Malpighiaceae plants like *acerola* (West Indian cherry) and *murici* (wild cherry), popular fruits from northeastern, where they visit the flowers to collect oil. *Centris* will be also a good pollinating agent of cashew trees (Anarcadiaceae) as detected by Freitas (1997) for *C. tarsata* in Ceara (NE), and tamarind (Caesalpineaceae) (Castro, 2002). From the species caught in trapnests we believe that *C. analis, C. tarsata* and *C. vittata* are the better candidates for pollination program, since they were relative constant and abundant in most studies (Gazola, 2003), furthermore they are wide distributed through the country.

The females of the orchid bees (Euglossina) would be a good pollinator of plants with poricide anthers, like Solanaceae (tomatoes, eggplant), because they can buzz the flowers very well and showed interest for such flowers (Minussi and Alves dos Santos in prep.). Maués (2002) pointed out for the importance of the Euglossine (specially *Eulaema nigrita*) and other big solitary bees like *Xylocopa frontalis* and *Epicharis* spp. for the pollination of Brazilian nut (*Bertholia excelsa*) in the Amazon region.

Xylocopa frontalis, and other big species of *Xylocopa*, as we known is a very good pollinator of passion fruit flowers (Passifloraceae) and also recognized as pollinator of guava (*Psidium guajava*) (Camillo, 2003; Alves, 2000 apud Freitas and Alves, 2003). According to Freitas and Oliveira Filho (2001) now is possible to manage species of *Xylocopa* rationally. The possibility to have nests of the carpenter bees in traps like bamboo cane and/or adapted honeybee hives would facilitate the manipulation of nests and make the management cheaper.

Among the leafcutter bees, the Megachilidae, we see great chances of using them for the increase of seed production in fields of Leguminosae and Compositae. Different from North American, Brazil does not have tradition of cultivate alfafa (*Medicago*). There are few growers cultivating alfafa in the south and not exactly for seeds. But we do have tradition of consuming seeds of other Leguminosae like beans (*Phaseolus*) and soya (*Glycine*). In the last decades huge fields of soya occupied the central part of the country, and beans are cultivated everywhere in small and big scales. Even knowing that both these Leguminosae produce seed without pollinating agents, it is also known that the presence of the right bee increase considerably the size of the pod and the number of seeds.

From results of bee surveys performed in different parts of the country, *Megachile* also show preference for Asteraceae flowers. So, seeds of many ornamental, comestible or medicinal plants could be benefited with the pollination of the leafcutter bees. The same is true for the Anthidiini that besides also visiting flowers of Fabaceae and Asteraceae show preference for Labiateae (Laminaceae). Then it will be possible to take advantage of them for seed production in field of flavors like *Ocimum*, *Rosmarinus*, *Mentha*, *Origanum*, *Basilicum*, and *Thymus*. Two species should be tested and considered for rearing: *Megachile guaranitica* and *Epanthidium tigrinum*. Both are constant caught in the traps and present a wide geographical distribution.

Among all the sampled bees in the trap nests there are some species, which the biology were already studied (Table 2). Data about their biology, nest structure and associated parasites are very important if we intend to use them in large scale.

Table 1. Bee species caught in trap nests in Brazil

APIDAE
Centridiini
Centris (Centris) aenea Lep.
Centris (Heterocentris) analis Fabricius
Centris (Heterocentris) bicornuta Mocsary
Centris (Heterocentris) labrosa Friese
Centris (Heterocentris) terminata Smith
Centris (Hemisiella) dichootricha (Moure)
Centris (Hemisiella) tarsata Smith
Centris (Hemisiella) vittata Lep.
Centris spp.

Euglossina
Eufriesea auriceps Friese
Eufriesea mussitans (Fabricius)
Eufriesea purpurata (Mocsáry)
Eufriesea surinamensis (L.)
Eufriesea theresiae (Mocsáry)
Eufriesea violacea (Blanchard)
Eufriesea violacens (Mocsáry)
Euglossa annectans Dressler
Euglossa avicula Dressler
Euglossa cordata (L.)
Euglossa fimbriata Rebêlo & Moure
Euglossa gaianii Dressler
Euglossa melanotricha Moure
Euglossa modestior Dressler
Euglossa pleosticta Dressler
Euglossa townsendi Cockerell
Euglossa truncata Rebêlo & Moure
Euglossa spp.
Eulaema nigrita Lep.

Tetrapediini
Tetrapedia amplitarsis Friese
Tetrapedia curvitarsis Friese
Tetrapedia diversipes Klug
Tetrapedia garofaloi Moure
Tetrapedia rugulosa Friese
Tetrapedia sp.
Lagobata ornata Spinola (*Tetrapedia*)

Xylocopini
Xylocopa frontalis (Olivier)
Xylocopa grisescens Lep.
Xylocopa suspecta Moure

MEGACHILIDAE
Anthidiini
Anthidium manicatum (L.)
Anthidulum spp.
Anthodioctes manauara Urban
Anthodioctes megachiloides Holmberg
Anthodioctes lunatus (Smith)
Anthodioctes moratoi Urban
Anthodioctes spp.
Cartolicola paraguayensis (Schrottky)
Epanthidium erythrocephalum (Schrottky)
Epanthidium maculatum Urban
Epanthidium tigrinum (Schrottky)
Epanthidium nectarinoides (Schrottky)
Epanthidium spp.
Dicranthidium arenarium Ducke
Dicranthidium luciae Urban
Duckeanthidium spp.
Saranthidium marginatum Moure & Urban

Megachilini
Megachile (Austromegachile) orbiculata Mitchell
Megachile (Austromegachile) sussurans Haliday
Megachile (Chrysosarus) guaranitica Schrottky
Megachile (Dactylomegachile) sp.
Megachile (Neochelynia) brethesi Schrottky
Megachile(*Pseudocentron*) *lissotate* Moure
Megachile (*Pseudocentron*) spp.
Megachile (Ptilosaroides) xanthoptera Schrottky
Megachile (Rhysochile) cara
Megachile (Sayapis) dentipes Vachal
Megachile spp.

COLLETIDAE
Colletes rufipes Smith

Hylaeini
Hylaeus spp.

Table 2. Studies about the biology of bee species caught in trapnests

Centridiini	
Centris analis	Morato *et al*., 1999; Jesus and Garófalo, 2000; Gazola and Garófalo, 2003
Centris bicornuta	Morato *et al*., 1999
Centris terminata	Morato *et al*., 1999
Centris dichootricha	Morato *et al*., 1999
Centris tarsata	Silva *et al*., 2000; Pires et al., 2002; Moreira *et al*., 2002
Centris vittata	Camillo *et al*., 1994; Pereira *et al*., 1999
Euglossina	
Eufriesea mussitans	Viana *et al*., 2001
Eufriesea violacea	Peruqueti and Campos, 1997
Eufriesea violacens	Garófalo *et al*., 1993; Peruqueti and Campos, 1997
Euglossa annectans	Garófalo *et al*., 1998
Euglossa cordata	Garófalo *et al*., 1985, 1992; Augusto and Garófalo, 1994,1996.
Euglossa townsendi	Peruqueti, 1998
Euglossa truncata	Braga and Garófalo, 1998; Augusto and Garófalo, 2002
Eulaema nigrita	Zucchi *et al.* 1969; Pereira and Martins, 1991; Santos and Garófalo, 1994
Tetrapediini	
Tetrapedia amplitarsis	Camillo, 2001
Tetrapedia curvitarsis	Camillo, 2000
Tetrapedia diversipes	Alves dos Santos *et al*., 2002
Xylocopini	
Xylocopa frontalis	Pereira and Garófalo, 2000; Queiroz and Freitas 2002
Xylocopa grisescens	Camillo and Garófalo 1982; Teixeira *et al*., 2002
Xylocopa suspecta	Camillo *et al*., 1986
MEGACHILIDAE	
Anthidiini	
Anthodioctes lunatus	Camarotti-de-Lima and Martins (submitted)
Anthodioctes megachiloides	Alves dos Santos (in press)
Anthodioctes moratoi	Morato, 2001
Cartolicola paraguayensis	Camillo *et al*., 1994
COLLETIDAE	
Colletes rufipes	Camillo *et al*., 1996

Perspectives and recomendations

The results of the performed studies in Brazil show that many species that use trap nests are potential for pollination programs. However, to manipulate these bees for the use in agriculture we still have to learn how to raise them in large scale and control the emergence for the periods we need. The diversity and abundance of bee species caught trap-nesting in an area depend on factors like the abundance of resources for provision and construction material, the need of nesting places and the conditions of the traps.

Excepting for the carpenter bees, until now no experience have taken place in Brazil to breed solitary species for the agriculture. And there are some important tasks we should care. We recommend and it is necessary:

- to define exactly which crops for each bees;
- to know how to keep cells and use them in the appropriate moment, in other words, to know exact the period of development and be able to control the emergence of adults;
- to know how to control the parasites and enemies;
- to control the use of pesticides with the growers;
- and finally, supply the bees with their needs besides the pollen grains, like: oil for *Centris*, leafs or petals for *Megachile*, or resin for the Anthiidini and Euglossina.

With the answers to these needs we will be able to standardizing the techniques of rearing the Brazilian solitary bees and build large populations of them. Then take advantage of their role in nature and agriculture.

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THE POPULATION DYNAMICS AND GENETICS OF SOLITARY BEES: A EUROPEAN CASE STUDY, *ANDRENA VAGA* (HYMENOPTERA, ANDRENIDAE)

Claudia Mohra
Martin Fellendorf
Robert J. Paxton

Abstract

Most temperate dwelling solitary bees have as single generation per year, with a short adult flight season at a species-specific time during spring or summer. For ground-nesting species, a female generally digs and provisions only one nest in its lifetime. Many such species nest in discrete aggregations. These traits facilitate analysis of their population dynamics and genetics. Large populations of non-social bees are rare in today's cultural landscapes. One of the few exceptions is the fossorial bee *Andrena vaga*, a typical insect species of river flood plains, which forms sizeable nesting aggregations along the high water dams of the Upper Rhine in SW Germany, each containing up to several tens of thousands of bees. Here we review our field and laboratory studies over three consecutive years of the solitary *A. vaga* at seven nesting aggregations in SW Germany. In particular, we highlight a catastrophic effect of high water in the river Rhine on aggregations nesting on this river's high water dams. Population genetic analysis using 14 microsatellite DNA markers remarkably revealed no differentiation among aggregations separated by more that 50 km within the Rhine river valley.

Key words: Mining bee, nest, aggregation, Rhine, flood, microsatellite DNA, population genetic differentiation.

Introduction

Wild bees are of enormous ecological and economic importance as pollinators of several wild plants and crops; hence they are often termed keystone species (LaSalle and Gauld, 1993). But due to dramatic losses of natural and semi-natural habitats, many species are considered rare or threatened; for example, 45% of bee species in Baden Württemberg, SW Germany, 52% of German species and 45% of Swiss species have been placed on their respective Red Data Book Lists (Amiet, 1994; Westrich *et al.,* 1998, 2000), a situation mirrored in the UK where 37% are similarly listed (Falk, 1991). Most bee species require open terrestrial habitats. The open landscapes that we now know in Europe are largely a result of human activity. It is assumed that the habitats originally used by bees were in an early succession stage, such as coastal and island sand dunes and dynamic floodplains (Westrich, 1990; Klemm, 1996). Nowadays, high water dams have often been considered to take over an important role by representing such early succession stage habitats in Central Europe and elsewhere, and are therefore seen as important habitat features for insect, particularly bee, conservation (Westrich 1985, 1990; Brechtel, 1987).

Andrena is a large genus (>1300 spp.) of fossorial bee with a primarily Holarctic distribution (Michener, 1979, 2000), most members of which are solitary, though a few nest communally (e.g. Paxton *et al.,* 1996). *Andrena vaga* Panzer 1799 is a conspicuous (Figure 1) solitary member of the genus, and characteristic of river floodplains (Müller *et al.,* 1997). In SW Germany it typically nests on high water dams of the Upper Rhine where aggregations of up to several tens of thousands of nests may be found (Fellendorf, 1995; Brechtel, 1987). Though the species is univoltine, nesting aggregations may exist for many years at the same location, at least 58 years in the case of one famous Berlin aggregation (Ulrich, 1956). In SW Germany adult bees are active from the middle of March to the middle of May when females provision the next generation in underground brood cells in their short adult life before they die. Larvae hatch after two to three weeks and develop quickly to imagines by August, then diapause until the next spring, when they emerge, mate, and each female then constructs and provisions one single nest. The species is strongly oligolectic, which means in the case of *A. vaga* that the females visit a narrow range of late blossoming *Salix* species to collect pollen and nectar (Westrich, 1990; Fellendorf, 1995). These are used to provision brood cells located at the bottom of an excavated tunnel circa 40 cm (range 20-70 cm) deep in the ground (Fellendorf, 1995). The species' main pollen source in SW Germany is *Salix alba*, a dominant tree species of lowland alluvial willow forests that grows in damp or wet sandy soil by lakes and rivers, often in soils that may be flooded for weeks at a time (Quinger, 1990).

Four traits of *A. vaga* facilitate measurement of the population size of its aggregations: i) it is univoltine, ii) females nest solitarily, iii) with one nest per female, iv) yet in spatially discrete nesting aggregations. Moreover, sampling of individuals and description of the population genetic structure of the species is also simplified because it nests in spatially discrete aggregations. That it is a species typical of early succession stage habitats, which are by definition ephemeral, would lead one to predict that *A. vaga* has either a high intrinsic rate of increase or is resilient to habitat perturbations. Consequences for its population genetic structure are not so easy to predict. Though adults are highly mobile, the existence of nesting aggregations over many years suggests limited gene flow amongst aggregations.

Here we document the change in population size of *A. vaga* at seven nesting aggregations in SW Germany across three years, and provide more detailed analysis of the cause of a catastrophic decline in two of these populations. Furthermore, we describe the population genetic structure of *A. vaga* in SW Germany and thereby infer patterns of movement of individuals between nesting aggregations.

Material and Methods

Field sites

The study was conducted in the years 1999-2001 around Karlsruhe, SW Germany, in the Upper Rhine valley (Figure 2). Seven nesting aggregations were selected that varied between 400 m and 75 km from one another. One aggregation, ET, was in the valley of the river Enz, a tributary of the Rhine, whereas the other six (AU, MH, PN, PP, PS and RB) were in the Rhine valley itself. Though we have not searched surrounding terrain exhaustively, these seven aggregations were the principal known locations for the species in the vicinity of Karlsruhe.

Estimate of population size

The fossorial nest entrances of *A. vaga* have obvious tumuli of excavated soil above them and are characteristic of the species (Figure 3). The number of nesting females per aggregation was calculated in 1999, 2000 and 2001 by counting nest entrances in 15 randomly selected areas of 1 m^2 per site and by measuring the total area in which *A. vaga* nests were located.

Effect of a severe Rhine flood in 1999

Two large nesting aggregations of *A. vaga*, PN and PS, decreased dramatically in size in 2000 following an extreme high water event in the Rhine in May 1999 (see results), after the period of activity of adults in 1999 and therefore when *A. vaga* were primarily in brood cells as eggs, larvae or prepupae. Both PN and PS aggregations are located on the east-facing, *landward* side of the Rhine flood plain's high water dam, in the 'Rastatt floodplain nature reserve' near Plittersdorf. A third aggregation, PP, was selected 6 km southeast of PS and PN on a wooded inland sand-dune sufficiently above the Rhine river basin never to be at risk of flooding. Here bees nest on a flat, near vegetation-free site at the edge of mixed woodland (Figure 3).

In 1999, two plots of 0.5 m^2 each were randomly selected at each site PN, PS and PP and the number of bee nests carefully recorded during the *A. vaga* flight season. Nests of other bee species in the plots were also noted. Plots at PN and PS were, respectively, 2.0 m and 1.3 m perpendicularly below the top of the high water dam. In 2000, an emergence net was placed over each plot from 7th March, before the emergence of any adults, till 12th April (PN and PS) or 20th April (PP), well after the last bee had emerged. Nets were inspected repeatedly throughout the day on all days suitable for bee emergence and bees were collected within 30 minutes of emergence. Bees could not enter or escape from the traps as they were made of fine insect mesh supported upon a wooden frame whose edges were buried into the soil. Neither could bees nest under these emergence traps in 2000. After all flight activity ceased in 2000, plots at PP were excavated on 28th May to a depth of 1 m to determine whether any offspring, provisioned in 1999, had not yet emerged.

For testing the permeability of *A. vaga* brood cells to water, brood cells were excavated at PP. Excavations were performed shortly after the flight season of the imagines (during the second week in May 2000), at the same time of year as the 1999 flooding event had occurred. Each brood cell was excavated intact using a small shovel and by carefully scraping away the sandy soil surrounding it with a spatula. Though many cells were excavated, only nine were retreaved intact due to the friable nature of the soil. The nine collected brood cells were brought back to the laboratory where they were submerged in water-filled shell vials for four days.

Genetic analyses

Analysis of *Andrena* population genetics has been hampered by their limited allozyme variability (Ayasse *et al.*, 1990). Microsatellites potentially make up this shortfall, though there are few loci described for this group of bees (cf Paxton *et al.*, 1996). We therefore developed a suite of microsatellite genetic markers for *A. vaga* (Mohra *et al.,* 2000) and used 14 of them

Figure 1. A female *Andrena vaga* on a catkin of willow *(Salix alba*), its primary pollen source in SW Germany.

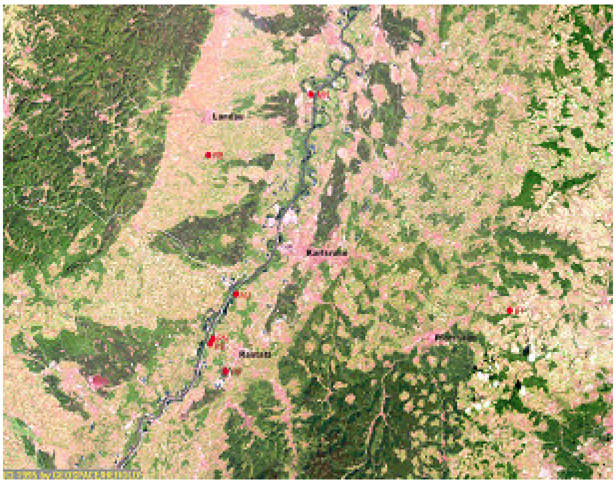

Figure 2. The seven studied nesting aggregations of *Andrena vaga* (each aggregation is located with a red dot and has a red two letter code) within the vicinity of Karlsruhe, SW Germany (N lies upwards). ET lies in the valley of the river Enz whilst the other six aggregations are in the Rhine valley, running SW to N. The distance from Karlsruhe to Pfortzheim is 25 km.

Figure 3. About 100 small tumuli of soil circa 5 cm in diameter can be seen, each representing the entrance of a single *Andrena vaga* female's nest at nesting aggregation PP.

to analyse 45 females collected from each of the seven nesting aggregations in 1999. Details of molecular genetic methods are given in Mohra *et al.* (2000). Population genetic parameters such as F_{st}, a measure of population genetic differentiation between pair of populations that varies between 0 (no structure, panmixia) and 1 (extreme differentiation; see Hedrick , 2000 for an overview), and its analogue R_{st} that is suited to the analysis of data generated by microsatellite markers (Rousset, 1996) were calculated using GENEPOP (Raymond and Rousset, 1995). We tested significance of the matrices of pair-wise R_{st} and geographic distances by Mantel tests (Mantel, 1967).

Results

Between year variation in population size

The number of nesting females per aggregation varied between 13,000 (PN in 1999) and 450 (MH in 2001). In general, there was little inter-annual variation in population size, but for the exception of sites PN and PS, where a large decrease from 1999 to 2000 was observed (Figure 4). There was a 77% and 44% decrease in the number of *A. vaga* nests at PN and PS respectively from 1999 to 2000 whereas there was, for example, a 20% increase at the adjacent site PP.

Cause of the population crash

In May 1999, the Rhine exceeded all levels since recording began, with a peak water level of 7.68 m (Plittersdorf, 13th May 1999); water levels of > 7 m over a period of four days (13th May to 17th May) emphasized the extreme character of the flood. This led to the water level on the high water dam at PN and PS to be 1.3 m perpendicularly below the top of the dam on the *river* side. On the *landward* side of the dam, where *A. vaga* nests are located, there was 10 cm of water at the foot of the dam at PN though none at PS. Moreover, the soil at PN where many *A. vaga* had nested, though not PS, was saturated with water during this high water period in May 1999.

Though numerous nests were located in all six plots of 0.5 m^2 each at PN, PS and PP in 1999, few bees emerged into traps in 2000 at PS and none at PN (Table 1). At PP, in contrast, many bees emerged into traps in 2000. Moreover, few other bee species emerged into traps at PN and PS. The data are also reflected in the population estimates for the respective sites (Figure 4) and demonstrate that the lack of emergence was not restricted to the sample plots but was rather a site-wide effect. The lack of emerging bees at PN and PS is further reflected in the location of emergence holes, small holes from which *A. vaga* adults emerge in spring from overwintering brood cells. At both PN and PS in 2000, none were found below a line approximately 1.4 m perpendicularly beneath the crown of the dam whereas up to 68 per m^2 were found above this line.

Few other fossorial bees (*Andrena ventralis* and *Colletes cunicularius*) emerged into traps at PS in 2000 (Table 1). Moreover, potential parasites of *A. vaga* that emerged into traps were numerous at PP plots but rare or absent at PS and PN (Table 1).

Brood cells of *A. vaga* excavated at study site PP were surrounded by a layer of consolidated sand, giving a degree of stability to the soil. There was no rigid or defined boundary to see between the periphery of a brood cell and soil. All excavated and experimentally submerged brood cells were waterproof over the tested period of four days, conditions mimicing those found during the May 1999 Rhine flood. The consolidated layer of sand surrounding each

Table 1. Numbers of hostbee nests in observation plots in 1999 (before slash) and their offspring that emerged into traps placed over these plots in 2000 (after slash). Bee parasites emerging into traps in 2000 are also given (adults of all named species were present in some number at all study sites in both years).

	Study site					
	PS		**PN**		**PP**	
	trap 1	**trap 2**	**trap 3**	**trap 4**	**trap 5**	**trap 6**
Host bees						
Andrena vaga	18/61	30/19	23/0	12/0	26/96	23/77
Andrena ventralis	1/2	2/14	0/0	2/0	-	-
Colletes cunicularius	2/5	1/2	1/0	1/0	-	-
Bee Parasites						
Nomada lathburiana	2	0	0	0	3	2
Myopa testacea	0	0	0	0	1	2
Bombylius major	0	1	0	0	2	1

brood cell became saturated with water during experimental submergence; however, it did not deteriorate or allow water to enter the brood cell itself. When we opened the cells, we found two containing eggs and seven with predefecating larvae of *A. vaga*.

Excavations under plots at PP at the end of May 2000 did not reveal a single *A. vaga*, either adult, pupa or immature stage. This suggests that all bees emerge in the year following their provisioning (univoltine) and do not undertake a prolonged diapause across two or more winters (parsivoltine).

Population genetic structure

Observed heterozygosity at the 14 microsatellite loci varied between 0.16 and 0.84 within individual aggregations, with 3-15 alleles per locus, indicating that the loci were variable and potentially able to detect any signature of population structure. There was statistically significant allelic and genotypic differentiation among aggregations using exact tests. However, for aggregations within the Rhine valley, F_{st} and R_{st} values were very low (for all pairs of Rhine valley aggregations, $R_{st} < 0.03$, see Figure 5), suggestive of considerable inter-aggregation movement and no or weak population structure. Furthermore, across all pairs of sites, there was no significant relationship between geographic distance and R_{st} (Figure 5).

Discussion

Aggregations of *A. vaga* in the vicinity of Karlsruhe remained relatively stable in population size (number of nesting females) across three years, but for those at two sites, PN and PS, where brood cells were probably subject to waterlogging in May 1999. Given that *A. vaga* is a species characteristic of river flood plains and that it nests in early succession stage habitats, it is all the more surprising that the Rhine flood of 1999 apparently led to a population crash.

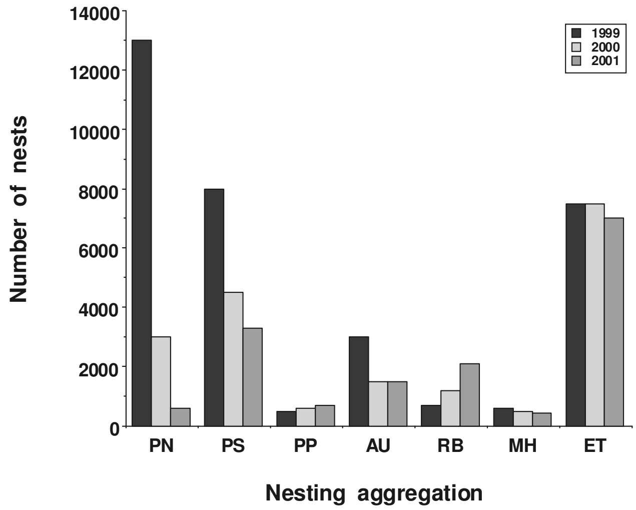

Figure 4. Number of nesting *Andrena vaga* females at seven sites in the vicinity of Karlsruhe, SW Germany, from 1999 to 2001.

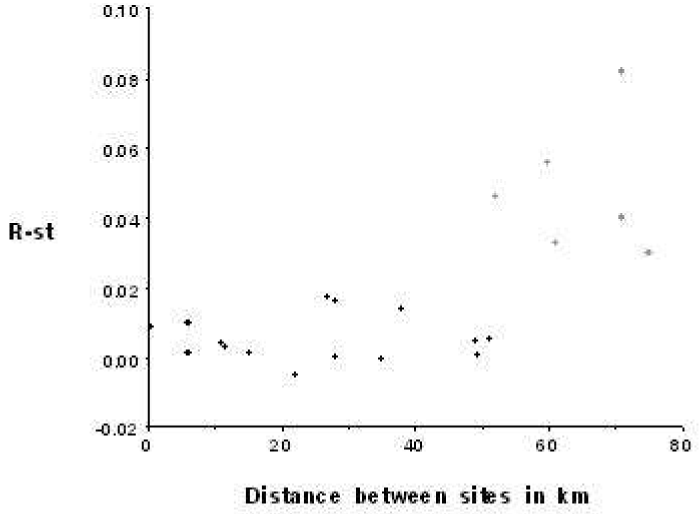

Figure 5. The relationship between genetic and geographic distance for seven aggregations of *Andrena vaga* in SW Germany. Black dots: pairs of aggregations within the Rhine valley; red dots: site ET versus the six Rhine valley aggregations.

Our results show that the immature stages (eggs and predefecating larvae) of *A. vaga* and their parasites inside host brood cells were not able to survive their probable submergence during the record Rhine flood of May 1999. That other bee species such as *C. cunicularius* and *A. ventralis* were similarly affected by the flood suggests a common cause.

Flooding may be a natural phenomenon for fossorial bee nests, and special features of their nest architecture have been interpreted as adaptations to avoid water entering nest burrows and damaging brood cells, their provisions and bee immatures (see review in Roubik, 1989). The nest entrances of many fossorial bee species, including those of *A. vaga*, are always filled with soil, which prevents rain and surface water from entering brood cells. However, these and other features may be of little utility against rising ground-water that may potentially flood a brood cell. The importance of flooding as a significant mortality factor for *A. vaga* was mentioned by Ulrich (1956); a large aggregation of *A. vaga* on the banks of the Großer Zug, a branch of the river Spree in Berlin, apparently became extinct following a high water event.

Paradoxically, though, Malyshev (1926) considered the cell coating of *Andrena ovina* Kl. [= *vaga* Pz.] to be completely watertight already prior to provisioning; our experiments confirmed this view that brood cell linings of *A. vaga* are impervious to water. Indeed, we could not determine the cause of the apparent mortality of *A. vaga* following flooding. For example, sublethal effects of submergence could not be observed. That brood cells were probably watertight over the entire period of flooding suggests that lack of oxygen was the most likely cause of death of the immatures and not, for example, fungal growth of brood cell provisions. That the flooding occurred when the bee larvae were at their smallest and likely had minimal absolute oxygen demand yet the greatest oxygen reserve inside the brood cell suggest that flooding at a later point in time would have had a similarly catastrophic effect on bees (see Fellendorf *et al.,* 2004 for a more complete discussion).

The lack of marked population genetic structure in *A. vaga* within the Upper Rhine valley needs to be interpreted with caution. Population genetic analysis can give a picture of the historical pattern of gene flow, and suggests that dispersal of *A. vaga* was formerly sufficient to erode any differentiation that may have arisen through founder effects or long-term isolation of aggregations, at least within the Upper Rhine valley around Karlsruhe. On the other hand, *A. vaga* aggregations have nowadays become separated by kilometres of inhospitable terrain through habitat fragmentation and conversion of semi-natural habitat for agricultural, industrial or other human needs; it seems unlikely that dispersing adults could locate another nesting aggregation. Direct observation of marked individuals is impractical over distances as great as those represented in this study, and estimates of present-day dispersal await the development of technologies capable of individually tracking small insects for several kilometres over vegetated terrain (see Riley *et al.,* 1996).

Conservation implications

Since 1820, technical conversion of the Upper Rhine has resulted in the loss of 86% of the Rhine floodplains and flood-prone areas; the consequences of this are that the flood risk and peak water levels for the areas downstream of the Upper Rhine have increased considerably. Indeed, during the last two decades of the 20th Century, ten extreme floods have been observed in Rastatt, just south of Karlsruhe, with peak water levels > 7.2 m; global climate change is likely to increase the frequency of such extreme events (Becker and Grünewald, 2003). The May 1999 Rhine flood exceeded all peak water levels recorded on the water-gauge at Plittersdorf in the past millennium, and even exceeded the catastrophic Rhine flood of 1882 by 21 cm. Further extreme flooding as a consequence of these anthropogeneic effects can be anticipated.

In the course of restoration of riverine landscapes, the view is often taken that measures aimed to increase habitat dynamics will provide a supply of habitats suitable for bees (e.g. Siepe, 1999; Mader and Völkl, 2002). However, our observations show very clearly that successful colonization and maintenance of populations of fossorial bees such as *A. vaga* and which are characteristic of riverine habitats require their nest-sites to be protected from flooding. Our results suggest that, on flood-threatened sites where the conservation of fossorial bees and their associated species is important, water levels should not be raised so high as totally to submerge brood cells. When this is not possible on grounds of human safety, then alternative or additional replacement habitats for nesting need to be made available if these keystone species are to be conserved over the longer term.

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LONG-TERM STUDIES OF SOLITARY BEES: WHAT THE ORCHID BEES ARE TELLING US

David W. Roubik

Abstract

Euglossines, ideally suited to study communities, permit 'rapid assessment' and quantification of diversity. Amazonian, Panamanian and Costa Rican wet forests are compared, highlighting Chao estimates and Simpson diversity. Reasonable estimates of both can be accomplished in a single day using five chemical baits, but only longer surveys can provide lists of valid species. In 50 years, the number of valid euglossine species known for Panama increased from 8 to 65. Deeper probes, such as DNA base sequences, haploid male frequencies, and extensive population sampling, complement taxonomic advances, and yield insights into population and community processes. A final theme concerns loose niches, ecological fitting, coevolution, and whether these categories—like 'generalist' and 'specialist'—help in understanding relations between bees and flowers that come from evolution, adaptive radiation, or opportunism, and may fluctuate substantially over time.

An orchid bee window

Orchid bees are unusually specialized. Many live in small colonies and all have long tongues. None is truly social in the sense of having castes or queens. Unusual specialization is inherent in the very long tongue of euglossines—except among certain *Eufriesea*—and in male collection of fragrant chemicals stored in hind tibiae. That trait, in particular, directs much interest and field work toward monitoring abundance, dynamics and other features of euglossine populations, both locally and regionally. The bees are a conservation biologist's 'dream come true' because males avidly seek fragrant chemicals that biologists can present in an appropriate way, with the resultant appearance of bees within minutes. Sometimes, the bees arrive with orchid pollinaria on them, which can often be identified.

But are orchid bees really so different from other solitary bees? Euglossines are monitored so efficiently, concerning orchids, that they reveal how unspecialized these supposedly specialized bees are. It also seems that they are similar to other solitary bees because there are generalists and specialists among them. Most important for population and community work, there are more species of euglossines in tropical American forests than most or all other bee groups, like centridines, xylocopines, halictines, meliponines and megachilids. In addition, chemical and physical means are available for capturing and monitoring female megachilid, halictid, and apid bees, among others. One chemical means is phenylacetaldehyde (S. Droge, personal communication), but this substance must be carefully stored in a cool temperature. Female orchid bees, like other solitary bees, are likely to pursue their nectar or pollen flowers just as avidly as the male euglossines pursue chemicals. Pollen analysis can be done with nest provisions or pollen on bees' bodies. The bottom line for euglossine manageability in quantitative field studies, however, is that there are many individuals and a large number of species, and collection with chemical lures is rapid. In this paper I will show how effective these means are (see also Roubik, 2001a; Tonhasca *et al.*,

2002) in formal diversity and richness estimates, examine what shortcomings may exist, and try to understand the implications of euglossine bee associations with orchids, regarding pollination and conservation.

Taxonomic questions and fine-resolution population study

Taxonomy will never be on the sidelines in tropical ecology. Fifty years ago, 17 euglossine species were listed for Panama; only eight of the names are valid (Michener, 1954). There were only three genera listed (*Exaerete*, *Eulaema* and *Euglossa*), and most species given of *Eulaema* were junior synonyms, while a few *Euglossa* that were not synonyms were simply wrong. Only the three species of the four *Exaerete* present in Panama still have the names applied a half century ago, although *Exaerete* is not classified in a separate tribe with *Aglae* (see Moure, 1950). Only ten years ago, with the advent of chemical baiting for male euglossines since the 1960s, 58 species of euglossines were listed from Costa Rica (Griswold *et al*., 1995). Currently, there are 70 known euglossine species in the two countries, 65 in Panama and 66 in Costa Rica (Roubik and Hanson, 2004).

Few euglossine subspecies (geographic variants) have been recognized. Some variation within a species is expected, for example tongue length (Roubik, 1993; Roubik and Hanson, 2004), but taxonomy is not applied uniformly to variation (Moure, 1967; Kimsey and Dressler, 1987; Ramírez *et al*., 2002, see Zink, 2004). For example, *Euglossa villosa* in Mexico is green, but red in Panama. *Euglossa ignita* varies from mostly red to all green, while Dressler (1978) designated red *Euglossa bursigera* and *E. gorgonensis* taxonomically, by naming subspecies.

Two relatively new lines of genetic investigation further our knowledge on population subdivisions within euglossines. Allozymes revealed that many males are diploid, therefore ‘failed females’ and unable to reproduce (Roubik *et al*., 1996; Zayed *et al*., 2004). As the proportion of diploid males increases (34% of male *Euglossa imperialis* are diploid in Panama), the effective population size is reduced. Indeed, a low effective population size, caused by inbreeding and loss of alleleic variation, is also the cause of diploid male production by Hymenoptera. How can euglossine populations persist, with an expected time to extinction of 60 generations, even in very large forests (Zayed *et al*., 2004)? In the absence of incoming alleles, none of the relatively inbred populations studied in Panama could continue, thus we must assume that dispersal or migratory ability of euglossines enable them to persist.

Genetic variation studies are of great utility in conservation (Packer and Owen, 2001), and there is known variation in different regions within the range of a single species (see Takahashi *et al*., 2001; Roubik, 2001b). At the broad geographic level, we found that widespread euglossine species show little differentiation that is geographically restricted, and suggested barriers were rare but some high-elevation ‘filters’ exist, preventing population circulation (C. Dick, D. Roubik, K. Gruber, E. Bermingham, unpublished data). However, *Euglossa ignita*, one very widespread species (Brazil to Mexico) has marked differences in populations separated by a short distance in Panama. From the preceding, a conclusion is that euglossine species richness can be measured adequately with taxonomic means, and that community processes can be studied accurately, but there are exceptional cases. In addition, many euglossine females are undescribed or cannot be identified or matched to their males, until further taxonomic advances are made (Roubik, in press). Microsatellite markers for euglossines have been studied with extensive population samples of one species within a relatively small transect, *Euglossa hyacinthina* in Panama, but revealed no variation (T. Giray, personal communication).

Euglossine diversity at the community level

How effectively can chemical baiting techniques be used to portray euglossine communities? Studies in Panama with a history of many years provide unique reference points. Often-cited figures of 50 euglossine speices at Cerro Campana, Panama and 57 euglossines for Central Panama do not mention that this area has at least three broad forest types, and considerable topographic diversity, including lower-elevation (ca. 1000 m) cloudforests. However, a single lowland site can have nearly 50 species. Forty-nine euglossine species are present on Barro Colorado Island. Five species found there (Roubik, personal observation), *Exaerete trochanterica, Exaerete dentata, Euglossa cyanura*, *Eufriesea purpurata* and *Eulaema leucopyga* were not included among the 44 species attracted to 16 chemical baits, placed at the center of the 16 km^2 island, during the course of a year (Ackerman, 1983a).

In Table 1, Ackerman's year-long survey is compared with another year-long study in the Ducke reserve near Manaus, Central Amazonia (Oliveira and Campos, 1995) and with short-term surveys in the western Amazon, in Costa Rica and Panama (Roubik, unpublished data), ranging from one to six days. Five chemical baits were used in the latter surveys (cineole, methyl salicylate, vanillin or methyl cinnamate, skatole, eugenol) and the attracted bees were captured and identified.

A conclusion is that any chemical baiting survey will miss some species. Another conclusion is that truly rich euglossine communities are very similar, with about 50 species and a Simpson diversity of around 0.9. It also seems that a survey of a single day has great utility, and may reveal almost as much about local community structure as studies lasting a full year. A 'RAP' or 'rapid assessment' study of bee diversity should be a potential invertebrate contribution to the vertebrate and plant groups now surveyed in such biological inventories (see Alverson *et al.*, 2000).

Table 1. Estimates of diversity and richness in six Neotropical forests. (At each site, a few euglossine species do not come to any bait).

No. individuals, spp.	**Baiting***	**Locality**	**No. Species$_{est}$, S.D., max****	**Simpson *D*§**
21,842, 44	56, 16, 365	PAN: BCI	44, 0; 44 (49)	0.853
2418, 38	26, 8, 365	BR: Manaus	39, 2; 43	0.837
1121, 43	18, 5, 6	EC: Yasuní	46, 3; 52	0.912
951, 30	4, 5, 5	BO: Madidi	38, 6; 50	0.829
200, 27	1, 5, 1	CR: Las Cruces	37, 5; 47	0.924
195, 33	2, 5, 2	PAN: Santa Rita	39, 2; 43	0.945

* Number of baiting days, number of different chemicals employed, and period during which the baiting studies were performed (days)

**Chao estimate of total species; given by $S_{est} = S_{obs} + (a^2/2b)$, where a = species observed only once, b = species observed only twice, S = total species (estimated and observed). The S. D. is derived from variance, given by $V_{est} = b\,[a/b/4)^4 + (a/b)^3 + (a/b/2)^2\,]$; Southwood and Henderson 2000. For BCI, the parenthetic maximum is a 'total evidence'observation, using methods besides chemical baiting.

§ Simpson Diversity, unbiased estimator; given by $D = (N/N\text{-}1)\ (1 - ?\ f^2)$, where f is the frequency of individual species in a collection of N individuals; Lande *et al.* 2000.

Categorizing pollinator niches

An empirical study in forest canopy for two or three successive seasons (Roubik *et al.*, 2003) indicated that loose niches are common among generalist flowering plants and pollinating bees. What is a loose niche? Loose niches vary with time and abundance of the participants, which may be specialists or generalists, but tend to be the latter. About half of plant species showed large variation in their pollinator species from year to year, and African honey bees visited many such flowers. An earlier study of specialization (termed 'tight' relationships) versus generalized relationships ('loose' relationships) among orchids and euglossines (Roubik,1992) showed that about two-thirds were tight. As 'ball park' estimates of what is going on in nature, these figures allow a model of loose niches, tightly-fitted, coevolved niches, and opportunistic "ecologically-fitted' (Janzen, 1985) plant-pollinator relationships (Figure 1).

What good comes from considering loose niches or ecological fitting, or supposedly close coevolution? In the case of orchid bees studied in central Panama, the mode average is visitation of five orchid species, and many apparently specialized species, at the taxonomic level, are not (review in Roubik and Hanson, 2004). Orchids use one to a few bees (Ackerman, 1983b). Loose niches can include either coevolution or ecological fitting, but coevolution and ecological fitting are mutually exclusive. The model allows the last two to vary in relative proportion, while maintaining loose niches at 50%. It is uncertain whether this figure applies equally well to all communities (Vazquez, in press), but the empirical data were taken in the primary forest of a biological corridor that connects South and Central America, along the Caribbean watershed, and is one of the most biodiverse regions in the hemisphere. An assumption made here is that generalization is a part of all plant-pollinator relationships, and that cryptic generalists, or variation in the degree of specialization from year to year, are common. In some cases there may also be very rapid turnover in the local population, reflecting extinction and immigration *within a species population*. If we divide the world up into specialists and generalists, and stable versus unstable populations, then the integrated niche theory (Figure 1) appears useful. In the future, we may recognize whether proportions of those species with loose niches that are tight mutualists or specialists vary, as a function of community complexity or longevity. One of this theory's basic premises comes from the theory of island biogeography (MacArthur and Wilson, 1963) which takes the view that extinction and immigration are a part of any community, and ultimately determine how many species coexist there. In two plant-pollinator communities compared in Fig. 1, one has more species, and a greater proportion of coevolved pollination interactions, than the other. If there were no extinction, the most complex community would be closed to immigrant species, maintaining itself solely through coevolved or loose niches, and adaptive radiation. Pollination relationships are more restrictive, and potentially more fragile, as communities become more complex. But they never arrive at a state where more than half of all relationships are of this kind—according to the graphical model (Figure 1). In contrast, a species-poor community will have fewer tight or restrictive relationships, and it may also be more open to immigrant species. The species that are participating are either diffusely-coevolved mutualists (similar to specialists, but possessing loose niches) or they are recent arrivals, with no evolutionary interactions with local species. There is a lack of ecological theory or data, so far, concerning what their eventual impact is, because all were once 'invaders' (Roubik, 2000; Vazquez and Simberloff, 2002).

Loose niches are the most common category in the most complex communities. This is because, as a hypothesis, diffuse coevolution has adjusted the relative abundances of different pollinators so that they are complementary. If one is very scarce, locally or regionally, another may take its place. Such ‘backup systems’ likely contribute to the sustainability of mutualist assemblages. In the case of tight coevolution, if the mutualist is locally very scarce (for example, a pollinator is unable to find its food or breeding site), local extinction may result, followed by re-colonization (Harrison, 2001).

Pollinators are often scarce and environments or habitats endangered. However, unless there are no flower visitors or pollinators, and human disturbance or the weather are consistently fatal, and the habitat is completely cut off from immigration by mutualists, the expected actions of opportunists and loose or tight mutualists will tend to guarantee essential pollinator services.

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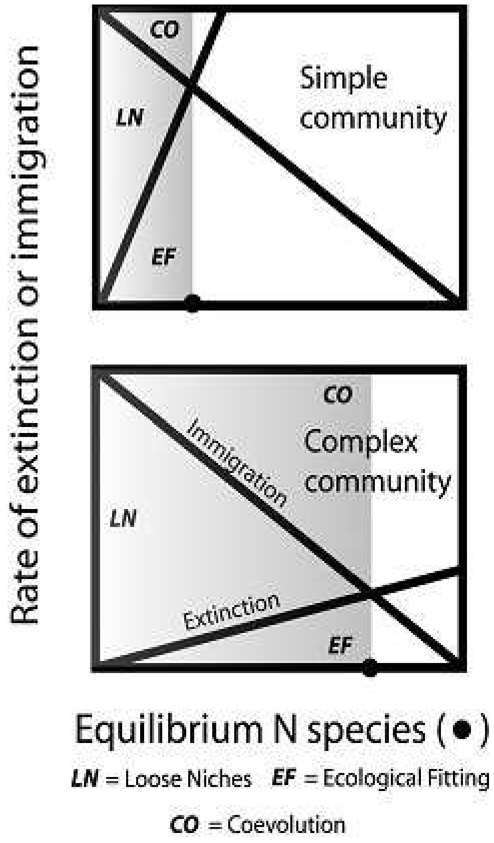

Figure 1. Two types of plant-pollinator communities, species-rich and species-poor, and predicted relative abundance of three kinds of plant-pollinator relationships (SHADED AREAS): co-evolved (tight relationships), loose niches (variation in relationship), and ecological fitting (opportunistic, non-co-evolved relationships), see text for further explanation.

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SESSION III

CONSERVATION AND ECONOMIC VALUATION OF SOLITARY BEE POLLINATION SERVICES

1. Gordon W. Frankie and S. Bradleigh Vinson. “Restoring Native Bee Pollinators: A Case History in Costa Rica”

2. Claire Kremen. “Pollination Services and Community Composition: Does it Depend on Diversity, Abundance, Biomass, or Species Traits?”

3. Adam G. Drucker. “Economic Valuation of Bee Pollination Services: Implications for Farm Management and Policy”

4. Hayo H.W. Velthuis and Adriaan van Doorn. “The Breeding, Commercialization and Economic Value of Bumblebees”

RESTORING NATIVE BEE POLLINATORS: A CASE HISTORY IN COSTA RICA

Gordon W. Frankie
S. Bradleigh Vinson

Abstract

Several countries have recently developed initiatives to conserve and protect pollinators. One of the main recommendations emerging from these initiatives calls for new research on pollinators with attention to restoration. Because they are one of the world's most important pollinators, bees, and especially native species, are emphasized in these research recommendations. To restore bees to an area requires certain baseline information and a realistic short and long-term plan of action. Examples of needed baseline information include: historical records on diversity of known or suspected bee species; preferred host plant records; year-round plant and bee phenological patterns; information on nesting requirements; standardized monitoring techniques for plant phenology and bee diversity and abundance; and historical and current records on human disturbance patterns. We present here a progress report of one project in Costa Rica that uses this baseline bee information to reestablish native bees to an area that has been disturbed by human activities such as fire, grazing, and partial deforestation.

Introduction

Several countries such as Brazil, Britain, Canada, S. Africa, and the United States have recently developed initiatives to conserve and protect pollinators, which are declining in many parts of the world (Stubbs and Drummond, 2001). In general, the initiatives address a variety of biological, ecological (incl. methodology), socioeconomic, educational, and political issues. Two important ecological issues are restoration and monitoring of pollinators.

In this paper we discuss restoration and monitoring in light of our ongoing research on native bee pollinators in the highly seasonal dry forest of Costa Rica. We begin with a series of considerations that should be made before a serious investment in resources and time is made to restore pollinators to an area. This is followed by a chronological account of our work since late 1999 to restore bees to a deforested site on a functional cattle ranch that still has a large amount of mostly intact natural vegetation. Evidence for bee decline in this and a nearby area was recorded in 1996 (Frankie *et al.*, 1997).

Considerations before starting Restoration

Restoring native bee pollinators in an area requires much planning, ongoing evaluation, and a realistic time frame to realize a productive and measurable outcome. Although a great amount of biological information is needed, several relevant social issues also must be addressed.

The major considerations are:

1. An appropriate site must be selected. It should have stable ownership, easy access by vehicle, nearby water supply, security against grazing animals and fire, and a cooperative and involved land steward.
2. Is there baseline information on the flora and bee fauna of the site being considered for restoration?
3. Is there information on habitat and/or vegetative types of the area?
4. Is there information on nesting requirements for the major bee groups?
5. Which quantitative methods available for monitoring bees to record changes in bee diversity and abundance through time would be most effective?
6. Is there historical information on human activities and disturbances in the study area over a several year period?
7. Is there a written plan for restoring bees with short and long term goals and an associated plan of action? Is site maintenance built into the plan?
8. Is there a committed group of individuals, including the landowner/steward, who will remain with the restoration work over the long term?
9. Is there an associated environmental education program with the restoration project? Does it include consideration of special status or protection of bees?
10. Are there adequate funds for the project?

The above considerations have been generated during the course of our native bee restoration project at a site in the seasonal dry forest of northwestern Costa Rica. The project started in late 1999 and is expected to be completed by 2010. Lessons learned each year have been used to reassess progress, make appropriate adjustments, and to continue focusing on short and long term goals. The following summary represents a progress report after almost five years of work.

Study Site

The center of the restoration work is at Hacienda Monteverde, located 8 km NNW of the town of Bagaces (pop. ca 5,000) in the Tempisque region of Guanacaste Province (see Frankie *et al.*, 2002 for map). In late 1999 we established a 3 ha plot at the Hacienda for the restoration. The site is a gently sloping hillside that had been deforested several years earlier and which had grown fallow since that time. We also used an earlier-established larger area surrounding the Hacienda as background habitat for monitoring plant diversity and phenology, bees, and human disturbance. The area, 10 km X 10 km, consists of mostly intact wooded savanna (~70%), several riparian corridors with perennial water (~8%), the small rural community of Bagaces (~2%), and pasture/regenerative forest (~20%). Most of the 100km^2 area is owned by private landowners. Just north of Hacienda Monteverde there is, however, a small section of public land owned by the National Park Service.

Hacienda Monteverde and the immediate land surrounding this site belonged to one family since 1923, and it is scheduled to continue under this ownership for at least another generation. Development of this property over the years has been slow, and most of it was done for cattle ranching prior to the start of our work. We began our initial biological studies there in the early 1970s and in early 2000 had the landowner's permission to restore the 3 ha site for pollinators (see Consider. 1 and 2).

Restoration Concept and Implementation History

In late 1999 the general concept of reforesting the 3 ha plot was developed (Consider. 7). It involved planting a diverse selection of local plants in close proximity to each other that are highly attractive to bees. The idea was to monitor bees visiting these plants with the expectation that a high diversity (and possibly high abundance) would be drawn to the plot.

There was much background information on plants, bees and their ecological relationships to support this predicted outcome of restoration (Consider. 2-4). For example, there were about 250 bee species known from the Tempisque region (Frankie *et al.*, 1976, 1983; Snelling, 1984), and some of their nesting requirements were also documented (Coville *et al.*, 1983; Vinson and Frankie, 1977, 1991, 2000; Frankie *et al.*, 1988, 1993). The flowering phenology of most of the ca 500 angiosperm species in the 100km^2 study area were also known, and 70% of them were adapted for bee pollination (Frankie *et al.*, 1974, 2004; see also Bawa, 1974). Finally, habitat and vegetation types were already classified for the site (Frankie *et al.*, 1988).

In early to mid 2000, seeds were collected from a wide variety of local plant species known to be highly attractive to bees. Some species, such as *Tabebuia rosea* (Bignoniaceae), known to be highly attractive and common to the area before cattle pastures were developed, were collected in proportionally higher numbers (Consider. 6). The seeds were planted in black plastic bags in a small nursery in the town of Bagaces. Once the young plants were of sufficient size and the rainy season began (around mid May), the more vigorous individuals were selected for planting in the field plot at Hacienda Monteverde (rainy season typically extends from mid May to early November). During this year about 400 plants representing about 80 species were planted in 1.5 ha of the 3 ha plot. The same procedure was repeated in 2001 when another 400 plants (and same 80 species) were planted in the second 1.5 ha section. In addition, resident plants in the plot known to be attractive to bees were conserved. In all, the 3 ha plot had about 100 bee-attractive plant species that represented trees, shrubs, vines/lianas, and herbs. As a group, these plants were known to attract a wide diversity of bees throughout the year (Frankie *et al.*, 1983 and unpub.).

During the period from May 2000 to July 2002, there were numerous activities implemented to keep the plants healthy and growing in the plot (Consider. 7). These included watering new plants during their first dry season, building and repairing fences to keep out grazing cattle and horses, constructing fire breaks outside the plot (Frankie *et al.*, 1997), and weeding around individual plants, especially to reduce the exotic grass, *Hyparrhenia rufa*, locally known as jaragua grass. This is a fire-adapted species from Africa, which was introduced to provide forage for cattle (note: native Guanacaste vegetation is not fire adapted. All wildfires in the region are human-caused).

By mid 2002, there was considerable flowering in the 3 ha plot, mostly of the lower plant forms. Pioneer tree species such as *Cochlospermum vitifolium*, *Muntingia calabura*, *Gliricida sepium*, and *Byrsonima crassifolia* also had begun to flower, but tree species of later seral stages had not yet flowered.

With this flowering diversity, an early bee-monitoring program was designed with the short term goal of learning about the capacity of the plot to attract bees to an early seral stage of development (Consider. 5). A 200m X 50m subplot was established within the 3 ha plot, and

30 pan traps (6 oz. plastic dishes – 10 sprayed with yellow fluorescent paint, 10 with blue fluorescent paint, and 10 left unsprayed cream color) were alternately spaced equidistantly through the middle of the 200m subplot. Pans filled with soapy water were placed in the field from 0900 to 1500 during one day in the middle of each month from August 2002 to July 2003 to trap bees. Bees were also lightly collected by two individuals using aerial nets at flowering vegetation inside and outside the subplot. Results of the work are still being analyzed, but preliminary data show that about 100 bee species came into the 3 ha plot and directly adjacent areas.

In 2004 most of the restoration project is being devoted to maintaining the 3 ha site for future monitoring of bees.

2005-2010 Future Plans

In 2007 we plan to monitor the bees again and expect that many of the tree species will be in flower by that time. The last planned monitoring of bees will take place in 2010 when most of the 100 plant species will be flowering. It is also the time when the plant vegetation will begin to change into a later seral stage, and the trees will begin to form a more closed canopy. Each year during the period 2005-2010 regular maintenance will be required to protect the site (Consider. 7 and 8).

Discussion

Restoring bees to a tropical dry forest has thus far proved to be a challenge because of the long time frames involved, numerous relevant logistics that need to be considered, and continuing ecological and social uncertainties. Perhaps the most limiting aspect of our work in this particular site is the long waiting period before most of the highly attractive bee trees come into flower and the understory plants are still flowering (probably at years 7-8). It is at this time that the greatest diversity and abundance of bees are expected.

One of the most demanding logistic aspects of a restoration project such as this is the need to establish a long-term maintenance program of the planted plants. In our study site, wildfires pose the greatest risk to plants, and much of the maintenance after the first year is directed at fire prevention measures such as constructing fire breaks and reducing combustible fuel loads, especially the exotic jaragua grass. Once the trees assume a larger size and their crowns shade the understory (probably year 9 or 10), the grass will largely disappear, thus greatly reducing the fire hazard (Frankie *et al.*, 1997).

One of the ecological uncertainties is monitoring bee pollinators, and doing it in a meaningful, quantitative way that can be standardized. Various methods have been proposed over the years, and recently the fluorescent pan trap method has received a lot of attention in North America among numerous bee ecologists (see above for pan trap description). We used the pan traps in our study and found them to be good for small bees, but poor in attracting large bees of the genera *Centris*, *Epicharis*, *Xylocopa*, and the Euglossini. To compensate and supplement the pan collections, we also used light netting, which helped to capture some big bee representatives. Even so, we could not reach large bees visiting the taller trees around the 3 ha perimeter. In the future, we will use bee-floral visitation counts in addition to pan traps and netting. These counts can be made on most plants except for very tall trees.

They involve recording numbers of bees and types (to genus or family) visiting flowers in a 2m X 2m patch of robust flowering vegetation for a 2 minute period and, after several replications on different plants, calculating an average for particular plant species. Binoculars are used for small to medium trees and, where possible, a voucher collection of bees is netted for confirming identifications (Frankie *et al.*, 2002).

Moist and wet tropical forests probably will pose many of the same kinds of bee restoration challenges that we experienced in the dry forest. In fact, the taller the forest trees, the greater the challenge because monitoring bees is much more difficult. However, other tropical vegetation types of lower stature may be easier sites in which to restore bee pollinators because the composition of plant types would likely contain a higher proportion of shrubs, vines, herbs, and perhaps a few small trees, which are easier to grow and monitor. In this regard, temperate environments may be easier areas for restoring bees because of the high proportion of bee-attractive annual plants that make up these communities. Smaller plants are easy to propagate, flowering shortly after they are planted, and could be monitored for bee visitations right away.

Several questions concerning bee populations attracted to the study site remain, regardless of vegetation type. A list of these include:

1. Is there an optimal size for an area for restoring bees? A minimal size?
2. Do bees visit attractive plants in a plot and nest elsewhere? If so, how far away?
3. What percentage of bees present in the original undeveloped site and surrounding area can be expected to visit a concentrated planting of attractive plants? (Based on the ca 100 bee species attracted to date (see above) and our experiences with previous bee collections in the surrounding Tempisque (N ˜ 250 species) region, we predict that the maturing plants in the 3 ha plot and immediately adjacent area will in 2007-2008 attract 150-175 bee species. This prediction assumes that local human disturbances will remain about the same).
4. How much monitoring of human disturbance is necessary to interpret changes in bee numbers in an area through time?
5. Are monitoring methods adequate for detecting changes in bee numbers through time? (We used pan traps plus netting and could have used additional methods such as wooden trap nests and bee visitation counts on selected plant species (Frankie *et al.* 2002 for methods).

There are other less tangible problems that must also be considered at some point in a restoration project. For example, will there be adequate funding for the life of the project (Consider. 10)? When do the bee experts begin turning over site maintenance and perhaps bee monitoring to other professionals (or paraprofessionals)? How should landowners/ stewards be kept involved? Is it necessary to pay them for conserving and protecting their bees? How much energy and time should bee (or other pollinator) experts commit to education and outreach in neighboring communities (Consider. 9)? What types of outreach are most effective in involving these communities to restore bees or other pollinators, for example, publications, websites, videos, radio talk shows, etc? It is relatively easy to restore plants to an area and see immediate results, but restoring and maintaining populations of associated bees is another matter.

To conclude, as bee experts, we have or can acquire the biological/ecological means to restore bees to an area, although there will certainly be unique problems associated with every restoration attempt. We are encouraged by our work to date to restore bees to a 3 ha plot in Guanacaste Province, Costa Rica, however, there are still many challenges ahead which will require new approaches (including social and economic). Not until completion of the next bee monitoring in year 2007 will we be able to fully evaluate our case study of bee restoration.

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POLLINATION SERVICES AND COMMUNITY COMPOSITION: DOES IT DEPEND ON DIVERSITY, ABUNDANCE, BIOMASS, OR SPECIES TRAITS?

Claire Kremen

Introduction

Insect pollination is required for 15 – 30% of food production from approximately 900 of the world's 1300 crop species ((McGregor ,1976; Roubik, 1995). Bees are the most important crop pollinating insects, and are thought to account for 75% of crop pollination requirements (Nabhan and Buchmann, 1997). Only a dozen bees are managed for pollination worldwide (Kremen *et al.*, 2002b); in contrast, hundreds to thousands of the world's 20,000 bee species may contribute to crop pollination as unmanaged populations (Free, 1993; Nabhan and Buchmann, 1997). Little is known about the extent to which wild bees contribute to crop pollination under various circumstances, which species contribute, the economic value of this contribution, or the influence of global changes (e.g. habitat alteration, invasive species, climate change) on wild bee populations (AllenWardell *et al.*, 1998; Kremen and Ricketts, 2000). Yet, it has often been observed that alternative bee species are either somewhat to greatly more efficient than the most widely-used managed pollinator, *Apis mellifera* (the European honey bee) in a variety of crops (e.g. alfalfa, blueberry, cranberry, sunflower, watermelon and many more) (Kevan *et al.*, 1990; Parker *et al.*, 1987).

Of great and immediate interest is the effect of agricultural practices themselves, particularly agricultural intensification, on wild bee communities and the services they provide. Agricultural intensification includes shifts to: (1) larger field sizes, (2) crop monocultures, (3) increased pesticide use, (4) intensive soil and water management practices including the use of synthetic fertilizers. Collectively, these practices tend to reduce the abundance and diversity of floral and nesting resources for wild bees by destroying or degrading natural habitat refugia, and removing alternative floral hosts (weeds, crops and native plants). In the industrialized world, a large proportion of agriculture is practiced intensively, while in developing countries, this trend is also underway. In a study in the Great Central Valley of California, a prime agricultural region, Kremen *et al.* (2002) found that agricultural intensification reduced the diversity and abundance of wild bee communities, with dramatic effects on the pollination services that these wild bee populations provided to watermelon (Figure 1). Organic watermelon farms near to natural habitat had, on average, two to four times as many visits per flower per day from twice as many species, compared to organic and conventional farms far from natural habitat. Accordingly, 80% of the organic near farms received sufficient pollination from the wild bee population, while only 50% of organic far and 0% of conventional far farms received sufficient pollination. These latter farms imported honey bees in order to have adequate fruit set for their crop. Since pollination function, species richness and aggregate bee abundance all declined on farms that were far from natural habitat (both types) relative to those that were near, it was difficult to disentangle the relative importance of different community attributes (richness, abundance, composition) to function.

Disentangling the roles of these different community attributes is important because of a growing realization that the relationship between biodiversity and ecosystem function is complex, including not only species richness, but also species identities (traits), distribution of species abundances, species interactions, aggregate abundances and the spatial and temporal variation of these community attributes (Symstad *et al.*, 2003). However most research has focused uniquely on the relationship between species richness and function, in highly controlled rather than natural settings (Hector *et al.*, 1999). In the California watermelon study, this gradient of agricultural intensification provides a “natural” community disassembly experiment. In this paper, I re-examine the original dataset on a site-by-site basis rather than by farm type, in order to examine the effect of community disassembly on pollination function, and to disentangle the effects of richness, abundance, biomass and species traits. I use the dataset to ask five questions. First, what is the relationship between pollination function and species richness? Second, what is the relative importance of aggregate abundance, biomass and species richness in explaining pollination function? Third, what is the relationship between diversity and abundance or biomass (either at the community or species level), and in particular, does density compensation occur with declining diversity? Fourth, are species losses ordered? Fifth, what species traits are correlated with extinction-proneness, and how does community composition contribute to function?

Methods

Studies of wild bee pollination were conducted on 14 organic and conventional watermelon farm sites in Yolo County, California, that varied in proximity to natural habitat. Bee abundance and diversity was assessed in watermelon patches along 50 m transects for 10 minutes each half hour from 7:30AM to 2:30 PM on a single day for each farm site during allowed weather conditions. Sampling effort was equivalent for all sites. Pollination efficiencies were studied by allowing individuals of each sex of each species to visit a previously unvisited (bagged) female watermelon flower, and then counting the pollen grains remaining on the stigma following the visit. Total pollen deposition per farm site was estimated by summing the products of efficiency by abundance (for each species and sex) over the time period studied (see (Kremen *et al.*, 2002b) for further detail). To estimate mean biomass for each species and sex, between 6 to 26 measurements of intertegular span were made on pinned, dried vouchers of female bees from our study sites (median = 14), and between 4 to 10 for male bees (median = 5). Intertegular spans(IT) were then converted to dry weights (mg) using the power function, IT = 0.77 (dry weight)^0.405 (Cane, 1987). Honey bees occurred on all farm sites, even those without managed colonies. I previously showed that their abundance did not influence the community composition of wild bees on watermelon farms and that competitive interactions between wild and honey bees were rare (Kremen *et al.*, 2002b). For these reasons, honey bees are not considered further here.

To examine the functional form of the relationship between species richness and pollination function, I used linear and non-linear regression (Larsen *et al.*, in manuscript). I used multiple regression to examine the relative contributions of richness, aggregate abundance and aggregate biomass to pollination function; regression to examine the relationship between richness and aggregate abundance or biomass, and non-parametric correlations (Spearman’s rank) to examine the relationships between individual species abundances or biomass with richness. I also used non-parametric correlations to examine the relationship between extinction proneness and species-specific traits, based on females only (average biomass, median pollination efficiency, pollination contribution), since females are the most important pollinators, and efficiencies vary greatly between males and females within a species (Kremen *et al.*, 2002b). Analyses were conducted in JMP 3.2.5 (SAS Institute). Finally, I used a nestedness calculator from http://www.fmnh.org/research_collections/zoology/nested.htm to determine the degree of order in species extinctions (Atmar and Patterson, 1993) across sites.

Results

Bee species richness significantly explained a substantial portion of total pollen deposition (Figure 2) when modeled either as an increasing power function ($y = 45.8x^{1.41}$, $R^2=0.74$, df=13, F=44.18, $p<0.0001$) or as a linear function ($y = 79.96 + 0.98x$, $R^2=0.77$, df=13, F=72.9, $p<0.0001$). A linear relationship means that the addition of each species increases pollination function equally; in other words, species are functionally interchangeable. An increasing power function means that at high diversity, additional species add disproportionately more function than at low diversity. This could result either from inter-specific interactions that enhance function, or because only the high diversity sites contain species that are functionally important. While we cannot distinguish between these two hypotheses from these regressions alone, it is clear that the data does not support a saturating power function, in which species added at higher diversity add increasingly less to function (redundant species). A saturating function is the most commonly proposed and observed diversity-function relationship in other systems (Schwartz *et al.*, 2000).

Importantly, however, when aggregate abundance and biomass were included with richness in a multiple regression, only abundance and biomass contributed significantly (Table 1), explaining most (97%) of the variation in the data. Thus, once the effects of aggregate abundance and biomass are accounted for, richness does not contribute any additional explanatory power.

Richness is nonetheless important in determining pollination function in this system, because both aggregate abundance and biomass strongly depend on it (Figure 3). In other words, density compensation is not occurring at the community level, because as communities are disassembled and species lost from sites, overall abundance and biomass also decline significantly. There was also no evidence for density compensation at the level of individual species. For each species, I calculated the correlation across sites between (1) its abundance versus species richness (2) its abundance versus abundance of remaining species, (3) its estimated biomass versus estimated biomass of remaining species. All correlations were either non-significant, or showed positive effects between the species' abundance and the community attribute (Table 2). Thus, at the species level, each species either did not respond to the factors that caused richness, abundance, and biomass to decline with increasing agricultural intensity (e.g. Figure 2, and (Kremen *et al.*, 2002b), or responded in a similar manner to the community–level attribute. If density compensation were occurring at the level of individual species, we would expect to see negative correlations for one or all of these tests.

Species composition was strongly nested among sites (temperature: 10.93°, randomized 48.63°, Monte Carlo $p = 0.000016$). In other words, species composition of communities at less diverse sites consistently represented subsets of species present on more diverse sites, suggesting that there is a non-random order to the loss of species (community disassembly). Figure 4 shows how species are distributed among sites. Extinction-prone species were larger (Spearman's rank test, $r = 0.83$, $p = 0.0009$) and more efficient (Spearman's rank test, $r = 0.69$, $p = 0.01$), but extinction-proneness was not correlated with abundance.

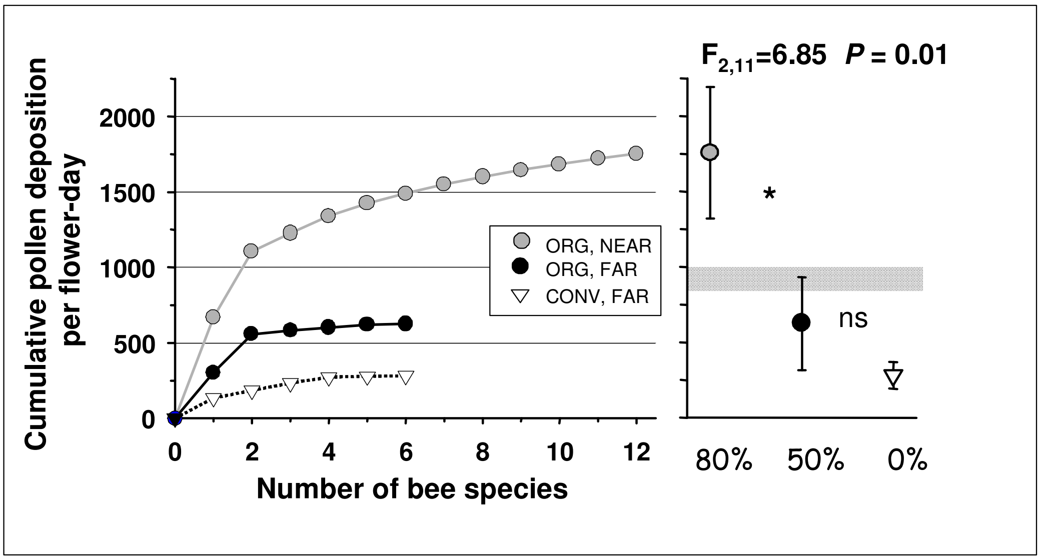


Figure 1. The effect of agricultural intensification on estimated pollination function provided by wild bees (adapted from Kremen *et al.*, 2002). The left panel shows the cumulative mean estimated pollen deposition contributed by each additional species in three farming conditions, organic near farms (gray circles), organic far farms (black circles), and conventional far farms(white triangles), as described in Kremen *et al.* 2002. Species are added in rank order of contribution, generating saturating functions. The right panel shows the means and standard errors of the total estimated pollen deposition by wild bee populations. Organic near farms had significantly higher total pollen deposition than organic or conventional far farms, but the latter were not statistically distinguishable. Watermelon requires deposition of 1000 pollen grains to set a marketable fruit; therefore, 80% of organic near, but only 50% of organic far and no conventional far farms, received sufficient pollination from wild bees alone (G = 5.9, df=2, p = 0.05).

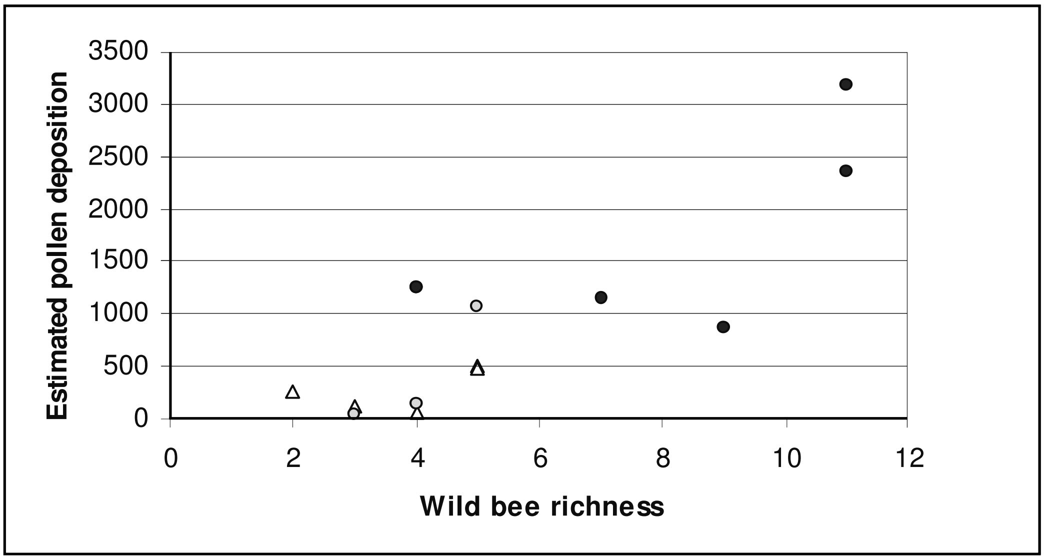


Figure 2. Total estimated pollen deposition against species richness of wild bees on different farm sites. Environmental characteristics of farms are indicated by symbol and color. Gray circles = organic, near; black circles, organic, far; white triangles; conventional far, as described in Kremen *et al.* 2002. Both linear and power functions (see text) fit well.

Table 1. Multiple regression of pollination function against species richness, aggregate abundance and aggregate biomass (overall model, F=140.2, df = 13, $r^2_{adj} = 0.97$, $p < 0.0001$).

Effect	Parameter estimate	Standard error	F Ratio	Probability
Species richness	32.27	25.67	1.6	0.24
Aggregate abundance	2.53	0.50	25.3	0.0005
Aggregate biomass	0.40	0.05	72.9	0.0001

Table 2. Correlations between individual species abundances and richness across sites, individual species abundances and abundance of remaining species, and invidual species biomasses and biomass of remaining species. All correlations were either significantly positive or neutral, showing that species abundance and biomass do not increase as community richness, aggregate abundance or aggregate biomass decrease. *: $p<0.05$, **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $P < 0.0001$. Species are listed in rank order of occurrence across sites. Note that significant positive relationships do not occur simply because of a large number of zero values; conversely, significant trends are also observed among commonly occurring species.

Species	Frequency of occurrence	Individual abundance versus richness	Individual abundance versus remaining species abundance	Individual biomass versus remaining species biomass
Lasioglossum (Dialictus) spp.	14	ns	ns	ns
Halictus tripartitus	14	<0.1	ns	ns
Halictus ligatus	11	<0.1	ns	<0.1
Lasioglossum (Evylaeus) spp.	8	****	ns	<0.1
Agapostemon texanus	5	**	**	***
Hylaeus spp.	5	***	ns	*
Lasioglossum spp.	5	**	***	<0.1
Melissodes spp.	5	****	<0.1	*
Bombus vosnesenskii	3	**	ns	ns
Peponapis pruinosa	3	ns	ns	ns
Bombus californicus	2	**	ns	ns
Halictus farinosus	2	*	ns	<0.1

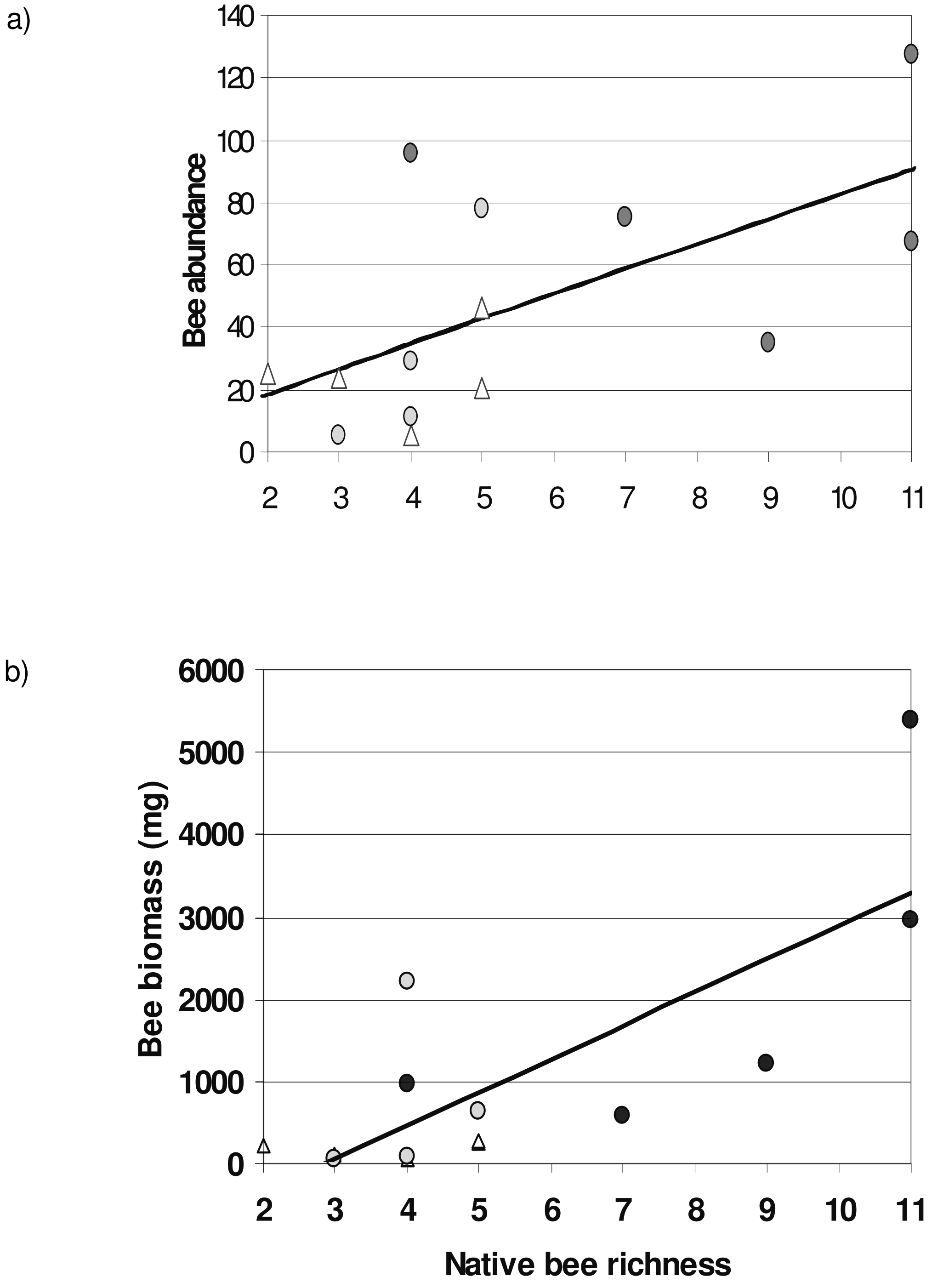
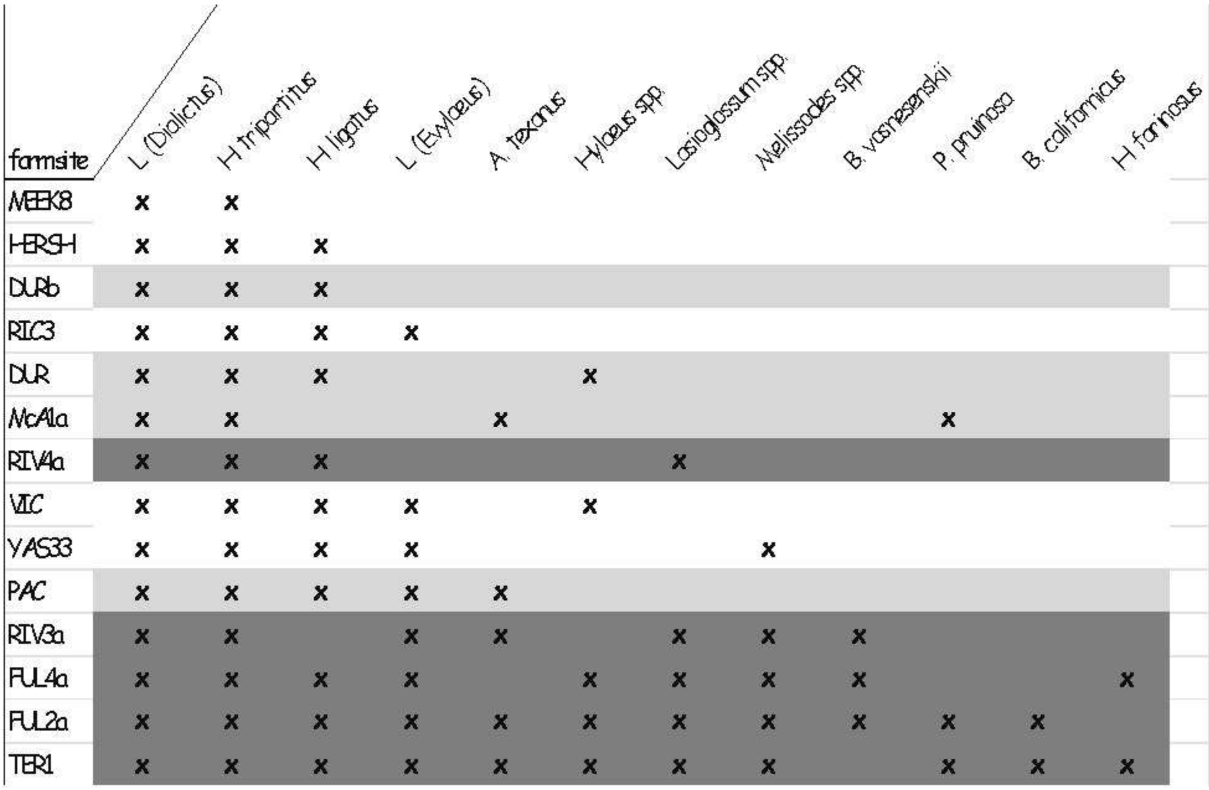


Figure 3. The aggregate abundance and biomass of wild bees regressed against species richness on different farm sites. Environmental characteristics of farms are indicated by symbol and color. Gray circles = organic near; black circles, organic far; white triangles; conventional far, as described in Kremen *et al.* 2002. (a) Aggregate abundance declines significantly with richness, F = 7.99, r^2_{adj}=0.40, p = 0.015; (b) Aggregate biomass declines significantly with richness, F=17.8, r^2=0.56, p=0.001.



farmsite	L (Dialictus)	H tripartitus	H ligatus	L (Evylaeus)	A. texanus	Hylaeus spp.	Lasioglossum spp.	Melissodes spp.	B. vosnesenskii	P. pruinosa	B. californicus	H farinosus
MEEK8	x	x										
HERSH	x	x	x									
DURb	x	x	x									
RIC3	x	x	x	x								
DUR	x	x	x			x						
McA1a	x	x			x					x		
RIV4a	x	x	x				x					
VIC	x	x	x	x		x						
YAS33	x	x	x	x				x				
PAC	x	x	x	x	x							
RIV3a	x	x		x	x		x	x	x			
FUL4a	x	x	x	x		x	x	x	x			x
FUL2a	x	x	x	x	x	x	x	x	x	x	x	
TER1	x	x	x	x	x	x	x	x		x	x	x

Figure 4. The distribution of species among sites. Sites are ordered from lowest to highest richness, while species are ordered from least to most extinction-prone. Site-type is indicated by color: gray = organic near, black = organic far, white = conventional far.

Discussion

Agricultural intensification, which included both a decline in the proportion of natural habitat, an increase in pesticide usage, and a decrease in the diversity of floral resources on farm sites, was responsible for a large decrease in wild bee species richness, aggregate abundance and biomass, and pollination function. While abundance and biomass proved to explain the majority of the loss in pollination function with agricultural intensification, both of these community properties depended upon species richness. In other words, as species dropped out along the gradient of agricultural intensification, other species did not respond by increasing in abundance or biomass. Thus, no density compensation occurred which could otherwise have mitigated the loss in pollination function. There is therefore little resistance (ability to retain community properties under disturbance) or resilience (ability to recover from disturbance) for pollination services when they are subjected to agricultural intensification. The most likely explanation is that the factors correlated with agricultural intensification are generally negative for all bee species, although certain species are far more sensitive than others (Figure 4). Thus the abundances of each species tend either to remain neutral or to decline along with declining diversity, aggregate abundance or aggregate biomass (Table 2).

Diversity is therefore important because each additional species adds its complement of pollinating individuals to this system. Diversity is also important, of course, because different bee species are differentially attracted to different crops, are differentially effective as pollinators within or between crops, or interact in a manner that enhances pollination efficiency (Greenleaf and Kremen, in manuscript, Kevan *et al.*, 1990; Kremen *et al.*, 2002a). Bee population abundances are highly variable in space and time (Williams *et al.*, 2001); thus a species that is an unimportant pollinator in one year may be important the next year (Kremen *et al.*, 2002b). Collectively across sites, diversity of the bee community was important in providing pollination services (Balvanera *et al.* in revision), and diversity buffers spatial and temporal heterogeneity of bee populations.

Communities are disassembled in a strongly non-random order (Figure 4), with the largest and most efficient species lost first. These larger species contribute to aggregate biomass disproportionately to their abundance, which explains why aggregate biomass so strongly complemented aggregate abundance in explaining pollination function (Table 1). The correlations between extinction-proneness and species traits also suggest that functionally important species tend to be most sensitive to agricultural intensification. Simulation studies supported this hypothesis. In a simple simulation, we randomly assembled communities while holding species richness at the maximum value for a given farm type, and then compared estimated pollination function of the simulated communities against the observed communities. We found that conventional far farm types had significantly lower estimated function then predicted from the simulation, suggesting that the most important contributors are selectively removed from these communities (Kremen *et al.*, 2002b). In a more complex simulation, we set the aggregate abundance and the richness according to that found at each site, but randomly selected species. We then estimated the total pollination function based on the efficiencies of selected species. Here we have found that most sites had lower pollination function then their site-specific simulations (mean of 250 measures), again suggesting that functionally important species are preferentially lost in response to anthropogenic disturbance. Thus agricultural intensification not only reduces diversity and abundance of pollinators, but also reduces pollination services by selectively killing the most important pollinators.

To date, there has been little appreciation of the role of community attributes other than richness in determining ecological function. The “diversity-function” debate has centered chiefly on whether diversity enhances ecological functioning because of niche complementarity (e.g. greater utilization of ecological resources through specialization), or simply due to sampling effects (e.g. the most functionally important species are included more often in more diverse communities simply due to random chance) (Huston, 1997; Loreau *et al.*, 2001; Tilman *et al.*, 2001). The evidence is overwhelming, however, that, communities are *not* randomly disassembled in response to disturbance (Atmar and Patterson, 1993; Petchey and Gaston, 2002); therefore what is relevant to conservation and the management of ecosystem services is the manner in which disassembly occurs and its effect on function. This study, along with several others on widely different ecological communities and functions (Duarte, 2000; Jonsson and Malmqvist, 2003; Ostfeld and LoGiudice, 2003), suggest that the selective loss of functionally important species in response to anthropogenic disturbance may not be uncommon.

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ECONOMIC VALUATION OF BEE POLLINATION SERVICES: IMPLICATIONS FOR FARM MANAGEMENT AND POLICY

Adam G. Drucker

I. Introduction

The demand for agricultural produce of good quality, low cost and predictable supply has led in the past century to massive transformations of rural environments as a result of the process of agricultural modernisation and the pursuit of increased food production. Modernisation has been characterised by externally developed packages of technologies that rely on externally produced inputs such as the use of "high yielding" varieties of crops and animal breeds, fertilisers, pesticides and machinery (Pretty, 1995). A range of environmental and health impacts have also been associated with such practices. These include, *inter alia*, land use changes, habitat destruction, deforestation, soil erosion, water and air pollution and the loss of biodiversity.

One particular aspect of the impact of modern agriculture on the environment has been the reduction in insect pollination. Such services have an estimated annual global value of US$ 65-70 billion (Pimentel *et al.,* 1997) and are growing given the demand by an increasing human population for food crops dependent on pollination. Modern agricultural systems therefore face a trade-off between the benefits of increasing production and the environmental costs associated with this increased production. Under such circumstances, environmental economic analysis can assist in ensuring that such trade-offs are made in such a way that social welfare is maximised.

II. Conceptual background to environmental economics and its application to pollination services

Economics is about the efficient allocation of scarce resources which have diverse alternative uses. By applying economics to environmental considerations, we can expect to gain some insights into the desirability of incurring environmental costs and benefits given the overall objective of increasing social welfare.

From a social welfare perspective, when calculating costs and benefits it is important to take any externalities into account. Externalities are the external costs of market exchange that exist when an activity by one agent causes an uncompensated gain or loss of welfare to another agent (Pearce and Turner, 1990). An example of a negative externality is nitrate run-off from a farm affecting nearby residential water supplies. When analysing the financial (i.e. taking an individual rather than a societal welfare perspective) benefit/cost of an activity, these externalities are usually ignored, leading to decisions that are not in the interest of society as a whole. A number of methods for overcoming such private/public divergences exist and may include regulations or taxes that aim to "internalise" such externalities. In the example given above, the farmer could be legally obliged to keep run-off below a certain concentration or be taxed per unit emission. In either case s/he would now be obliged to take the run-off into consideration in the farm-production process. Unfortunately, existing national accounting methods in most countries do not provide adequate incentives to do so as they tend to count only (farm) outputs but do not subtract any value for the associated decline in environmental quality (i.e. natural capital)

Approximately 30% of 1,500 crop plant species worldwide depend on pollination by bees and other insects (Buchmann, 1996). Pollination services can therefore be understood as a positive externality since, with the exception of commercial pollination services, farmers do not pay for this service which results in increased production. By contrast, the destruction of pollination services by modern farming practices can be understood as a negative externality. Not only does the wider farming community have to find an alternative means of pollination, but the significant worldwide decline in both honey bees and native bees currently being experienced (Dias *et al.*, 1999), also affects the pollination of wild species and has other ecosystem impacts which reduce society's overall welfare (given that a change in environmental quality can be considered to constitute a change in human welfare). Hence, in order to understand the economic importance of pollination services, it is necessary to consider this wide range of benefits which they provide and in order to do so an understanding of how such values can be calculated and the limitations/difficulties faced is required.

III. Methods for the valuation of pollination services

A variety of pollination services valuation methods exist and have been applied across a range of crops and countries. The methods used have, *inter alia*, considered (Carreck and Williams, 1999): the market value of all (Matheson and Schrader, 1987) or some (O'Grady, 1987) of the insect pollinated crops grown; others only the proportion attributable to honey bees (Borneck and Merle, 1989). Some have included the value of crops grown from seed derived bee-pollinated plants (Martin, 1975), the legume crops and livestock products dependent on them, or even those legumes that fix nitrogen and thereby reduce nitrate fertiliser requirements (Levin, 1984, 1983). A relatively more sophisticated consumer surplus approach (i.e. one that measures changes in gains to consumers resulting from pollination induced price changes and thereby accounting for the effect of the existence of potential substitute crops) was also developed by Southwick and Southwick (1992, 1989).

IV. Constrains to valuation of pollination services

Unfortunately, there is insufficient information to allow such estimates to be made with great accuracy. In particular, more precise information is required, *inter alia*, on i) the pollination needs of species and varieties of crops; ii) the effectiveness of particular pollinators; iii) the value of locally marketed crops which are usually ignored in national accounts; and iv) a much clearer understanding of the farm-level costs and benefits of different crop and pollination systems (Dias *et al*, 1999). Considering the urgent need to address the issue of worldwide decline of pollinator diversity, the Conference of the Parties (COP) to the Convention on Biological Diversity (CBD) established an International Initiative for the Conservation and Sustainable Use of Pollinators in 2000 (decision V/5, section II) and requested the development of a plan of action. This action plan explicitly recognises the importance of an improved understanding of the economics of pollination and, in particular, calls for "comprehensive analyses in selected production systems of the costs and benefits of alternative management practices and technologies on pollinator conservation and effectiveness, and the valuation of the goods and services provided by pollinator diversity including the pollination requirements and best pollinators of each crop species and the impact of pollinator presence/absence on fruit and seed yield" (CBD/SBSTTA-7, 2001)

V. Results of existing pollination services valuation studies

Nevertheless, despite these existing constraints to valuation, identifying the economic value of pollination services, even in terms of rough orders of magnitude, is important as supporting conservation by determining economic value is an effective approach for protecting ecosystem services. Those studies that have been carried out to date consequently provide useful "ballpark" estimates of the value of pollination services in a number of countries. These are summarised in Table 1. With few exceptions, these studies concentrate on the value of honey bees to agriculture using the following formula:
V x P x D where –

V = annual value of the crop
D = dependency of the crop on insect pollinators
P = proportion of (-effective-) insect pollinators that are honey bees

Given the scarcity of information available, only the main agricultural crops for which national statistics are available are usually considered in calculating "V" and "P" is widely assumed to be 80%. The dependency variable "D" is calculated in a number of ways, varying from rough estimates of high (D=0.9), medium (D=0.5) or low (D=0.1) or that of farmers' opinions to more scientific estimates based on actual research.

The selected studies presented in Table 1 reveal that:

- Most of the calculations are based on the pollination service contribution of honey bees, as it is argued that bees are responsible for the pollination of some 73% of the world's crops (Roubik, 1995) and that the most widely used species in crop pollination is the honey bee.
- Despite the rough nature of the estimates, it is clear from the magnitude of the results that the value of pollination services is significant. Furthermore, they are frequently many times that the value of bee products such as honey/wax, even though only the latter tend to be properly considered in national accounts. The value of commercial pollination services is also small compared to that provided by nature, suggesting that the market for pollination services has to date only been able to capture a small fraction of the total value of pollination services.
- Commercial pollination services have revealed that the benefit/cost ratio of renting pollination services can be very high for the farmer.
- The overall value of pollination services has grown significantly over the past decade, as a growing human population increases the demand for crops which happen to be dependent on pollination. Commercial pollination services are also likely to benefit in the future, as modern farming systems become more dependent on such services as natural pollinators decline.
- In addition to crops, seed production, livestock/pasture production and soil fertility can also be significantly influenced by pollination.
- Aesthetic/existence values for pollinators and the agroecosystems that they maintain through wild species pollination is also likely to be substantial (perhaps even larger than the values for agricultural crops) but have not been calculated to date.

- The more sophisticated measures of pollination service value (e.g. the consumer surplus approach) recognise that a loss of bee pollinators may result in alternative crops being produced/consumed, thereby reducing the actual costs of such a loss. The actual cost would therefore depend on: i) the extent to which crops are dependent on bees; ii) the profitability of the current crop compared to the next best (non-pollinator dependent) crop; and iii) the impact on market prices. There is therefore a need to consider both long-term costs and those that are only incurred during the transition period. It is also interesting to consider the incidence of costs. In countries such as Australia, costs would fall almost equally on consumers and producers.

VI. Conclusions and ways forward

Identifying the economic value of pollination services is important as supporting conservation by determining economic value is an effective approach for protecting ecosystem services. In particular, taking the value of such pollination services into account can:

i) provide farmers with a better understanding of the relative costs and benefits of undertaking certain agricultural practices (e.g. agrochemical use, planting of monocultures, habitat conservation);

However, a range of information constraints need to be overcome before existing pollination service valuation approaches can provide more precise estimates of these values of the type that will be better able to orient the decision-making process. In particular, this will require further research and capacity building with regard to the realisation of activities related to determining the pollination needs of different species and varieties of crops, the effectiveness of particular pollinators and an in-depth understanding of the farm-level costs and benefits of different crop and pollination systems.

ii) support the identification of conservation priorities and the design of cost-efficient bee diversity conservation programmes.

The above economic valuation of pollination services together with an improved understanding of pollinator diversity and conservation costs could be used to design a cost-effective diversity maximizing conservation programme based on the Weitzman (1993) approach. Weitzman's original approach for combining information on the genetic distances between species of wild animal with their extinction probabilities and conservation costs was recently adapted by Simmianer *et al.* (2003) to livestock breeds. Applying such a decision-support tool to pollinator species would allow a given conservation budget to be allocated in such a way as to maximise the diversity of pollinators conserved.

iii) support the design of policy instruments and mechanisms that promote the sustainable use of bee pollinators.

If the essential pollination services provided by bees are to be maintained, the recent decline in pollinator populations must be reversed. For national agricultural policies that aim to promote sustainable farming and meet consumer demand to be achieved, there must be greater appreciation of the role of pollinators in agriculture and conservation. This also requires the development of policies to halt the erosion of resources, particularly nest sites and food plants that bees require for their survival (Carreck and Williams, 1998).

In this context, the development and dissemination of techniques and guidelines to promote agricultural practices that permit the conservation and sustainable use of pollinators need to be developed. Such guidelines should, *inter alia* (Dias, 1999):

- promote pollination as an integral component of sustainable agricultural systems and cropping practices
- promote the maintenance and management of natural areas for pollinators in different farming systems and interfaces between ecosystems, such as forest crop margins, protected areas, etc.
- promote the restoration of pollinators and pollinator habitats following degradation an natural calamities.

Nevertheless, while improved awareness among the principal actors (farmers, extensionists, researchers, policy-makers, etc.) influencing pollinator conservation and sustainable use may lead to improved practices, there is also a need to deal with the underlying economic incentives that currently lead to the loss of pollinators. Much clearer incentives for sustainable agriculture need to be provided (not only because of pollinator loss) by ensuring that modern agricultural practices internalise their environmental costs as much as possible. Land use restrictions and obligations to maintain natural habitats within agroecosystems need to be better enforced and the subsidies provided to intensive farming systems need to be reduced (e.g. agrochemical and fuel subsidies, cheap loans for farm machinery purchase, etc.). By contrast, farmers could be subsidised for adopting environmentally-friendly practices that generate positive externalities (e.g. habitat and wildlife conservation, watershed management, reforestation, bee-keeping). This is also likely to require a significant change in the way national accounts are compiled, so that they properly consider changes in environmental quality and reflect these in indicators of national welfare (unlike the present measure of gross domestic product).

The development of niche markets for sustainably produced farm products can also assist this process. This will require the identification of appropriate marketing (e.g. certification practices) and trade policies, and legal and economic measures which support beneficial practices. (CBD/ SBSTTA, 2001).

Table1. Summary of selected pollination valuation studies

Location	Methodology	Plant Species	Key Assumptions	Value of Pollination Services	Other Key Findings	Reference
United Kingdom	Proportion of pollination attributable to honey bees	Arable, tree, soft fruit and seed crops	· Honey bees responsible for 80% of total pollination · Dependency is assumed to be high (0.9), medium (0.5) or low (0.1)	· GBP172 million p.a. for outdoor crops (of which GBP137.8 related to honey bees) · GBP29.8 million for glasshouse crops		Carreck and Williams, 1998.
England	Proportion of pollination attributable to honey bees	>13 Orchard and field crops	·Honey bees responsible for 80% of total pollination · Dependency is assumed to be high (0.9), medium (0.5) or low (0.1)	GBP120 million p.a. (2000)	· Based on farmers' estimates of dependency value is GBP54 million · Valuation of pollination in home gardens important but not calculated as unlikely to be significantly dependent on only honey bee pollinators · Proportion of value of pollination internalised through pollination charges is small (1.7%) · Value of pollination to the existing 1,800 wild species likely to be substantial but dependency is unknown. 27% of 321 endangered species are pollinated by bees and declining wild bee populations mean honey bees are likely to play an increasingly important role. · Impact of bee pollinated plants on other animals and the ecosystem in general should also be accounted for.	Temple *et al.*, 2001.

Location	Methodology	Plant Species	Key Assumptions	Value of Pollination Services	Other Key Findings	Reference
USA	Proportion of pollination attributable to honey bees	Major crops		Honey bee pollination value increased from US$9.3billion in 1987 to US$14.6billion in 2000. 20-25% due to inflation, rest because of increased demand for pollinated food by an increasing population	· Leads to conclude that under modern farming methods, most growers will depend more on honey bees · 1/3 of total human diet is derived directly/indirectly from insect pollinated plants · Production of most beef and dairy products consumed in the US is dependent on insect-pollinated plants (e.g. alfalfa, clover, etc.)	Morse and Calderone, 2000.
USA	Consumer surplus approach	62 crops dependent on honey bee pollination	Losses defined as increased costs from additional inputs such as land/ labour required to maintain yields.	US$1.6 - US$8.3 billion p.a.	· Losses associated with other pollinators are in the range of US$4.1-US$6.7 billion p.a. · Aesthetic values and impact on perfume and pharmaceutical industries also likely to be substantia	Southwick and Southwick, 1992.
Yucatan, Mexico	Proportion of production dependent on pollination by any type of pollinator	Principal agricultural crops dependent on pollination	Pollinator loss scenarios generated by assuming losses to be high (0.9), medium (0.5) or low (0.1)	US$ 1.1 million to US$9.6 million p.a. (average between 1986 –1992)	Average honey and wax production during this period was US$7.6 million	Drucker and Magana, 1997.

Location	Methodology	Plant Species	Key Assumptions	Value of Pollination Services	Other Key Findings	Reference
Canada	Proportion of pollination attributable to honey bees	Commercially grown insect pollination dependent crops (excluding seeds)		C$782 million in 1998 up from $444 in 1990	· Honey and wax production valued at Can$93.5 in 1998 · Cost/benefit of pollination services: Each dollar spent in rental fees realised a gain of C$185 (Quebec, apples, 1998) and C$41 for blueberries (1990)	Anonymous, 2001.
Australia	Proportion of pollination attributable to honey bees	35 horticultural and agricultural crops	Speed of adjustment to pollinator loss depends on: i) extent to which crops are dependent on honey bees; ii) profitability of current crop compared to next best (non-pollinator dependent) crop; and iii) impact on market prices and changes in import controls	· A$1.7 billion (1999-2000) · Additional A$2billion in adjustment costs.	· Bee products (A$60 million) are small compared to value of pollination services. · Costs of any loss would be divided equally between producers and consumers	Gordon and Davis, 2003.
Australia	Proportion of pollination attributable to honey bees Total value of production	Major crops		A$1.2 billion p.a.		Gibbs and Muirhead, 1998.
New Zealand	Total value of production			NZ$3.1 billion	· Replacement of nitrogen from pollinated pasture legumes (NZ$1.9 billion) · Total value of fruit crop production (NZ$1 billion) · Total value of vegetables/seed production (NZ$0.2 billion)	Cited in: Gibbs and Muirhead, 1998.

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THE BREEDING, COMMERCIALIZATION AND ECONOMIC VALUE OF BUMBLEBEES

Hayo H.W. Velthuis
Adriaan van Doorn

Introduction

For the general public, bumblebees are among the most appealing insects. They are large enough to be seen, and they are attractive because of their colourful furry coat and the low tone of their buzzing when flying and visiting the flowers in the garden. Their industrious activity, observed when sitting in a shady spot, and sipping a nice cool drink, deepens the relaxation of many of us on a late Sunday morning.

When visiting a botanical garden in the spring or summer, a keen observer will note differences in the preferences that the four or five common species of bumblebees have for various flowering plant species. Honeybees can also be included in this comparison. It is soon clear that 'the bee and the flower story' is more complicated than initially was comprehended. Further inquiry into pollination biology will reveal that there are rather specific preferences, relationships, and matchings and that these three terms are by no means synonymous.

The value of bumblebees as pollinating insects in agriculture has been recognized for a long time. Their tongues are longer than those of honeybees, so they are much better at pollinating flowers with deep corollas. This recognition was followed by the manipulation of their population densities. Not only were refuges and nest boxes provided near and in the fields containing the crop to be pollinated, but queens, or incipient colonies, were frequently transported to these fields, sometimes over great distances. In 1885, for example, four species of European bumblebees were introduced in New Zealand for the production of red clover seeds; the species have settled since and thrived there (Hopkins, 1914; Goulson, 2003). A century later, one of these species was brought to Chile for the same purpose (Arretz and Macfarlane, 1986). The bioindustrial breeding of colonies, out of the natural season, for use in a very technical agricultural setting, the greenhouse, has now been added to this stimulation of a natural development of colonies in a seminatural-agricultural environment. This transition in the application of bumblebees took place in 1987, when dr. R. de Jonghe started the company Biobest, producing colonies of *Bombus terrestris* for the pollination of greenhouse tomatoes. Currently, there are a number of companies that together produce almost a million colonies annually. These colonies are used on all continents.

The currently known 250 species of bumblebees form a monophyletic group, that is subdivided into 35 subgenera (Michener, 2000). Of these, species of the subgenus *Psithyrus* (45 species, Goulson, 2003) are inquilines, that parasitize on the incipient colonies of other bumblebees; each *Psithyrus* species exploits one or a few other species of bumblebees. The genus *Bombus* is often subdivided into the sections *Odontobombus*,which has a spine at the hind apex of the middle basitarsus of the females, and the *Anodontobombus*, in which such a spine is lacking (Krüger, 1920). This subdivision largely coincides (Alford, 1975; Sakagami,

1976) with the behavioural subdivision made by Sladen (1912): i.e., pocket makers and pollen storers. The brood cells of the pocket makers, which each contain a number of eggs or larvae, have pockets at their base into which the pollen is pressed and from which the larvae take their food. In contrast, the pollen storers transfer the pollen from storage pots to the brood cells, where it is fed directly to the larvae by the nurse bees. The *Odontobombus* and *Anodontobombus* subgenera can be roughly distinguished by the difference in their tongue length, the tongue being longer in the *Odontobombus* (Sakagami, 1976).

This difference between pollen storers and pocket makers is a factor of importance when attempts are made to breed them. Pollen storers accept pollen that is placed anywhere in their nest box and carry it to the larvae, which are subsequently fed. In the case of the pocket makers, the pollen has to be put very close to the brood cells; otherwise, the nurse bees will not use it in the preparation of the pollen mass inside the pockets. The production of a well-developed colony of pocket makers, therefore, requires much more and rather frequent attention from the breeder.

Not surprisingly, the five species that are presently available on the commercial market are all pollen storers. In fact, they belong to only two of the subgenera.

The exclusive commercialization of the pollen-storing species implies that there is no mass rearing of those long-tongued bumblebees that belong to the pocket-maker group, species that could also be profitably used in the pollination of flowers with deep corollas. For these crops, there only remains the traditional attraction of the queens of the long-tongued species to the fields by providing nest boxes with adequate nesting material in them. However, in such cases pollination greatly depends on the abundance of queens of suitable species in the vicinity, and is therefore, unpredictable. Moreover, due to the expansion of agricultural activities, there has been a strong decline in the densities of bumblebees and in the occurrence altogether of species in the semi-natural environment.

The history of breeding

Before the industrial breeding could start, an accumulation of knowledge on the biology of bumblebees and on the conditions that favour the initiation and development of their colonies had to take place. We owe much to interested naturalists who took colonies home or attracted queens to artificial nest sites and then reported on their observations and experiences. Sladen (1912), for example, was successful in stimulating queens to start breeding in such nest boxes. After collecting queens in spring, he would place them in pairs in a nest box. He observed how the interactions among the queens led to aggressiveness and subsequently to one of them starting to breed. He was able to obtain this stimulating effect by using workers. Interspecific stimulation was also successful: Sladen used workers of species that start early in the spring to stimulate queens of other species that appear later in the season. This technique, in modified versions, is now incorporated in the industrial production of colonies.

Many others since Sladen have made important observations and developed methods that have added to the success of breeding. Such methods include the kind of nest boxes to be placed in the field, the material inside it that serves as insulation once the colony has made its start, the places where to put the nest box, how to provide some food inside the box, how to introduce captured queens in them, etc. A number of agricultural institutes in Europe attempted to attract bumblebees to field crops in order to achieve better pollination (Bilinski, 1976; Bornus, 1975; Hasselrot, 1960; Holm, 1960; Zapletal, 1961), mostly to conclude that their attempts gave promising results. Reviews of these trials were given by Holm (1966) and Free (1970).

Meanwhile, methods were developed to breed bumblebees under laboratory conditions. Pomeroy (New Zealand), Plowright (Canada) and especially Röseler (Germany) provided the elements that allowed the start of commercial breeding. Under laboratory conditions, queens apparently need to be stimulated in order to start breeding. Species that naturally start breeding at the relatively low ambient temperatures characteristic of early spring needed to be placed in climate rooms with temperatures of 28 ºC. In these early years, two queens were placed together in a nest box. The resulting aggressiveness, often leading to the death of one of them, causes the dominant queen to initiate the construction of a food storage cell. If a pollen ball is provided at the proper moment, she will construct an egg cell on it and lay her first batch of eggs in this egg cell.

Leaving aside the many interesting results of these studies, we need to mention some of the discoveries leading to the commercial breeding technique. They include mating under artificial conditions, how to hibernate queens, and how to stimulate them again. To obtain mated queens, young males are taken from the colonies and placed in flight cages, where they attain sexual maturity at an age of about 14 days (Duchateau, 1985; Duvoisin *et al.*, 1999). Queens, placed in these cages, will be mated by them from an age of 6 days onwards (Tasei *et al.*, 1998). Mating takes place mainly during the morning hours and bright illumination favours success. Because mating takes at least half an hour (Röseler, 1973; Duvoisin *et al.*, 1999), checking the mating cages every 15 min is sufficient for the collection of all the copulating pairs. In this way, the inseminated queens are separated from those that are still unmated. In almost all species, a queen mates only once (Röseler, 1973; Estoup *et al.*, 1995; Schmid-Hempel and Schmid-Hempel, 2000).

An important discovery made by Röseler (1985) was that the obligatory hibernation period could be circumvented by anesthetizing mated queens with CO_2. This method allows breeding throughout the year and was widely used in the initial years of commercial colony production. One disadvantage of the narcosis is that the queens often produce male offspring among the females of the initial brood, a waste because males do not take part in the further development of the colony.

Zapletal and Ptacek (see Ptacek, 1991) used honeybee workers, instead of using a second queen or workers from another bumblebee species, in order to activate a queen in spring. Honeybee workers are more readily available in early spring, and the use of these honeybee workers circumvents the loss of a number of the queens (in case they were put in pairs) due to the resulting fights among them. This modification certainly has its advantages, especially if it concerns an occasional breeding attempt. This method was used by some companies in the early phase of commercial breeding. However, once there was a continuous production of bumblebee colonies, most of the companies considered the use of bumblebee workers or male cocoons a much simpler procedure.

Commercial breeders soon refrained from CO_2 narcosis, because the demand for colonies fluctuates over the seasons. It is much more economic to keep hibernating queens in stock than to breed bumblebees uninterruptedly. Hibernation methods, already applied by Holm (1960), Zapletal (1961) and Pouvreau (1970), were refined by Duchateau in 1985. Basically, queens are given the opportunity to dig themselves into a layer of moist peat dust after mating.

Once enough queens make their hibernaculum, the box containing the peat dust is placed in a storage room. Queens may also be stored in other sorts of containers. The queens are kept at 3-5 ºC for 2-6 months before they are reactivated by placing them at room temperature or in the climate room.

The commercial breeding

The many attempts to stimulate the establishment and growth of bumblebee colonies in or near agricultural field plots are indicative of the hope researchers had concerning the applicability of these bees. However, there was also pessimism concerning the economic feasibility of breeding them for the purpose of pollination (Free, 1970; Röseler, 1979). The relatively high labour costs and the often low success rate were considered major barriers for economic success. It seemed that these costs could only be compensated for by crops that were difficult to obtain, such as high-value hybrid seeds that needed hand pollination. These included the crops that were considered potentially good targets for research (e.g. Minderhoud, 1950; Sneep, 1952; Kraai, 1958; Velthuis and Cobb, 1991).

Quite unexpectedly, in 1985, the Belgian veterinarian and amateur bumblebee researcher Dr. R. de Jonghe, discovered the value of bumblebees for the pollination of greenhouse tomatoes. Up to that moment, the Belgian and Dutch tomato flowers were pollinated mechanically by vibrating the plants 3 times a week. The labour involved in this process exceeded • 10,000 per ha per year (van Ravestijn and Nederpel, 1988). In other countries the tomato flowers were either pollinated in the same way, or treated with hormones (mainly in Southern Europe and South-East Asia) with a comparable frequency. Bumblebees are capable of buzz-pollination and, in doing so, vibrate the flowers at the moment they are physiologically prepared for pollination. As a result, the production levels increased and the tomatoes are of a better quality (the Netherlands: van der Sande, 1990; van Ravestijn and van der Sande, 1991; UK: Banda and Paxton, 1991; Spain: Molina Herrera and Garcia Espinosa, 1992; Japan: Wada, 1993; Italy: Fiume and Parisi, 1994; Turkey: Abak *et al.*, 1995; Canada: Dogterom *et al.*, 1998; Israel: Presman *et al.*, 1999), and thus gave a better price. Moreover, even in the early days, bumblebee pollination was slightly cheaper than mechanical pollination (• 9100 per ha per year in The Netherlands and Belgium).

In 1987 Dr. de Jonghe founded the company Biobest for the breeding of bumblebees. This was followed in the next year by Koppert Biological Systems (the Netherlands), a producer of biological control agents since 1967.

Expansion of Bumblebee Pollination

By 1988, Biobest supplied bumblebee colonies to some 40 hectares of tomato crop (van Ravenstijn and Nederpel, 1988). In the years thereafter, a few more companies in addition to Koppert were founded in Western Europe, mainly in the Netherlands and Belgium. In 1991, almost all tomato growers in those two countries were using bumblebees for pollination (van den Boogaard, 1991). This rapid introduction of bumblebee pollination was facilitated by the fact that many of the tomato growers in those countries had already become very restrictive in the use of chemicals for crop protection: instead of pesticides, they used the parasitic wasp Encarsia formosa to fight their main pest, the whitefly Trialeurodes vaporariorum.

During these first years, the bumblebee producers, especially the bigger companies, not only sold their colonies to the local market, but also started exporting colonies to neighboring (Western European) countries that had high-standard tomato crops comparable to those in the Netherlands and Belgium. From 1992 onwards, they also started exporting colonies to growers in the huge tomato-growing areas in Southern Europe (Spain and Italy), where tomatoes are grown in plastic tunnels. These growers usually lacked sophisticated equipment and used hormones for fruit set and chemicals for crop protection. To be able to use bumblebees for pollination, the growers in these countries had to become restrictive with respect to the pesticides that could be used (listed in so-called Side Effect Lists issued by the bumblebee producers). Thus, within a few years, the application of bumblebees led to a change in the growers' thinking about crop protection and opened up the way to biological control methods. The transition to biological control methods does not, however, always proceed as quickly as desired: the presence of new pest insects and differing climatic conditions often demand the development of new 'answers'. This takes time. Moreover, procedures for the admission and registration of biological agents often take a very long time (Japan for instance). Nevertheless, the transition is taking place all over the world, so that it is safe to say that the use of bumblebees has caused a strong reduction in the use of pesticides in tomato (and other) crops and has thus made the products safer and the environment cleaner.

In 1990, Dr. R.C. Plowright started the Canadian company Bees-under-Glass. This company was taken over by Koppert one year later. Since then, the North American market has been controlled by two European companies, Koppert and Biobest, using local breeding units. These companies have also expanded their activities from North America to Central America (Mexico). As far as South America is concerned bumblebees are only used in Chile (since 1999). These bumblebees are imported from Europe.

In South-East Asia the pollination of greenhouse crops by bumblebees started in 1992. This occurred first in Japan, followed some years later by South Korea. From the beginning, the colonies were imported from Europe. However, some breeding of *B. terrestris* has been conducted in Japan too for the past few years.

In the Middle East an Israeli company started rearing bumblebee colonies with European technological support in 1991; some years later, a second company followed. Initially, these companies produced for the local market only, but, after some years, they started exporting colonies. In Turkey, bumblebee production started in 1997 in order to satisfy the local demands.

Bumblebees have been used in the Northern part of Africa (Morocco) since 1993. These colonies are imported from Europe.

Finally, in the Southern hemisphere, local bumblebee breeding has occurred since 1991 in New Zealand. Bumblebees were accidentally (?) introduced to Tasmania in 1991 or 1992 (Stout and Goulson, 2000). Since then, some local production of bumblebees has occurred there as well. Bumblebees are not yet present on or allowed to be introduced to the Australian continent.

The bumblebee species that are currently in use

The main bumblebee species used for commercial pollination is *Bombus terrestris* (Table 1). This species has a wide distribution. It is found all over Europe, to the coastal area of North Africa and the Middle East, as well as in the Western part of Asia. Within this area, it is represented by a number of subspecies that differ in their coloration (Rasmont *et al.*, in prep.). The wide distribution was an important reason for bumblebee breeders to choose this species. Other aspects taken into consideration were that the species was known to produce relatively large colonies and that it adapts quite well to artificial conditions. Moreover, it appeared that *B. terrestris* queens occurred in large densities in certain areas. In the early years of commercialization, this made it relatively easy to collect from the field the large number of queens that were needed.

Table 1. Bumblebee species commercially cultivated for pollination purposes.

species	geographic origin	used in	number of colonies	population size
Subgenus Bombus				
B. canariensis Pérez	Canary Islands	Canary Islands	30,000	150-300
B. ignitus Smith	Japan, Korea	Japan	2,500	100-200
B. terrestris (Linnaeus)	Europe, N. Africa, Middle East	Europe, Asia, N. Africa, Chile, N. Zealand, Middle East	850,000	200-400
Subgenus Pyrobombus				
B. impatiens Cresson	East N.America	North America and Mexico	70,000	300-500
B. occidentalis Cresson	West N.America	West N. America	1,000	200-400

Nowadays, *B. terrestris* is used not only in countries belonging to its original area of distribution (Europe, Israel, Turkey, Morocco), but also in South-East Asia (Japan, South Korea), as well as New Zealand (where the species had been introduced by Charles Darwin, see Hopkins, 1914), Tasmania and Chile. To date, the total sales of *B. terrestris* are estimated to be around 850,000 colonies per year.

A second 'species' is used in Europe: *B. canariensis*. It is, in fact, a subspecies of *B. terrestris*, endemic on the Canary Islands and used exclusively there. Currently, there is scientific discussion concerning the status of *B. canariensis*. Geographically and based on its colour patterns, it can indeed be considered a separate species (Erlandsson, 1979; Rasmont, 1984a). However, in Linnean terminology, it is definitely a subspecies of *B. terrestris*. Mitochondrial DNA analysis (Estoup *et al.*, 1996) and the fact that it readily mates with other subspecies of *B. terrestris*, leading to fertile offspring (van den Eijnde and de Ruijter, 2000), support this view. The breeding and use of *B. canariensis* started in 1994. To date, some 30,000 colonies of *B. canariensis* are used per year.

The main species cultivated in North America is *B. impatiens*. This species has a wide distribution east of the Rocky Mountains. In the past, a second species, *B. occidentalis*, was bred for use in the states west of the Rocky Mountains. The use of the two species had its origin in the desire not to use a species outside its natural geographic area. However, the recurrent heavy infestations of *B. occidentalis* colonies with the protozoan *Nosema bombi* caused severe problems, so that the mass breeding of this species was discontinued. Nowadays, *B. impatiens* is also used west of the Rocky Mountains. There are, however, some restraints for its application: for example, there is the obligatory use of a so-called 'queen-excluder', that prevents young queens from escaping. This species is also used in Mexico. To date, the total yearly sales of *B. impatiens* amount to 70,000 colonies.

As stated before, *B. terrestris* colonies are used in the Asian market (Japan, Korea). These colonies are mainly imported from Europe. Forced by the discussions about the possible ecological impact of the use of *B. terrestris* colonies, bumblebee breeders have decided to start breeding a native species. The species chosen is *B. ignitus* (Asada and Ono, 1996). Although this species has turned out to be the most suitable one, there are some clear disadvantages. In comparison to *B. terrestris*, the full-grown *B. ignitus* colonies are much smaller, having about half the number of workers, and the breeding success rate is much lower. Therefore, using *B. ignitus* colonies costs at least twice as much as using *B. terrestris* colonies. The Japanese growers are not yet willing to pay that price, a condition which strongly hampers the breakthrough of this species. For experimentation purposes small numbers of *B. ignitus* colonies became available on the market since 1999. In 2003, some 2,500 *B. ignitus* colonies were used in a total Japanese market of 70,000 colonies. In Korea the use of bumblebees (*B. terrestris* only) is estimated to involve 9,000 colonies.

Crops pollinated by bumblebees

The main crop on which bumblebees are used is the tomato. It comprises over 40,000 hectares of greenhouse tomatoes. Although these plants typically grow and flower during several months, the length of the period bumblebees are needed depends upon local circumstances. In the Netherlands, for instance, the growing season lasts 11 months from January through November, while in Spain it is shorter, 7 to 9 months from September through March-May.

During the flowering period of the tomatoes additional bumblebee colonies are introduced on a regular basis. Again, this depends upon local circumstances: colonies are added every 2 weeks in the Netherlands, compared to every 4 weeks in Spain. The idea behind this is to build up, in synchrony with the increase in the number of flowers, a population of colonies with an overlap in the developmental stages. Depending upon crop type and location, the number of colonies during the growing season may add up to 50 per hectare.

The number of colonies that are first introduced, as well as the successive ones, strongly depends upon the type of crop (many more on small cherry tomatoes versus large beefsteak tomatoes) and climatic conditions. The numbers at first introduction range from 3 to 10 per hectare while the numbers at successive introductions range from 1 to 4 per hectare.

Other greenhouse crops pollinated by bumblebees include sweet pepper, eggplant, courgette, melon, pumpkin, red and black currant, raspberry, and strawberry. In the open field, bumblebees are used for apple, pear, peach, apricot, plum, cherry, kiwi, strawberry, blueberry, and cranberry. Honeybees can also serve the purpose for most of these crops, but they are often less efficient. Which pollinator is economically preferable depends on the local costs and on the climatic conditions. In the open field, bumblebees are to be preferred when the temperatures are low;

low temperatures are uncommon in the greenhouse; however, bumblebees often outcompete the honeybee at higher temperatures.

Instead of using entire colonies, it is sometimes favourable to use a number of individual bees. For example, Minderhoud (1950), Sneep (1952), and Kraai (1958) used queens and males for the production of hybrid Brassica seeds. Nowadays, males are used for seed production in onion, cabbage, and leek. These instances always concern small greenhouses or cages. Bumblebee males and queens, adapted to solitary ways of life, survive better than solitary workers.

Aspects of the commercial breeding

All bumblebee producers have developed their own systems, which are primarily kept secret. However, since these systems probably do not differ very much, a general overview is given below.

In the early years, queens were collected in the spring from the field. This concerned populations in the Netherlands, Belgium, France, Spain, Italy, Greece, Turkey and even New Zealand. However, with increasing insight into the colony developmental processes, it soon became possible for the major bumblebee producers to rely completely on their own production. Today, a small proportion of the produced colonies is set apart for the production of queens and males. Hibernated queens are taken from the stock according to a production plan that is related to the sales forecast. Breeding occurs year round since sales do also. For *B. terrestris*, breeding is characterized by two peaks; one occurring in January (start of the new growing season in Western Europe) and one occurring in September (start of the new growing season in Southern Europe, i.e. in Spain and Italy). At Koppert, over 10,000 colonies leave the company every week during these peak periods; this is about 3 times more than in the low season.

After the installation of young queens in the breeding rooms, it typically takes about 8 weeks until the colonies are ready for sale. During this period, the colonies are fed a sugar solution (supplied by a sugar company) and pollen (bought from beekeepers). It can be estimated that, per year, approximately 3 million kg of sugar-water (1 million kg in the breeding itself, 2 million kg as food stores in the greenhouses) and over 200 tons of pollen are used by the bumblebee producers.

Colonies meant for sale are typically selected when they reach a size of around 50 workers. Nowadays, most bumblebee suppliers breed their colonies in relatively small, transparent hives. After a colony is selected for ‘expedition’, the bottom of the breeding hive is clicked inside a bigger ‘expedition hive’ and the cover of the breeding hive is removed. This method enables a quick and safe transfer. The expedition hive usually consists of a plastic inner box and a cardboard outer box. The bees have access to a supply of sugar solution underneath the inner box. Usually, 2 liters of the solution is provided. This is needed because the flowers of the main target crop (tomatoes) do not produce any nectar. This amount is sufficient for the entire lifespan of the colony in the greenhouse, which is typically between 8 and 12 weeks. During this period, the worker force first increases, reaching a peak of 200-300 individuals about 3 to 5 weeks after introduction into the greenhouse. Thereafter, queens and males emerge and the worker force starts to decline. Less sugar-water is added to the colonies when nectar-producing crops are to be pollinated.

As mentioned before, a small proportion of the colonies is set apart for the production of sexuals (queens and males). By controlling these colonies, a parasite-free queen stock can be built up. The main parasites which, in the artificial environment of the breeding, may cause problems are the protozoa *Nosema bombi* and the tracheal mite *Locustacarus (Bombacarus) buchneri* (Alford, 1975; van den Eijnde, 2000; van der Steen, 2000). If managed properly, these 'set-aside' colonies produce, on average, over 200 queens each. Usually, males are produced in abundance in the same colonies and do not need to be reared in extra colonies. Nevertheless, it is important to prevent brother-sister matings, because this would cause inbreeding, expressed by the occurrence of diploid males instead of workers (Duchateau *et al.*, 1994).

A parasite-free status is not only important for the breeding itself, but also for exporting colonies to other countries. Breeding operations are checked by the national veterinarian services and veterinarian certificates are issued when needed. It is curious to note that some of the importing countries request the absence of honeybee parasites and diseases rather than those of bumblebees (which are clearly different species).

Economic value of bumblebees and the crops pollinated by them

Most growers in the Netherlands and Belgium buy 'pollination service', which means that, for the set price, the bumblebee producers deliver as many colonies as needed to achieve full pollination of the crop. The producers usually work with standard introduction schemes, from which is deviated when necessary. According to these schemes, which differ per type of crop (e.g., beef tomato *versus* cherry tomato), new colonies are introduced every two weeks. In most other countries, however, colonies are sold individually. In this case, the grower decides when new colonies are introduced. Generally, these growers follow a scheme with regular intervals, but the intervals are often somewhat longer. In both cases, the nest-boxes are usually not returned to the producers (after the death of the colony), because of the high transport and cleaning costs and because of the risk of importing parasites. This means that the nest-boxes are disposed of locally.

The prices of colonies differ between countries, depending upon the species that is bred, the volume of the market, transport costs, and so on. The prices have come down considerably during the past 15 years since the producers succeeded in improving the success rate of the breeding and in lowering the production costs through mechanization. In the first years (1988-1990) growers in the Netherlands paid about • 200 per colony. Today they pay less than one third of that (around • 60 per colony).

Because of the strong interrelationship between bumblebee pollination and biocontrol, all bumblebee producers have started to sell not only bumblebees for pollination, but also insects, mites and microbials for crop protection. The smaller companies usually do not breed these biologicals themselves. The total turnover of this industry (producers and distributors) can be estimated at • 100 million per year; some • 55 million of this can be attributed to bumblebees.

Most of the bumblebee colonies (95%) are used in greenhouse tomato crops. The value of these bumblebee-pollinated tomato crops can be estimated at • 12,000 million per year.

We refrain from estimating the crop value of the minor crops, because the use of bumblebees or honeybees varies from year to year. The value of bumblebees for hybrid seed production, mainly in onion, leek, and cabbage, also remains incomparably low considering the value of tomatoes, even though such seeds are quite expensive.

Environmental concerns

In the earlier years of commercial production, field-collected queens gave much better results than queens produced in the breeding programs. These queens, therefore, were collected in large numbers. This occurred not only in the Low Countries, i.e. at relatively near distance to the breeding site, but also in several countries in Southern Europe. Besides the subspecies *Bombus terrestris terrestris* from Western and Central Europe, *B. t. ferrugineus* was collected from Spain and Portugal, *B. t. sassaricus* from Sardegna, and *B. t. dalmatinus* from Greece and Turkey. These distant populations had the advantage that the bumblebee queens emerged from hibernation several months earlier than those of the more northern populations. In addition some of the queens aestivate in these southern populations, while others hibernate (depending upon the geographical and climatical conditions). Therefore, colonies from these queens could become available for pollation much earlier than colonies from queens of Western European populations and they could be placed in the greenhouse as soon as January and February. Generally speaking, these two populations, aestivating and hibernating, are isolated subpopulations (located on islands, for instance), although in some cases there may be some exchange of genetic material (mountain versus lowland population). There are also some indications that, in a certain area, two generations may occur per year (one being of minute extent) (Rasmont, 1984b, for *Bombus terrestris*; Peeters *et al.*, 1999 for *Bombus jonellus*). This subject clearly needs further study.

Today, the main subspecies used by the commercial breeders is *B. t. dalmatinus*. It is preferred because of its superiority with respect to colony size.

The massive collection of these queens ‘from the wild’, however, invoked protests among the citizens of these countries (e.g. Ösbek, 1993), a factor that made the companies search more intensively for the alternative: an efficient scheme for breeding and hibernating queens from their own stock. This scheme soon became economically successful and field collection was abandoned, first by the larger companies and then later by most of the others.

The use of geographic races and their subsequent use in other regions led to another concern (e.g. Ornosa, 1995): what would the consequences be if males or queens, escaping from the greenhouse, mix with the local races? Laboratory experiments showed that there was no difficulty in obtaining hybrids (de Jonghe, 1985; Duchateau, 1996; van den Eijnde and de Ruijter, 2000). However, since males and queens produced in greenhouse colonies do not appear in synchrony with those of the local population, it was considered quite improbable that interracial matings would take place. Also, foreign races, adapted to the climates of far-away countries, would probably hardly have a chance to establish themselves in the new environment. Nevertheless, these kinds of concern made the government of the Canary Islands demand that only *B. canariensis* be used in the greenhouses in their territory. Breeders and tomato growers found a way out: *B. canariensis* colonies are produced in Belgium and the Netherlands and then returned to the Canary Islands.

Apart from introducing a *B. terrestris* race in an area where it can potentially meet another race, there is also the exportation to areas where *B. terrestris* does not occur at all. *B. terrestris* could establish itself in these areas or perhaps even hybridize with another closely related species or occupy the niche of such a species (Ono, 1997; Dafni, 1998). In theory, it is also possible that *B. terrestris* imports carry a bumblebee disease or parasite not yet present in the area and that in the new environment these organisms may find a new host species (Goka *et al.*, 2001). These considerations have made people, and governments, oppose to importations. However, the interests of farmers are also at stake. Thus, the European bumblebee is now used in Japan and Korea and has entered Tasmania (accidentally???), where it interferes with the local bee fauna (Stout and Goulson, 2000; Hergstrom *et al.*, 2002).

It would have been possible, technically, to breed colonies locally, in quantities, and at times whenever they are needed. This type of breeding, however, would have had a rather seasonal character and starting the production anew takes much more effort than its continuation. Furthermore, since the dearth period for selling needs also to be compensated, locally produced colonies would become much more expensive than those that come from a year-round regular producer. This is the economic mechanism that has prevented the emergence of a network of local production of colonies, with the consequence that the potential risks have had to be implicitly accepted. From the viewpoint of safety and nature protection, however, local production, preferably of a local pollinating species, should be encouraged. In any country, the local tomatoes can most probably be pollinated by those bees that prefer the native Solanaceae; if there is concern about the dangers adhering to importation, the gouvernment as well as the farmers should invest in research if they want to compete on the international market (e.g. Hogendoorn *et al.*, 2000; Estay *et al.*, 2001). This takes time, but there is no shortage of biologists capable of doing the job.

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SESSION IV

REARING AND MANAGING SOLITARY BEES: *OSMIA* AND *MEGACHILE*

1. Jordi Bosch and William P. Kemp. “The Life Cycle of *Osmia lignaria*: Implications for Rearing Populations”

2. Antonio Felicioli, Miloje Krunic and Mauro Pinzauti. “Rearing And Using *Osmia* Bees for Crop Pollination: A Help From A Molecular Approach”

3. Anthony Raw. “Ambivalence Over *Megachile*”

4. Rogel Villanueva-Gutiérrez and David W. Roubik. “Pollen Sources of Long-Tongued Solitary Bees (Megachilidae) in the Biosphere Reserve of Quintana Roo, Mexico”

THE LIFE CYCLE OF *OSMIA LIGNARIA*: IMPLICATIONS FOR REARING POPULATIONS

Jordi Bosch
William P. Kemp

Abstract

The solitary bee *Osmia lignaria* has been developed as a manageable orchard pollinator in North America. Adult *O. lignaria* are active in the spring, building and provisioning nests. By early summer, eggs laid during the current year complete development to fifth instars, which spin cocoons and enter an apparent dormant stage. Pupation occurs by late summer, followed by adult eclosion within one month. Eclosed adults remain in their cocoons for the winter, and emerge in the spring as temperatures increase. Thus, *O. lignaria* are univoltine and winter in the adult stage. Respiration rates indicate that *O. lignaria* undergoes two diapausing periods, one in the prepupal stage in the summer, and another in the adult stage during autumn and winter. Exposure to warm and/or fluctuating temperatures is required for summer diapause completion. Exposure to cold temperatures is necessary to complete winter diapause. The timing between adult eclosion and the onset of winter temperatures is critical to *O. lignaria* survival. Pre-wintering adults exposed to warm temperatures for too long use up their fat body reserves, loose weight and vigor and are more likely to die during the winter. In this study, we discuss the implications of these results for the establishment of appropriate rearing methods for *O. lignaria* populations used in orchard pollination.

Key words: *Osmia lignaria*, development, wintering, diapause, orchard pollination

Introduction

Osmia lignaria (Hymenoptera: Megachilidae) is a North American solitary bee that flies in the spring and is a very effective pollinator of fruit trees. For these reasons, it has been developed as a manageable orchard pollinator (Torchio, 1982a, 1985; Bosch and Kemp 1999, 2001). *Osmia lignaria* females nest in pre-established cavities (mostly beetle burrows in dead wood). Each female flies for approximately one month, during which time she may build one or more nests. Nest contain several (1-10) cells, each with a pollen-nectar provision onto which an egg is deposited. When the last cell is completed, females seal the nest entrance with a mud plug. *Osmia lignaria* are univoltine. Development from egg to adult takes place during the spring-summer and adults do not emerge until the following spring.

When managed for orchard pollination, *O. lignaria* nests containing wintered adults are placed adjacent to empty nesting materials in nesting shelters. Typical nesting materials are wood blocks with drilled holes that females use as nesting cavities (Torchio, 1982b; Bosch and Kemp, 2001). Nesting shelters provide protection from rain and direct sunlight. Females mate shortly after emergence and, after a short pre-nesting period (2-3 days) during which their ovaries mature, initiate nesting activities (Bosch and Kemp, 2001). After petal fall, when most nesting females are old or have died, nesting materials containing nests with eggs and young larvae are removed from the orchard and taken to a storage area to avoid excessively hot summer temperatures, predation (by birds, rodents, ants and various scavenger beetles) and parasitism (by chalcid wasps) (Bosch and Kemp, 2001). Then, nests need to be wintered (exposed to cold temperatures) during the winter. Populations wintered adequately are ready to emerge and nest the following spring as fruit trees begin to bloom.

In many areas, *O. lignaria* populations can be easily reared under natural or close-to-natural conditions. However, when managed on a large scale, a certain degree of manipulation is desirable, not only to avoid parasitism and predation as mentioned above, but also to better time emergence and nesting with the bloom of a variety of target crops, ranging from almonds in February to apples in May. Timing is important because orchard crops bloom for a short period (3 weeks approximately). Therefore, a thorough understanding of the life cycle of *O. lignaria* is essential to establish adequate rearing and management methods, and ensure an adequate supply of populations prepared for pollination (Bosch and Kemp, 2002).

Here, we review a series of recent ecophysiological studies on the development, wintering and emergence of *O. lignaria*. We address the effect of temperature regimes on developmental rates, respiration rates, diapause development, fat body condition, survival and vigor. We emphasize timing between development and environmental conditions, especially during the critical pre-wintering period. We also address differences in developmental rates and temperature requirements in populations from different latitudes. Finally, we discuss the implications of these studies for the establishment of adequate rearing protocols for managed populations.

Development

Osmia lignaria populations from northern Utah, USA, nest in April-May. Eggs hatch within a week and develop through five instars (Torchio, 1989). By early summer (June), after completing the consumption of the pollen-nectar provision, the fifth instar spins a cocoon with silk strands from the salivary glands (Torchio, 1989). Pupation occurs approximately in July, followed by adult eclosion a month later. Respiration rates (O_2 consumption and CO_2 production) drop from ~ 0.5 ml/g·h during the cocoon-spinning stage, to ~ 0.05-0.1 ml/g·h 7-10 days after cocoon completion. From then on, respiration rates in the cocooned larva (prepupa) steadily increase up to ~ 0.2 ml/g·h at pupation through to adult eclosion (~ 0.35 ml/g·h) (Figure 1; Kemp *et al.*, 2004). In populations from northern Utah, prepupae exposed to 22 °C or higher pupate within a month of cocoon completion (Bosch and Kemp, 2000). However, when exposed to 18 °C some prepupae fail to pupate. These prepupae may remain alive for over a year, but do not pupate. The prepupal stage takes ~ 29 days in individuals exposed to a continuous 22 °C temperature, compared to ~ 17 days in individuals exposed to a fluctuating thermoperiod (14:26 °C; mean: 22 °C). The duration of the prepupal stage is greater in populations from southern latitudes than in populations from northern latitudes (Bosch *et al.*, 2000, Bosch and Kemp, 2001). Southern populations also have higher temperature thresholds for pupation. For example, in contrast to northern Utah populations, populations from Texas do not readily pupate at 22 °C (Bosch and Kemp, 2001). The progeny of northern populations forced to fly ahead of their natural time in a warmer area maintain the developmental rate of their parental population (Bosch *et al.*, 2000). Thus, developmental rates appear to have a strong hereditary component.

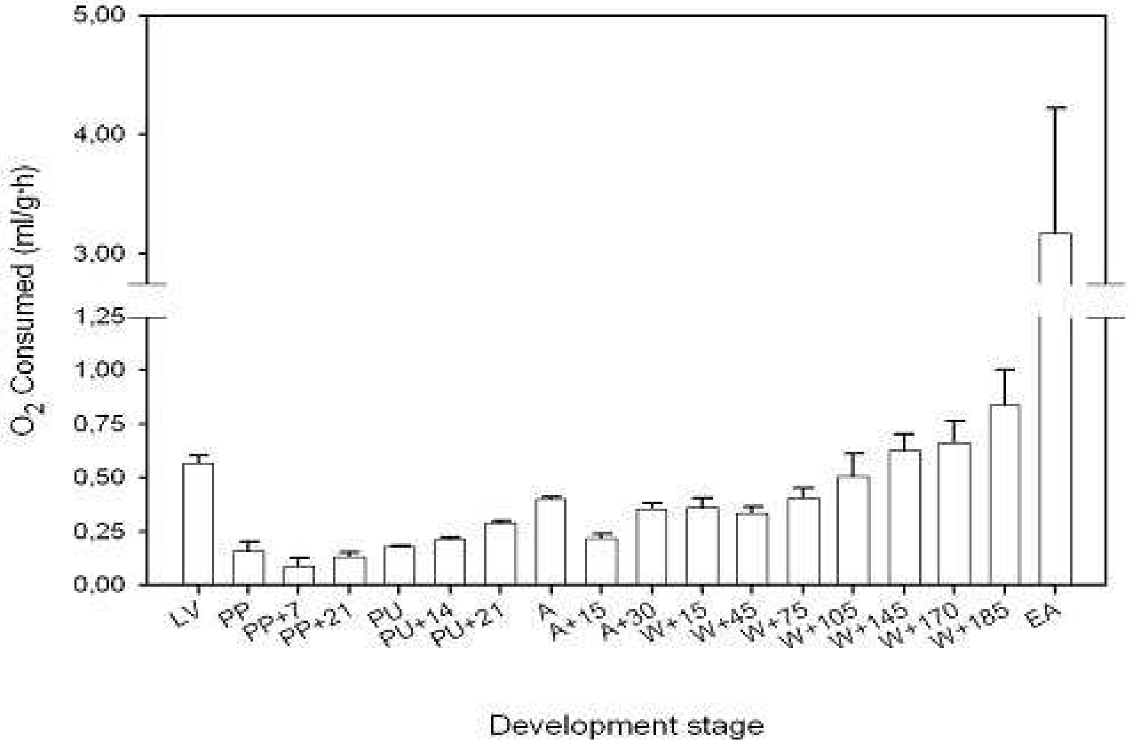


Figure 1. Oxygen consumption (mean + 95% CI) of *O. lignaria* males at selected developmental stages. LV: cocoon-spinning fifth instar larva; PP: prepupa; PP+7: prepupa + 7 days; PP+21: prepupa + 21 days; PU: pupa; PU+14: pupa + 14 days; PU+21: pupa + 21 days; A: adult; A+15: adult + 15 days; A+30: adult + 30 days; W+15: adult wintered for 15 days; W+45: adult wintered for 45 days; W+75: adult wintered for 75 days; W+105: adult wintered for 105 days; W+145: adult wintered for 145 days; W+170: adult wintered for 170 days; W+185: adult wintered for 185 days; EA: emerged adult. (From: Kemp *et al.*, 2004. *Ann. Ent. Soc. Am.*, **97**: 161- 170).

These results indicate that *O. lignaria* enters a summer diapause shortly after cocoon completion, and diapause development requires exposure to sufficiently warm and/or fluctuating temperatures. After petal fall, *O. lignaria* nests obtained during the flowering period need to be stored in a protected area with temperatures high enough for diapause completion, yet low enough to avoid premature adult eclosion (see below). For northern Utah populations, adequate constant temperatures are 22 to 29 °C. However, best results (lower developmental mortality) are obtained with fluctuating temperature regimes (Bosch and Kemp, 2000). Populations with different geographic origins have different developmental thresholds and developmental rates, and need to be reared accordingly.

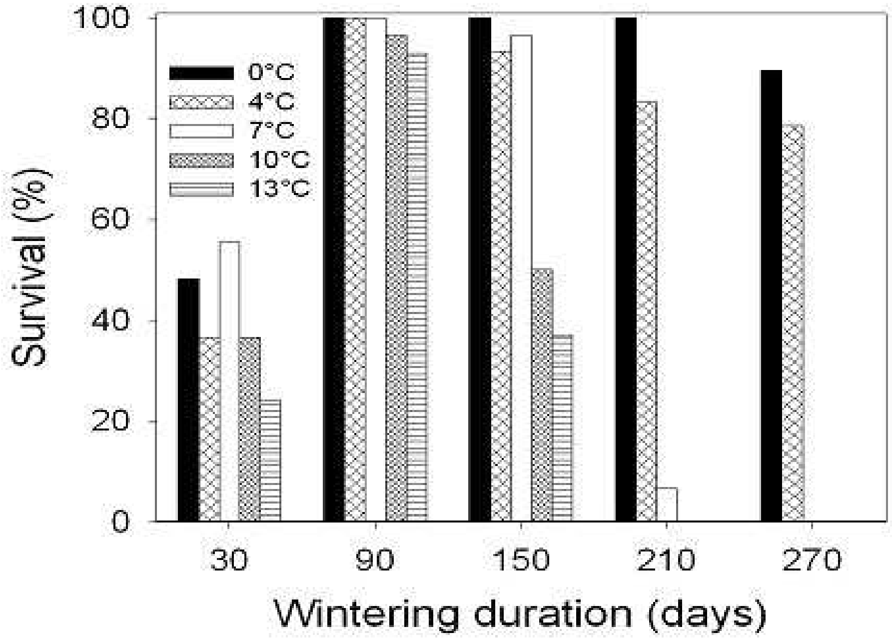
Pre-wintering

Eclosed adults remain in their cocoons through the end of summer, autumn and winter. Respiration rates drop from ~ 0.35 ml/g·h within a week from adult eclosion to ~ 0.1 ml/g·h approximately 3 weeks afterwards (Figure 1; Kemp *et al.*, 2004, and unpublished results). From then on respiration rates steadily increase through the winter to reach levels of ~ 0.7 ml/g·h right before emergence out of the cocoon. We define pre-wintering as the period during which cocooned adults, still exposed to warm temperatures in late-summer and early fall lower their respiration rates; and wintering as the period during which respiration rates increase following exposure to cold temperatures. Individuals pre-wintered for too long (that is, the initiation of wintering occurred too late), rapidly loose weight, and use up their fat body reserves, as evidenced from X-ray pictures in which the abdomen of these bees appears partially depleted (Bosch *et al.*, 2000, Bosch and Kemp, 2001). These individuals are more likely to die during the winter and to show decreased vigor at emergence. Because adult eclosion in a population may span more than a month, the first individuals to reach adulthood (early-developing individuals) are exposed to longer pre-wintering periods than individuals reaching adulthood shortly before the onset of wintering temperatures (late-developing individuals). Both in populations reared under artificial and natural temperature regimes, early-developing individuals show more extensive signs of fat body depletion and decreased vigor after wintering compared to early-developing individuals (Bosch *et al.*, 2000, and unpublished results).

Timing between adult eclosion and the onset of wintering temperatures is critical to ensure good survival in populations managed for crop pollination. Adult eclosion needs to be monitored towards the end of the summer. This is easily accomplished by periodically dissecting a few cocoons with a razor blade. No later than one month after the last individuals in the population have become adults, nests should be exposed to either natural winter temperatures or artificially refrigerated (Bosch and Kemp, 2001). Otherwise, winter mortality will increase and bees will be weaker at emergence. On the other hand, bees wintered before becoming adults (as pupae or prepupae) die or do not develop into viable adults when incubated in spring (Bosch and Kemp, 2000). Due to differences in developmental rates between populations from different geographic areas, monitoring adult eclosion becomes particularly critical when populations are reared in an area different from their area of origin. The progeny of a northern Utah population reared in the Central Valley of California took ~5 months to reach adulthood compared to ~7 months for a California population (Bosch *et al.*, 2000). Utah bees were thus exposed as cocooned adults to hot August-September California temperatures. These bees showed extensive signs of fat body depletion and had 67.6% adult (pre-wintering + wintering) mortality, compared to 0.2% in the locally-adapted California population.

Wintering

Wintering duration and winter temperature have a strong effect on survival, vigor at emergence, and emergence time (Bosch and Kemp, 2003). Individuals not exposed to winter temperatures or wintered for only 30 days are not likely to emerge (Figure 2). On the other hand, some individuals exposed to excessively long wintering (270 days for northern Utah populations) may emerge without additional incubation. Time to emerge following incubation decreases with increased wintering duration. For example, males wintered for 90 days at 4 °C have high survival rates, but take ~12 days to emerge compared to ~3 days and ~1 day for males wintered for 150 and 210 days, respectively (Figure 3; Bosch and Kemp, 2003). Southern populations require shorter wintering periods for prompt emergence than northern populations (Bosch and Kemp, 2001). These results indicate that winter diapause completion in *O. lignaria* requires exposure to low temperatures for sufficiently long periods, and that the necessary chill unit accumulation varies with latitude. At least for long wintering durations (beyond 150 days), emergence time following incubation decreases with increasing wintering temperature. However, bees from northern Utah wintered at 7 °C or higher use up their fat body reserves and exhibit elevated mortality rates if wintered for longer than 150 days (Bosch and Kemp, 2003).



The Life Cycle of Osmia lignaria

Figure 2. Percent survival of adult male *O. lignaria* wintered under various duration/temperature treatments and incubated at 20 °C. (From: Bosch and Kemp, 2003. *Environ. Entomol*. **32**: 711-716).

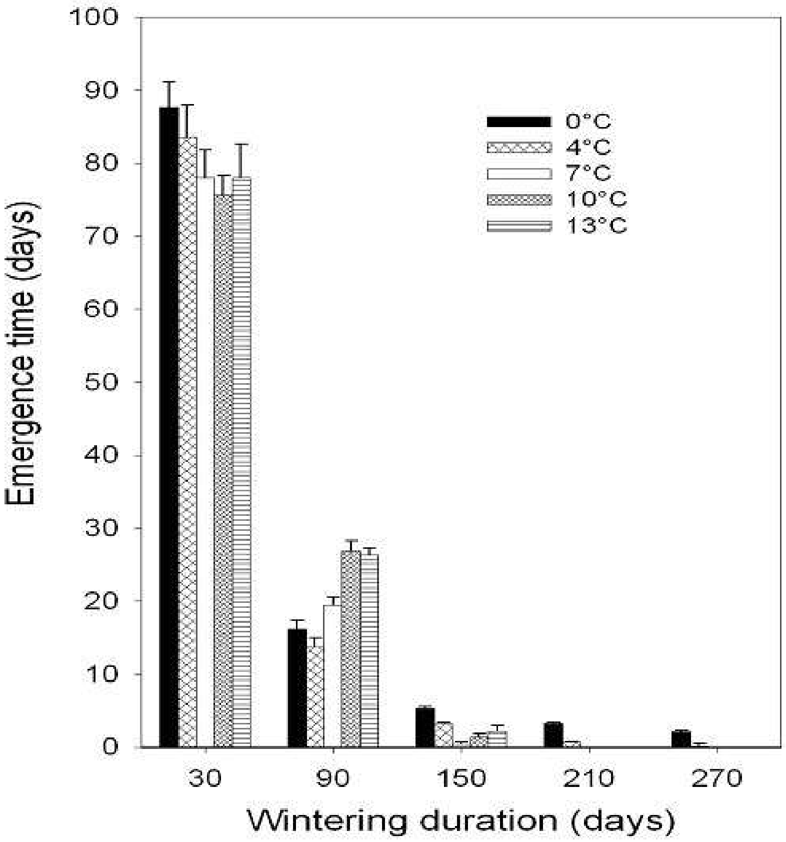


Figure 3. Mean ± SE emergence time (days from incubation at 20 °C to emergence) of adult male *O. lignaria* wintered under various duration/temperature treatments. (From: Bosch and Kemp, 2003. *Environ. Entomol.*, **32**: 711-716).

Osmia lignaria populations from northern Utah should be wintered for 150 to 210 days at 0-4 °C. Because orchard crops bloom for a short period, timing *O. lignaria* emergence with bloom initiation of the target crop is essential for optimal pollination and bee reproduction. Populations not exposed to sufficiently long wintering or to excessively cold temperatures take longer to emerge and show increased emergence time variability. These populations are difficult to manage, because many bees may emerge when bloom is well advanced. Increasing wintering temperatures will shorten emergence time, but can only be done within limits in order to avoid decreased survival and vigor. The best way to provide sufficiently long wintering periods and timely emergence is to monitor adult eclosion in late summer and avoid unnecessarily long pre-wintering periods.

Incubation and emergence

Osmia lignaria adults start chewing their way out of the cocoon and nest as temperatures increase in spring. Most males emerge 2-3 days before females start to emerge. As mentioned, emergence time following exposure to warm temperatures decreases with increasing wintering duration. In other words, individuals exposed to long winters need to accumulate fewer heat units for emergence than those exposed to short winters. For long wintering periods, individuals exposed to warm wintering temperatures emerge faster. Even in populations wintered for sufficiently long periods, complete emergence takes at least one week. Although, once mated and established, *O. lignaria* females forage at temperatures as low as 12 °C, emergence from the cocoon and nest proceeds slowly at temperatures lower than 20 °C (Bosch and Kemp, 2001; and unpublished results). Emergence time declines as incubation temperatures increase, but temperatures above 30 °C are detrimental when applied continuously. There is no difference in emergence time between bees incubated to 20 °C and bees exposed to a 10:30 °C thermoperiod (mean: 20 °C) (unpublished results).

Upon emergence, *Osmia lignaria* females require 2-3 days to mate, mature their ovocites, and select a nesting cavity. This time needs to be accounted for when releasing populations in orchards. If ambient temperatures are expected not to exceed 20 °C, populations may require artificial incubation, for example in a heated room. Populations can be released with a good proportion of males already emerged without compromising mating or female establishment. The use of nesting materials and shelter designs favoring heat retention is another way to provide additional incubation. Emergence is faster in shelters oriented South or East.

Concluding remarks

The life cycle of *Osmia lignaria* includes two dormant periods, one in the prepupal stage and one in the adult stage. Respirometry results indicate that both periods are diapause-mediated (*sensu* Tauber *et al.*, 1986). Most other Megachilidae overwinter in the prepupal stage. *Megachile rotundata* is another solitary bee that has been developed as a crop (alfalfa) pollinator, and whose life cycle has been studied with some detail. *Megachile rotundata* have a long prepupal, diapause-mediated, dormancy that spans from late-summer to spring of the following year (Kemp *et al.*, 2004). Wintered prepupae complete development during spring, and adults are ready to emerge by early-summer. As with adult dormancy in *O. lignaria*, time to resume development following incubation in *Megachile rotundata* decreases with increasing wintering duration (Johansen and Eves, 1973; Taséi and Masure, 1978; Richards *et al.*, 1987). Also, as with prepupal dormancy in *O. lignaria*, diapause completion in *M.*

rotundata takes less time at fluctuating temperatures (14:27 °C, mean: 22 °C) than at constant temperatures (22 °C) (Kemp and Bosch, 2000, 2001). Phylogenetic evidence indicates that adult wintering as found in *Osmia* is a derived trait within the Megachilidae (Bosch *et al.*, 2001). Wintering in the adult stage allows *Osmia* species to be ready to emerge and nest in early-spring, in contrast to other Megachilidae, which fly in the summer. A similar situation is found in other solitary bee taxa. For example, spring-flying *Colletes* and *Anthophora* overwinter as adults in contrast to summer-flying congeneric species, which overwinter as prepupae (Westrich, 1989).

Those interested in rearing *O. lignaria* populations need to follow a few simple rules and adjust their rearing protocols according to the geographic origin of their populations and the blooming phenology of the target crop. The least amount of manipulation is required when managing local populations, as they are adapted to the local climatic conditions. In small-scale operations or when the blooming period of the target crop coincides with the natural emergence of the population, *O. lignaria* can be reared under natural or close-to-natural conditions. Rearing under artificial or semi-artificial conditions is advisable when managing large populations or when emergence time needs to be adjusted to bloom time of the target crop. Delaying emergence can be accomplished by wintering bees at a colder temperature or by maintaining bees under winter conditions for some extra weeks. Advancing emerge can be accomplished by using slightly higher winter temperatures or by incubating artificially in the spring. In any case, only populations with short emergence periods (those having received sufficiently long wintering periods) can be optimally used for orchard pollination. A critical step in *O. lignaria* management is the monitoring of adult eclosion in the late summer. Adequate timing between adult eclosion and the onset of winter temperatures (short pre-wintering periods) will result in healthy, vigorous individuals that are more likely to survive the winter. At the same time, appropriate pre-wintering will allow for sufficiently long wintering durations and, therefore, prompt emergence in the spring. This will facilitate the timing of actively nesting adult bees with bloom initiation, thus maximizing pollination and bee production.

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REARING AND USING *OSMIA* BEES FOR CROP POLLINATION: A HELP FROM A MOLECULAR APPROACH

Antonio Felicioli
Miloje Krunic
Mauro Pinzauti

Introduction

Up to now the releasing-and-rearing strategy has been the most common method to develop an osmia bee population obtained by nest-trapping. It is based on releasing a batch of osmia imagos in a suitable environment where artificial nests are present. The suitable environment could be either an agro-ecosystem, so that the increase of the population and crop pollination are directly linked, or a natural environment so that the increase of the population is completely independent from using the bees for crop pollination (Bohart, 1972; Torchio, 1985; Pinzauti, 1991a; Bosh, 1994a, b; Krunic' *et al.*, 1995).

The RR strategy is based on the SWOT system (Strength, Weakness, Opportunity and Threat) that focalises on the critical phases of a process. The Strengths of the RR strategy phases are gregarism, the ease of acceptance of artificial nests and the presence of a diapause period. The Weaknesses are the biotic and abiotic limiting factors on the populations, dispersion after release and the lack of available technology. The Opportunities include the diapause modularity, availability of rearing sites, nest handling and management of food sources. The Threats are represented by pesticides and parasite proliferation (Pinzauti, 2002).

This way of developing osmia populations has the disadvantage to be restricted in time and space. It is restricted in time because the field conditions and the life cycle force rearing only during Spring and restricted in space because the “suitable environment conditions” force rearing only in open field or at least in big greenhouses.

Osmia bees rearing, their management in pollination, their economic values and their diffusion as a commercial available pollinator tool in agriculture could be improved by their year-round rearing that could be obtained with the development of a laboratory rearing conditions. In particular, food availability, luminosity conditions, population density, fat metabolism during diapause, diapause plasticity, sex-ratio and aging are the critical limiting factors when trying to rear osmia bees in laboratory conditions.

Reared osmia bees are used in both open field and confined environments, and the success in increasing yield is due to both crop and bee management as well as the optimisation of the bee-plant syndrome.

Efforts to direct the osmia bee rearing strategy from nest-trapping (NT) and releasing-and-rearing (RR), towards more controlled conditions such as the laboratory (captivity), whilst optimising their use for crop pollination in both open field and confined environments, can be improved by a molecular approach.

The releasing and rearing single step description

The man-made nests

A good rule to develop osmia populations in open field cultivations is to build a shelter in the middle of the field and place a large number of artificial nests inside together with aerated boxes containing cocoons with insects ready to emerge (Pinzauti, 1991a; Bosh, 1994c). Of course it is essential that adult bees emerge and start their activity when trees or plants are in full blossom. In this way it is possible to obtain a progeny output of two to three times the parental population (Felicioli, 2000).

Man-made nests are bundles of cane segments (*Arundo donax* or *Phragmites australis*) tied together and hung from any support. Two months to one year old canes are better accepted by the bees than the fresh and still green newly cut ones. Paper tubes implanted in wooden or plastic blocks are also well accepted. Building both these types of artificial nests is time consuming and they are not recyclable (Felicioli and Pinzauti, 1994a). The Assembled Artificial Trap-Nest (AATN) (Felicioli and Pinzauti, 1994b), are differently accepted as nesting site by the bees according to the species. All the offered tunnels will be filled if the nest is placed above the bundle of cane segments as these bees tend to nest the upper tunnels first or if no other suitable nests are available. This type of nest facilitates internal inspection during the different phases of the life cycle and can be built with different types of material, such as wood, hardboard, recycled plastic, pressed polystyrene and terracotta in a very fast and cheap way. The diameter of the grooves is chosen according to the function of the species of osmia to nest. Once the boards are assembled these grooves become tunnels of the right size. This type of man-made nest could retain humidity causing almost all cocoons to mould in the tunnels so ventilation must be taken into account (Krunic *et al.*, 1995).

The food availability

Because of the great quantity of pollen needed to develop every single larva (up to one cm^3 of pollen for 5 to 30 trips) they tend, when possible, to prefer flowers producing a great quantity of pollen per time unit (Felicioli, 2000). For a good bee progeny output it is important then to choose environments with a large trophic source (Maeta, 1987; Marquez *et al.*, 1994; Bilinski, 2003). In the case of a natural environment nesting site must be taken into account that the pollen of anemophilus trees such as *Salix* sp., *Acer* Sp., and *Quercus* Sp. (Felicioli *et al.*, 1998) is particularly appetising for *O. cornuta* and *O. rufa.* In this way it is possible to increase the parental population from five to ten times. A good rule to develop osmia populations in open field cultivations (agro-ecosystem) is to choose a vast orchard blossom or some fields of oil-rape (*Brassica napus*) and to build a shelter in the middle of the field and place a large number of artificial nests inside together with aerated boxes containing cocoons ready for the emergence of the insects. Of course it is essential that adult bees emerge and start their activity when trees or plants are in full blossom and that the blossom period is at least one month. In this way it is possible to obtain a progeny output of two to five times the parental population (Pinzauti and Felicioli, 2002).

Food type and quality is still one of the limiting factors in rearing osmia bees in captivity conditions (laboratory). In this context neither the choice criteria, nor the post-gathering treatment of the pollen by the bee, or the enzymatic digestive pool through the ontogenetic stages are known.

Timing and temperature

The release of the cocoons with osmia bees ready to emerge must be done during winter time (february to the first week of April) if the nesting site is at sea-level otherwise must be done in Spring not later than the first week of May if the nesting site is at 1000m a.s.l. If the temperature rises over 30 ° C during the day bees will not give progeny (Felicioli, 2000). The emergence of the bee must be synchronised with the orchard blossom or with the presence of the maximum level of pollen availability. Accidental exposure of the cocoons to direct sunrays causes the immediate death of the adults inside the cocoons. This is due to suffocation caused by the carbon dioxide resulting from the rapid increase of the metabolism of the bee caused by the sudden transfer from cold to heat (Felicioli, 2000).

Emergence, nesting activity and gregarism

Osmia cornuta is a polylectic, gregarious lodger bee that builds allocellous and linear odal nests (Banaszak, 1998). Males emerge before females, the time between male and female emergence ranges 10-0 days depending on how long (days) the cocoons have been kept at cold temperatures (0-15 ° C). Once the males have emerged they start to feed on flowers close to the nests and keep flying in the vicinity until the females emerge. During the night the males colonize the old open tunnels but if new tunnels are available they will readily accept them first. After females have emerged mating occurs and trophic and nesting activity begins. Females also prefer new tunnels, if available, to the old ones to nest. The optimal diameter of the nest cavity entrance varies according to the species (Tasei, 1973; Jacob-Remacle, 1990; Rust, 1993a,b). Each osmia nest contains a linear series of pedotrophic cells each built at a rate of one cell per day for about a month, however a single female can complete more than one cell within a single day. When the whole nest is finished the osmia seals the entrance with a consistent mud closure. It then often goes on to colonize another tunnel. Sometimes a single female colonizes more than one tunnel at the same time (personal observation). Osmia bees perform their nesting activity in collective nesting sites. Nesting gregarism in these bees is one of the bases in rearing them and some speculation has been done concerning the advantages and disadvantages of this way of nesting (Rosenheim, 1990) but its mechanism is still unknown.

Dispersal reduction

Since a single female can nest in at least four tunnels at the same time it is a good general rule to allow at least 4 tunnels for every female released. In the case of releasing in an open field orchard, in order to avoid the dispersion of females ready to nest it is advantageous to distribute additional artificial nests in the field. Good results have been obtained with ten artificial nests each containing a hundred tunnels per hectare (Krunic' *et al.*, 1995). Recently it has been shown that the presence of a nesting osmia female in a given area can be a visual stimulus for other co-specific females to nest in the same area (Felicioli *et al.*, 1995; Felicioli *et al.*, 1996). For this reason in some particularly adapted ecosystems it could be useful to leave some colonized nests in loco to guarantee a natural diapause for part of the population, in this way the newly emerged bees will start their activity in a nesting site where some females are already performing nesting activity (Felicioli, 2000). In this case the old artificial nests must be substituted at least every two years in order to control parasites.

Adequate humidity condition of the soil will help in reducing dispersion allowing the bees do not consume energy searching for a suitable site to collect mud to seal their nests. Nests that has been occupied by males before females emergence seems to be accepted readily than the others by females, however no information's are available concerning nest marking behaviour by males and the possible role of males in avoiding female dispersal is unknown. Data concerning the visual and olfactory systems at a molecular level could help to reduce dispersion.

Prevention of dispersion is reached also by good management of the diapause period that implicates the releasing of non aged bees. Also in this case the knowledge of the mechanisms of diapause, fat-metabolism and aging at a molecular level would strongly help the rearing of osmia bees.

Mating system and sex ratio

Offspring sex is determined by the haplo-diploid mode of reproduction. Females develop from diploid eggs while males develop from unfertilised haploid eggs. A successful courtship and mating behaviour, leading to diploid eggs, is certainly of interest to perform the correct management of this species for pollination purposes, since only females perform pollen gathering. Within a mating the released osmia bees perform a pre-copulation courtship characterized by a rhythmic downwards movement of the antennae towards the females ones without touching them that leads to copulation. Copulations are of two types, termed "long copulation" and "short copulation" (Felicioli *et al.*, 1998). If the female, after courtship, becomes receptive, i.e. available for accepting a long copulation, with the possibility of sperm transfer, a single long copulation occurs, followed by the post-copulatory courtship. Otherwise, the copulation is interrupted early and the mating sequence enters the recovery loop until detachment without sperm transfer occurs. The short copulation events became detectable with aging. (bees that are coming from a long storage in a cold temperature). Knowledge of molecular basis of sex-recognition and aging could be useful in increasing the achievement of insemination and prevent unsuitable sex-ratio of the progeny.

Parasite control

At the end of the nesting season (September) the artificial nests are removed, opened and inspected in order to detect parasites such *Chetodactilus osmiae*, *Cacoxenus indagator* and *Trichodes apiarius*. Then the nests are stored at room temperature until they are put into a thermostatic cell at "cold temperature"(0-8 ° C). During this period it is extremely important not to contaminate the environment, and therefore the nests, with chemical vapours, especially pesticides, that could be mortal for the insects. Also unused artificial nests must never be placed close to toxic substances, as osmia bees will not colonize any contaminated nesting material (Krunic *et al.*, 1995). If some nests have been left *in loco* a good control of *Monodontomerus obscurus*, *Leucospis dorsigera* and *Anthrax anthrax* is gained by destroying the old nests just after the female bee have emerged and the parasites have not yet emerged. However is a good rule to renew them at least every two years. These nests filled up with the pedotrophic cells can also be destroyed by birds such as Picidae, Paridae and Corvidae that feed on the bee larvae or imagos This type of damage is quite little during summer but could become intense during winter so the nests should be protected by wire mesh (Krunic' *et al.*, 1995).

Diapause

Osmia bees are generally monovoltine but can be found in the genus parsivoltine and bivoltine species (Torchio and Tepedino, 1982; Banaszak and Romasenko, 1998). These insects spend the critical period of the year (winter) in diapause. Even today the diapause of the monovoltine osmia is considered to be of the para-pause type (obliged diapause apparently genetically determined and free of environmental factors, because factors inducing this particular physiological state are still unknown) (Saunders, 1982; Hodek, 1983).

Osmia bees spend diapause as imago and can tolerate temperatures even lower than -20 °C. The term "low temperature" refers to a temperature not higher than 15 °C. In fact studies carried out at different temperatures on the mortality of imago in the cocoon show that there is a high degree of survival if the temperature does not exceed 15 °C (Bosch and Blas, 1994; Felicioli, 1994). Experiments on diapause of the osmia show that once the cocoons containing imagos are exposed from November at 8 °C, adults can emerge from the cocoons (end of diapause) without external stimulus as from the month of May when they are still inside the fridge. The same phenomenon happens in August if the exposure temperature is 2 °C. This phenomenon induces us to believe that the end of diapause is due to the completion of the horotelic metabolism and the start of tachitelic metabolism at a specific time (Felicioli, 1994).

Normally a temperature of 4 °C, typical of normal kitchen fridges, is enough to guarantee an optimal survival and strenght of the diapause populations of osmia bees used such as *Osmia cornuta* Latr. and *Osmia rufa* L. It could be useful to recall here that no exposure to these temperatures for more or less long periods normally causes high mortality and weakness in the surviving population (Felicioli, 1994). Integration between the length and the temperature of exposure favours the modulation and synchronization of the time and duration of emergence of the insect from the cocoon.

This mechanism favours a predisposition of the number of multiple releases in time in a single place or a single release in many places. The time interval for the release of the insects, therefore, varies according to the combination time and temperature of exposure and osmia species.

In the case of *O. rufa* and *O. cornuta* it is important not to wait beyond the 1st of December to expose the cocoons to low temperature, or release the cocoons over the period after the 15th of May (Felicioli, 1994).

The presence of an imago Diapause is the limiting factor in managing osmia bees in periods of the year other than Spring as well as managing them all year round.

If modulating the diapause of these insects enables to manage the bees in a time ranging from February to May. Plasticity of diapause (induction of diapause in an ontogenetic stage different from the imago) could help to manage them in a period other than Spring. Moreover knowledge of the diapause induction and termination mechanisms could help to manage the bees all year-round.

Some new data for rearing osmia from a molecular approach

Abiotic and biotic factors still influence field rearing of bees. Field rearing of bees is still necessary due to lack of knowledge in diapause and nutrition molecular mechanisms.

Artificial diet

Investigations have been made in setting an artificial diet for *Osmia cornuta* and *Osmia rufa* rearing (Steen van Den, 1997; Ladurner *et al.*, 1999; Maccagnani *et al.*, 2002) and results indicate that something missing in the artificial diet prevented the larvae from completing their development suggesting that *O. cornuta* females produce and add something to the pollen provision, that is called "maternal factor", essential for the progeny survival. In particular the higher content in glucose and fructose and the lower content of sucrose in osmia pollen provisions than that of pollen gathered directly from flowers might be related to the presence of the enzyme saccharase in the saliva of the female which could be the maternal factor. Glandular secretion and pollen digestion mechanisms need to be investigated in order to understand the presence of the enzyme saccharase, if this is the only "maternal factor" involved or if a set of substances is added to pollen provisions.

Digestive proteases

The expression profile of several soluble digestive proteinases of *Osmia cornuta* is different from that of Megachile rotundata (Felicioli *et al.*, 2004) and honeybees (Dahlmann *et al.*, 1978) moreover it varies during ontogeny. The maximum of active band appears in the I-IV instar larvae characterised by a high rate of growth and pollen intake. Our results suggest that both the number and the intensity of the band is related to growth and pollen intake. The study of these proteinases (characterization by inhibitors or sequencing) could help in setting a suitable artificial diet.

Chemoreception

SDS-PAGE of protein extracts from different parts of the insect, revealed electrophoretic bands that were antenna- and sex-specific. In particular, an antenna-specific 14 kDa band, is revealed in both sexes. A 20 kDa and 21 kDa band protein are detected only in antennae of males and females, respectively.

Both the 20 and 21 kDa band detected in the SDS-PAGE for male and female respectively are each resolved in only one spot by the 2D-PAGE. The isoelectric point of the male's spot is basic, while the female's one is acidic. In addition, in the 19 kDa region the female shows an exclusive spot and in the 23 kDa region, two exclusive proteins are found only in the male while, in the same region, three proteins are more expressed in the female (Felicioli *et al.*, 2003).

The proteomic approach, in particular the use of the 2D-PAGE could be an useful tool to reach a deeper insight into sex differences of molecular mechanism of chemoreception and sexual recognition.

Diapause

The beginning of diapause also seems to occur at a specific time. In fact, from experiments carried out on *Osmia cornuta* (Felicioli *et al.*, 2000) it has been shown that even if the number of degrees per day is insufficient to complete the development of the larvae and the metamorphosis in adults, the osmia could enter diapause at the mature larval or at a pupa stage. These larvae will complete their development only the next spring once diapause is over. In constant temperature conditions (18 °C) the diapausing adult bees clearly show a discontinuous gas exchange cycle (DGC) while the pre-imago stages show a continous pattern in their gas exchange. At the beginning of recording the DGC of diapausing adult bees shows four cycles every 10 hours. The cycles increased to 6 on the 10th of December, to 8 on the 24th of January and reaches 12 on the 10th of February. The concentration (ppm) of CO_2 in the pyrex cell gradually increases throughout the experiment. At the beginning of recording the concentration rise of CO_2 was 4.5 ppm every hour. On the 10th of December it was 7ppm, on the 24th of January it was 8ppm and on the 10th of February it reached 22ppm every hour. The concentration of CO_2 in the pyrex cell containing the artificially induced diapausing pupae increases throughout the experiment. At the beginning of recording the concentration rise of CO_2 was 7.5 ppm every hour. On the 20th of December it was 8ppm, on the 25th of January it was 8ppm and on the 14th of February it reached 27ppm every hour. Physiological and biochemical studies on metabolism and CO_2 role in ending the diapause could help to manage diapause and rear osmia bees year round.

Use of osmia bees in crop pollination

The relative facility with which osmias can be induced to nest in pre-prepared artificial nests and their strong gregarious nesting is the base of the management and multiplication of these insects in controlled pollination. The use of these bees in the pollination of open field cultivations has been very successful especially for early flowering fruit trees (Balana *et al.*, 1983; Asensio, 1984; Torchio and Asensio, 1985; Krunic' *et al.*, 1989; Bosh, 1994a,b,c) and good results have recently been obtained also for the pollination of confined environment cultivations (Felicioli, 1996; Pinzauti *et al.*, 1997).

Open field pollination

Since the bees collect pollen and nectar from flowers that are near the place where nests are placed and they will not fly more than 100m away from the nest if it is not necessary, in order to achieve good pollination it become important to placed nests and cocoons (protected from direct sunlight and in an aerated dark box) in the orchard with spacing of about 20 to 50 m. As a general rule two to five females per fruit tree in blossom is sufficient to achieve 50 to 90 % of pollination depending on the orchard (Krunic' *et al.*, 1995). Bilinski and Teper (2003) indicate a number of females ranging from 926/ha for apple trees, to 3500/ha for cherry trees.

Apricot - At our latitude this tree blossoms from the middle of February onwards, when there are still great differences in temperature with frequent night frost.

Many varieties of this plant are self-pollinating but they also use cross-pollination (Guerriero and Monteleone, 1988; Guerriero, 1990; Felicioli and Pinzauti, 2000).

The use of osmia bees on apricot trees can be extremely profitable (Pinzauti, 1991b; Pinzauti and Rondinini, 1991; Lepore and Pinzauti, 1994) for the following reasons: osmia bees also fly at 13 °C and well tolerate night temperatures below zero; in this period there are very few competitive flowers. The activity of a hundred females per hectare is sufficient to increase setting by 30 to 50%. In particular we have tested two cultivar The Pisana one (autofertile) and the Aurora one (partial autosterile). In both cases we obtain a fruit set that varies from 62,3% in the vicinity of the nesting site (the shelter) to 41,2% at a distance of 50 m. Fruit set was lower (30%) for the Aurora cultivar although bouquet of flowers were utilised one every 5 trees.

***Chinese-Japanese plum* cv *"*angeleno" –** At our latitude Central part of Italy the cultivation of this plant has two major difficulties due to little self pollination and the reduced production of nectar that cause these plants to be unattractive for pronubial insects. *Osmia cornuta* and *Osmia rufa* are good pollinators for these plants since they are more attracted by pollen than by nectar like honey bees and bumble bees. They gather great quantities of pollen as a food reserve for the nest and enough nectar to produce the metabolic energy for flight. For this they need relatively little sugar substance (the presence of spontaneous flowers is very useful).

The reward for the pollination service is therefore only pollen that osmias gather up in great quantities with the hairs of the ventral part of their abdomen and carry back to their pedotrophic nest. Also in this case a hundred or so females per hectare are sufficient to obtain an optimal percentage of fruit setting.

***Oil-rape* –** This cultivation is very attractive to bees due to the large amount of nectar and pollen. Mason bees pollinate this plant with an efficiency comparable to that of honeybees. This cultivation is a food source for developing osmia populations since a large amount of pollen is associated with a good scalarity in blossom so that the flying and nesting activity could be extended to at least one month. With this plant cultivation Osmia population could increase five times (Felicioli and Pinzauti, 2002).

Cultivation in greenhouses

Recently it has been shown that osmia bees are well adapted for pollination in greenhouses, where however it is not yet possible to reproduce or multiply them successfully. Is very important that the structural poles (generally mouldings or hollow iron tubes) of the confined environments have no holes opening into the greenhouse as osmias are attracted by them and risk certain death inside the tubes. Often osmia bees are not the elective pollinator for certain plants but having to select seeds using a low number of plants in a reduced space (few square meters) in a very bad climate (very low or very high temperature), the possibility to modulate the number of specimen and time of flight makes these bees better pollinators than the elective ones (Piano *et al.*, 1998; Pinzauti and Felicioli, 2002; Piano *et al.*, 2002).

***Blackberry, Strawberry and raspberry* -** The flowers of these plants have abundant nectar and are generally very attractive to bees. There are three main types of advantage and benefit from the use of osmia in these cultivations. The first is the increase in both weight and number of drupeoles (for blackberries and raspberries) probably due to a better mass effect of the pollen laid by gastrolegid rather than podilegid insects on the female structures of the pluricarpellar flower. The second is a more uniform fruit due to a more balanced setting of the single drupes or, in the case of the strawberry, achenes (Pinzauti, 1987) that results in a reduction of badly formed fruit. The third important benefit is the reduction of the number of treatments against *Botrytis cinerea*, in the case of raspberry cultivation the treatments are reduced by five to one. The explanation of this phenomenon is the decrease of the nectar volume due to the foraging activity of the bees. In the particular greenhouse environment the nectar in excess stagnates and becomes sticky thus often causing the sticking together of the sepals and the small drupes and creating a suitable substrate for *Botrytis cinerea* (Felicioli, 2000).

Blueberry - Investigation concerning pollination of the American giant blueberry (*Vaccinium corymbosum*) by Osmia cornuta in confined environments show that this bee did not have the pollination efficiency reached by honeybees and bumblebees (Pinzauti and Felicioli, 2002). This result is the opposite of those obtained by Sampson and Cane (2000), concerning the pollination of the blueberry *Vaccinium ashei* using *Osmia ribifloris* in open field conditions this bee showed an higher efficiency in pollinating the blueberry flowers than the honeybee. It is quite interesting that the morphology of the flower is different in the two species of flower. The *V. ashei* shows the stigma coming out from the corolla while the *V. corymbosum* keeps the stigma inside the corolla. The Osmia bees are gastrolegid and gather pollen directly with the ventral brush so it is quite difficult for them to collect pollen from the *Vaccinium* sp. that has a very small and deep corolla. So pollination happens only during nectar sucking visits and in this case flowers with exposed stigma gain more from osmia visits than those with inside stigma.

Seed and red cabbage **-** Investigation carried out with *O. cornuta* demonstrate that both these plants are well pollinated by the bee under confined environment also in bad weather, seed yield was the double of that obtained with honeybees. These plants are also good candidates in osmia rearing since the population increases three to five times also in confined environments (Ladurner *et al.* 2000; 2002).

Lucerne and White clover **-** *Osmia cornuta* and *Osmia rufa* in a density of 0.50 females/m^2 has been utilised for pollination of both lucerne and white clover breeding material grown in isolation by mesh cages (10 m^2) in summer. For lucerne pollination *O. cornuta* give a yeld of 8.1 kg/ha while *O. rufa* give a yeld of 5.3 kg/ha. Similar results has been obtained for white clover. Both species did not reach the efficiency of *Megachile rotundata* (250kg/ha) probably due to the high summer temperatures. However *O. cornuta* show a great efficiency (175kg/ha) in pollinating off-season generation in white clover grown in a growth cabinet during winter (24 °C/18 °C day/night and 16 hours photoperiod) if utilised in a density of 5 females/m^2 (Piano *et al.*, 1998, 2002).

New data concerning osmia-flower syndrome from a molecular approach.

Inhibition of pollen germination

Normally the reproductive process of plants involves flower pollination and fertilization. The mechanism of pollen germination, and of fertilization process, is complex and conditioned by many biochemical events which occur in more or less brief timeframes (Frediani, 2000; Rondinini and Pinzauti, 1994; Rondinini *et al.*, 2000).

1) the transported pollen must be vital on the stigma.
2) pollen grains reach the top of the stigma in great number (mass effect).

Osmia cornuta gather pollen from the flower anthers directly with her ventral *scopa* without manipulating it first. After visiting several flowers, when the *gastrum* brush is filled up, the female bee flies towards her nest in order to discharge the pollen in the pedotrophic cell.

Honeybees and bumblebees (both podilegids) inhibit the germination of the gathered pollen (Harriss and Battie, 1991). They start inhibiting the gathered pollen germination during its placing in the curbicula. In both insects the germinability of pollen take from the *curbicula* is the 50% of the anther one (Pinzauti *et al*., 2002). Investigations has been made to detect pollen germinability after being gathered by *Osmia cornuta* (gastrolegid) (Pinzauti and Marroni, 2003). Results indicate that this Mason bee female deactivates pollen only after discharging it in the nest during the placing and pressing phases. The osmia female pressed the newly gathered pollen on the pollen provision with her *clipeus*. Head extrats of both diapausing and nesting osmia females show to inactivate pollen germinability by 75% indicating that the heads of this insects contain substances capable of deactivating the germinating potential of the pollen. The inhibitor substance could be secreted by mandibular glands but we cannot discount eventual facial sprays.

Conclusions

Field rearing of bees is still necessary due to lack of knowledge in nutrition and diapause molecular mechanisms.

The knowledge of the aminoacid sequence of the different pattern of digestive proteases showed by Osmia throughout its larval phases could help in detecting the “maternal factor” and in preparing a suitable artificial diet for larvae which permits to rear the bees indoors.

The proteomic approach of the mating system and aging of these bees could permit an understanding of the sex-recognition mechanisms, to manage sex-ratio and to avoid dispersion.

Understanding the role of CO_2 within tachitelic and horotelic metabolisms could allow the control of the diapause modularity and plasticity of solitary bees, permitting rearing all year round in spite of their usual annual rhythm.

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AMBIVALENCE OVER *MEGACHILE*

Anthony Raw

Abstract

The bee genus *Megachile* is common almost everywhere there are flowering plants and it is a relief for the entomologist or pollination botanist to identify the genus on sight and know something of the bees' biology. He knows they are solitary, nest in existing cavities including trapnests and many species cut leaves to line their cells. Many are important and efficient pollinators of crops and other plants. Their major nest predators in most regions are species of *Coelioxys*. But there is much ambivalent feeling over the genus. How to identify the species? What was a godsend turns out to be a nightmare. There are hundreds of species and most of them look much the same. Currently 524 species are recorded from the Americas. Several subgenera have been created to deal with this problem. My hope is that a combination of identification to subgenus or species group coupled with knowledge of the geographical distribution will reduce the difficulties of identification of the species to feasible proportions. Many species are easy to manage in trapnests – as *M. rotundata* has clearly demonstrated. Easier identification should stimulate new studies on the bees' role as pollinators and on management of their populations.

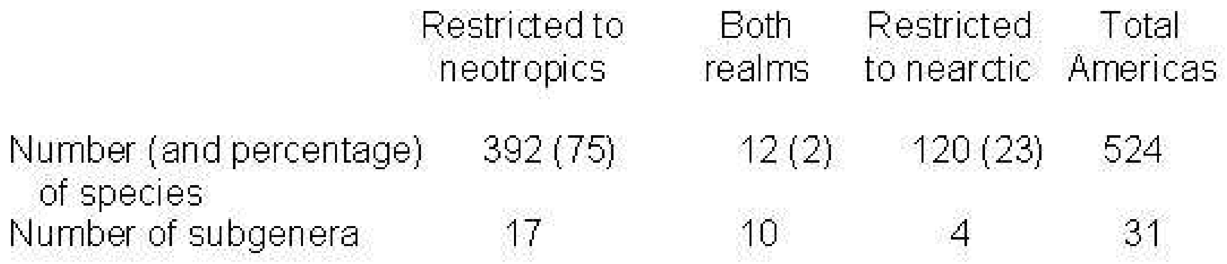
Key words: Leafcutter bees, Mason bees, Hymenoptera, Megachilidae, Identification, Americas.

The ambivalence

There is much ambivalent feeling among entomologists, pollination botanists and even bee biologists over leafcutter and mason bees. These bees comprise the genus *Megachile* (Hymenoptera; Megachilidae). On the positive side, the bees occur almost everywhere there are flowering plants and it is a relief to be able to identify the genus easily and know something of their biology. It is well known that all the species are solitary and most nest in existing cavities, including trapnests. The leafcutter species cut leaves to line their cells, while some use chewed leaves. The mason bees use mud and resin to build the partitions or even the entire cell. Major nest predators in most regions are species of the bee genus *Coelioxys.* Many species of *Megachile* are important pollinators of crops and other plants.

Then there is the negative side; how to identify the species? What was a godsend turns out to be a nightmare. There are huge numbers of species and most of them look much the same. According to Professor Michener (2000) *Megachile* comprises at least 1,320 species world-wide. He was being cautious. At the start of this work I compiled a list of 2,645 names. Here I have concentrated on the species of the Americas. A total of 524 species have been placed in their subgenera (Table 1) (Durante and Abrahamovich, 2002; Raw, 2002, 2003, In press A, In press B; Genaro, 2003) and an additional 90 names have been recorded from the region, but the whereabouts of the types are not known (pers. data).

Table 1. The species of *Megachile* in the Americas

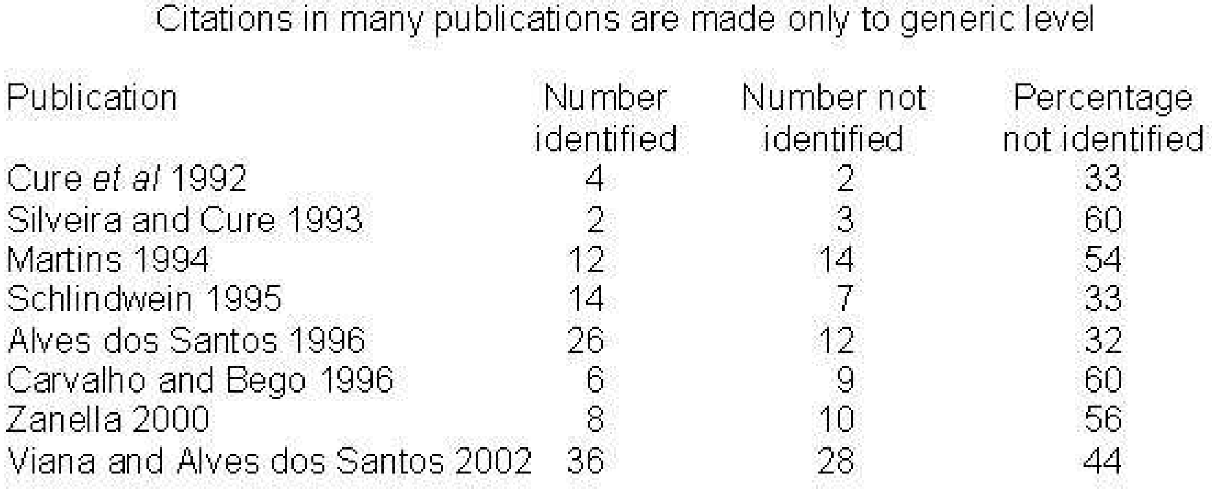


	Restricted to neotropics	Both realms	Restricted to nearctic	Total Americas
Number (and percentage) of species	392 (75)	12 (2)	120 (23)	524
Number of subgenera	17	10	4	31

Identification

The major hurdle to studying the biology of *Megachile* is in the identification of the species. Despite their acknowledged ecological importance, citations in many publications are made only to generic level. A survey of recent publications on Brazilian bees demonstrates this difficulty (Table 2). From 32% to 60% of the species were determined only to morpho-species by the best specialists in bee taxonomy reflecting the difficulties existing in the genus.

Table 2. Identification of *Megachile* species in Brazil



Citations in many publications are made only to generic level

Publication	Number identified	Number not identified	Percentage not identified
Cure *et al* 1992	4	2	33
Silveira and Cure 1993	2	3	60
Martins 1994	12	14	54
Schlindwein 1995	14	7	33
Alves dos Santos 1996	26	12	32
Carvalho and Bego 1996	6	9	60
Zanella 2000	8	10	56
Viana and Alves dos Santos 2002	36	28	44

For identification even to subgenus, the bees must be adequately prepared. In both sexes it is essential to open the mandibles to expose the teeth and the inner surface. In males it is necessary to spread the fore and mid legs to examine their lower surfaces. In order to determine the subgenus of females the apex of the abdomen must be opened to allow examination of the sixth sternite. The simplest method to discover how to prepare the bee is to identify freshly caught specimens. Furthermore, the sexes of many species of *Megachile* are very different in appearance and a frequent question, as with many species of Hymenoptera, is to associate the sexes. Fortunately, many *Megachile* species are adventive nesters and it is often possible to rear both sexes from a nest.

Geographical distribution

One of the most basic manifestations of the difficulties in identification is the number of species recorded from the countries and regions of the Americas (Figure 1). Latitude must influence species richness (MacArthur and Wilson, 1967) with temperate countries (marked in black on Figure 1) having relatively fewer species. The faunal lists of some temperate countries (Canada, U.S.A. and Argentina) are more or less complete. However, one should expect the species richness of tropical countries and regions (marked gray on Figure 1) to be closely related to area and this to be manifested in their values being close to a line from the Caymans (with a single species; Genaro, 2003) to Brazil (the richest country with 158; Raw, 2003). The records from tropical countries are artificially low; a result of lack of identification of the species rather than under-collecting. For example, only 2 species have been recorded from Venezuela,16 from Ecuador, 17 from the Guianas and 19 from Colombia. For this reason the species-area curve for Figure 1 has not been calculated. Even in Brazil undescribed species are coming to light (Raw in press A). A similar situation obtains within Brazil where no records of *Megachile* exist for eight of the states (Figure 2).

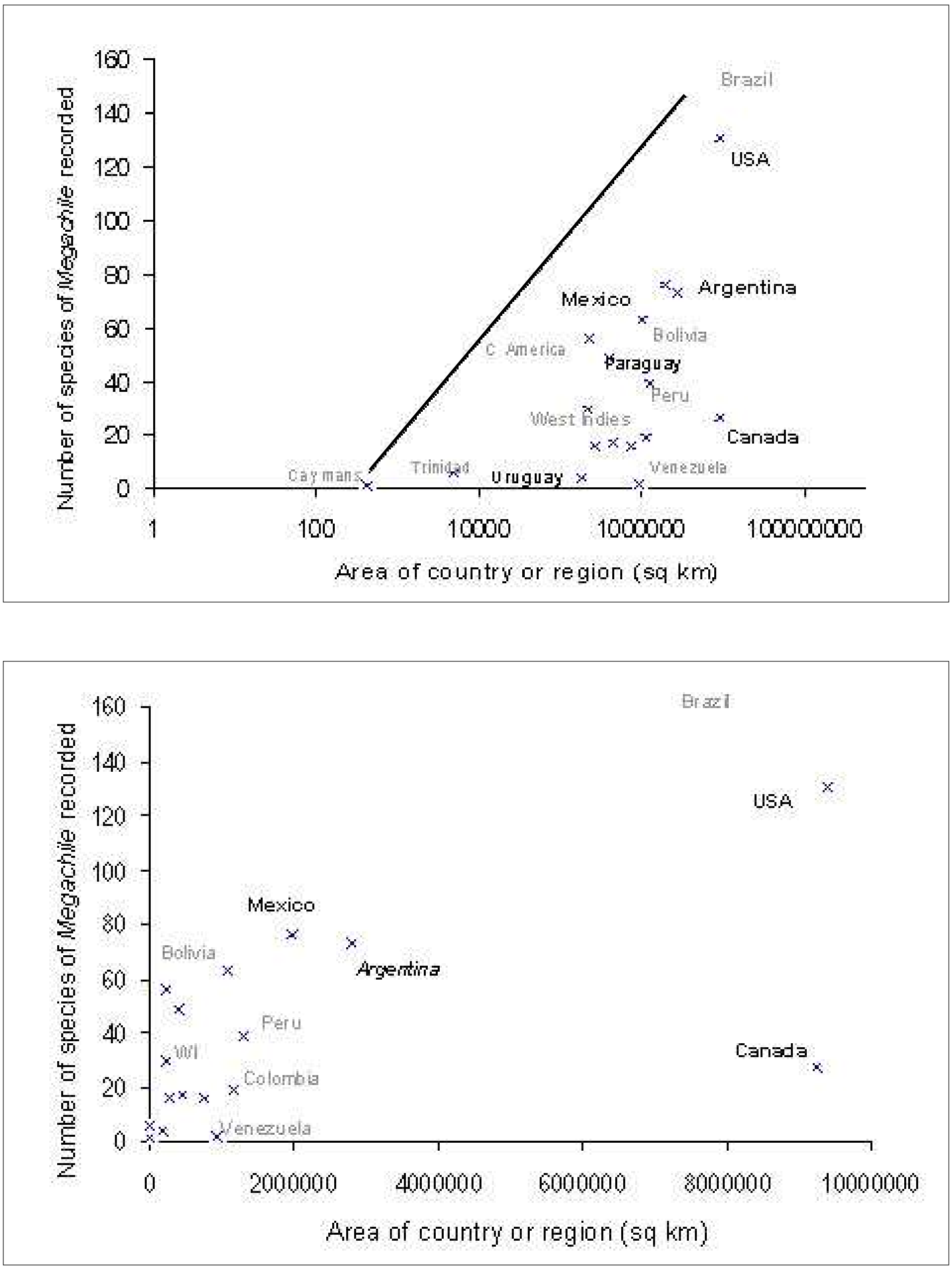


Figure 1. Number of species recorded from the countries and regions of the Americas.

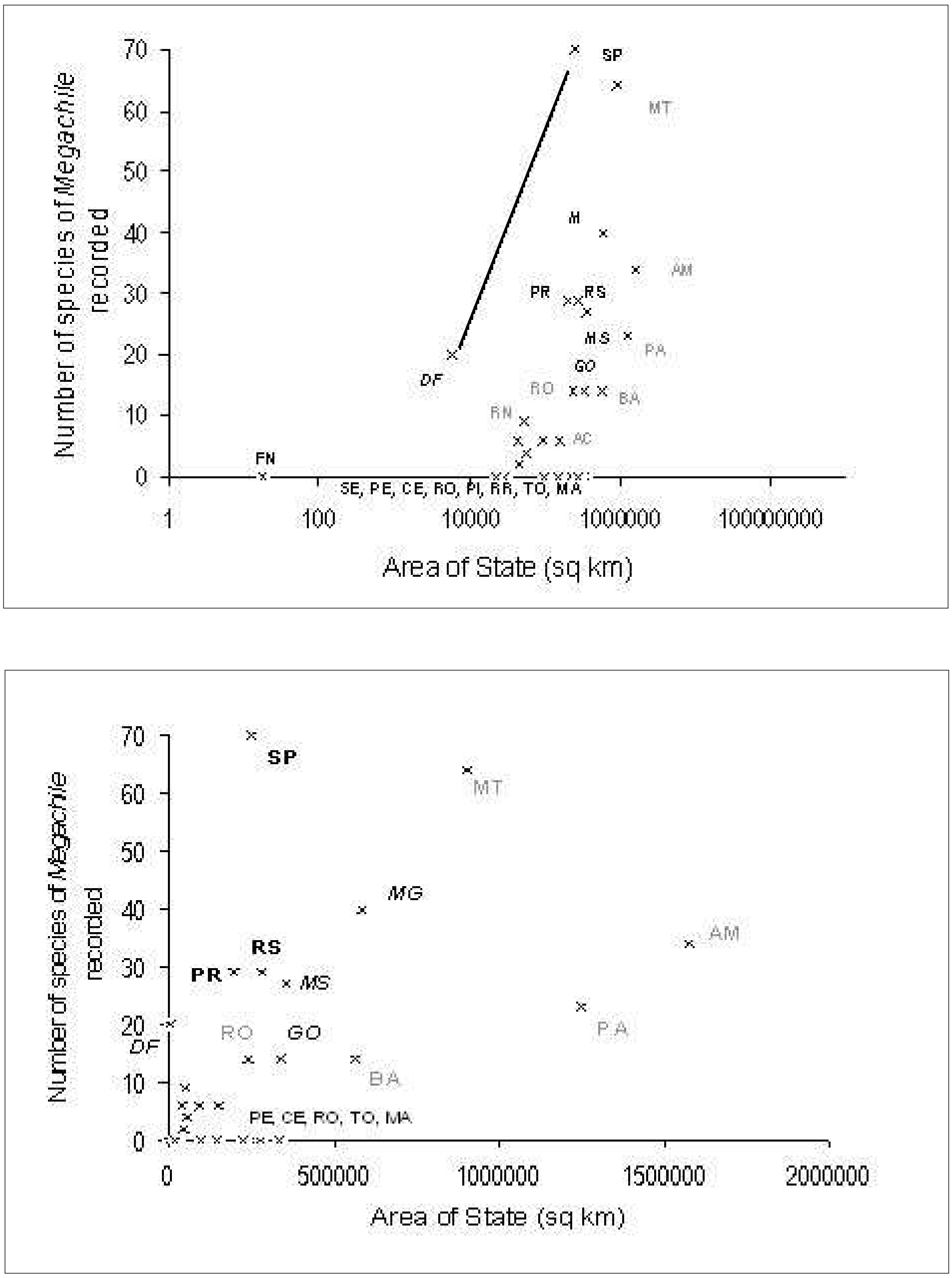


Figure 2. Number of species of *Megachile* recorded per Brazilian state.

Megachile at flowers

Innumerable scattered publications refer to the plant taxa that *Megachile* species visit. Many species are polylectic. In southern England *M. centuncularis* averaged more than seven plant families per cell (Raw, 1988). However, many bees prefer particular plants like members of the families Compositae and Leguminoseae. Polylectic species visit flowers near the nest so often they collect pollen from a few plant species. In contrast, some species restrict their choices to a few species. In the Neotropics members of the subgenus *Ptilosarus* are adapted to visit *Piper* flowers (Michener, 2000; pers. obs). The scopa is modified to scrape off the pollen as the bee runs up and down the *Piper* inflorescence.

The females of *Megachile* are well adapted for pollination. She does not transfer the pollen to a leg, but carries it on her abdominal scopa. As she alights directly on the anthers and stigma of a flower the pollen grains are easily transferred from one flower to another.

The success story of *Megachile* is the pollination of alfalfa. In most of U.S.A. *M. rotundata* is the most important pollinator. It provides yields of 2,200 kilos of seed per hectare; a twenty-fold difference over its absence. However, *M. rotundata* is not the recommended pollinator in regions with severe climates where several other species pollinate the crop. The use of *M. rotundata* in regions with a severe winter is complicated because the overwintering stages must be stored in frost-free conditions. In southern Alberta two native species, *M. dentitarsus* and *M. perihirta* fill the role (Hobbs and Lilly, 1954). The developmental stages of *M. rotundata* cannot survive high daily temperatures (>42°C). In warmer climates (southern U.S.A. and the West Indies) *M. concinna* pollinates alfalfa (Butler and Wargo, 1963; pers. data).

These other species are effective pollinators of the crop. An individual female of *M. perihirta* tripped 372 flowers per foraging trip, a nesting female averaged 75 foraging trips per cell and provisioned 15 cells so each foraging bee may trip 418,500 flowers to produce two kilos of seed (Hobbs, 1956). For a yield of 1,300 kilos of seed per hectare in the absence of other pollinators, a density of 500 foraging females of *Megachile* per hectare of flowering crop is recommended (Hobbs, 1956).

Some species can be adapted to small enclosures. *M. rotundata* has been used to pollinate alfalfa in glasshouses (Aubury and Rogers, 1971) and *M. concinna* nested successfully in a flight room of 12 m^3 so is a candidate as a pollinator in small enclosures (Butler and Ritchie, 1965).

An important revelation of the above findings is the great dependence of pollination on individual bees and, hence, the need to exercise great care when applying pesticides on the crop so as not to kill the pollinators. This dilemma is exacerbated because *M. rotundata* is more susceptible to pesticides on alfalfa than are honeybees (Johansen *et al.,* 1963) possibly because the bees cut leaves as well as visiting the flowers. If this be so, a similar threat would apply to many members of the genus.

Historical introductions

Many species nest in abandoned beetle burrows in wood and cracks in timber. This has given them great vagility and some are among the most widely dispersed of any bees. *Megachile* occurs on many oceanic islands, presumably because their nests were transported. Notable recent introductions are *M. gentilis*, native to western U.S.A. which has become established in Hawaii (Mitchell, 1935), the Eurasian species, *M. rotundata* arrived, apparently accidentally, in U.S.A. in the 1940's and is now widespread (Hurd, 1979) and the Chinese species, *M. sculpturalis* Smith has recently appeared in eastern U.S.A. (Mangum and Brooks, 1997). In order to improve the pollination of alfalfa *M. rotundata* has been deliberately introduced into Chile (Stephen, 1972), Australia (Winn, 1988; Woodward, 1994, 1996) and New Zealand (Donovan, 1980).

The situation in the West Indies is of considerable biogeographical interest. Ten palaeotropical species have become established there (Raw, 2003). Nine are African and *M. lanata* is Indian, but has spread to Africa. Undoubtedly nests were carried to the Caribbean on slave ships on the "Middle Passage" (Raw, 1985). However, it is strange that no palaeotropical species has been recorded from Brazil. In the West Indies, males of *M. lanata* often hold territories at flowering *Crotalaria* (Raw, 1985). I have searched the same species of *Crotalaria* in many parts of Brazil, but have never seen *M. lanata* there.

The Catalogue

For several years I have been compiling information for a catalogue on neotropical *Megachile* which currently includes 404 neotropical species (Raw, 2003). For each species all nomenclatural changes are given with information on types. The available information is summarized under the following nine headings.

D = Geographical distribution.
F = Flowers visited (including pollination studies).
K = Keys to identification.
L = Life history (including mating, development of young, sex ratios, dormancy, emergence, life-tables).
M = Morphology and anatomy.
N = Nesting (nest sites, nest architecture, building and provisioning cells, building materials).
P = Predators (inquiline bees and flies, predators on the developmental stages, predators on adult bees, fungal diseases and other pathogens).
R = Redescription of type sex or description of allotype. Female = (R-F); male (R-M) and both sexes = (R-FM).
T = Taxonomic information (includes locations of types).

The letters are cited after each reference to indicate the kind of information it contains and for each species the information is summarized under the nine headings. Where information on geographical distribution has been obtained from collections its name is cited in curved brackets. The follow examples illustrate the information presented for two species.

4. ***Megachile*** **(*Leptorachis*)** ***aureiventris*** **Schrottky**

Megachile aureiventris Schrottky 1902: 441. Female. Type locality: Jundiahy, S. Paulo [state], Brazil, 1897 (Schrottky). Type repository: MZSP. Examined.

Megachile (*Leptorachis*) *aureiventris* : Mitchell 1943b: 663.

ADDITIONAL REFERENCES. Graf 1967a: 127-130 (M); 1967b: 131-133 (M). Moure 1948: 326 (K). Schrottky 1913a: 141 & 144 (K), 175 (D, R-F). Silveira *et al* 2002: 213 (D).

DISTRIBUTION. BRAZIL: DF- Brasilia (Raw), Planaltina (Raw); MG; PR; RS; SP- Campinas, Ipiranga, Jundiaí.

MORPHOLOGY. Anatomy of head glands.

24. ***Megachile*** **(*Leptorachis*)** ***paulistana*** **Schrottky**

Megachile paulistana Schrottky 1902: 440-441. Female. Type locality: São Paulo [state], Brazil. Type repository: MZSP. Examined. [Type is missing right antenna and right fore leg.]

Schrottky (1913a: 215-216) believed *M. paranensis* Schrottky to be the male of this species. *Megachile subita* Mitchell 1930: 232-233. Female. Type locality: Chapada [dos Guimarães, MT], Brazil (H. H. Smith). Type repository: ANSP 4153. Examined. Synonymy of Mitchell 1943b: 663.

Megachile (*Leptorachis*) *paulistana* Mitchell 1943b: 663.

ADDITIONAL REFERENCES. Ihering 1904: 469-470 (N). Michener 1954: 100 (D, L). Moure 1941: 94 (R-F); 1948: 325-326 (K). Schrottky 1913a: 143 (K), 184-185 (D, R-F); 1913b: 247 (D).

DISTRIBUTION. PANAMA: PA- Chilibre, Tocumen. BRAZIL: DF- Brasilia (Raw); MT; SP- Campinas, Ipiranga, Itú, Jundiaí, São José do Rio Pardo. PARAGUAY.

LIFE HISTORY. Adults fly November to May.

NESTING. Nest of rolled leaves among books in a library.

Public catalogues must be produced with the co-operation and advice of the people who will use them. Furthermore, the presentation should be standardized because they need to be produced for many genera of bees throughout the world. In order to evaluate the presentation of the information in a catalogue the user should consider the following questions.

1. Is the information presented in an easily accessible form ?
2. Do the nine categories (cited above) adequately reflect the scope of information ?
3. How should geographical distributions be cited ? Often only the name of the country or state is known and these vary enormously in size. Geographical co-ordinates are known for few localities.
4. The catalogue would be best made available on an Internet site, but the question is where to place it ?

Promoting *Megachile* conservation

In order to conserve bees the interest of lay people needs to be aroused. Many people are fascinated with the idea of providing trapnests for wild bees in their gardens so it is an effective way to involve people directly in conservation. An additional aspect is that it is convenient, easy and cheap for the amateur entomologist to study the bees' nesting biology.

Many species are easy to manage in trapnests – as has been so clearly demonstrated with *Megachile rotundata*. A few comments on the use trapnests are worth repeating. They are more likely to attract bees when they contain large numbers of tunnels. Also, it is easier to examine the nest contents when the tunnel is lined with paper. It may take a few seasons to attract numbers of bees. For example, females of *M. rotundata* prefer trap-nests used by the previous generation to new tunnels. Apparently they detect aromas secreted left by the previous occupants rather than nest residues. Apparently the bees are not confused when several females use a trapnest simultaneously because a nesting female (at least of *M. centuncularis*) distinguishes the scent of the interior of her own nest from that of her conspecifics (Raw ,1992).

People want to know the names of the bees they are living with. *Megachile* is one of the most common genera to use trapnests in gardens so every effort should be made to identify the species. The present plan is to combine identification to subgenus or species group with knowledge of the geographical distribution. This approach will reduce the difficulties of identification of the species to feasible proportions. We need to produce workable keys for particular localities for use by the amateur conservationist and professional researcher. Easier identification should stimulate new studies on the bees' role as pollinators and on management of their populations.

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POLLEN SOURCES OF LONG-TONGUED SOLITARY BEES (MEGACHILIDAE) IN THE BIOSPHERE RESERVE OF QUINTANA ROO, MEXICO

Rogel Villanueva-Gutiérrez
David W. Roubik

Abstract

Pollen reference collections and palynological keys were developed for the biosphere of Sian Ka'An, in the south of Quintana Roo state, Yucatan Peninsula, Mexico. Our aim was determining the impact of invasive African honey bee on resident bees. Before African *Apis mellifera* became common in Yucatan, we studied the pollen provisions of five megachilid bee species that readily came to wooden block trap nests, retrieved each three months from forest sites over two years. All five were generalists on the resources of megachilids, which comprised trees, vines, herbs and shrubs of the Anacardiaceae, Sapotaceae, Euphorbiaceae and legumes in particular. Among the bees, a mean of 75% of their pollen species were also used by other megachilids. Uneven sample sizes for nests of different bee species made clear that the number of "unique" species grew with sample size. Approxoimately 4% of the local flora was utilized by megachilds, and 18 plant species were used by more than one species, while 12 were seemingly used by single species. We suggest that megachilid flora comprise a diverse yet small group of species.

Introduction

To investigate the impact of immigrant African honey bees on native bees in Mexico, we initiated a study of solitary bee floral resource use in the Yucatan Peninsula of southern Mexico. Ninety native bee species were registered but as many as twice this number may occur in the study area (Roubik *et al.*, 1990). The study took place in Quintana Roo state, located in the eastern part of the Yucatán Peninsula. No pollen studies had been made to determine food sources and dietary specialization of solitary bees in this tropical region. However, other studies that include pollen analysis were made with honey or pollen collected by *Melipona beecheii* and *Apis mellifera*. Roldan-Ramos (1984) analyzed and described the pollen grains found in honey samples taken from European honey bees and *Melipona beecheii* in Tixcacaltuyub, Yucatán. Later, Villanueva-G. (1994) made comparisons of the nectar sources used by the European and Africanized honey bees, and also compared pollen and nectar flowers visited by bees. Recently, Villanueva-G. and Roubik (in press) compared the quantitative pollen diets and foraging patterns of European apiary bees and naturalized Africanized honey bees in Sian Ka'An (see also Villanueva-G., 2002). It was found that the invading bees made resident generalist bees (apiary *A. mellifera*) expand their diet breadth, while at the same time their degree of specialization increased and the Simpson diversity decreased. This conclusion could only be reached because the amount of each pollen source in bee diets was quantified, and the 171 pollen species in this two-year study could be identified.

Methods

Background

According to the classification of Koeppen (1936), the type of climate that exists in the Yucatán Peninsula is *Aw*, which is defined as hot subhumid, with a mean annual temperature over 22°C and an annual precipitation between 700 and 1500 mm, with rainfall during the summer season. According to the precipitation we can divide the year into two periods, the wet season (June to October) and the dry season (November to May). This classification is very useful in understanding the phenology of flowering plants and foraging choices of bees.

The most common types of vegetation, according to the classification of Miranda and Hernández-X. (1963), are 'selva mediana subperenifolia' (medium stature sub-evergreen forest), 'selva mediana subcaducifolia' (medium stature sub-deciduous forest), 'selva baja subcaducifolia' (low stature sub-deciduous forest), 'manglar '(mangrove), 'selva baja inundable' (low stature sub-deciduous forest that floods during the wet season, with *Dalbergia glabra*, *Bucida spinosa*, *B. buceras* and *Haematoxylon campechianum* as characteristic elements), and secondary vegetation in different successional stages. The flora, the vegetation and the climate are similar throughout the Yucatán Peninsula.

Trap-nest Studies

The field work took place from September 1988 until 1990— approximately one year after the arrival of the feral Africanized honey bee. Four sites were chosen along a transect of 40 km within the Biosphere Reserve of Sian Ka'an, each surrounded by natural, continuous vegetation, accessible by a narrow road. Sites were designated as "St. Teresa" (site 1), the "Aguada" (site 2), "Yuras" (site 3), and "Ramonal" (site 4). St. Teresa is located 23 km NE, and Ramonal 63 km NE, of Felipe Carrillo Puerto.

Wooden trap nest blocks, of untreated pine wood 2 x 4" sections of 6-7" in length, with holes drilled of three diameters, were hung on small trees and left for approximately three months in the study sites. The traps were collected and brought to outdoor rearing cages, when new ones were placed in the field. Thus, trap nests were present throughout the year. To collect emerging bees from the trap nest blocks, a small cage or plastic tube was glued over the entrance of each nest hole. This procedure allowed us to capture young adult bees as they emerged, and to associate their nests provisions with them. The bees were identified and deposited in the collections of ECOSUR and STRI .

After adult bees had emerged, usually within three months of removing the trap nests from the field, the wooden trap nest blocks were opened to remove pollen and pollen faeces from nests. This pollen represented a sample of all the pollen deposited by the female bee in the progeny cells. The combined pollen and fecal meconia were acetolyzed (Erdtman, 1943) and mounted on microscope slides. Subsequently pollen grains were identified, counted, and photographed. Each nest sample yielded 600 to 800 pollen grains that were counted and identified along random transects on the microscope slides (see Villanueva and Roubik, in press).

A palynological reference collection of the area, with more than 500 pollen species, was used to aid identification of the pollen grains. In order to identify pollen in the region of the Biosphere Reserve of Sian Ka'an, a pollen atlas was prepared by Palacios *et al*. (1991).

The pollen grains from the bee nest sample were further desegregated using a sonicator cell disrupter (O'Rourke and Buchmann, 1991). The pollen was sonicated for 5 minutes at 24 kHz using a probe 'ultrasonic disintegrator' (M.S.E. SONIPREP) adjusted to medium power setting.

In this way, each bee pollen sample composition could be analysed in terms of (a) pollen percentage frequency, (b) mean percentage frequency and (c) occurrence.

As with the honey samples, 600 pollen grains were counted from each pollen load sample.
a) Pollen percentage was calculated as:

$$\text{pollen \% by taxon} = \frac{\text{number of pollen grains counted by taxon}}{\text{sum of all pollen grains counted}} * 100 \qquad (1)$$

b) Mean percentage frequency was calculated by obtaining the mean of the percentage of each pollen species in all the samples either from European or Africanized honeybees:

$$\text{mean percentage frequency} = \frac{\sum \text{ percentage frequency of each taxon}}{\text{total number of samples}} \qquad (2)$$

c) The occurrence refers to the percentage of each pollen species in the total number of samples either from European or Africanized honeybees. Considers only the presence or absence of a pollen species in a pollen load sample.

Results

From the total of 16 species that have been reported for Megachilidae in the Sian Ka´an reserve (Roubik *et al.*, 1990), only five made nests in the block traps: *Megachile zapatlana*, *Megachile quadridentata*, *Megachile pseudocentron*, *Megachile* sp. and *Anthodioctes* sp. From these, *Megachile zapatlana* was the most abundant. It was collected from 48% of the nests, while the least abundant was *Megachile quadridentata* with only 7 %.

A total of 70 nest provisions and 181 pollen-bee samples were analyzed for Megachilidae during the wet and the dry seasons at the four sites. The most abundant pollen species were *Metopium brownei* (Anacardiaceae, a tree), *Pouteria* sp. (Sapotaceae, a tree), Leguminosae 1, *Dalbergia* sp.*, Centrosema* sp. (leguminous trees and shrubs), *Chamaesyce* sp. (Euphorbiaceae, an herb), *Laetia thamnia* (Flacourtiaceae, a tree), *Euphorbia* sp. 2 (Euphorbiaceae, a tree), *Bursera simaruba* (Burseraceae, a tree) and *Coccoloba* (Polygonaceae, a tree). From these, the first five species were the sole pollen source in some nests. *Acacia* sp. (Leguminosae, a tree) was found only in five nests, but in one of them its percentage was 100%. Other plants with very high (>60%) representation in bee nests, but present in only one or two samples were *Eugenia* (Myrtaceae, a tree) *Terminalia* (Combretaceae, a tree), *Euphorbia* (a tree), *Gliricidia* (Fabaceae, a tree), *Vigueira* (Asteraceae, an herb), and an unidentified Sapotaceae (probably a shrub).

Certain plant pollens, in particular, were utilized by more than one megachilid species, with 18 species used by a mean and mode of ca. two megachilid species. Plant pollens harvested by only one megachilid species were 12 in number, with the most (5 species) found in nests of *Megachile zapatlana*. Among the four other megachilids, one had five unique species and three had one apiece. The statistics for shared pollen species between all five bees were a mean of 75% of their total pollen diet taxa (range 54 to 88 %).

Discussion

This group of bees seems to be very selective. Only a small proportion of all the flowering species were visited by Megachilidae. There are at least 1200 plant species in Sian Ka'an Reserve (Durán and Olmsted, 1987; Villanueva-G. and Cabrera, 1990; Palacios *et al.*, 1991) and the flora and vegetation from the four study sites are very similar, so the total number of species found in the nest pollen samples probably represents around 4% of the total Angiospermae present in the study area.

Only 48 pollen species were identified from the pollen samples, which belong to 17 different plant families. Among five species of megachilids studied intensively within large tracts of natural vegetation in Quintana Roo, there was a high reliance on only a few pollen sources. The five most frequently used pollens could comprise 100% of the pollen diet of the brood found in trap nest blocks. Those most important pollen sources were *Metopium brownei, Pouteria*, *Chamaesyce*, Leguminosae 1, *Dalbergia,* and *Centrosema.*

Families that contributed to megachilid pollen provisions with the largest number of pollen species were Leguminosae, Anacardiaceae, and Sapotaceae. Other studies of neotropical megachilids, in seasonally dry forest types, often cite many legumes, mints and composites as principal resources (eg. Martins, 1995; Carvalho and Bego, 1997), which are used as both nectar and pollen sources. In addition, megachilids can sometimes be very specialized in pollen use (Horne, 1995; Williams, 2003). Our limited knowledge of tropical megachilid pollen choice, at least in Quintana Roo during the study period, does not seem to closely fit the expectations from other work. However, beans constitute major pollen supplies.

None of the pollen species were present in all the pollen samples from megachilid nests, and *Metopium brownei* and *Pouteria* were the pollen species with the largest percentage in the nest samples. However, a great majority (75%) of the pollen of each species was also used by other megachilids. Moreover, the number of pollen species that appeared unique to a species was a function of the number of nests sampled, as seen in the pollen of *Megachile zapatlana*, with 13 unique species but nearly half (90 of 181) of the species samples identified. It appears, therefore, that more extensive sampling will reveal more pollen species utilized by megachilids in such tropical forests. The apparent specialization indicated by unique species associated with a particular bee will largely disappear with more extensive studies.

During the wet period we found almost no Megachilidae nesting in the wooden trap nests. Thus, a period of four months (May to August) shows no reproductive activity by these species. It is unknown whether a lack of nectar sources also occurred at this time.

In comparison, from a melissopalyologycal study in the same area and in the same period, food sources for *Apis mellifera* (nectar and pollen) represent around 20% of the total angiosperm flora (Villanueva-G, 1994, 1999). Nonetheless, the megachilids, as a group, generalize on many of the same species, and the plants, in turn, are probably generalists with regard to pollinating megachilids. These relationships do not, however, establish which species are the most significant mutualists for their corresponding plant or pollinator. From the bee perspective, quantitative nest pollen studies, especially those which quantify the actual volume of different pollen types (Villanueva and Roubik, in press) seem to be the most satisfactory available research tool. That quantitative research represents the fullest and most significant challenge to the continuing study of relationships between bees and flowers.

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SESSION V

SOLITARY BEES IN AGRICULTURE SYSTEMS: *CENTRIS* AND *XYLOCOPA*

1. Breno M. Freitas and Julio Otávio P. Pereira. “Crop Consortium to Improve Pollination: Can West Indian Cherry (*Malpighia emarginata*) Attract *Centris* Bees to Pollinate Cashew (*Anacardium occidentale*)?”

2. Stephen L. Buchmann. “Aspects of Centridine Biology (*Centris* spp.) Importance for Pollination, and Use of *Xylocopa* spp. as Greenhouse Pollinators of Tomatoes and Other Crops”

3. Katja Hogendoorn. “On Promoting Solitary Bee Species for Use as Crop Pollinators in greenhouses”

CROP CONSORTIUM TO IMPROVE POLLINATION: CAN WEST INDIAN CHERRY (*MALPIGHIA EMARGINATA*) ATTRACT *CENTRIS* BEES TO POLLINATE CASHEW (*ANACARDIUM OCCIDENTALE*)?

Breno M. Freitas
Julio Otávio P. Pereira

Abstract

Recent researches have shown that *Centris* bees can be relevant pollinators to some cash-crops largely cultivated in the tropics. However, while these bees play essential role in pollination of wild and cultivated varieties or closely related species, there are other economic important species in which only wild variety/type have benefited from their pollinating services. We focused on the cases of two cash-crops in NE Brazil: cashew (*Anacardium occidentale* L.) and West Indian cherry (*Malpighia emarginata* L.). We observed that while flowers of wild growing cashew found in its natural habitat of sand dunes in coastal areas of the state of Ceara, Brazil, were visited and largely pollinated by *Centris tarsata*, these bees were very rare in cashew plantations and did not contribute to the pollination of cultivated cashew varieties. However, *Centris* bees such as *C. tarsata*, *C. aenea* and *C. bicolor* were common visitors of both native nance (*Malpighia crassifolia* L.) and cultivated West Indian cherry. Considering that *Centris* bees need a source of oil for nest provisioning, which was provided in the sand dunes by the nance but not in the cultivated cashew plantation, and the lack of any known method for mass-rearing *Centris* species for introduction in cashew cropping areas, we suggest that cashew growers adopt a consortium of these two cash-crops, cashew and West Indian cherry, to attract *Centris* bees to their plantations and to promote some biodiversity increment in their agricultural land.

Key words: *Byrsonima crassifolia*, *Centris*, crop consortium, crop pollination, solitary bees.

Introduction

Oil collecting bees of the genus *Centris* are common in the Neotropics. Adults feed on nectar and pollen like most of other bee species, but nest cells are provided with a mixture of pollen and vegetable oil, collected from oil-secreting flowers, as food for the larvae (Raw, 1979; Frankie *et al.*, 1988; Freitas *et al.*, 1999). Because there is a great number of *Centris* species, they are common throughout the tropics and adults visit a range of flower species to collect pollen, nectar and oils, these bees are important pollinators of many tropical plant species (Pereira, 2001; Castro, 2002; Toro, 2002). Recently, some *Centris* species were also implicated as potential pollinators of crop species such as West Indian cherry (*Malpighia emarginata*), Barbados cherry (*M. glabra*), cashew (*Anacardium occidentale*) (Freitas and Paxton, 1998; Freitas *et al.*, 1999; Buchmann, 2004).

Many authors have pointed out the honey bee *Apis mellifera* as the main pollinator of cashew plantations around the world (McGregor, 1976; Free, 1993; Freitas, 1994; Freitas and Paxton, 1996). But this bee species is not indigenous to the native range of cashew in NE Brazil, and studies by Freitas (1997), Freitas and Paxton (1998) and Freitas *et al.* (2002) suggest that *Centris* bees, particularly *C. tarsata*, are the efficient native pollinator of wild cashew growing in coastal areas of the state of Ceara, by visiting its flowers regularly to collect nectar. Despite very common there, these bees are rare in areas cultivated with cashew, even though the trees of most plantations are wild types or varieties of cashew and spraying insecticides or any other chemical is not a common practice. Since natural pollination levels in cashew fields are low and have limited production, cashew growers dependent on honey bee pollination to ensure adequate pollination of their trees (Freitas and Paxton, 1998; Holanda-Neto *et al.*, 2002).

Honey bees can be used to pollinate cashew, but they are very difficult to direct to this crop since cashew produces few flowers per day, its flowers are poor in pollen and most cashew varieties produce little nectar and/or poor sugar content nectar (Freitas, 1995a). Those characteristics favor competition from wild plants, like *Borreria verticillata* in NE Brazil, which grows in large patches within and in the surroundings of cashew plantation and diverts most honey bees from the cashew flowers (Freitas, 1995b). Considering Africanized honey bees, there is also the risk of stinging and the need to adapt farming management to allow their safe use.

If solitary bees of the genus *Centris* were present to the cashew plantations in adequate numbers, the use of the exotic and difficult to manage *Apis mellifera* could not be needed. However, there is no known method for mass-rearing *Centris* species for introduction in agricultural areas (Buchmann, 2004) and natural populations of these bees seem not to be attracted to cashew plantations as they are to wild grown cashew. There is a need to develop techniques to rear and manage *Centris* bees and/or to attract feral populations to agricultural areas. In both cases, these plantations will have to provide the bees with adequate environmental conditions to match their nesting sites, mating, sheltering and food requirements.

***Centris* bees as pollinators of wild grown cashew**

The development of mass rearing *Centris* bees, although promising, is still a bit far away. Attracting these bees to cropped areas seems more feasible nowadays. However, it is necessary to find out how to attract and fix feral populations in the plantations. A comparison between the coastal sandy dunes areas where cashew grows wild and *Centris* bees are common visitors/ pollinators of its flowers, and the agricultural areas where commercial plantations of cashew are found but not regularly visited by *Centris* bees, shows the former as a hostile environment, with tougher natural conditions and much fewer live diversity than the latter. Coastal areas are covered by sandy dunes which are very poor soils with little consistence and poor water retention, winds over 20 km/h, intense sunlight and high temperature (33 °C), while agricultural cashew areas have deep well structured soils with good fertility and water retention levels, weak winds (around 4 km/h), intense sunlight but temperatures are around 27 °C. Even so, *Centris* bees inhabit the coastal areas but not the inland cashew cropping fields. There should be a limiting factor that allows *Centris* bees to survive in the harsh conditions of coastal areas but not in the milder environment of agricultural cashew areas.

A recent study by Pereira (2001) carried out in the same areas where Freitas (1997) and Freitas and Paxton (1998) identified *C. tarsata* as an efficient pollinator of cashew, have shed some light on the puzzling attractiveness of *Centris* bees for wild grown cashew but not for the commercially grown trees. What Pereira (2001) found out was that *Centris* bees of the studied coastal area also forage intensively on a wild bushy plant species belonging to the

Malpighiaceae family, the nance (*Byrsonima crassifolia*), which fruits are much appreciated by the local population for juices and a variety of dishes. A remarkable feature of this plant species, like others of the Malpighiaceae family, is the fact that its flowers do not secrete nectar, but do produce edible oils in special glands called elaiophores.

According to Pereira (2001), two *Centris* bee species, *C. bicolor* and *C. tarsata*, the latter being the same species registered by Freitas (1997) and Freitas and Paxton (1998) for cashew, visited nance for pollen and mainly for floral oils. Considering that this bee species also visits cashew for nectar, it seems that nance and cashew flowers are complimentary parts of the bee's diet in coastal areas, since the bee needs nectar, pollen and oil sources to survive and to reproduce successfully. Cashew flowers can only provide the bee with nectar, while the nance does not produce nectar but offers pollen and oils.

Foraging strategies of *Centris* bees on coastal areas of Ceara

Investigation on the floral biology and foraging behavior of *Centris* bees on flowers of *B. crassifolia* have shown that the nance concentrates its blooming from October to January, although small amounts of flowers can be found almost at any time of the year. Its inflorescence is a terminal raceme to 11 cm long containing an average of 32 flowers open at any time (Pereira and Freitas, 2002).

Flower anthesis take place from 5:00 to 17:00 h, except at the hottest part of the day, from 13:00 to 15:30. Most flowers open at the coolest hours of the day between 6:00 and 8:00 h (Figure 1) and 85% are already open by 12:00 h (Pereira, 2001).

Anther dehiscence also occurs throughout the day, in many flowers even before the anthesis.
However, as for the anthesis, pollen present
more than 97% of flowers have presented the

The main floral visitors of nance in the coa
tarsata and *C. bicolor*. Although they visit flo
nance flowers concentrated from 05:00 to 08:0
and pollen presentation (Figure 3).

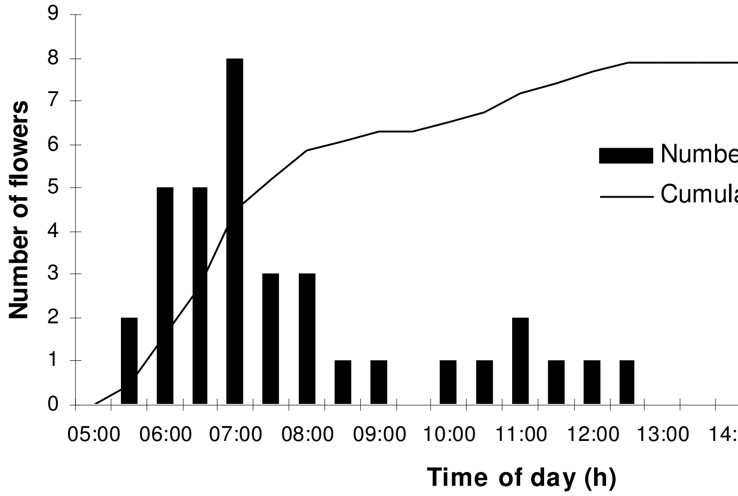

Figure 1. Anthesis pattern in nance (*Byrsonima crassifolia*).

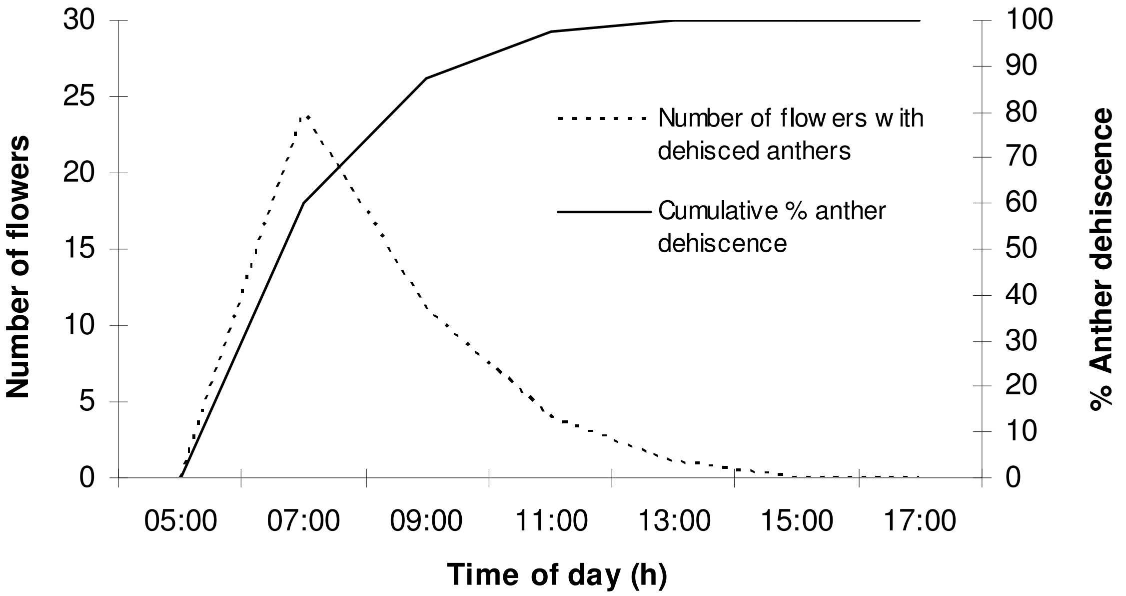

Figure 2. Pattern of pollen presentation in nance (*Byrsonima crassifolia*).

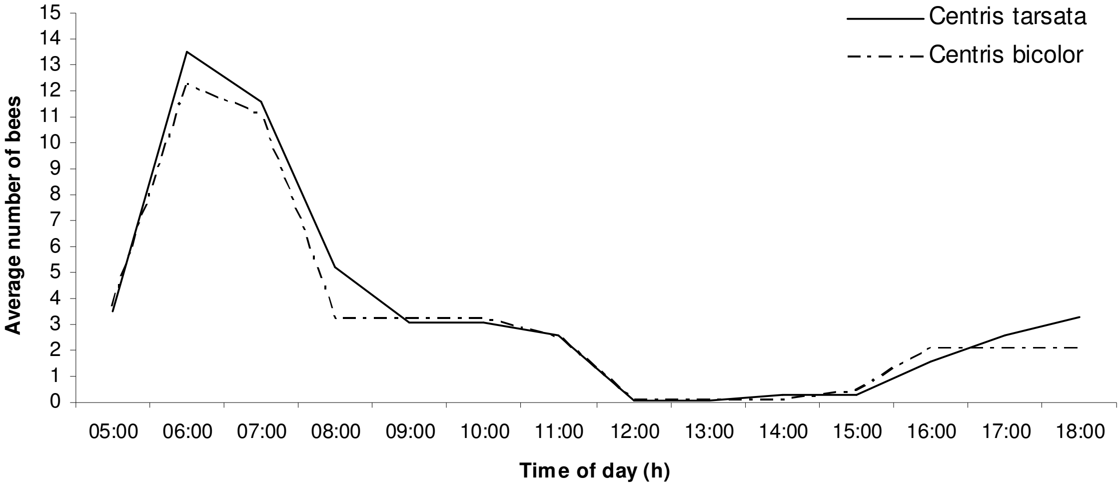

Figure 3. Foraging pattern of *Centris tarsata* and *Centris bicolor* on flowers of nance (*Byrsonima crassifolia*).

Wild cashew growing in the same area blooms from July to October and presents anthesis of male flowers from 06:00 h onwards with 82% of flowers open at 10:00 h, while the anthesis of hermaphrodite flowers begins at 10:00 h and 97% are open by 12:00 h. In both type of flowers, anther dehiscence takes place from 9:30 h onwards and 89% anthers are dehisced by 12:00 h (Freitas, 1995a; Freitas and Paxton, 1998), as shown in figure 4.

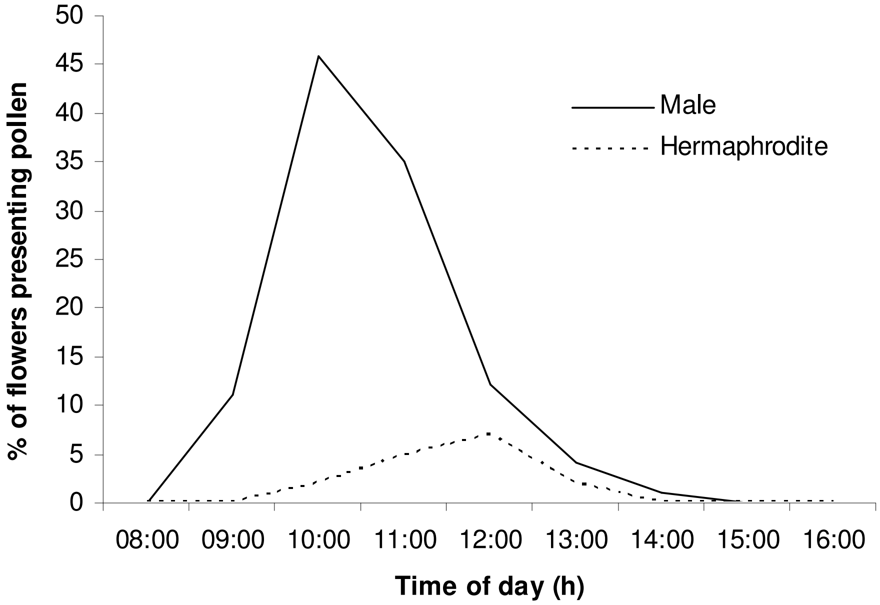

Figure 4. Pollen presentation pattern of male and hermaphrodite wild cashew flowers.

The coincidence in time of resource presentation by both plant species allows the bees to procreate. The adult bee satisfy its own need of energy feeding on cashew nectar, but it can only reproduce if nance flowers are available because its larvae feeds on a mixture of pollen and floral oils, both collected from this plant species.

When both flower species are present in the area, the bees alternate trips of nectar feeding in cashew and pollen and oil collection in nance until noon. At the hottest part of the day, the bees stop foraging on the nance and reduce considerably any feeding at cashew. Flower visits are resumed on nance after 15:00 h (Figure 5).

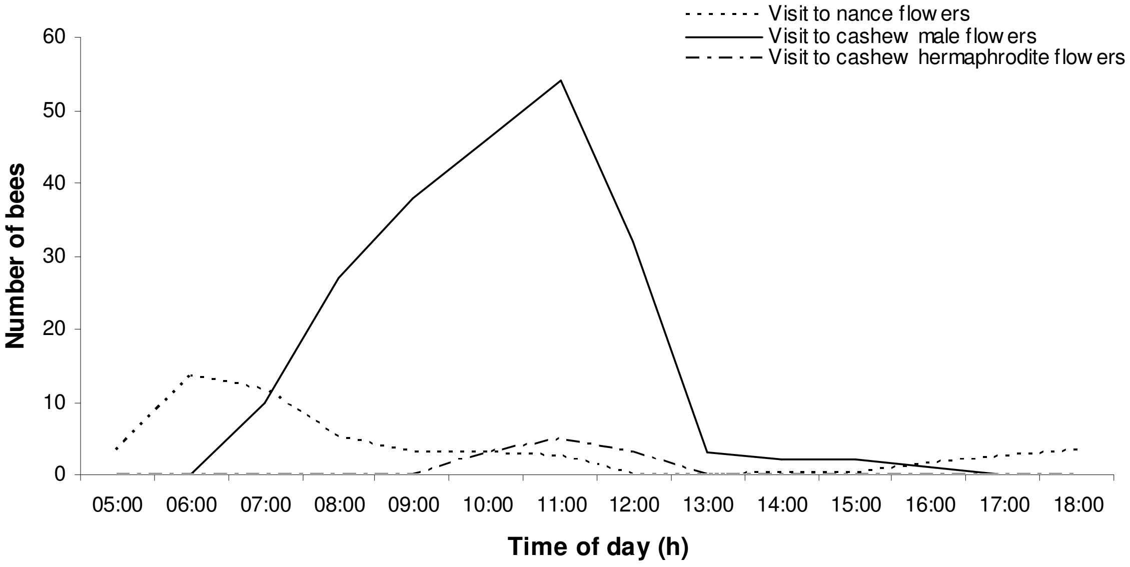

Figure 5. Foraging pattern of *Centris tarsata* on flowers of nance and wild cashew trees in coastal areas of Ceara, Brazil.

The absence of *Centris* bees in cashew plantations

It seems that the presence of both a nectar source, represented by the cashew trees, and a pollen and oil source, corresponded by the nance, associated with adequate environmental conditions, can explain a stable feral population of *C. tarsata* and *C. bicolor* in the costal sandy dune areas. On the contrary, nance is not native to cashew cropping areas and cannot be found within or in the surroundings of plantations. Therefore, despite the abundance of cashew flowers, *Centris* bees cannot establish wild populations in cashew agricultural settings.

There is little information on the existence of other native oil-producing plant species in the region where most cashew plantations are set. However, these areas are much more densely populated than coastal areas and human intervention is much stronger. Extensive deforestation for cropping huge fields of cashew and other tree species of economic value, farming, coal production, road developments and intensive transit of people and goods have changed considerably the landscape, and affect strongly the presence and frequency of plant species in the vegetal community.

Whether there were other oil-producing plant species different of nance in the region of cashew plantation or not, is yet to be proven. Cashew itself is not native to most regions where it is cropped now in NE Brazil, particularly inland areas, making clear that the nance – *Centris* bees – cashew relationship observed in coastal areas did not occur in these areas in the past. What is known for sure nowadays, is that there is no established population of any given oil-producing plant in the cashew cropping areas that could support wild populations of *Centris* bee big enough to make any difference in cashew pollination. *Centris* bees are rare on cashew flowers in this region but can be found, meaning that they are present in the environment although their numbers are limited by one or more limiting factor. The most obvious limiting factor one can see is the lack of oil sources, because there are other sources of pollen on the surroundings and cashew produces nectar enough for the *Centris* demands.

Is it possible to attract *Centris* bees to cashew plantations?

Apparently, the main reason for the absence of a well established *Centris* population in cashew plantations is the lack of oil sources used to feed the brood. Therefore, it seems possible to attract these bees to live in the agricultural area providing them with oil-producing plants. Planting nance on the surroundings of cashew fields could be a good attempt to attract *Centris* bees.

But Pereira (2001) has shown that nance are significantly more visited and set more fruits when they are in groups apart 10 to 15 m from each other, possibly because longer distances make nest provisioning too expensive in energy to the bees (Figure 6). Therefore, in order to have the same attractiveness in cashew plantations, growers would have to crop wild trees among their cashew trees.

Planting nance among cashew trees does not seem very interesting to growers. Besides the fact that introducing this plant species to the plantations will reduce the number of cashew trees per hectare, it will increase costs/area and demand changes in agricultural practices. Although nance produces edible fruits which are appreciated by local people, its commercial value is low due to the amount picked from wild plants, there is no market for large scale production and fruits are extremely perishable. However, except for these commercial reasons, cropping nance could work as well to attract *Centris* bees to cashew plantations as the native nance does to wild cashew in coastal areas.

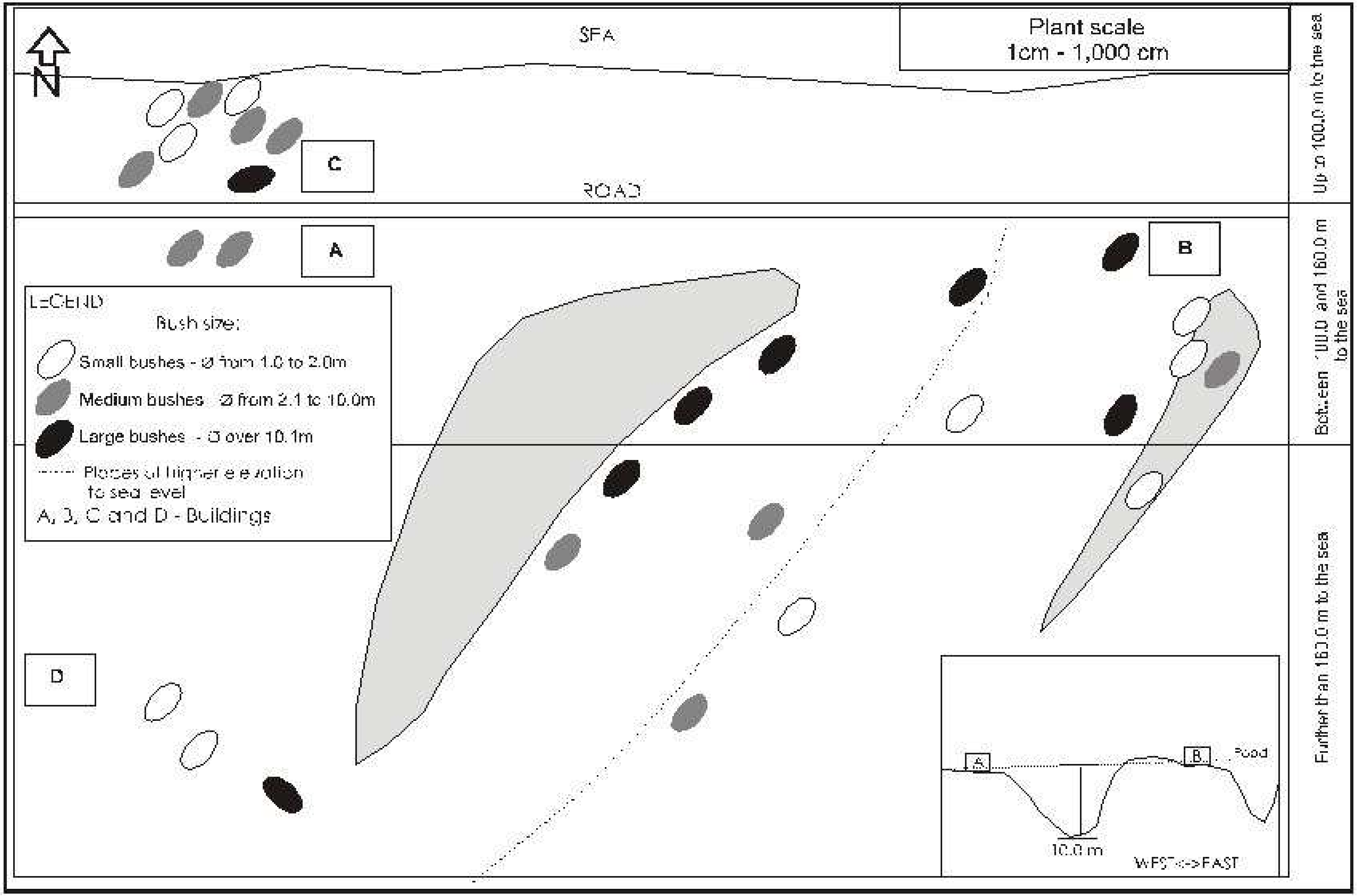

Figure 6. Map showing the distribution, size and distance to the sea of nance (*Byrsonima crassifolia*) bushes in a coastal area of Ceara, Brazil (Adapted from Pereira, 2001).

Proposing a crop consortium of cashew and West Indian cherry to improve pollination

An alternative for nance cropping we believe is viable and growers will not make objections is a consortium between cashew and West Indian cherry (*Malpighia emarginata*). The West Indian cherry, like the nance, belongs to the Malpighiaceae family and also produces floral oils. According to Freitas *et al.* (1999), floral and pollinating biology of the West Indian cherry is similar to that of nance, with flower anthesis and pollen presentation early in the morning and being visited by a variety of *Centris* bee species, inclusive *C. tarsata* and *C. aenea*.

The West Indian cherry is largely cultivated in NE Brazil and there are no reports of productivity limited due to pollinator shortage. Investigations carried out with this plant species have always observed great number and variety of *Centris* bees visiting their flowers, like *C. tarsata*, *C. aenea*, *C. fuscata*, *C. spousa*, *C. spilopoda* and *C. bicolor* (Carvalho *et al.*, 1995; Melo *et al.*, 1997; Freitas *et al.*, 1999). In opposition to what happen with the nance, its fruits are greatly demanded due to its high content of natural ascorbic acid, mainly for fresh consumption and to be processed into pulp or juice (Buchmann, 2004). Therefore, growers are already familiar with this crop and can increase their income per area by cultivating two cash crops simultaneously, and both contributing and benefiting from this consortium by making possible the presence of their pollinator in the area. Besides that, the consortium of cashew and West Indian cherry can promote some biodiversity increment in cropped land and contribute to reduce negative agricultural impacts.

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ASPECTS OF CENTRIDINE BIOLOGY (*CENTRIS* SPP.) IMPORTANCE FOR POLLINATION, AND USE OF *XYLOCOPA* SPP. AS GREENHOUSE POLLINATORS OF TOMATOES AND OTHER CROPS

Stephen L. Buchmann

Abstract

Centris is a large genus of fast-flying moderate to large bees found in forested and desert habitats of the New World. These bees play important keystone mutualist roles by providing essential ecosystem services (pollination, nutrient cycling, bioturbation and themselves as food for other organisms). Centridine bees are unusually hairy, thus capable of transporting thousands of pollen grains and are highly effective pollinators of native plants and crops. *Centris* species are important pollinators of oil-producing (elaiophore-bearing) plants in the pantropical family Malpighiaceae. They visit, harvest floral oils and pollinate *Byrsonima* ("Nance") and *Malpighia glabra* ("Barbados Cherry") grown as crops. Some of the smaller *Centris* can be readily managed and deployed in trap nest bundles within plantings of managed crops. Like *Centris*, the large genus *Xylocopa* is an important genus just now being developed as a pollinator of certain crops. Both genera harvest pollen from angiosperms with poricidal anthers. These bees utilize floral sonication ("buzz pollination") to eject and rapidly harvest pollen grains from these specialized poricidal anthers. The syndrome of buzz pollination, and those bee taxa utilizing this pollen harvesting strategy are discssed. Preliminary information on the use of carpenter bees as pollinators of greenhouse hydroponic tomatoes in Arizona, are also presented.

Introduction

The tribe Centridini is a speciose bee group distributed largely throughout the tropical and even desert regions, of the Western hemisphere. These are medium to very large robust, extremely hairy bees that are anthophoriform to eruciform in body shape. They are extremely agile bee aeronauts, very fast flying and difficult to capture on the wing as any melittologist will confirm. Best to wait for them to land, then swing your net!

Their scopae, the pollen transport apparatus is confined to the hind tibiae and basitarsi. These pollen-holding setal fields are huge, usually dark brown or black in coloration and composed of long dense hairs. The setae are relatively unbranched and coarse, especially in scopae adapted for sopping up and transporting floral lipids.

The tribe is composed of two closely related genera, *Centris*, a large genus (approximately 144 species distributed among 12 sugbenera) and the smaller genus *Epicharis* (23 species in 9 subgenera). For this paper, I follow the taxonomic designations of C. D. Michener (Michener, 2000) for centridines and xylocopines.

Nesting Biology-

Most centridines nest in the ground (odalous), their underground burrows can be found in either horizontal substrates or vertical banks. Some females locate pre-existing holes, usually buprestid or cerambycid beetle burrows in branches, upright or downed logs that aren't decomposed (eg. Heterocentris and Xanthemisia). A few species nest in the outermost carton layers of arboreal termite nests in tropical rainforests. Nesting habits for the genus were reviewed recently by Frankie *et al.* (1993) and by others (Coville *et al*, 1983; Vinson *et al.*, 1987). In Costa Rica and Panama, certain Centris (Ptilotopus) regularly construct their nests within arboreal termitaria of the termite genus Nasutitermes. Some smaller tropical Centris are easily attracted to and readily accept wooden boards drilled to form trap nests, thus can be easily managed compared to soil-nesting congeners.

Extremely large Centris nesting sites can form. One nesting site of *C. caesalpiniae* (near Sahuarita, AZ in 1989) covered and area of 1,290 square meters, had an estimated 423,000 females actively nesting and likely produced 1.68 million cells (Rozen and Buchmann, 1990). *Ericrocis lata* cleptoparasites were reared from excavated *Centris* cells. *Centris caesalpiniae* species has a shifting nest strategy, perhaps to evade predators and parasites. Although we've searched each year, this immense nesting site and *Centris* population has not been relocated.

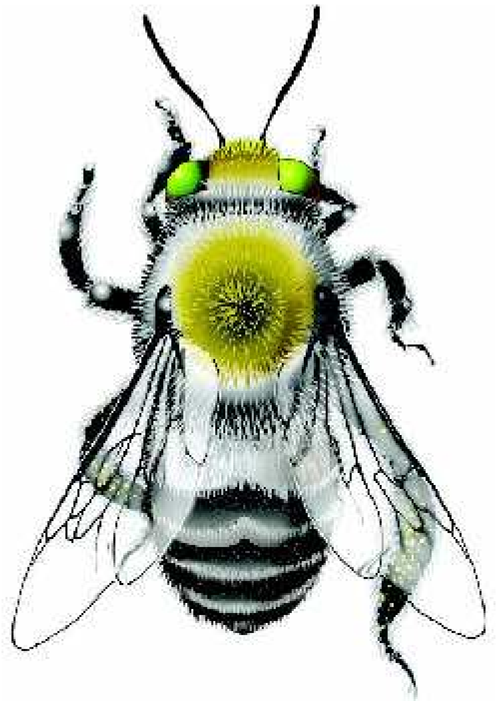

Figure 1. A nonmetandric male of *Centris pallida* Fox from the Sonoran desert near Tucson, AZ. Illustration by Paul Mirocha.

Floral Oils and Cell Linings

The nidal cells within the soil are typically located at shallow depths and usually in a vertical orientation. The interior walls and cell cap are smoothed by the females and often lined with a thick exocrine gland lining similar to that found in Anthophora. Floral resins may be admixed with sand and soil particles to form the cells of other species. *Centris* females dig solitary nests in a scattered fashion, or some, especially desert species, can be clumped and highly gregarious. *Centris* pallida exhibits both patterns.

Floral oils from *Byrsomina* and other malpighiaceae cap the outermost cells of twig or trap nest block utilizing species including *C. analis* in Panama (Roubik, pers. comm.). Perhaps this sticky layer serves as an ant repellent or tanglefoot barrier.

Buchmann and Roubik (unpubl. observations.) studied a gregarious nesting site of *Epicharis rustica* in a steep ravine with vertical banks on Taboga Island off the Pacific coast side of the isthmus. The *Epicharis* cells were lined with large amounts of *Bryrsonima* greenish-yellow floral oils. Recently provisioned cells containing pollen masses with eggs, had cell very thick cell wall linings that dried (polymerized with a bee-added enzyme?) initially to light olive color in a period of several days. Cells containing mature larvae, presumably several weeks old, had hardened to a wax-like texture, reacting like wax when scored with a knife blade, and darkened to a light chocolate brown in color. We have not yet performed definitive chemical analyses on these cell wall constituents, comparing them to *Byrsonima* oil or bee Dufour's gland exocrine secretions. Cells of *Epicharis zonata* are so well water-proofed that they remain below the water table in waterlogged soils for many months without damage (see Roubik and Michener, 1980).

Mating Biology

The genus *Centris* has a amazing repertoire of mating biologies and especially alternative male mating strategies (Thornhill and Alcock, 1983). Males of some *Centris* are territorial and patrol faithful routes among flowering plants searching for potential mates, while others patrol emergence sites, especially gregarious ones.

Other species can't seem to wait and dig for love. This unusual behavior was studied in the Sonoran and Chihuahuan omnipresent desert bee *Centris pallida* Fox. by Alcock and Buchmann in numerous papers (e.g. Alcock; Jones and Buchmann, 1976,;Alcock, 1980, 1984; Rozen and Buchmann, 1990). Other authors have studied the reproductive and mating biology of the genus *Centris* (e.g. Coville *et al.* 1983).

Centris caesalpiniae has huge "metander"males which fly low over nest sites, alight and dig in spots directly over where virgin females are digging out of their natal cells. Mating is rapid and forceful with much scramble competiton among males. A polymorphism occurs in this species, for smaller "normal" males look for females at the periphery of nest sites at flowering *Cercidium* trees or *Larrea* bushes.

Even stranger, is the case of mating tactics used by *Centris caesalpiniae*. This bee seems to have a balance polymorphism of not one but two male morphs. Small males are female impersonators in body coloration and flight behavior. They pursue escaped virgins among blooming Larrea bushes. Larger males are metanders and have an entirely different coloration pattern from the other males. The two male morphs were not originally identified as belonging to the same species until Rozen and Buchmann (1990) reared both types from natal cells. The large males dig up pre-emergent females and mate with them on the ground.

Anthecology and Oil-harvesting

Floral lipid (oil) production by certain flowering plants is a widespread phenomenon, especially in desert and tropical regions. It occurs in at least 10 families, 79 genera and over 2400 species (see review by Buchmann, 1987). Specialized lipid-secreting glands (elaiophores) are produced in water blister-like calycine glands within the large tropical family Malpighiaceae. Nesting females of *Centris* and *Epicharis* (along with a select group of other bee genera) utilize the oils admixed with pollen and some nectar in their brood provisions. Oil-harvesting species ("oil baron bees") have modified foreleg basitarsal scraper setae and coarse scopal hairs on their hind legs for collecting and transporting the lipids. These oils are the highest energy content floral rewards known (Buchmann, unpubl. data).

Along with numerous wild plants, several species of oil-bearing malpighs have been domesticated, brought under cultivation or wild-harvested for their edible fruits. These plants are visited by centridines and include *Byrsonima, Malpighia* and *Bunchosia* species.

Managing *Centris* for Crop Pollination (*Byrsonima* and *Malpighia*)

In Central and South America, the fruits of several genera and species of Malpighiaceae are harvested for their edible fruits and derived products sold in markets.

"Nance"- Byrsonima crassifolia is a shrub or small tree up to 9m in height. Its distribution extends from southern Mexico through northern South American. It is adapted to various soils, climates and vegetation types from coastal to semi-desert arid and humid tropical forests. Trees can withstand prolonged droughts and even occasional freezes. The small yellow rounded fruits (2-5 cm diamter) are produced in dense clusters. They are oily with an acidic flavor much prized by locals. Trees are usually not planted but wild-harvested. Often, they are left standing in pastures following conversion from forests. The fruits of Nance are made into a refreshing cold drink, a refresco in Costa Rica, Panama and Brazil. In Brazil, this tree and fruit is known as "murici."

Small plantations of Nance (*B. crassifolia*) have been established in several Brazilian states as an economic crop, but the majority of the fruits are still harvested from the wild. At present there are no efforts underway to use *Centris* or *Epicharis* as managed pollinators of *Byrsonima* although several researchers are contemplating this (A. Raw and S. Buchmann).

"Field Nance"- Another Byrsonima species is B. basiloba which has a small yellow-orange fruit very similar to Nance. It is a small tree, to 10m, native to scrub-lands of northern Brazil. Its fruits are eaten fresh or made into drinks. It is not cultivated at present.

"Acerola" or "Barbados Cherry"- Malpighia glabra is a bushy shrub up to 6 meters. Its native range is from South Texas through the Caribbean, through Central America and into Brazil. It is a prolific bearer of bright red fruits. Malpighia glabra is grown on a much larger commercial scale than Byrsonima. Commercial production supplies beverage makers and use in nutritional products. Fruits can be eaten fresh or used as flavorings for drinks. Commonly used in South America to flavor ice creams, drinks and cocktails. The red fruits have a Vitamin C concentration up to 65 times more than Citrus fruits. A single fruit contains the minimum daily nutritional requirements for this vitamin.

"Peanut Butter Fruit"- The small shrub or treelet Bunchosia argentea is native to Central and South America. Its small red-orange fruits are sticky with a dense-pulp. Its flavor is said to resemble dried figs or even peanut butter. Its fruits are eaten fresh or used as a flavoring in drinks. Fruits are wild-harvested.

Recommendations for Pollinating Cultivated Byrsonima and Malpighia with Artificially Managed Populations of Centris.- Many Centris and Epicharis species visit oil-bearing flowers of the large genus Byrsonima (about 120 spp.) in Central, South America and the West Indies. Unlike many crops visited by non-adapted exotic pollinators, Centris fits the crop, is in fact the typical and best pollinator to use. Byrsonima does not however, provide floral nectar, only pollen and floral oils. Centris females require other non-malpigh blossoms for their nectar supply. If commercial plantings of Byrsonima trees are kept small enough (e.g. 1 ha) then surrounding vegetation probably contain abundant nectar plants for foraging females. If largescale plantings of *Byrsonima* are created, then interplanting with nectar-bearing trees/ shrubs, or providing a mixed floral ground cover of plants Centris visit for nectar (eg. Hoffmanseggia or other legumes) would be advisable.

Many mid-sized *Centris* species of Central and South America (e.g. *C. analis, C. inermis*, *C. trigonoides*) are ideal candidates for research on their use as managed pollinators of malpigh or other tropical crops. This guild of small to moderate sized *Centris* look similar, having brown thoraces and reddish abdomens. These bees readily accept trap nest blocks (holes drilled into lumber, blocks or planks) attached to buildings or placed in a shelter structure near their host plants. Commercial sized populations of these bees can be built up from wild caught seed stocks, if managed properly for various parasites and predators.

Sturdy nesting shelter boxes (attached to metal fence posts) for bees can be placed along the periphery, or even inside files of *Malpighia* or *Byrsonima* orchards. Large wooden or metal boxes (1m X 0.5m X 0.5m0 can be used to create nesting boxes. Boxes should secured tightly to the posts, approximately one meter above ground.

The boxes should be oriented on their sides, the opening facing out allowing easy bee foraging. They should face east or southeast but not due south for optimal internal temperatures. If bird predation on adults or larvae is a problem, a sheet of coarse metal mesh (e.g. poultry fencing) can be nailed across the shelter box opening to dissuade avian predation.

Holes drilled into sold lumber should be a mixture of sizes, with diameters ranging from one-fourth to three eighths (6.4 to 9.5 mm). Long drill bits should be used. Ideal nest depths should be from 5 to 10cm, but not with holes breaking through the back side of the wooden blocks.

Table 1. Advantages and disadvantages of using *Centris* spp. as *Malpighia* and *Byrsonima* pollinators.

Advantages	**Disadvantages**
1. Natural pollinators of oil plants	1. Nectar plants must grow nearby or be provided.
2. Long-lived individuals and flight periods.	2. Bees are solitary, relatively low nos. compared to social bees
3. Easily trap-nested from wild popls.	3. No guiding documents for management techniques.
4. Populations can be managed and built up in drilled wood blocks with or w/out paper drinking straw inserts.	4. Populations aren't commercially available, must be wild-trapped.
5. Shelter boxes are moveable	5. Unknown flight ranges
6. Floral constancy for oil flowers	

Carpenter Bees as Managed Pollinators of Tomato

The large carpenter bees (not *Ceratina*) in the tribe Xylocopini are are large to very large robust bees. They are often confused with bumblebees by non-specialists. Within the genus *Xylocopa*, includes approximately 463 described species in 30 currently recognized subgenera. A few species nest in soil substrates (e.g. *Proxylocopa*) but the major have powerful mandibles and create two or three dimensional nests or extensive galleries in sound dead wood that has not decomposed. In Arizona, certain species under study nest in the relatively narrow diameter one to two year old flowering/fruiting scapes of common desert plants (e.g. *Agave, Dasylirion* or *Yucca*). Other species prefer to nest in Populus or introduced Melia logs and these log-nesters can be semi-social with nest tunnels re-used by succeeding generations of bees over several decades (Buchmann, pers. observ.).

Figure 2. An artists illustration of a female Arizona carpenter bee (*Xylocopa varipuncta*) While foraging. Illustration by Pa ul Mirocha.

Buchmann and Donovan (unpubl.) have used Arizona species of carpenter bees (e.g. *X. californica arizonensis* and X. *varipuncta*) in preliminary pollination trials of hydroponically grown greenhouses in Tucson.

Because of their large size, long lives as individuals, polylectic diet breadth for pollen and lengthy seasonal activity periods, carpenter bees have begun to attract the attention of researchers looking for crop plant pollination solutions, especially in glass or plastic greenhouses (e.g. Hogendoorn, Buchmann and others). The need for viable greenhouse pollinators is exacerbated because honey bees (*Apis mellifera*) perform poorly when used in greenhouses.

At present, a large industry has developed around providing *Bombus* colonies to pollinate high cash crops (e.g. tomatoes, peppers, eggplants) grown under glasshouse conditions. Some countries/continents, such as Australia, do not have native *Bombus* and it would be unwise to import them and risk their establishment there. This makes carpenter bees an intriguing alternative pollinator for greenhouse situations.

All of the above plants require floral sonication (buzz pollination) to set optimal fruit levels, or produce the highest quality fruits. Again, honey bees cannot be used to effectively pollinate these pollen-only buzz-pollinated crop plants.

Using carpenter bees for field or indoor pollination is not without problems, however. There are no currently available management guidelines for the use of *Xylocopa* species as pollinators of field or glasshouse crops. Research on their use for crop pollination is in its infancy. Within such a large genus, the nesting, mating and nutritional requirements of most species are unknown. Thus, their utility as managed crop pollinators will vary across subgenera and from species to species. The numbers of individuals in a “pollination unit”, especially solitary species that utilize flowering scapes, is limited compared even to the relatively low numbers of individuals in commercially-produced Bombus colonies. This is less of a problem for semi-social larger tropical species which nest in logs.

New domiciles to manage carpenter bee nests for pollination or observation need to be developed. One especially interesting and ingenious “book hive” has been developed, tested and used by Dr. Makhdzir Mardan at the Universiti Putra Malaysia in Kuala Lumpur (see Roubik, 1995). Although difficult to trap nest, foraging and already nesting *Xylocopa* females can sometimes be moved into new habitations. These new nests can take advantage of nesting substrates including soft woods (e.g. sugar pine or balsa wood) or even dense styrofoam plastics.

Table 2. Advantages and disadvantages of using Carpenter Bees (*Xylocopa* spp.) as Managed Pollinators of Tomatoes.

Advantages	**Disadvantages**
1. Long-lived individuals and nest associations of females.	1. Must be wild-trapped
2. Polylectic pollen usage	2. Nectar sources must be provided in the form of flowers or feeders.
3. Can buzz pollinate	3. Mating possible in captivity?
4. Will nest in artificial substrates	4. Low population numbers per nest
5. Can forage and live under greenhouse conditions	

Buzz Pollination in Angiosperms

Certain plants have anthers that dehisce and shed their pollen through small to minute apical pores. This restricts access to the nitrogen and protein-rich pollen grains by bees. Only certain bees have the necessary configuration of indirect flight muscles and physiology, and can learn how to exploit them, enabling them to harvest pollen from flowering plants with poricidal anthers, using buzz pollination (floral sonication).

Approximately 72 plant families, 544 genera and almost 20,000 species of monocots and dicots have porose anther dehiscence along with small light/dry pollen grains, therefore necessitating buzz pollination by bees. Among crops plants, blueberry, cranberry, chile peppers, eggplant, kiwi fruit and tomato have this form of pollen release and must be vibrated by bees for optimum fruit set (Buchmann, 1983).

Certain bees, especially the genera *Bombus, Melipona, Centris, Anthophora, Amegilla, Xylocopa, Colletes, Ptiloglossa, Caupolicana, Agapostemon* and many other halictid have the appropriate indirect flight musculature, physiology and learned floral behaviors to work flowers with pored anthers. Notable exceptions, bees that cannot buzz pollinate, include *Apis* and *Trigona* and almost all megachilids and andrenids are incapable of sonicating flora anthers to release pollen.

The fact that both *Centris* and *Xylocopa* can use floral sonication for pollen harvesting, makes these taxa especially valuable for pollinating crops requiring this specialized intrafloral behavior. Whether *Centris* can be manages as an indoor pollinator is not known. A comprehensive biophysical investigation of floral sonication by *Bombus* and *Xylocopa* was recently conducted by researches in New Zealand and Arizona (King and Buchmann, 2003).

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ON PROMOTING SOLITARY BEE SPECIES FOR USE AS CROP POLLINATORS IN GREENHOUSES

Katja Hogendoorn

Abstract

The use of locally abundant bees as crop pollinators should be preferred over the introduction of alien species. An impediment to the use of native solitary species is the fact that husbandry practices for breeding the bees are generally unknown. In this paper, species from two geographically widespread taxa, Xylocopini and Anthophorini, are compared for their usefulness as crop pollinators in greenhouses. It is argued that once husbandry for both these groups is in place, locally abundant species can be promoted for use as a crop pollinator, both inside glasshouses and in the open. The Xylocopini have long-lived females that nest in wood. They may have relatively long periods of inactivity, and their mating system may require special provisions in the greenhouse. The representative of the Anthophorini that is currently under study for use in greenhouses in Australia, *Amegilla*, nests in the ground or in mud cliffs. Females are short-lived and there is no overlap of generations. Females therefore continue to reproduce throughout their adult lives without any periods of inactivity. Mating readily occurs in greenhouses. Both species are highly amenable for use in greenhouses, once husbandry practices are in place, and specific difficulties are overcome.

Introduction

Local bees for local jobs

When selecting species to develop for use as agricultural pollinators whether for pollination in greenhouses or in the open, species that are locally abundant should have preference over the introduction of exotic species. The advantages of locally abundant species are that their inadvertent escape from agricultural settings into the wild (e.g. Ruz, 2002; Goulson, 2003) will cause minimal disruption of the natural ecosystems. In contrast, introductions of exotic bees are very likely to disturb the ecosystem through long-term effects on plant composition. These effects can be direct and indirect. Indirect effects can occur through interference with native pollinators, which can affect the seed production in native plant species. For example, presence of honeybees cause reduced seed set in *Melastoma affine* (Gross and Mackay, 1998) and poor seed set in *Mimosa pudica* coincides with relative high frequency of honeybees in French Guyana (Roubik, 1996). Direct effects are to be expected because introduced species can be pollinators of non-native plant species, and therefore a vector for the propagation of weeds, such as honey bee pollinated gorse in New Zealand (MacFarlane *et al.*, 1992) and in New South Wales (Gross pers. com.), or bumble bee pollinated *Lupinus arboreus* in New Zealand and Tasmania (Stout *et al.*, 2002). Evidence of such impacts of introduced bees on native ecosystems has only just been recognised (Goulson, 2003).

Promotion of locally abundant bee species as pollinators of agricultural crops is a more sustainable option: it supports ecosystem biodiversity by promoting local ecologically important plant and bee species and by impeding the progress of the 'global ecology' (Low, 1999).

Solitary bees as crop pollinators

Social species are generally preferred over solitary species as crop pollinators, partly because husbandry practises are already in place, and partly because a relatively high density of pollinators can be maintained in a limited area. However, for some crops solitary bees may deliver better pollination services than social species, they may be better adapted to the local climate, or a native social alternative may not be available (Cane, 1997). For example, honey bees and stingless bees are close to useless in pollination of alfalfa and passionfruit, several bumblebee species function badly at high ambient temperatures and in Australia no native social buzz pollinators occur. Solitary bees that are successfully managed at high densities as pollinators of several outside crops, including *Osmia species* for orchard apples, plums and almonds, *Megachile rotundata* and *Nomia melanderi* for alfalfa (e.g. Torchio, 1985, 1990, 1991; Batra, 1994, 1995; Cane, 1997), but to date the use of solitary bees inside greenhouses has been limited, and apart from *N. melanderi*, ground-nesting bees have been neglected altogether (Cane, 1997).

Several representatives of two groups of solitary bees are very amenable for use as pollinators of tomato in greenhouses: Carpenter bees of the genus *Xylocopa* and Anthophorid bees. These groups contain large buzz pollinating species, which can be locally abundant and have a widespread distribution. Both *Xylocopa* and Anthophorid bees can be found in all Mediterranean, tropical and subtropical areas around the world.

This paper focuses on the potentials for the use of *Xylocopa* and *Amegilla* as tomato pollinators in greenhouses. For each of these genera, I outline the suitability for pollination of tomato, some intrinsic advantages and disadvantages of the two groups, and possible ways to overcome challenges involved in propagating the species and keeping them active in greenhouses.

***Xylocopa* and *Amegilla* as greenhouse pollinators**

Both *Xylocopa* and *Amegilla* have been successfully reared in a greenhouse environment (Hogendoorn *et al*., 2000; Bell pers. com; Hogendoorn pers. obs.). Both species will easily learn to visit sugar water feeders, which is necessary, as tomato flowers do not provide any carbohydrates. In addition, there is no doubt that both *Xylocopa* and *Amegilla* are excellent tomato pollinators. For *Xylocopa* (subgenus *Lestis*) an increase in tomato weight by 10% was found relative to a combination of wind and insect pollination (Figure 1; Hogendoorn *et al*., 2000), which is comparable to what bumblebees achieve. For *Amegilla*, pollination performances have not yet been published, but the information will soon become available (Bell pers. com.; Hogendoorn, in progress).

Bees can only be used in a greenhouse if a breeding protocol is in place. There are several pronounced differences in the biology of *Xylocopa* and *Amegilla*, which is the main reason why the two taxa present us with different types of challenges when it comes to development of a breeding program and use of bees in the greenhouse. Below, I will outline some of the more and less beneficial traits of the two genera.

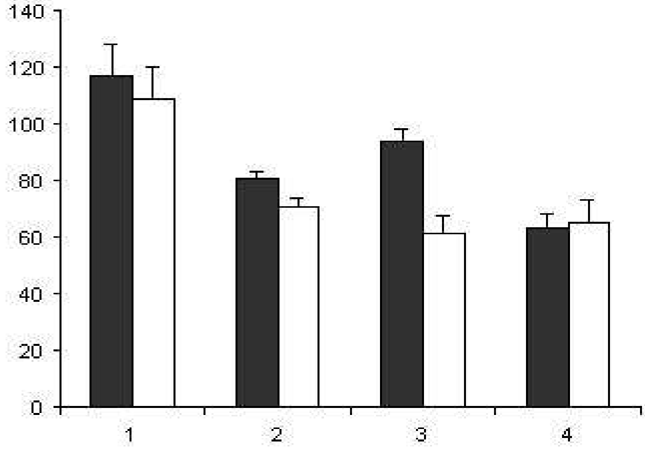

Figure 1. Average weight (± s.e.) of tomatoes pollinated by *Xylocopa* (*Lestis*) in a flight cage (black bars) and of wind pollinated tomatoes outside the flight cage (white bars). **1**: Initial control. The weight of tomatoes of the two groups of plants did not differ before the onset of the experiment; **2**: First experimental stage. Tomatoes reared through in flight cage (black bars) are significantly heavier than those reared through outside (white bars); **3**: This difference remains significant when the tomatoes of the two groups of plants are reared together in a greenhouse; **4**: Final control. The weight of tomatoes of the two groups of plants did not differ after the experiment was completed. Modified from Hogendoorn *et al.* 2000.

Xylocopa

This genus contains about 300 species and representatives can be found on all continents (Hurd and Moure, 1963; Michener, 2000). Strictly speaking carpenter bees are not completely solitary, because social nests of variable composition frequently occur (e.g. Sakagami and Laroca, 1971; Camillo and Garófalo, 1982, 1989; Gerling *et al.*, 1989). However, the number of females inhabiting nest is always low, and frequently only one active forager is present at a time (e.g. Gerling *et al.*, 1989; Hogendoorn and Velthuis, 1993). Thus, in the context of crop pollination, these bees can be classified as solitary.

Carpenter bees nest mostly in dead wood or branches. Some species make linear nests, while others make branched nests provided the substrate allows them to do so. The possibility to lure these bees into suitable artificial nesting material allows provision of nesting material that can be easily used in agricultural settings and moved to places where pollination services are needed (e.g. Gerling *et al.*, 1983; Freitas and Oliveira Filho, 2001; Oliveira Filho and Freitas, 2003). One advantage of this group is that hibernation occurs as adults, and females will start foraging whenever temperatures reach high enough values. This means that it is relatively easy to manipulate the onset of foraging in the greenhouse. Another important advantage is that the genus has a worldwide distribution. This implies that species of *Xylocopa* have the potential to be used globally in Mediterranean, subtropical and tropical areas once husbandry protocols are in place and local adaptations of these protocols have been developed. In addition, most species are polylectic and can therefore be applied to work on a variety of crops.

Most difficulties encountered when breeding local carpenter bee species for pollination purposes can be solved, but some require research and experimentation. Some of these problems are intrinsic to the species' behaviour and life history, while others are a consequence of the need to propagate bees at high densities and in artificial circumstances. In the following, I discuss some of the problems, and suggest how we may be able to counteract them

(a) Reduced reliability of pollination services due to periods of adult inactivity

Carpenter bee females are long-lived, and are sometimes not very productive or may be inactive for extended periods of time (Camillo and Garófalo, 1982; Camillo *et al*., 1986). There are two main reasons for such periods of inactivity:

Firstly, the circumstances may not favour brood production either because of low temperatures or temporary lack of pollen and nectar supplies. The importance of these factors in shaping activity is bound to vary locally, and so do the measures to prevent inactivity. When carpenter bees are used as pollinators outside, additional food plants can be propagated around the crops, in particular for periods when the crop is not in flower. Sugar water can be provided in feeders. In greenhouses, bees may need to be active during winter, which could require heating.

Secondly, females may go through periods of inactivity after production of a number of brood cells. This is particularly the case for species that make linear nests. The reason for this period of inactivity in linear nests is that the last made cell needs to contain a pupae when the young adult from the first made cell ecloses, otherwise the brood is damaged while the young adult digs its way out. Species that nest in linear nests seem to be aware of this limitation, and cease reproduction within a set number of days after closure of the first cell (Velthuis, 1987). Introducing asynchronicity between nests by replacing the nest by empty substrate 10 days after initiation of reproductive activity may overcome this problem. The brood in the nests that are removed can then be reared through in absence of the mother, and re-introduced in the greenhouse.

However, periods of inactivity are not unique to species nesting in linear nests. Species using branched nests with several tunnels may also be inactive for lengthy periods of time, even when ample pollen and nectar may be available. Research in Israel has shown that during periods of intense competition for nesting substrate, solitary females may produce only a limited number of cells, and then wait until their brood ecloses (Hogendoorn and Velthuis, 1993). This does not only reduce the probability of a take-over of the nest by an intruder, it also reduces the risk of losing pollen to a conspecific pollen-robbing female. Pollen robbing occurred in situations of high competition for nesting substrate, when solitary females minimise the time away from their nest by robbing from their neighbours rather than collecting pollen from flowers. Thus, ample nesting substrate should be supplied, to prevent periods of inactivity and promote nest founding by newly eclosed females.

(b) Parasites and predators are likely to cause problems during mass breeding

Carpenter bees suffer from a range of parasites and predators (Watmough ,1983; Gerling *et al*.,1989). Many species carry phoretic mites, some of which are beneficial (e.g. Okabe and Makino, 2002), while others can be detrimental (e.g. Krombein, 1962; Watmough, 1974) and can greatly reduce health and productivity in greenhouses (Steen PhD thesis). In addition, brood parasites of carpenter bees can cause major brood loss. The most important parasites include meloid beetles (Watmough, 1983) and encyrtid wasps (Gerling *et al*., 1989; Hogendoorn and Velthuis, 1993). When used in the greenhouse, methods should be developed to keep track of such infestations and to remove infested brood and parasites. Ants are among the most important brood predators of *Xylocopa* (Gerling *et al*., 1983; Freitas and Oliveira Filho, 2001). In the greenhouse environment, this is easily prevented by protecting the nests using tangle foot.

(c) Mating territory requirements may hard to fulfil in a greenhouse setting.

Carpenter bees display a variety of mating strategies (e.g. Eickwort and Ginsberg, 1980; Gerling *et al*.,1989). Males of some species defend territories at spots that contain resources for females, *i.e.* flowers or nesting sites. However, males of other species maintain non-resource territories or dispersed leks, which have as yet unknown qualities but are frequently high up in trees or at hilltops. It may be hard to achieve the requirements needed for such leks in greenhouses (pers. obs.). Thus, if choice is available, it may be easiest to choose species that have a resource defence system and will mate in the greenhouse.

Amegilla

The genus Amegilla has 253 species and a distribution throughout the Mediterranean basin, Africa, Asia, and Australia (Brooks, 1988; Eardley, 1994; Michener, 2000). Recent measures to protect the Australian mainland from introduction of bumblebees have spiked an interest in the development of Australian native buzz pollinators. Because carpenter bees have become rare in southern Australia and are extinct in Victoria and the mainland of South Australia (Leys, 2000), the focus has shifted to the blue-banded bees in the genus *Amegilla* (Anthophoridae), subgenera *Zonamegilla* and *Notomegilla*. Recent research has shown that these bees have a strong preference for buzz pollinated flowers, and are adequate pollinators of tomatoes (pers. obs.). An important advantage of this genus is that these bees are among the most common solitary bees in Australia, so there is abundant breeding stock at most localities.

Blue-banded bees nests in the ground or in mud cliffs. Because their nests are very shallow, nesting substrate can be relatively easily provided. In contrast to carpenter bees, they have a short and active lifespan of about four – five weeks, and will breed rapidly and prolific throughout their lives. They mate and reproduce readily in the greenhouse. Brood development takes approximately six weeks, and several generations per year are possible.

Current research in collaboration with tomato growers focuses on:

a) Breaking the winter diapause

Because tomatoes are grown throughout the year in Australia, breaking the hibernation period is an important aspect of research. The brood hibernates in the cells as prepupae, and current research using both light and heat is underway to investigate how the onset of winter diapause can be prevented.

b) Development of a nest-attractant

For a successful propagation program, it is necessary to regulate the choice of nest substrate. The bees readily use mud brick to nest in, but prefer to nest in aggregations. Cardale (1968a) has argued that this might be the result of an odour emanating from old casts of cells, as these are attractive to females searching for nests. We are currently performing chemical analysis of the substances involved. If such substances are indeed acting as attractant, they then can be used both to lure females into artificial substrate in the field, and to regulate substrate choice in the greenhouse.

c) Developing practical nest substrate that can be transported and protected

Nest substrate must be developed in such a way that it is easy to relocate, that it can be protected and closed off when growers spray, and that it allows inspection of brood cells for infections. The preferred design for the substrate is investigated.

d) A propagation protocol

Most solitary bees used as pollinators in the open are reared on site, ie where they provide their pollination services. Rearing brood on site will also be the preferred method for *Amegilla,* as it is difficult to provide pollen artificially. Depending on the benefits and costs, the brood can then be harvested, checked for parasites and artificially reared through, as is frequently done for hibernating brood of *Megachile rotundata* (Richards, 1984), or it can be left in place.

e) Amegilla has relatively few parasites

No mites have been found on *Amegilla,* which is fortunate. However, *Miltogramma* (Sarcophagidae) may cause problems, a cuckoo bee (*Thyreus*) may parasite cells (Cardale, 1968b), and some beetles have been found developing in cells (Hogendoorn pers. obs.). Most importantly, there may be severe chalkbrood infestations by an as yet undescribed *Ascosphaera* species (Bell, pers.com) that is specific to the genus. Infestations by the larger parasites can be regulated in a greenhouse. To prevent chalkbrood infestations, effectivity of fungicides in either the sugar water or the nesting substrate is being investigated.

Conclusion

Breeding solitary bees for use as pollinators can be successful provided detailed knowledge of the various aspects of the life cycle and general biology are available. Both *Xylocopa* and *Amegilla* contain several species that are promising candidates for buzz pollination in greenhouses and in the open. The species that should be preferred depends on local abundance and on the nature of the pollination services required.

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SESSION VI

WILD PLANT POLLINATION SYSTEMS INVOLVING SOLITARY BEES

RICOCHET POLLINATION IN CASSIAS – AND HOW BEES EXPLAIN ENANTIOSTYLY

- PRELIMINARY COMMUNICATION -

Christian Westerkamp

Abstract

The pollen-only flowers of some cassias have a strange morphology. The anthers are below the visiting bee, while the stigma touches her back – where also pollen is carried. It arrives there by an astonishingly circuitous route. The pollen jet buzzed from the anther is ricocheted several times before arriving on the bee's rear side. Pollen for bee-collecting is provided at reach of their legs, so there is a perfect division of labour between pollen for pollination and that for bee attraction. As the pollen-foraging bee usually lands atop the reproductive parts, the style that originates from the same area as the stamens has to surround the bee laterally to reach the pollen on her back. To avoid lateral adaptation, the lateral position has to alternate between flowers. Thus, pollen foraging is responsible also for enantiostyly.

Introduction

Pollen-only flowers face a serious problem with their pollen-collecting bee pollinators: both struggle for the very same few pollen grains. The flowers have to ensure at least part of them for pollination (Westerkamp, 1997, a, b). This pollen must be placed out of the immediate reach of the bee – but accessible to the stigma of the next flower.

One means to separate pollen functions between pollination and bee-use observed in many specialized pollen-only flowers is a division of labour between different stamens (Müller, 1881, 1882/3; Müller and Müller, 1883; Vogel, 1978).

In the Cassiinae (Caesalpiniaceae = Leguminosae-Caesalpinioideae), especially in the genus *Senna*, there is an enormous diversity in the androecium: up to five functionally different sets of stamens exist within the same flower; those that still produce pollen are poricidal, and thus depend on buzz-foraging bees (Buchmann, 1983).

As pollination is a chancy affair, preconditions for successful pollen transfer are a standardized position of the visitor and a regular contact of pollen and stigma with the very same spot of the pollinator. In *Senna*, however, this rule is violated in several ways: the gynoecium (1) has about double the length of the pollinating stamens, (2) touches the bee on the back while the stamens are on the ventral side, and (3) is deflected to the left while the anther is on the right (or vice versa) – alternating in a regular pendulum-like manner within the inflorescence, a behaviour typical for enantiostyly.

How do these flowers circumvent these violations in order to ensure pollination, and how does this behaviour explain the existence of enantiostyly?

Material and methods

Field observations were carried out on the Campus Umuarama of the Universidade Federal de Uberlândia, Minas Gerais, Brazil (48º17' W, 18º55' S), where the species studied grew as ornamentals. Additional observations were gained on related (incl. native) species and in other localities in Uberlândia, especially in the Cerrado reserve of the Clube Caça e Pesca Itororó de Uberlândia.

In the lab, flowers and insects were studied using a dissecting microscope Wild M5 with camera lucida for documentation. To imitate bee vibrations in the lab, I used a tuning fork (current standard pitch a'=440 Hz) modified with one prong lengthened with an entomological needle in order to locate vibrations more specifically.

Macro-photos were taken with Nikon F90X, Sigma 105 mm macro-lens and Nikon ring-flash (SB-29). Movements of at least some of the visitors were video-taped (Sony CCD-TRV 58).

Results and discussion

Ricochet

In the stamens of the Cassiinae we observe a great diversity of forms and functions – production of pollen for pollination, production of pollen for bee foraging, offering of a handle for gripping, offering of optical signals for bee orientation, and reduced forms that no longer have any function. The anthers that still produce pollen are equipped with ejecting tubes with an extremely smooth inner surface resulting in a directed pollen jet instead of an irregular pollen cloud. Corresponding to the conditions described by Buchmann and Hurley (1978), these narrow tubes let only those grains pass that are accelerated in their direction. The anthers offering pollen for bee collection, deposit their load on the lower side of the bee, either in the midline of the thorax or directly on the pollen-harvesting feet. The anthers destined at pollination eject their pollen jet more or less parallel to the bee's lower surface, evading initial contact. After leaving the anther pore, the pollen jet follows the same laws as a light beam. When hitting a surface, the pollen stream is ricocheted respecting the laws of reflection (e.g., angle of incidence equals angle of reflection) – with an important difference from light hitting a mirror: as the initial energy of the pollen beam is not sufficient for a simple rebound, new energy has to be provided by the reflecting surface. This energy has the same source as that powering the original pollen jet: the vibrating thorax of the pollen-foraging bee. It is transmitted – by the thorax itself or by other parts of the bee – to almost all parts of the flower. Thus, the flower is vibrated in its entirety, not only the anthers.

The pollen jet ejected from the anther tube hits a first petal and is ricocheted from its vibrating surface. After that, it meets the same or another petal - depending on flower species and its construction and is ricocheted again before arriving on the upper surface of the buzzing bee. At least two ricochets are needed to surround the bee without meeting other parts of their body than that contacted by the stigma in the reciprocal flower. The flower offers a narrow space around the visiting bee where the pollen can be ricocheted and where it can surround the bee until finally arriving at its destination.

As other organs than the anther are involved in pollen presentation – although admittedly only in passing by – this is a special type of secondary pollen presentation (Westerkamp, 1989; Yeo, 1993) that we might call virtual secondary pollen presentation.

In the past, there already had been hints for pollen ricocheting in Cassiinae that, however, remained unperceived as an indication of a new pollination mechanism: Todd (1882) described in *Chamaecrista fasciculata*, that "some [pollen] grains are dropped on the incurved petal, and by it made to adhere to parts of the bee." Delgado and Souza (1977) gave further details when depicting the ricocheting of pollen in *Senna multijuga* (as *Cassia doylei*) as "efecto de aspersión por rebote." Gottsberger and colleagues (Gottsberger *et al.*, 1988, Gottsberger and Silberbauer-Gottsberger, 1988) finally described the guiding of the pollen jet by a tubular petal in *Chamaecrista hispidula*.

Division of labour in the androecium has a much more important meaning than simply characterizing unlike stamens with different roles of their pollen: the dusting of different parts of the bee's body. Pollen destined at bee collecting is offered within reach of the collecting organs (usually forelegs, Westerkamp, 1987, 1996) on the lower side of the bee; pollen for pollination. on the other hand, dusts the bee on her back (a tendency already mentioned by Pijl, 1954), usually unregistered by the foraging bee. Above the insertions of the legs, pollen is only reached with difficulty. It is safe here during the actual flower visit (because of the flower parts touching the bee here) as well as during flight (because of the impeding wings). Moreover, it is outside the field of perception of the bee: the bee does not see nor feel the pollen sticking to her back. Even after accumulating a large amount of pollen on her back, it does not seem to affect her. In *Cassia fistula*, for example, *Xylocopa* sp. (Apidae) did not remove nor even touch the pollen spot on her back for a long time during at least one foraging bout – so distracted she was by the pollen offered in front of her and in reach of her legs. We thus observe a perfect separation of the different roles of pollen in these flowers. While the bees are busily and actively collecting pollen with their legs, they are distracted from the (usually much larger) pollen dose they are receiving on their back, where it usually remains unperceived for a long time – a perfect adaptation for securing pollen for pollination!

What is strange at first view is the fact that it is the uppermost group of anthers that provides pollen to the collecting legs at the lower side of the bee while the lowermost anther(s) make pollen adhere to the upper side of the bee. But as pollen collecting bees usually land atop of the anthers they are going to exploit, anthers for pollination can only be located (hidden!) at a lower level.

Enantiostyly

Enantiostyly, mirror-image flowers in which the style bends either to the left or to the right side of the floral median, is observed in several families of mono- and dicotyledons (see Jesson and Barrett, 2003 for an overview). In the majority of the families, right- and left-styled flowers are observed on the same plant (monomorphic enantiostyly), the separation of left-styled from right-styled plants (dimorphic enantiostyly) is a rare phenomenon, restricted to three closely related monocot families (Jesson and Barrett , 2003). There exist two mutually exclusive functional groups of enantiostylous flowers that might have had a different origin, blossoms using nectar as attractant vs. pollen-only flowers. In both sets, there are species with an exclusive reciprocity of stigma and pollen as well as those that present pollen on either side, *i.e.*, on the side of the gynoecium as well as on the other side.

Several reasons already have been cited for the existence of enantiostyly, e.g., a contrivance for cross-fertilization (Todd, 1882), outcrossing (Irwin and Barneby, 1976), a reduction of geitonogamous self pollination (Barrett, 2002a), or the protection of female parts from injuries originating from insect vibrations (Dulberger, 1981), but even the most intense students of enantiostyly had to admit that the functional significance is still poorly understood (Jesson and Barrett, 2002).

All of the afore-mentioned reasons are simply refuted, at least in Cassiinae. First, plants are rich in flowers open on a given day, including right-styled and left-styled flowers; there are even species (e.g., *Senna alata*) that dust both sides of the bee while the stigma contacts only one. Pollen foraging bees, on the other hand, remain faithful to the plant worked at the moment. So, an improved outcrossing cannot have been the reason for the evolution of enantiostyly. Second, if buzz foraging were destructive to the flowers, flowers adapted to it would not have evolved to such diversity and perfection as exists today. Buzz flowers destructed by their pollinators have never been reported. So, protecting the flowers from injuries also has not been the reason for the evolution of enantiostyly.

Thus, the cause for the evolution of enantiostyly has to be sought elsewhere. In the Cassiinae, at least, it is found in the division of labour described above. With the bee perching on top of the joint complex of reproductive organs (androecium and gynoecium) and pollen aimed at pollination located in a safe place on the bee's back, the stigma has to follow to this new position. In the majority of plants, there is a single pistil with a single style; in legumes with their sole carpel, this is even mandatory. In a median position, the style would obstruct the flight path of the bee; it also would have to be extremely long when attempting to reach around the bee's abdomen. Both facts would require a great investment in stabilizing elements, which would render such a style extremely expensive.

To remove the style to a lateral position would not only save costs for the plant, but also promote protection of the gynoecium against bee activity. Collocating the style always on the same side in a widely open flower with anther(s) on one and the style on the other side, however, would open a way for specialized bees to adopt a position that results in a greater pollen removal but is unfavourable for pollination. Totally asymmetrical flowers thus are extremely rare and restricted to groups that are able to force the visitor into a unique standardized fixed position adequate for pollination; this is observed, for example, in certain highly specialized fabaceous flowers as in the genera *Lathyrus* (Westerkamp, 1993) and *Vigna* (pers. obs.). Another solution often encountered in asymmetric plant organs is the so-called "pendulum symmetry" (Goebel, 1928) or "pendulum asymmetry" (Barrett, 2002b). It is regularly observed in monomorphically enantiostylous plants; within inflorescences the right- and left-handed flowers are regularly alternating: flowers on the left have their style to the right, and flowers on the right have their style to the left.

Conclusions

Again, it became obvious, that an understanding of floral morphology and functioning requires a thorough knowledge of bee morphology and behaviour. Once more, floral morphology is explained as an adaptation against bees. Like keel blossoms (Westerkamp, 1997a), buzz-flowers of the Cassiinae are an adaptation against pollen foraging bees – that at the same time are the sole pollinators. With division of labour and the detour of pollen for pollination towards the back of the bee, the style has to reach there, too. As the median option is too expensive and would obstruct access to the flower, the singular style can only approach the rear side of the bee laterally. Right- and left-styled flowers have to alternate regularly, as they are wide open and thus have no means to force the bee into a standardized position adequate for pollination. Without this pendulum-like alternation, the pollen-foraging bee could specialize on the pollen side of the flower – and thus annihilate the “intentions” of the flower. Thus, adaptation against bees also gives an explanation for enantiostyly, that was an enigma in an adaptationist perspective until recently.

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ARE OLIGOLECTIC BEES ALWAYS THE MOST EFFECTIVE POLLINATORS?

Clemens Schlindwein

Introduction

Bee-plant community studies always reveal a big difference in the number of relationships which the surveyed bee species show to the plant species of the community. While highly eusocial bees seem to not discriminate among melittophilous species - in Brazilian studies worker bees of *Trigona spinipes* and *Apis mellifera* visit flowers of up to more than 50 % of the local melittophilous flora - other bee species show relationships restricted to a few or locally only one plant species (see for example Aguiar *et al.,* 1995; Schlindwein, 1998; Wilms, 1995; Alves-dos-Santos, 1999; Aguiar, 2003; Moura, 2003). It is to be expected that these oligolectic species, which feed their larvae with pollen of only a few plant species of the same genus or family, show morphological and behavioral adaptations to collect pollen from these flowers more efficiently than polylectic species. Then, the oligolectic species would have an advantage over their generalist competitors. When both partners, oligolectic bee and plant, show adaptations and benefit from the close relationship, the interaction may be the result of co-evolution. Effective pollen transfer to stigmas is directly related to the reproductive success of the plant. Therefore, selection may have favored relationships with those pollen collecting bees that are effective pollinators. Are oligolectic bees, in fact, effective pollinators of their specific food plants and better pollinators than polylectic bees? Here is given an overview of the oligolectic bee species of Brazil and information on their effectiveness as pollinators.

Oligolectic bees in Brazil

Information on oligolecty in the Brazilian bee fauna is still riddled with gaps. In most cases it is deduced from bee-plant community studies and detailed information on the relationship between the partners and especially on pollinator effectiveness of the oligolectic species is limited to a few case studies.

Oligolectic bees occur in 12 tribes: especially Andrenidae-Panurginae, non-corbiculate Apidae, and Colletidae-Paracolletini (Table 1). All oligolectic species are solitary. Oligolecty is most common in Onagraceae (*Ludwigia*: 10 bee species), Cactaceae (8 bee spp.), Malvaceae (8), Pontederiaceae (*Eichhornia, Pontederia:* 7), Asteraceae (7), Convolvulaceae (*Ipomoea:* 5), Apiaceae (*Eryngium:* 3), Iridaceae (*Sisyrinchium:* 3), Loasaceae (*Cajophora-Blumenbachia:* 3), Oxalidaceae (*Oxalis:* 3) and Solanaceae (*Petunia-Calibrachoa:* 3). Monocotyledons are only represented by Pontederiaceae and Iridaceae.

The plant species related to oligolectic bees are nearly exclusively herbs and shrubs. The only exceptions are *Jacaranda* and *Tabebuia* trees: their flowers are visited by males and females of the oligolectic monotypic *Niltonia virgilii* (Colletidae, Paracolletini) besides numerous medium- to large-sized polylectic species (Laroca and Almeida, 1985).

Numerous species of food plants of the oligolectic bees are common at ruderal sites in Brazil like *Sida, Ludwigia, Ipomoea, Eryngium, Sisyrinchium, Oxalis, Petunia, Turnera* and Asteraceae species. An adequate habitat management, therefore, may increase the local bee diversity.

Distribution of oligolectic bees in Brazil

The surveys of bee-plant communities in Brazil indicate that the number of oligolectic species increases from Northern to Southern Brazil. In various regions with a short favorable season, oligolectic bees are species-rich (Michener, 1979; Feinsinger, 1983; Westrich, 1989). Partitioning of the abundant pollen resources among specialized bees, which are able to locate and explore the floral resources more efficiently than polylectic species, may be a reason for the high diversity of oligolectic species in these regions. However, this is not the case for the highly seasonal *caatinga*, a succulent, thorn-shrub savannah with deciduous leaves occurring in NE-Brazil and the Central Brazilian *cerrado*, a tree-grass savannah with evergreen sclerophyllous leaves (see compilation of bee species in Silveira and Campos, 1995; Zanella, 2000; Zanella and Martins, 2003). Tropical rainforests seem to house only a few species of oligolectic bees. However, there have been only a few studies and information on the bee fauna, especially in the canopy, is scarce. In these habitats, bees of the genera *Centris* and *Euglossa* (Apidae) are very common. They are not oligolectic but show preferences to collect pollen from poricidal anthers (Buchmann, 1983) and nectar from flowers with long corolla tubes, as well as visit oil flowers, perfume flowers and resin flowers. These bees are highly effective pollinators of numerous plant species and may have a key role as pollinators in neotropical ecosystems (Schlindwein, 2000). In addition, stingless bees are very abundant in these tropical ecosystems. This could be the reason for the small number of oligolectic species. In Southern Brazil, the apifauna shows a strong influence of temperate elements: Paracolletini and Panurginae, which are mainly oligolectic, are common in Southern South America and various species show their northern distributional limits in Southern Brazil (Schlindwein, 1998; Alves-dos-Santos, 1999).

Oligolectic bees as pollinators

With the exception of *Sisyrinchium*, which in addition to pollen offers floral oil to the females of *Lanthanomelissa*, all plants that attract oligolectic species are also nectar resources (Table 1). This might prevent the pollen-collecting females from looking for nectar in other flowers, thereby enhancing their flower constancy and, furthermore, also attracting the males to visit these flowers. In almost all cases the male bees patrol the flowers searching for females (Table 1). Alves-dos-Santos (2002) stresses the contribution of patrolling males of oligolectic bees to cross pollination: the males are highly flower-constant, visit often just a few flowers on an individual plant, carry pollen in small quantities, generally touch the stigmas during their legitimate visits, show higher frequencies of flower visits than females, and often perform large transects during their patrolling trips.

In several species, however, *e.g.* in the Emphorini *Ptilothrix fructifera, P. plumata, Ancyloscelis apiformis, A. fiebrigi, A. gigas, Melitoma segmentaria,* and *Melitomella grisescens*, copulation in the flowers is extremely rare in spite of the abundant presence of females. Perhaps females of these species copulate only during a short period after hatching. Alternatively, males of these species may look for females at their nesting sites.

Numerous plant species visited by oligolectic bees have extraordinarily large pollen grains, *e.g.* those of *Ludwigia, Pavonia, Sida, Opuntia* and *Ipomoea* which require a specialized scopa of long, unbranched bristles (Thorp, 1979; Gimenes, 1991) and adapted pollen handling. In the flowers of two *Opuntia* species in S-Brazil, for instance, females of most of the numerous polylectic species collected exclusively nectar, while the oligolectic bees *Ptilothrix fructifera* and *Lithurgus rufiventris* collected most of the produced *Opuntia* pollen and were the most effective pollinators (Schlindwein and Wittmann, 1997a).

Other morphological adaptations are found in females of *Ancyloscelis gigas, A. ursinus* and *A. turmalis* which show hooked hairs on their proboscis, appropriate to collect hidden pollen from short-level anthers in the long flower tubes of tristylous *Eichhornia* and *Pontederia* spp. (Alves-dos-Santos and Wittmann, 1999, 2000; Alves-dos-Santos, 2003). These oligolectic bees are the most effective pollinators of these flowers, especially for the short-styled morphs. Moreover, with their extraordinarily long proboscides these bees reach the nectar in the long flower tubes. Very long mouthparts occur also in the “short-tongued” *Niltonia* bees who show unusual prolonged labial palpi involved in nectar uptake from the base of the nectar chamber of *Jacaranda* and *Tabebuia* flowers. Prolonged labial palpi are also found in the related *Hexantheda missionica*, *H. eneomera* and *Albinapis gracilis* which also are oligolectic on flowers with nectar chambers (*Petunia* spp.*, Calibrachoa* spp.) or a deep, funnel-shaped corolla (*Oxalis* spp.), respectively.

A highly specialized foraging strategy characterizes the oligolectic females of *Bicolletes pampeana* in S-Brazil. They establish micro-foraging routes among annual herbs of *Blumenbachia* and *Cajophora* (Loasaceae) to collect pollen liberated in small portions after stimulation of unique nectar scales during the previous flower visit (Schlindwein and Wittmann 1997b; Schlindwein, 2000b). *Bicolletes pampeana* is the most effective pollinator of these Loasaceae species and competes only with females from a second species of the genus (B. franki). Polylectic competitors do not occur.

The pollination studies performed in Brazil that involve oligolectic species show that these bees are always effective pollinators. Several case studies carried out in species of *Opuntia, Parodia, Gymnocalycium, Pavonia, Eichhornia, Pontederia, Ipomoea, Merremia, Ludwigia* and *Petunia* (see references in Table 1) point out that oligoleges are the most effective pollinators. This corroborates the experiences of Neff and Simpson (1992). Nevertheless, in some cases oligolectic bees are not better pollinators than polylectic bees (Feinsinger, 1983; Neff and Simpson, 1992; Michener, 2000). In Paraíba, NE-Brazil, the seed- and fruit-set of a population of distylic *Turnera subulata* remained high in the absence of its narrowly oligolectic pollinator *Protomeliturga turnerae.* The introduced *Apis mellifera* and other polylectic species were no worse pollinators than *P.turnerae* (Medeiros and Schlindwein, n. publ.). Oligolectic pollen-thieves like those visiting sphingophilous flowers of *Oenothera* (Onagraceae) (Gregory, 1963-64 *apud* Feinsinger, 1983), or females of *Perdita texana* (Andrenidae) which complete a pollen load in one flower visit (Barrows *et al.*, 1976; Neff and Danforth, 1992) are not yet reported among Brazilian oligolectic species.

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Table 1. Oligolectic species recorded in Brazil and their related food plants (Aguiar and Martins, 1994 (1); Alves-dos-Santos, 1999 (2) - *Actenosigynes fulvoniger* as *Leioproctus fulvoniger*, *Hexantheda eneomera* as *H. petuniae;* Alves-dos-Santos, 2002 (3); Alves-dos-Santos and Wittmann, 1999 (4); Alves-dos-Santos and Wittmann, 2000 (5); Ducke, 1912 (6); Gaglianone, 1999 (7); Gaglianone, 2000 (8); Gimenes, 1991 (9); Hurd and Linsley, 1966 (10); Laroca and Almeida, 1985 (11); Medeiros and Schlindwein, 2003 (12); Morato and Campos, 2000 (13); Pinheiro and Schlindwein, 1998 (14); Schlindwein, 1998 (15) - *Albinapis gracilis* as *"Pentantheda"* sp.; Schlindwein n. publ. (16); Schlindwein, 2000b (17); Schlindwein and Martins, 2000 (18); Schlindwein and Moure, 1998 (19); Schlindwein and Wittmann, 1995 (20); Schlindwein and Wittmann, 1997a (21); Schlindwein and Wittmann, 1997b (22); Silva-Pereira *et al.*, 2003 (23); Silveira *et al.*, 2002 (24); Stehmann and Semir, 2001(25); Vieira and Lima, 1997 (26); Wittmann *et al.*, 1990 (27); Wittmann and Schlindwein, 1995 (28); Zanella, 2000 (29).

Bee species	**Related plants**	**Nectar collection**	**Patrolling males**	**effective pollinator**	**Reference**
ANDRENIDAE					
PROTANDRENINI					
Anthrenoides meridionalis (Schrottky, 1906)	*Oxalis* (Oxalidaceae)	yes	yes	?	2,15
Cephalurgus anomalus Moure and Oliveira, 1962	*Gaya, Modiolastrum, Sida, Wissadula* (Malvaceae)	yes	yes	yes	7
Panurgillus flavitarsis Schlindwein and Moure, 1998	*Ludwigia* (Onagraceae)	yes	?	?	2, 19
Panurgillus formosus Schlindwein and Moure, 1998	*Eryngium* (Apiaceae)	yes	no	?	15,19
Panurgillus hamatus Schlindwein and Moure, 1998	*Acicarpha* (Calyceraceae)	yes	?	?	2,19
Panurgillus malvacearum Schlindwein and Moure, 1998	*Abutilon, Modiolastrum* (Malvaceae)	yes	no	?	15,19
Panurgillus minutus Schlindwein and Moure, 1998	*Eryngium* (Apiaceae)	yes	no	?	2,19
Panurgillus pereziae Schlindwein and Moure, 1998	*Pamphalea, Perezia* (Asteraceae)	yes	yes	?	15,19
Panurgillus plumosulus Schlindwein and Moure, 1998	*Oxalis* (Oxalidaceae)	yes	no	?	15,19
Panurgillus reticulatus Schlindwein and Moure, 1998	*Oxalis* (Oxalidaceae)	yes	no	?	2,15,19
Panurgillus vagabundus (Cockerell, 1918)	*Abutilon, Modiolastrum Sida* (Malvaceae)	yes	no	?	15,19
PROTOMELITURGINI					
Protomeliturga turnerae (Ducke, 1907)	*Turnera* (Turneraceae)	yes	yes	yes	6,12
CALLIOPSINI					
Arhysosage cactorum Moure, 1999	*Parodia, Gymnocalycium*, *Opuntia* (Cactaceae)	yes	yes	yes	15,20,21
Callonychium petuniae Cure and Wittmann 1990	*Petunia* (Solanaceae)	yes	yes	yes	2,15,27
APIDAE					
EMPHORINI					
Ancyloscelis apiformis (Fabricius, 1793)	*Ipomoea, Merremia* (Convolvulaceae)	yes	yes	yes	2,3,4,15
Ancyloscelis fiebrigi Brèthes, 1909	*Gymnocalycium, Opuntia, Parodia,* (Cactaceae)	yes	yes	yes	2,4,15,20
Ancyloscelis gigas Friese, 1904	*Eichhornia* (Pontederiaceae)	yes	yes	yes	2,3,4,5,15

Ancyloscelis turmalis Vachal, 1904	*Pontederia* (Pontederiaceae)	yes	yes	yes	2,4,15
Ancyloscelis ursinus Haliday, 1836	*Pontederia* (Pontederiaceae)	yes	yes	yes	2,4,15
Diadasina distincta (Holmberg, 1903)	*Ludwigia* (Onagraceae)	yes	?	?	2
Diadasina riparia (Ducke, 1908)	*Ludwigia* (Onagraceae)	yes	?	?	2,15
Melitoma ipomoearum (Ducke, 1913)	*Ipomoea* (Convolvulaceae)	yes	?	?	29
Melitoma segmentaria (Fabricius, 1804)	*Ipomoea* (Convolvulaceae)	yes	yes	yes	2,3,14,15
Melitomella grisescens (Ducke, 1907)	*Ipomoea* (Convolvulaceae)	yes	?	?	29
Melitomella murihirta (Cockerell, 1912)	*Ipomoea* (Convolvulaceae)	yes	?	?	29
Ptilothrix fructifera (Holmberg, 1903)	*Opuntia* (Cactaceae)	yes	yes	yes	15,17,21
Ptilothrix plumata Smith, 1853	*Pavonia* (Cactaceae)	yes	yes	yes	3,18
Ptilothrix relata (Holmberg, 1903)	*Ludwigia* (Cactaceae)	yes	?	?	2,15
EUCERINI					
Florilegus condignus (Cresson, 1878)	*Pontederia* (Pontederiaceae)	yes	?	?	15
Florilegus festivus (Smith, 1854)	*Pontederia* (Pontederiaceae)	yes	yes	yes	2,3
Florilegus fulvipes (Smith, 1854)	*Eichhornia*, *Pontederia* (Pontederiaceae)	yes	yes	yes	2,3,5,15
Florilegus riparius Ogloblin, 1955	*Pontederia* (Pontederiaceae)	yes	yes	yes	2,
Gaesischia fulgurans (Homberg, 1903)	*Vernonia* (Asteraceae)	yes	?	?	2
Gaesischia nigra Moure, 1948	*Vernonia* (Asteraceae)	yes	?	?	2
Gaesischia sparsa (Brèthes, 1910)	*Vernonia* (Asteraceae)	yes	?	?	2
Gaesischia trifasciata Urban, 1968	*Vernonia* (Asteraceae)	yes	?	?	2,15
Melissoptila bonaerensis (Holmberg, 1903)	*Abutilon, Modiola, Modiolastrum* *Wissadula* (Malvaceae)	yes	?	?	2,15
Melissoptila fiebrigi Brèthes, 1909	*Abutilon* (Onagraceae)	yes	?	?	2
Melissoptila paraguayensis (Brèthes, 1909)	*Ludwigia* (Onagraceae)	yes	?	?	2
Melissoptila thoracica Smith, 1854	*Sida* (Malvaceae)	yes	yes	yes	8,23
Melissoptila cnecomala (Moure, 1944)	*Sida, Malvastrum* (Malvaceae)	yes	no	yes	13
Peponapis fervens (Smith, 1879)	*Cucurbita* (Cucurbitaceae)	yes	?	yes	10
Santiago mourei Urban, 1989	*Vochysia* (Vochysiaceae)	?	?	?	24
TAPINOTASPIDINI					
Lanthanomelissa clementis Urban, 1995	*Sisyrinchium* (Iridaceae)	no	no	yes	15
Lanthanomelissa discrepans Holmberg, 1903	*Sisyrinchium* (Iridaceae)	no	no	yes	15
Lanthanomelissa pampicola Urban, 1995	*Sisyrinchium* (Iridaceae)	no	?	?	15
COLLETIDAE					
PARACOLLETINI					
Actenosigynes fulvoniger (Michener, 1989)	*Cajophora* (Loasaceae)	yes	yes	yes	2,17,28
Albinapis gracilis Urban and Graf, 2000	*Oxalis* (Oxalidaceae)	yes	yes	yes	15
Bicolletes pampeana Urban, 1995	*Blumenbachia, Cajophora* (Loasaceae)	yes	yes	yes	15,22,28
Bicolletes franki (Friese, 1908)	*Blumenbachia, Cajophora* (Loasaceae)	yes	yes	yes	15,22,28
Cephalocolletes isabelae Urban, 1995	*Opuntia* (Cactaceae)	yes	yes	?	2,17
Cephalocolletes rugata Urban, 1995	*Opuntia* (Cactaceae)	yes	yes	yes	15,21
Hexantheda eneomera Urban and Graf, 2000	*Petunia* (Solanaceae)	yes	yes	?	2,16
Hexantheda missionica Ogloblin, 1948	*Calibrachoa, Petunia* (Solanaceae)	yes	yes	yes	2,25

Niltonia virgilii Moure, 1964	*Jacaranda, Tibouchina* (Bignoniaceae)	yes	?	?	11
Nomiocolletes arnaui Moure, 1949	*Eryngium* (Apiaceae)	?	no	?	15
Perditomorpha brunerii Ashmead, 1899	*Abutilon, Sida, Malvastrum* (Malvaceae)	yes	yes	yes	7,15
Protodiscelis echinodori Melo, 1996	*Echinodorus* (Alismataceae)	?	?	?	26
Sarocolletes guaritarum Urban, 1995	*Abutilon, Modiolastrum* (Malvaceae)	yes	yes	?	15
Tetraglossula anthracina (Michener, 1989)	*Ludwigia* (Onagraceae)	?	?	?	2
Tetraglossula bigamica (Strand, 1910)	*Ludwigia* (Onagraceae)	yes	yes	yes	2,9,15
HALICTIDAE					
HALICTINI					
Pseudagapostemon brasiliensis Cure, 1989	*Ludwigia* (Onagraceae)	yes	yes	?	2,3
Pseudagapostemon pruinosus Moure and	*Ludwigia* (Onagraceae)	yes	yes		

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THE IMPORTANCE OF SOLITARY BEES ON THE REPRODUCTIVE BIOLOGY OF TIMBER TREES AT THE TAPAJÓS NATIONAL FOREST, BRAZIL

Márcia Motta Maués
Milene Silva de Souza
Milton Kanashiro

Abstract

The Amazon forest comprises an area of 4.9 millions km^2, with the largest extent of tropical ecosystems in the world. The expansion of logging activities, cattle ranching and agricultural systems is an outstanding reality, resulting in approximately 10,000 to 15,000 km^2 of forest being cut down per year. Habitat loss and forest fragmentation may affect the reproductive health of native trees, considering that it reduces the effective population of the woody population, decreasing the number of pollen donors and the possible amount of compatible pollen deposited on the stigma, which may lead to low fruit set and genetic drift. These processes may also go along with a decline on the pollinator's population. This study contributes to the recommendations of the "International Initiative for the Conservation and Sustainable Use of Pollinators" (São Paulo Declaration on Pollinators, October 1998), concerning the maintenance of the pollinator's ecosystem services for the sustainability of Amazon timber trees. This study is a component of the Dendrogene project, coordinated by Embrapa Amazônia Oriental and several partners. It was focused on floral biology, reproductive system and pollination of climax trees at the Tapajós National Forest, Para State, Brazil. Among the species selected in the project, we are presenting data on *Jacaranda copaia* (Aubl.) D. Don (Bignoniaceae), a pioneer canopy or understory species with regular spatial distribution and *Dipteryx odorata* Willd. (Leguminosae-Papilionoidae), an emergent light demanding species, randomly distributed. The flowers of both species were attractive to bees in colors and rewards. *J. copaia* flowers' are violet and *D. odorata* presents pink flowers with a conspicuous sweet odor. Nectar was the primary reward for the visitors. Controlled pollination tests revealed that both species were allogamous. The legitimate pollinators were mainly native bees (e.g. *Euglossa, Epicharis, Eulaema, Bombus* and *Centris*), thus, the pollination system was mellitophilous. These bees use to fly long distances, hence it is expected that once their habitat is well preserved, there will not be major impacts on the reproductive success of those native trees. This information is also very important for simulation studies of logging impact through scenario analyses done with Eco-gene, for these two timber species. Given that solitary bees were the most important pollinators of the studied species, their preservation within the remnant stands of native forests is vital for the reproductive health of the woody upper canopy stratum of tropical forests. Therefore, in practical terms, it is important to open a discussion related to those bees´ living habitats, so that during the process of trees selection for wood extraction, part of potential trees hosting a bee colony, be maintained in the remaining forest.

Key words**:** Pollination, floral biology, *Jacaranda copaia*, *Dipteryx odorata*, forest management, Amazon, Sporophitic self-incompatibility (SSI), Late-acting self-incompatibility (LSI).

Introduction

The Brazilian Amazon covers an area of 4.9 million km^2 (Kitamura, 1994), with the largest extension of tropical ecosystems of the world, comprising approximately 21,000 species of higher plants (Gentry, 1982). A single hectare may present an assemblage of 280 woody species (Oliveira and Mori, 1999). The amount of timber resources within this forest represents about 60,000,000 m^3 in logs, which is almost 30% of the world tropical forests (Barros and Veríssimo, 1996).

Human occupation increased considerably during the last three decades, and the expansion of logging, cattle ranching and agricultural activities are an outstanding reality within the Amazon forest (Kitamura, 1994; Barros and Veríssimo, 1996). Throughout 1996 and 1997, approximately 10,000 to 15,000 km^2/year of standing forest was felled (Nepstad *et al.*, 1999). According to INPE (2003), until 2000 the total deforested area represented 600,000 km^2, corresponding to 10 times the Costa Rican territory.

Habitat loss and forest fragmentation promotes biodiversity decline, due to microhabitat loss, isolation, as well as changes in migration and dispersion patterns (Laurance *et al.*, 2002). Logging promotes up to 37% of canopy gaps, modifying the microclimate conditions and increasing the chances of flammability of the remnant stands (Nepstad *et al.*, 1999), directly killing bee colonies (Eltz *et al.*, 2003). These processes may affect the reproductive health of the native trees, considering that it reduces the effective population of the woody population (Cascante *et al.*, 2002; Fuchs *et al.*¸2003), decreasing the number of pollen donors and the possible amount of compatible pollen deposited on the stigma (Quesada and Stoner, 2003), which may lead to low fruit set and genetic drift (Bawa, 1990; Hamrick and Murawsky, 1990; Cascante *et al.*, 2002; Quesada and Stoner, 2003). These processes may also lead to a decline on the pollinator's population (Aizen and Feisinger, 1994; Cascante *et al.*, 2002).

Forest fragmentation resulted in a decline of bee visitation, and, therefore, a medium decrease of 20% on fruit and seed set in a dry forest (Aizen and Feisinger, 1984), indicating a negative effect on the community health of fragments in comparison with continuous forests. The type of pollinator or dispersal agents influences pollen and seed dispersion ratios. Euglossinae bees may fly up to 23 km (Janzen, 1971), conversely, meliponinas (e.g. *Cephalotrigona capitata* and *Melipona panamica)* forage up to 1.2 to 1.5 km (Roubik and Aluja, 1983).

This study contributes to the recommendations of the "International Initiative for the Conservation and Sustainable Use of Pollinators" (São Paulo Declaration on Pollinators, October 1998), concerning the maintenance of the pollinator's ecosystem services for the sustainability of Amazon timber trees. The main purpose was the identification of the pollinating agents of two important Amazonian timber species and linking the kind of these pollinators with potential disruption of the reproductive process due to forest fragmentation caused by logging operations.

Material and methods

The main study area is located at the Tapajós National Forest (FLONA Tapajós), in eastern Brazilian Amazon (2.89ºS 54.95ºW), which comprises an area of 600,000 hectares of lowland native forest. It is situated almost 90 m above the Tapajós River water level. The predominant soil type is Oxisol (Haplustox), dominated by kaolinite clay minerals and is free of hardpan or iron oxide in the upper 12 meters (Nepstad, *et al.*, 2002). The climate, according to Köppen classification, is **Ami**, characterized by annual dry period of 2-3 months and average rainfall of 2,000 mm (600 mm to 3,000 mm). The average annual air temperature is 25 ° C (18,4ºC to 32,6 ° C) (Carvalho, 1992).

This forest may experience severe drought during El Niño events (Nepstad *et al.*, 2001) and has been submitted to controlled timber extraction and sustainable forest management studies (Silva *et al.*, 1985; Carvalho, 2001; Kanashiro *et al.*, 2002). The only perturbation before the creation of the National Forest in 1974,was caused by hunting activities, fruit harvesting, latex extraction of *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. and selective logging of *Manilkara huberi* Standley, *Cedrela odorata* L., *Cordia goeldiana* Hub. and *Aniba duckei* Kostermans, approximately 50 years ago (Carvalho, 2001). Currently, a low-impact selective logging project is being conducted under supervision of the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) and the International Tropical Timber organization (ITTO).

The study plot is a 500 ha area (Figure 1), where seven target species, with different ecological growing conditions and life history strategies, are being studied on their genetic structure, reproductive process and regeneration. A commercial tree inventory was accomplished in the area, including all target species individuals with a diameter at breast height (DBH) ^{3} of 20 cm. In a 100 ha subplot the inventory was further extended to DBH ^{3} of 10 cm (Kanashiro *et al.*, 2002). These studies are components of the Dendrogene project, coordinated by Embrapa Amazônia Oriental and several partners.

Complementary studies on floral biology and reproductive systems were also carried out in adult trees (> 20 years) planted at the experimental area of Embrapa Amazônia Oriental in Belém, Pará State (1º27'S 48º29'W). The climate according to Köppen, is **Afi**, characterized by an average annual temperature of 25,9ºC (21º to 31,6ºC) and annual average rainfall of 2,900 mm.

Jacaranda copaia (Aubl.) D. Don (Bignoniaceae) is a pioneer canopy, upper canopy or understory species (Ribeiro *et al.,* 1999) with regular spatial distribution (Parrota *et al.*, 1995). This species promptly colonizes gaps in the forest and may be used in agroforestry systems and reforestation (Guariguata *et al.*, 1995). Its wood is largely used in plywood industries (Loureiro *et al.*, 1979). The trees may grow up to 30-35 m in height and 75 cm in diameter (Silva *et al.*, 1985) under natural conditions. The flowering period occurs annually in the dry season (August to October), displaying conspicuous violet flowers. The flowering period extends up to 3-4 weeks within the population (Maués *et al.*, 1999a).

Dipteryx odorata Willd. (Leguminosae-Papilionoidae) is an emergent light demanding species (Ribeiro *et al.,* 1999), randomly distributed in primary forests (Parrota *et al.*, 1995). The flowering period occurs in the middle of the dry season, with numerous violet-pink flowers, which releases a conspicuous sweet odor (Perry and Starret 1983). The species is an important timber tree, due to the resistance and durability of the wood. It grows up to 30-35 m in height and 115 cm in diameter (Silva *et al.*, 1985). An asynchronous flowering pattern was noticed, as some trees may flower in the wet season (May to June), although the majority blooms in the dry season (September to November) (Maués *et al.*, 1999b).

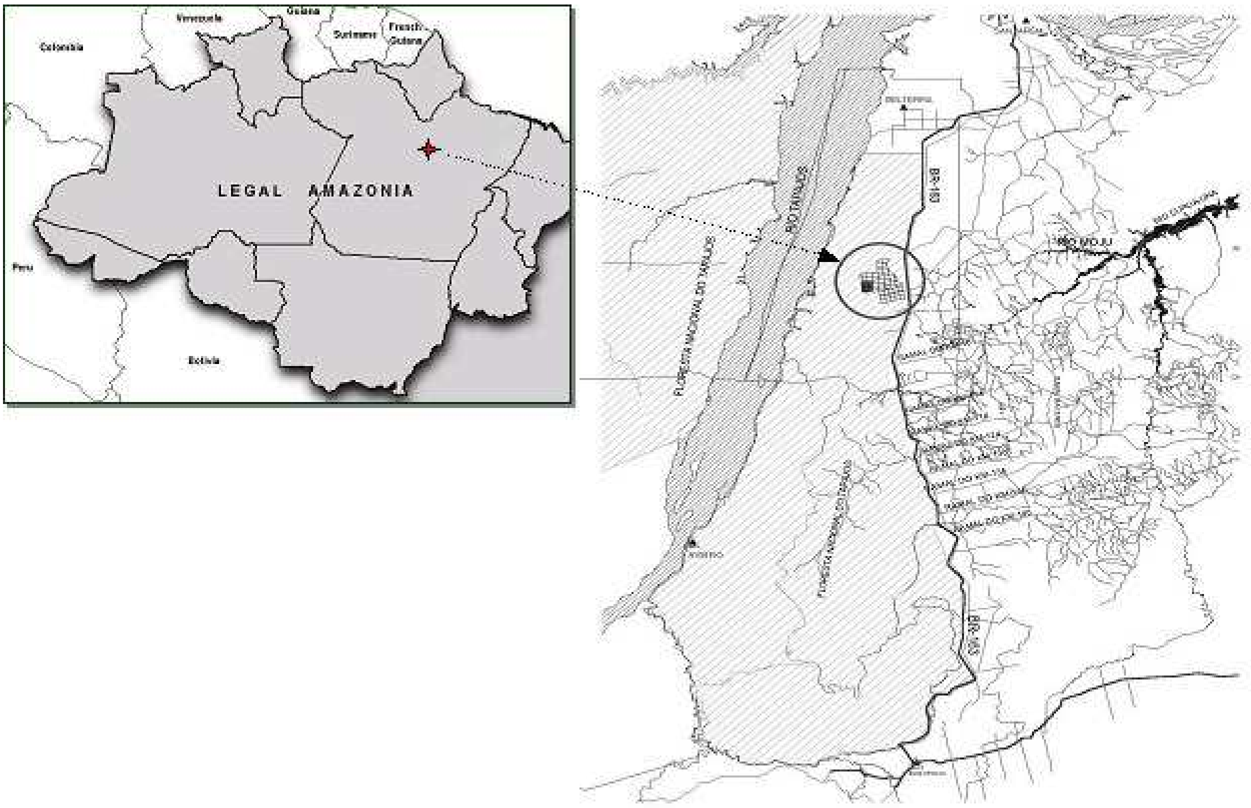

Figure 1. Study site at the Tapajós National Forest, Pará State, Brazil. The circle indicates the selective logging area of the Sustainable Forest Management Project (IBAMA/ITTO). The gray square is the 500 ha plot of the Dendrogene Project.

To describe the inflorescence structure, flower morphology and aspects of the floral biology (anthesis, number of flowers opened/day, flower longevity, duration of flowering/plant, pollen/ovule ratio), five trees of each species were monitored in a plantation at the Embrapa Amazônia Oriental Experimental Area in Belém, from August to October 2002. Hand pollination experiments in pre-bagged flowers were also performed in order to assess the breeding system. The following treatments were carried out: (1) cross-pollination; (2) self-pollination; (3) spontaneous (automatic) self-pollination; (4) control – tagged flowers left to natural pollination. Fruit set was evaluated for each treatment until the complete fruit development.

To have access to the crowns of the trees, wood towers with 32-34 meters were used. During the flowering period, direct observations about the behavior of the insect visitors were accompanied by specimen collection and photographic documentation, in order to support the identification of the legitimate pollinators. The observations were performed since the anthesis until the end of flower visitation (from 7:00 to 18:00 h). Most insects were identified by comparison with previously identified specimens in the Entomological Museum of Embrapa Amazônia Oriental. Dr. David W. Roubik, from the Smithsonian Tropical Research Institute in Panamá, identified the euglossine bees. The following data about the insect visitors were recorded: (1) species; (2) if there was contact between the visitor body and the reproductive organs of the flowers; (3) if pollen or nectar was collected/consumed. These observations were carried out during the main flowering season of 2002 (Sep-Oct).

Results and discussion

Jacaranda copaia displays large panicles up to 37 cm long, with an average of 1,900 flowers (n=9) and 93 flowers opened/day. The flowers are opened from the basis to the apex of the inflorescence, and this process took an average of 35±11 (n=5) days. The flowers are hermaphroditic, violet and tubular shaped, with four stamens and one optically attractive staminode (Figure 3a) with glandular hairs (osmophores), a single central style, shorter than the staminode, with a bifid tactile stigma (Figure 2a). The ovary contains an average of 243±33 ovules (n=20) and the number of pollen grains/flower was 30,425 (n=4). The pollen/ovule ratio was 125.2, indicating facultative autogamy *sensu* Cruden (1977). The anthesis occurs from 7:30 to 8:30 a.m. The flowers last for one day with all the floral organs. By the end of the first day, the corolla detaches and falls down, but the pistil remains functional one day more. The stigma maintains the receptivity for 48 h. The fruits contain an average of 245±26 (n=25) seeds.

Dipteryx odorata presents violet-pink flowers with a conspicuous sweet odor in ferruginous panicles up to 12.9 cm long. There was an average of 53 (n=6) flowers/inflorescence and an average of 14 opened flowers/day. The blooming period lasted an average of 17.5±2.5 days (n=4). The flowers are hermaphroditic, papilionoid (flag-flower) (Figure 3b), with hidden floral resources, androecium with 10 diadelphous stamens (Figure 2b). The unicarpellate gynoecium bears a single ovule (n=20) and at anthesis the stigma slightly protrudes beyond the longest whorl of anthers. Pollen/ovule ratio was 15,525 (n=4), indicating an obligate xenogamy reproductive system *sensu* Cruden (1977). Nectar was the primary reward for the visitors. Anthesis occurred from 5:00 to 6:00 a.m. Floral buds may be attacked by larvae of Cecidomiiydae and Curculionidae, promoting abortion or floral malformation, affecting the fruit set. Most fruits contain a single seed.

Controlled pollination tests revealed that both species were basically self-incompatible (Table 1). Most tropical trees are out-crossers due to genetic incompatibility systems, dioecy and heterostily (Bawa *et al.*, 1985).

In *J. copaia*, fruit set from open pollination was 1.06% (n=6,932). Hand pollination using self-pollen did not set fruits. Cross-pollination resulted in 6.54% fruit set (n=2,524). Flowers excluded to insect visitation (automatic self-pollination) did not set fruits. Self-pollen rejection is related to self-incompatibility (SI) system. In *J. copaia*, abscision of selfed pistils within two days indicated homomorphic sporophitic self-incompatibility (SSI), by the inhibition of pollen germination at the stigma surface (Seavey and Bawa, 1986).

The pollination tests in *D. odorata* presented similar results. Firstly self-pollination resulted in a reasonable number of initiated fruits and, simultaneously, high rate of fruit abortion was observed during the first five weeks, although the fruit abortion lasted until the 17th week. This is a strong evidence of a “late-acting self-incompatibility” (LSI) system, as referred in Seavey and Bawa (1986). However selfing resulted in 0.16% fruit set (n=3,206), the highest fruit set resulted from cross-pollination with 4.10% (n=3,091); while those flowers exposed to insect visitation (open pollination) resulted in 0.29% fruit set (n=6,477), thus xenogamous cross-pollination resulted in over ten times the number of developing ovaries compared to the self-pollinations. Despite that, in the automatic pollination tests a very low percentage of fruit set was registered and only two fruits (0.02%; n=8,255) reached the complete maturation, but could be the result of accidental contamination during the pollination handling and not a indicative of agamospermy. Similar results were presented by Perry and Starrett (1980) in *D.*

panamaensis, which showed a low selfing rate (0.018%; n=109) compared to cross-pollinated flowers (0.277%; n=77). The flowers exposed to insect visitation (unbagged) resulted in 0.044% (n=68) fruit set.

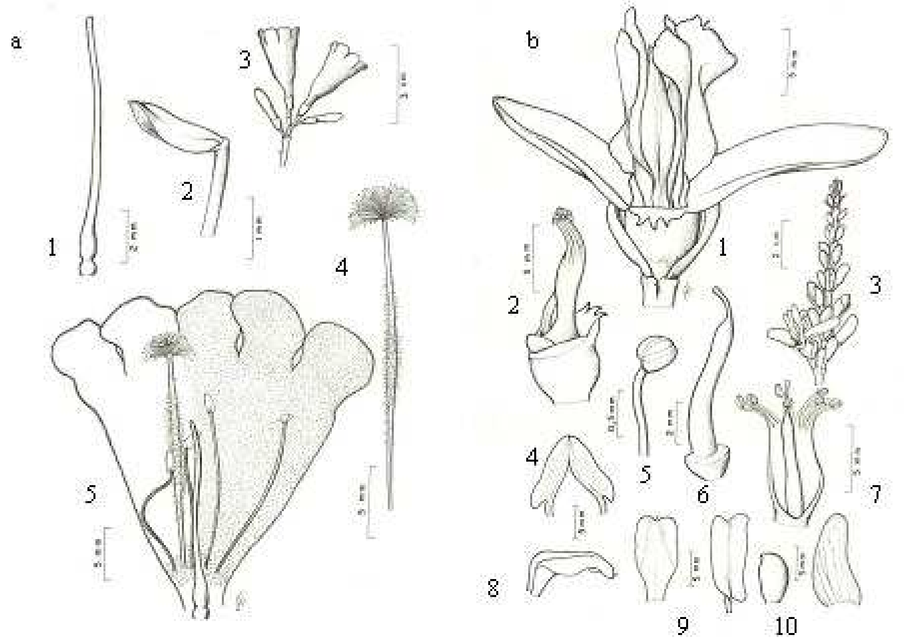

Figure 2. a) *Jacaranda copaia*: 1. Pistil; 2. Anther; 3. Flowers; 4. Staminode; 5. Opened flower showing the reproductive organs and staminode position. b) *Dipteryx odorata*: 1. Flower; 2 and 7. Stamen; 3. Inflorescence; 4. Vexillum; 5. Anther; 6. Pistil; 8. Carina or Keel; 9. Alae; 10. Petaloid lobes (calyx).

Table 1. Percentage (%) of fruit set from hand-pollination treatments and open pollination flowers (control) in *Jacaranda copaia* and *Dipteryx odorata*. Data correspond to initiated and mature fruits (number of fruits/number of flowers per treatment).

Species	*Jacaranda copaia*		*Dipteryx odorata*	
Treatments	**Initiated fruits**	**Mature fruits**	**Initiated fruits**	**Mature fruits**
Cross-Pollination	21.7% (469/2,524)	6.54% (173/2,524)	15.04% (465/3,091)	4,10% (127/3,091)
Self-pollination	0.06% (1/2,099)	-	8.36% (268/3,206)	0,16% (5/3,206)
Automatic SP	-	-	0.19% (16/8,255)	0.02% (2/8,255)
Control	4.99% (414/6,932)	1.06% (91/6,932)	3.52% (228/6,477)	0,29% (19/6,477)

"Late-acting self-incompatibility" has been also reported in several other tropical species including *Dalbergia retusa, Dipteryx panamaensis* and *Myrospermum pubescens* (Seavey and Bawa, 1986), *Hymenaea stigonocarpa* (Gibbs *et al.*, 1999), *Dalbergia miscolobium* (Gibbs and Sassaki, 1998), *Dolichandra cynanchoides* and *Tabebuia nodosa* (Gibbs and Bianchi, 1999). A list of species with "late-acting self-incompatibility" system was provided by Seavey and Bawa (1986) and revised by Gibbs and Bianchi (1999). According to Gibbs and Sassaki (1998), early inbreeding depression could be the reason for the rejection of selfed pistils in species with LSI, as observed in cases where pistils/young fruits are abscised within a period of weeks or months like in *D. odorata*.

In both species, there was a plethora of flower visitors, including medium to large-sized bees, butterflies, moths, wasps and hummingbirds. Considering the species separately, *J. copaia* the flowers were mainly visited by *Euglossa* spp. and *Centris*, which initiated the visits at the anthesis, sometimes promoting the flower opening by forcing the entrance at the pre-anthesis flower stage. The visits of *Centris* were very fast lasting from 3 to 6 seconds (n=46), and the main reward was nectar. The euglossine bees were also very active and their visits lasted 8 to 12 seconds (n=55). These bees together were considered the legitimate pollinators, due to the compatibility between their body sizes with the petal hood, direct contact with the reproductive organs and frequency of visits (Table 2).

The staminode "selected" the legitimate visits reducing the perianth chamber, therefore large-sized visitors were not able to enter and contact the stigma and anthers. Flowers were also visited by *Xylocopa frontalis* with an illegitimate pattern, grasping the whole corolla and making a hole in the base, to collect nectar, and was classified as a nectar-robber. Butterflies used the openings made by *X. frontalis* to act as nectar-robber too. Other bees such as *Apis mellifera, Epicharis rustica, Bombus brevivillus, Eufriesea mussitans, Centris flavifrons, C. similes,* as well as a few species of Halictidae and Meliponina were occasional pollinators. Hummingbirds were also occasional flower visitors and did not contributed with the pollination success.

In *D. odorata* the pollinators were mainly Apidae (*Eulaema nigrita, Eulaema cingulata, Epicharis rustica, Epicharis (Hoplepicharis) affinis, Bombus transversalis* (Figure 3c) and *Bombus brevivillus*). These bees promoted the anthers exposure by forcing the keel petals, and doing this, their body was covered with pollen. Other important pollinators were Scarabaeidae – Rutelinae beetles, which visited the flowers regularly, sometimes using it as a *rendezvous* (Figure 3d). *Xylocopa frontalis,* Meliponina, Chrysomelidae, Pieridae, Nymphalidae, Papilioidae, Heliconidae, Amatidae and Trochilidae were occasional pollinators (Table 3). Perry and Starrett (1980) found similar groups of pollinator in *D. panamaensis* flowers.

Table 2. Flower visitors and pollinator agents collected in *Jacaranda copaia* at the FLONA Tapajos and Belem, in the Brazilian Amazon.

Flower visitors	Locality	Category*	Resource+
INSECTA			
HYMENOPTERA			
Apidae			
Aparatrigona impunctata (Ducke, 1986)	Bel/Tap	O	P
Bombus brevivillus Franklin, 1913	Bel/Tap	P	N
Bombus transversalis (Olivier, 1789)	Bel/Tap	P	N
Centris flavifrons (Fabricius, 1775)	Bel	O	N
Centris similis (Fabricius, 1804)	Bel	O	N
Centris spp. (5 species)	Bel/Tap	P	N, P
Epicharis (Hoplepicharis) affinis Smith, 1874	Bel	P	N
Epicharis rustica (Olivier, 1789)	Bel	O	N
Epicharis spp. (2 species)	Bel	O	N
Eufriesea mussitans (Fabricius, 1787)	Bel	O	N
Euglossa chlorina (Dressler, 1982)	Bel	P	N, P
Euglossa spp. (2 species)	Tap	P	N, P
Eulaema meriana (Olivier, 1789)	Bel	O	N
Eulaema nigrita Lepeletier, 1841	Bel	P	N
Exomalopsis sp.	Bel	O	P
Melipona compressipes (fabricius, 1804)	Tap	O	N, P
Meliponina (6 species)	Tap	O	P
Paratetrapedia spp. (2 species)	Bel	O	P
Xylocopa frontalis (Olivier, 1789)	Bel	R	N
Megachilidae			
Megachile sp.	Bel	O	N
Andrenidae (1 species)	Tap	O	N
Halictidae (5 species)	Tap	O	N
Vespidae (1 species)	Bel/Tap	O	N
DIPTERA			
Bibionidae (1 species)	Tap	O	P
Syrphidae			
Ornidia obesa Fabricius, 1775	Bel	O	P
COLEOPTERA			
Chrysomelidae (4 species)	Tap	O	P
Scarabaeidade – Rutelinae (3 species)	Tap	O	P
LEPIDOPTERA			
Pieridae (1 species)	Tap	R	N
BIRDS			
Trochilidae (3 species**)**	Bel/Tap	O	N

Bel = Belém; Tap = Tapajós National Forest

*p = legitimate pollinator; o = occasional pollinator; r = pollen/nectar robber

+Floral resources used by the visitors/pollinators (N = nectar; P = pollen)

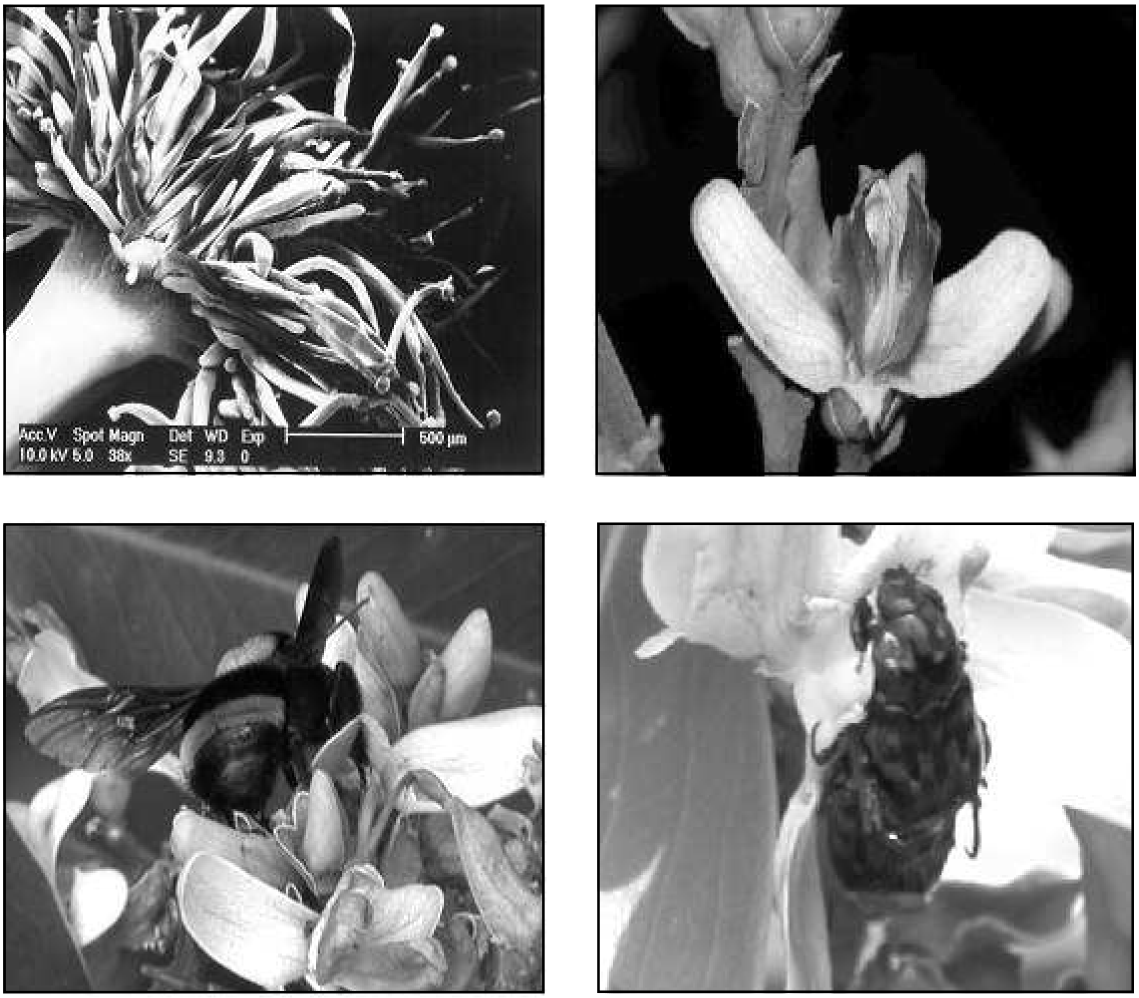

Figure 3. a. Scanning Electron Microscope photography (33x) of the *Jacaranda copaia* staminode; b. *Dipteryx odorata* flower; c. *Bombus transversalis* in *D. copaia* flower; d. Scarabaeidae – Rutelinae beetles foraging and mating in *D. odorata flower*.

Table 3. Flower visitors and pollinator agents collected in *Dipteryx odorata* at the FLONA Tapajós and Belém, in the Brazilian Amazon.

Flower visitors	Locality	Category	Resource
INSECTA			
HYMENOPTERA			
Apidae			
Bombus brevivillus Franklin, 1913	Bel	P	N
Bombus transversalis (Olivier, 1789)	Bel/Tap	P	N
Centris sp.1	Bel	P	N, P
Epicharis (Hoplepicharis) affinis Smith,1874	Bel	P	N
Epicharis rustica (Olivier, 1789)	Bel	P	N
Epicharis sp. (2 species)	Bel	P	N
Eulaema meriana (Olivier, 1789)	Bel	P	N
Eulaema nigrita Lepeletier, 1841	Bel	P	N
Meliponina (5 species)	Tap	O	N, P
Trigonisca sp. 1	Tap	O	P
Xylocopa frontalis (Olivier, 1789)	Bel	O	N
Vespidae (1 species)	Bel	O	N
COLEOPTERA			
Chrysomelidae (2 species)	Bel/Tap	O	P
Scarabaeidae – Rutelinae (3 species)	Bel/Tap	P	P
LEPIDOPTERA			
Heliconidae (1 species)	Tap	O	N
Nimphalidae (1 species)	Tap	O	N
Papilionidae (2 species)	Tap	O	N
Pieridae (1 species)	Tap	O	N
BIRDS			
Trochilidae (2 species)	Bel/Tap	O	N

Bel = Belém; Tap = Tapajós National Forest
[*]p = legitimate pollinator; o = occasional pollinator
[+]Floral resources used by the visitors/pollinators (N = nectar; P = pollen)

Considering the legitimate visits, both species presented a mellitophilous pollination syndrome. The pollinators of *J. copaia* were less diversified than in *D. odorata.* Non-social bees tend to show higher oligolecity species-specific predilections (Cane, 2001). Large-bodied euglossinas may fly over many kilometers (Janzen *et al.,* 1982), and also fly in canopy or open sun conditions to a far greater extent than most members of the genus *Euglossa*, which comprise most local species (Roubik, 1992). Selective logging modifies the spatial structure of a group of target species, reducing the effective population of pollen donors. The removal of trees with different spatial distribution patterns may affect in different ways the reproductive health of target species. Information of this kind are very important for scenario analyses using Eco-gene simulation model for logging impacts (Kanashiro *et al.*, 2002). Also, logging activities may affect directly the population of native bees by removing nesting places or killing bees colonies living in felled trees. In Borneo 51% of bee nests were found in commercially important trees (Eltz *et al.*, 2003). Indirectly, the removal of trees may increase the distances of pollen flow mediated by pollination agents (Aizen and Feisinger, 1984). Furthermore, in a deforested area of the central Brazilian Amazon, was observed a decline on the number of species of euglossine bees (Powell and Powell, 1987; Becker *et al.*, 1991). Considering the importance of these bees as legitimate pollinators of timber trees, efforts should be addressed in order to improve the forest management techniques to preserve adequate conditions for nesting and foraging of native pollinators for conservation purposes, of pollinators themselves, and as a means of maintenance of genetic diversity in the tree population for allowing the continuity of its own adaptation process. Disruption of pollination services in fragmented forests is a major concern for the maintenance of the sustainability of forest management through low impact logging.

Final remarks

Jacaranda copaia and *Dipteryx odorata* are self-incompatible species, with different incompatibility mechanisms: Sporophitic Self-Incompatibility (SSI) and Late-acting Self-Incompatibility (LSI), hence both species are obligate out-crossers, as most tropical woody species. Pollen flow is mainly mediated by medium to small-sized bees, e.g. *Centris, Euglossa, Epicharis* and *Bombus*. Given that native bees were the most important pollinators of the studied species, their preservation within the remnant stands of native forests is vital for the reproductive health of the woody upper canopy stratum of tropical forests. Therefore, in practical terms, it is important to open a discussion related to those bees´ living habitats, so that during the process of trees selection for wood extraction, part of potential trees hosting a bee colony or adequate nesting conditions to solitary bees, will be maintained in the remaining forest.

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OIL-COLLECTING BEES AND RELATED PLANTS: A REVIEW OF THE STUDIES IN THE LAST TWENTY YEARS AND CASE HISTORIES OF PLANTS OCCURRING IN NE BRAZIL

Isabel Cristina Machado

Oil-flowers

The great majority of the zoophilous flowers developed a series of mechanisms and floral specializations to attract its pollinators, facilitating the pollen transfer and gene flow among individuals of the same species (Faegri and van der Pijl, 1979; Richards, 1985; Proctor *et al.*, 1996). The zoophilous Angiosperms, in a general way, attract their pollinators through floral rewards. The nature of these rewards is varied, and they can be distinguished from non- nutritious, as local for rest or mating, materials for nest construction (waxes and resins) and sexual attractants (perfumes and- volatile oils), to those nutritious (Simpson and Neff, 1981, 1983; Armbruster, 1984). For a long time, nectar and pollen were considered as the most important, or even the only nutritious rewards. The discovery by Vogel and his works (1969, 1971, 1973, 1974, 1976a,b, 1981, 1990a) revealing the existence of floral glands that secrete oils (lipids), opened a vast research field, involving some genera of bees and families of plants.

Eight families (Cucurbitaceae, Iridaceae, Krameriaceae, Malpighiaceae, Orchidaceae, Primulaceae, Scrophulariaceae and Solanaceae) have species with flowers that offer exclusively oils to its visitors, or offer oils in addition to pollen and/or nectar (Buchmann, 1987; Vogel, 1988, 1989; Vogel and Cocucci, 1995; Sérsic and Cocucci, 1999; Machado, 2002). The families Melastomataceae and Gesneriaceae were previously included in this list, based on references of floral oil secretion, respectively in *Mouriri* (Buchmann and Buchmann, 1981) and *Drymonia* (Steiner, 1985). However, the meaning of the floral oil produced in glandular trichoms in the corolla of *Drymonia serrulata* (Gesneriaceae) is not still clear, and it seems to act as a sticker (accessory pollenkitt), facilitating the adherence of the pollen grains to the thorax of *Epicharis* bees (Steiner, 1985), thus not functioning as a reward for the bees. In *Mouriri*, the oil produced in the anther conectives and collected by *Trigona* spp. seems also to play an adhesive function for the pollen. Corolla glands occurring in some *Caesalpinia* spp. with a supposed function of elaiophores (Vogel, 1988) were studied later on by this author who does not confirmed this function (personal communication). The meaning of the floral oils in the systematic of Malpighiaceae and other families and its relationship with pollen morphology was discussed by Lobreau-Callen (1983).

The lipidic material secreted by the oil-flowers consists of saturated fatty acids (Simpson *et al.*, 1977, 1979; Cane *et al.*, 1983; Roubik, 1989) and also free fatty acids (Vogel, 1974; Seigler *et al.*, 1978). The oil flowers secrete these lipids in specific glandular structures, denominated by Vogel (1974) as elaiophore. These elaiophores can, morphologically, be of two types: epithelial and trichomatic (Table 1). The epithelial elaiophore consists of areas of epidermis secretory cells, where large amounts of secreted lipids accumulates being protected by a cuticle. The trichomatic elaiophore consists of areas covered by hundreds or thousands of glandular oil secreting trichomes. The trichomatic elaiophore generally occurs in different areas of the corolla, but they can also be located in the androecia, as in *Lysimachia* (Vogel, 1976, 1986), or in parts of the ovary (Simpson and Neff, 1981).

Oil-collecting bees and structures for collection

The floral oil-collecting bees related in the literature are solitary bees, distributed mainly in two families (*sensu* Michener, 2000): Melittidae and Apidae (Vogel, 1974; Buchmann, 1987).

The family Melittidae is found mainly in Africa and in Holartic region. Only the two genera *Macropis* and *Rediviva* have oil-collecting species. There are references that *Macropis* is a specialist genus in the oil collection from flowers of *Lysimachia* (Vogel, 1976, 1986; Simpson and Neff, 1983; Cane *et al.*, 1983). In turn, *Rediviva* is considered as being, probably, an example of co-evolution involving the South-African genus *Diascia* (Scrophulariaceae) (Vogel, 1984; Whitehead *et al.*, 1984; Whitehead and Steiner, 1985; Manning and Brothers, 1986; Steiner, 1990, 1992; Steiner and Whitehead, 1987, 1988, 1990). Later on, visits of several *Rediviva* species were registered in flowers of *Disperis* spp. (Orchidaceae) in Africa (Steiner, 1989, 1994; Steiner and Johnson, 1989), and authors suggested this could be a secondary oil source for most of the bee species in almost all observed places.

The family Apidae contains the great majority of the genera and species of bees collecting flower oils, which are grouped in four tribes: Centridini, Ctenoplectrini, Tapinotaspidini and Tetrapediini (nomenclature following Michener, 2000). Centridini and Tetrapediini were previously included in the family Anthophoridae; Ctenoplectrini constituted the family Ctenoplectridae, and the tribe Tapinotaspidini belonged to Exomalopsini. In these tribes, the oil collection behaviour was already documented for several species of *Centris* and *Epicharis* (Centridini), as well as for some species of *Paratetrapedia*, *Arhysosceble*, *Chalepogenus*, *Lanthanomelissa*, *Tapinotaspis* and *Monoeca* (Tapinotaspidini) (Tables 1 and 2) (Vogel, 1974; Simpson *et al.*, 1977, 1990; Sazima and Sazima, 1989; Cocucci, 1991; Sérsic, 1991; Vogel and Machado, 1991; Vogel and Cocucci, 1995; Cocucci and Vogel, 2001; Teixeira and Machado, 2000; Machado *et al.*, 2002; Gaglianone, 2003). Since 1974, Vogel, based in morphologic characters, also suggested that the oil collection could be made by representatives of *Tetrapedia* (Tetrapediini), that was confirmed further by Neff and Simpson (1981), Gottsberger (1986), Vogel (1988), Sazima and Sazima (1989), Vogel and Machado (1991), among others. Ctenoplectrini is an Asian and African tribe, consisting of two genera, one of which *Ctenoplectra*, uses the abdomen for oil collection (Vogel, 1981, 1990a). These species were only observed in flowers of Cucurbitaceae (*Momordica* and *Thladiantha*).

The floral lipids, mixed with pollen, seem to be used by bees basically as constituent of the larval food (Simpson and Neff, 1981). However, Cane *et al.* (1983) mentioned oil collection by *Macropis* from flowers of *Lysimachia* used also as part of the cell wall recovering in nests. There are also indications that floral oils can be possibly also used for adults' nutrition (Buchmann, 1987).

Simpson *et al.* (1983) comment that the main advantage of the oil in relation to the nectar (that is generally the substance used by bees in the mixture with pollen) is its largest amount of energy for unit of weight. According to Vogel (1989), the floral oil is about eight times richer in calories, when compared with the same amount of nectar.

Centridini collects and use floral oils of Malpighiaceae (Vogel, 1974, 1988; Sazima and Sazima, 1989; Barros, 1992; Santos, 1996; Teixeira and Machado, 2000; Gaglianone, 2003), Krameriaceae (Simpson, 1987; 1989a; Simpson, *et al.*, 1977, 1979; Machado *et al.*, 1997), Scrophulariaceae (Vogel, 1974; Cocucci, 1984; Vogel and Machado, 1991; Sérsic and Cocucci, 1999; Machado *et al.*, 2002) and of other families (see Tables 1 and 2). Only representatives of Centridini, tribe restricted to the New World, seem to be involved. The largest group of oil-collecting bees, in Centridini, is represented by the genera *Centris* and *Epicharis* (Hiller, 1991) (cf. Tables 1 and 2).

Almost all the female bees of *Centris* possess special structures, located in the basitarse of the fore and mid- legs that act in the collection of floral oils (Vogel, 1974; Roberts and Vallespir, 1979; Neff and Simpson, 1981; Roubik, 1989). These bees generally use the fore and mid-legs for oil collection, except Ctenoplectridae and some Tapinotaspidini, that use the specialized bristles located in the ventral part of the abdomen (Vogel, 1990a).

The structure of the collector organs is correlated with the different elaiophore types. According to Vogel (1974) and Neff and Simpson (1981), in Centridini generally exists basitarsals combs, formed by arrays of specialized bristles. Most of the species of *Centris* and *Epicharis* exhibit that structure, with some variations, in the two first pairs of legs, fore and mid, and this pattern is considered as being primitively associated to the oil collection in epithelial elaiophore, as in Malpighiaceae, where the four legs are used in the exploitation of the calicinal glands (Vogel, 1974; Neff and Simpson, 1981; Sazima and Sazima, 1989). Among Tapinotaspidini, *Monoeca* spp. use the two first pairs of legs for oil collection, as observed by Vogel (personal communication) in *Banisteriopsis* (Malpighiaceae).

This relationship (the use of the four legs in oil collection) is not restricted and constant, once, in the visits to flowers of *Angelonia (*Vogel and Machado, 1991; Machado *et al.*, 2002), all the observed *Centris* species use only the first pair of legs, although almost all bee species, excepting *Centris hyptidis*, have the barsitarsal combs in the fore and mid-legs. On the other hand, species of *Tapinotaspis* have oil-collecting structures restricted to the mid-legs, which are more prolonged (Cocucci, 1991; Roig-Alsina, 1997, 1999; Machado *et al.*, 2002). These bees have been registered pollinating flowers of *Angelonia* (Machado *et al.*, 2002; Gimenes *et al.*, 2002), *Calceolaria* (Sérsic, 1991), *Nierembergia* (Cocucci, 1984, 1991) and *Sisyrinchium* (Cocucci and Vogel, 2001).

While most zigomorphic nectar flowers have its nectaries in a medium position, accessible to the tongue and proboscides of the pollinator insects, the spurs or depressions with elaiophores, as for example in *Angelonia* and *Diascia* (Scrophulariaceae), are presented in pairs, side by side, according to flower exploitation, made with the two forelegs (Vogel, 1984; Vogel and Machado, 1991; Machado *et al.*, 2002).

Oil-collection behaviour and specificity of the visitors

According to Vogel (1988, 1990b), the morphology of the oil-flowers of the Malpighiaceae can be better understood when it is related with the behaviour of Centridini bees. Species of *Epicharis* show oil-collecting structures in the two first pairs of legs and they are observed collecting oil exclusively in flowers of Malpighiaceae. Despite most of the *Centris* species have oil-collector structures in the four first legs (Vogel 1988), the possible relationship between species of this genus and flowers with epithelial elaiophores is not so exclusive, as mentioned by Neff and Simpson (1981) and Vogel (1988) and reinforced by Vogel and Machado (1991), Cocucci (1991), Vogel and Cocucci (1995), Sérsic and Cocucci (1999) and Machado *et al.* (2002), that registered visits of *Centris* in flowers with trichomatic elaiophores.

Specificity in relations between species of *Centris* and representatives of Malpighiaceae is not registered (Vogel, 1988). Bees of different sizes are observed in the same flower, as well as, a same species of *Centris* is found in different plant species (see Table 2). In the same way, specificity between species of *Rediviva* and flowers of *Diascia* does not exist, despite the existence of relation between the length of the forelegs of the bees and the length of the spurs of the flowers (Whitehead and Steiner, 1985; Steiner and Whitehead, 1988). Steiner and Whitehead (1988) demonstrated that a certain species of *Diascia* is visited indiscriminately by several species of *Rediviva*, showing different lengths of the forelegs, at the same time that, a same species of *Rediviva* can pollinate different species of *Diascia*, with different spur lengths. On the other hand, the strong correlation between the spur length of *Diascia* and the foreleg length of *Rediviva "pallidula"* suggests that the length of the spur should have determined the evolution of the legs length (Steiner and Whitehead 1991), similar to *Rediviva neliana* (Steiner and Whitehead 1990), through a diffuse coevolution.

Steiner (1993) and Steiner and Whitehead (1996), on the other hand, revealed the importance of the specialist bee (*Rediviva gigas*) in the oil collection and the reproductive effort of *Ixianthes retzioides* (Scrophulariaceae).

Another example of close relationship between an oil-collecting bee and a related plant species was documented by Vogel and Machado (1991), involving *Centris hyptidis* and *Angelonia pubescens*. Later on, Aguiar *et al.* (2003), in another Caatinga region, also observed flowers of *A. pubescens* being visited only by *C. hyptidis*, although in their study site others *Centris* spp. occur. The forelegs of this bee are prolonged, when compared with the habitual pattern found in another *Centris* spp. and they are related with the length and position of the two flower spurs. Vogel and Machado (1991) discussed a possible parallelism of *Centris hyptidis* and *Angelonia pubescens* with species of *Rediviva* and *Diascia*, in Africa. Prolonged forelegs also happen in *Rediviva* spp., which pollinate *Diascia* flowers by collecting oil from trichomatic elaiophores occurring inside two prolonged spurs, whose length varies between 2 and 22 mm (Whitehead and Steiner, 1985; Steiner and Whitehead, 1988). In 1974, Vogel, analysing the structural floral characteristics and the lenght of the spurs of *Diascia* and comparing with the flowers of *Angelonia*, presumed the existence of a bee, whose forelegs were long enough to find the floral oil located inside the *Diascia* floral spurs, including the longer ones. Then years later, this extraordinary supposition was confirmed (Vogel, 1984; Whitehead and Steiner, 1985; Manning and Brothers, 1986). In some species of *Rediviva*, the length of the forelegs exceeds the length of its own body, as in *R. longimanus*, whose forelegs have lengths of 17 to 20 mm and *R. emdeorum*, with forelegs showing about 25 length mm (Vogel, 1984; Whitehead and Steiner, 1985).

The pattern of the oil-collector apparatus of the forelegs of *Rediviva emdeorum* resembles that registered to the legs of *Centris hyptidis*, which consists of fine absorptive hairs and of some spatulate ones (Vogel and Machado, 1991). The reduction of the comb of the forelegs and its complete absence in the mid-legs, had been already mentioned by Neff and Simpson (1981) for some species of the subgenera *Paracentris* and *Wagenknechtia*. Both subgenera were mentioned as exclusively associated to oil-flowers of *Calceolaria* (Scrophulariaceae) (Vogel, 1974; Neff and Simpson, 1981; Sérsic, 1994).

How the knowledge on oil-flowers and oil collecting bees evolved in Brazil

In Brazil, studies on pollination of oil-flowers begun with Vogel (1974), who made observations in various species of different families. I observed a concentration of studies on Malpighiaceae species (42 spp., representing 79% of the published studies) (Vogel, 1974; Sazima and Sazima, 1989; Barros, 1992; Albuquerque and Rego, 1989; Rego and Albuquerque, 1989; Teixeira and Machado, 2000; Bezerra, 2004), with few works focusing on species of Scrophulariaceae (7 spp, exclusively referring to the genus *Angelonia*, representing 13 % of the studies), Iridaceae (2 spp) and Krameriaceae (2 spp) (Vogel, 1974; Cocucci and Vogel, 2001; Vogel and Machado, 1991; Machado *et al.*, 1997, 2002). (See Table 3).

Regarding the bees, when analysing tables 2 and 3, it is observed that Centridini is responsible for 73 % of the total of registered bees on oil-flowers. The genus *Centris* is dominant, with 39 spp, representing 52 % of the total. I also noticed that there are some generalist species, as *C. aenea, C. fuscata* and *C. tarsata*, which were observed in flowers of different plant species, genera and families. On the other hand, species, like *C. hyptidis* were observed only in flowers of two *Angelonia* species. The genus *Epicharis*, responsible for 21 % (16 spp) of the total of observed visits, has a similar behaviour with generalist species like *Epicharis affinis, E. analis* and *E.bicolor*, while others species are more specialists or even rare.

Concerning to the plant families, studies on Malpighiaceae show that pollination is mediated by several species of *Centris* and *Epicharis* bees (Vogel, 1974; Buchmann, 1987; Barros, 1992; Teixeira and Machado, 2000; Aguiar *et al.*, 2003; Gaglianone, 2003). Vogel (1990) discusses that the changes observed in the floral morphology of New and Old World Malpighiaceae are part of the interaction process of those plants with Centridini bees. Some of the researches commented above showed the relationship between the specialized structures in the legs of oil-collecting *Centris* spp. (Neff and Simpson, 1981; Simpson and Neff, 1981) and the oil collection in epithelial elaiophores occurring in Malpighiaceae.

In this family, which is one of the most studied plant families concerning on these interactions, a concentration of papers is observed with species of the genus *Byrsonima* (including 35% of the studies in the family and representing 28% of the total of studied oil-flower species in Brazil) (Gottsberger, 1986; Albuquerque and Rego, 1989; Rego and Albuquerque, 1989; Sazima and Sazima, 1989; Barros, 1992; Vinson *et al.*, 1997; Gaglianone, 2000; Teixeira and Machado, 2000). *Malpighia* is another well studied genus, perhaps due to the economic value of some species, as the “West Indian cherry” (Raw, 1979; Magalhães *et al.*, 1997; Melo *et al.*, 1997; Freitas *et al.*, 1999). The other genera of Malpighiaceae are less studied, with restricted punctual information to only one species, or they are just mentioned in articles with check-list of bee species (especially Centridini) and the flora associated to floral oil-collection (Martins, 1995; Aguiar *et al.*, 1995, 2000, 2003; Aguiar and Martins, 1997; Martins and Moura, 1995; Alves-dos-Santos, 1999; Silva and Martins, 1999; Zanella, 2000; Gaglianone, 2002, 2003). (Table 3). Recently, Bezerra (2004) carried out a study about the pollination of *Stigmaphyllum paralias* in two populations in “restinga” and in a “caatinga” area in Pernambuco, Northeast of Brazil, indicating species of *Centris* and *Epicharis* as the main pollinators. The only previous reference involving the pollination ecology of a species of this genus is that of Haumann-Merck (1913), which describes the pollination of *S. littorale*, with the misinterpretation that nectar was the substance secreted by the calicinal glands. Further, Vogel (1974) shows the pollination of *S. littorale* by *Centris trigonoides*.

Regarding the Iridaceae, except for the book of Vogel (1974), that mentions the pollination of some species of *Cypella*, *Sphenostigma*, *Alophia* and *Sisyrinchium* outside Brazil, I found only one paper which shows details of the pollination of species of *Sysirinchium*, in South America, referring to two species occurring in Brazil (Cocucci and Vogel, 2001).

Concerning to the Scrophulariaceae, the studies are limited to species of *Angelonia* (Vogel, 1974; Vogel and Machado, 1991; Machado *et al*., 2002; Aguiar *et al*., 2003), focusing on the relationships with *Centris* species and the specializations of the flower morphology and the elaiophore structures related to the legs of the pollinating bees. Machado *et al*. (2002) also discuss the hole of a new species of *Tapinotaspis,* besides *Centris hyptidis,* on the pollination of *A. cornigera* in Caatinga.

About *Krameria* spp. (Krameriaceae), I only found a punctual work related to the capture of *Centris* bees in flowers of *Krameria bahiana* in sandbank areas in Bahia (Gimenes *et al*., 2002) and a summary about the pollination of *Krameria tomentosa*, in areas of caatinga/ campo rupestre in Pernambuco state (Machado *et al*., 1997). In both works the exclusiveness of *Centris* bees visiting flowers of these two species of *Krameria* is in accordance with other studied species outside Brazil (Simpson *et al*., 1977).

To the other plants families with oil-flowers occurring in Brazil (Cucurbitaceae, Orchidaceae and Solanaceae) we found no registers about the pollination ecology of oil-flowers species, indicating that there is still a lot to be made in this interesting research field.

Concluding remarks

The oil-collecting bees, especially Centridini, belong to the most effective group of pollinators in Neotropics. They are not only indispensable to the reproduction of the oil-producing plants, but also for other plant species co-occurring in a same community that offer nectar and pollen as reward and are visited by them. In a similar way these bees also depend on these substances: nectar, for their own feeding in both sexes, and pollen, for the females. Thus, the presence of the floral oil-producing plants and oil-collecting bees in a community is indirectly important for the survival of several other non-floral-oil-producing plants and vice versa.

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Table 1 – Families and genera of plants and bees with references of visits in oil-flowers.

Families/Genera of plants	**Elaiophore type**	**Genera/Families of bees (*sensu* Michener 2000)**	**Site of observations**	**References**
CUCURBITACEAE	**Trichomatic**			
Momordica		*Ctenoplectra*/ Apidae	Africa, Malasia	Vogel, 1981; Vogel, 1990a
Thladiantha		*Ctenoplectra*/ Apidae	Asia	Roman'kova, 1989; Vogel, 1990a
IRIDACEAE	**Trichomatic**			
Alophia		*Paratetrapedia*/ Apidae	Argentine	Vogel, 1974
Cypella		Chalepogenus/ *Apidae*	Argentine	Vogel, 1974
		Centris/*Apidae*	Argentine	Vogel, 1974
Sphenostigma		*Arhysosceble*/ Apidae	Argentine	Vogel, 1974
Sisyrinchium		*Chalepogenus*/ Apidae	Argentine, Brazil and Chile	Vogel, 1974; Schlindwein, 1998; Roig-Alsina, 1999; Cocucci and Vogel, 2001
		Lanthanomelissa/ Apidae	Argentine, Brazil	Roig-Alsina, 1997; Schlindwein, 1998; Cocucci and Vogel, 2001
KRAMERIACEAE	**Epithelial**			
Krameria		*Centris*/Apidae	Brazil, Chile, Mexico, USA, Honduras, Curaçao	Simpson *et al.*, 1977; Simpson and Neff, 1987; Simpson, 1989a; Machado *et al.*, 1997; Gimenes *et al.,* 2002

MALPIGHIACEAE	**Epithelial**			
Banisteria		*Centris*/Apidae	Brazil	Vogel, 1974
Banisteriopsis		*Centris*/Apidae	Brazil	Gottsberger, 1986; Sazima and Sazima, 1989; Rebêlo 1995; Albuquerque and Mendonça, 1996; Gaglianone, 2000, 2003
		Epicharis/Apidae	Brazil	Gottsberger, 1986; Sazima and Sazima, 1989; Gaglianone, 2000
		Monoeca/Apidae	Brazil	Gottsberger, 1986; Vogel com. pess.
		Paratetrapedia/ Apidae	Brazil	Sazima and Sazima, 1989
		Tetrapedia/Apidae	Brazil	Sazima and Sazima, 1989; Rebêlo, 1995
Bunchosia		*Epicharis*/Apidae	Brazil	Vogel, 1974
		Paratetrapedia/ Apidae	Brazil	Vogel, 1974
		Trigona/Apidae	Brazil	Vogel, 1974
Byrsonima		*Centris*/Apidae	Brazil, Costa Rica	Vogel, 1974; Gottsberger, 1986; Albuquerque and Rego, 1989; Rego and Albuquerque, 1989; Barros, 1992; Vinson *et al*., 1997; Teixeira and Machado, 2000; Gimenes *et al*., 2002; Aguiar *et al*., 2003; Gaglianone, 2000, 2003

		Epicharis/Apidae	Brazil	Vogel, 1974; Gottsberger, 1986; Albuquerque and Rego, 1989; Rego and Albuquerque, 1989; Barros, 1992; Pedro, 1994; Gaglianone, 2000; Teixeira and Machado, 2000; Neves and Viana, 2001; Gimenes *et al.*, 2002
		Paratetrapedia/ Apidae	Brazil	Vogel, 1974; Gottsberger, 1986; Teixeira and Machado, 2000
		Tetrapedia/Apidae	Brazil	Albuquerque and Rego, 1989; Rego and Albuquerque, 1989
		Trigona/Apidae	Brazil	Albuquerque and Rego, 1989; Rego and Albuquerque, 1989
Dinemandra		*Centris*/Apidae	Chile	Simpson, 1989b
Dinemagonum		*Centris*/Apidae	Chile	Simpson, 1989b
Heteropterys		*Centris*/Apidae	Brazil	Sazima and Sazima, 1989; Aguiar *et al.*, 2003; Gaglianone, 2003
		Epicharis/Apidae	Brazil	Sazima and Sazima, 1989; Gaglianone, 2000
		Paratetrapedia/ Apidae	Brazil	Sazima and Sazima, 1989
		Tetrapedia/Apidae	Brazil	Sazima and Sazima, 1989
Janusia		*Centris*/Apidae	EUA	Simpson and Neff, 1987
Lophanthera		*Centris*/Apidae	Brazil	Gaglianone, 2003
Macvaughia		*Centris*/Apidae	Brazil	Aguiar *et al.*, 2003

Malpighia		*Centris*/Apidae	Brazil, Jamaica	Raw, 1979; Magalhães *et al.,* 1997; Carvalho *et al*., 1995; Lorenzon *et al*., 1995; Melo *et al*.,1997; Freitas *et al*., 1999; Aguiar *et al*., 2003
Mascagnia		*Centris*/Apidae	Brazil	Ducke, 1910; Aguiar *et al*., 2003; Gaglianone, 2003
		Epicharis/Apidae	Brazil	Gaglianone, 2000
Peixotoa		*Centris*/Apidae	Brazil	Gaglianone, 2003
		Epicharis/Apidae	Brazil	Gaglianone, 2000
Ptilochaeta		*Centris*/Apidae	Brazil	Aguiar *et al*., 2003
Stigmaphyllum		*Centris*/Apidae	Argentine, Brazil	Hauman-Merck, 1913; Vogel, 1974; Aguiar, 2003a b; Aguiar *et al*., 2003; Gaglianone, 2003; Bezerra, 2004
		Epicharis/Apidae	Brazil	Gaglianone, 2000
		Trigona/Apidae	?	Steiner, 1985
Tetrapteris		*Epicharis*/Apidae	Brazil	Gottsberger, 1986
		Paratetrapedia/ Apidae	Brazil	Gottsberger, 1986
Tricomaria		*Centris*/Apidae	Argentine	Jörgensen, 1909
ORCHIDACEAE	**Trichomatic/ Epithelial**			
Disperis		*Rediviva*/Melittidae	Africa	Steiner, 1989
Oncidium		*Centris*/Apidae	?	Dodson, 1965 (Apud Vogel, 1974); Pijl and Dodson, 1966
Ornithocephalus		*Paratetrapedia*/ Apidae	Costa Rica, Ecuador	Dodson, 1965 (Apud Vogel, 1974)

PRIMULACEAE	**Trichomatic**			
Lysimachia		*Macropis*/Melittidae	EUA, Europe	Popov, 1958; Vogel, 1976; 1986; Cane *et al*., 1983; Simpson *et al*., 1983
SCROPHULARIACEAE	**Trichomatic**			
Alonsoa		*Rediviva*/Melittidae	Africa	Steiner, 1989
Anastrabe		*Rediviva*/Melittidae	Africa	Steiner and Whitehead, 1987
Angelonia		*Centris*/Apidae	Brazil	Vogel, 1974; Vogel and Machado, 1991; Gimenes *et al*., 2002; Machado *et al*., 2002; Aguiar *et al*., 2003
		Paratetrapedia/Apidae	Brazil	Vogel and Machado, 1991
		Tapinotaspis/Apidae	Brazil	Gimenes *et al*., 2002; Machado *et al*., 2002
		Tetrapedia/Apidae	Brazil	Vogel and Machado, 1991
Basistemon		*Paratetrapedia*/ Apidae	Argentine	Vogel and Cocucci, 1995
Bowkeria		*Rediviva*/Melittidae	Africa	Steiner and Whitehead, 1991a,b
Calceolaria		*Centris*/Apidae	Argentine, Chile	Vogel, 1974; Sérsic, 1991
		Tapinotaspis/Apidae	Argentine	Vogel, 1974; Molau, 1988; Sérsic, 1991
Diascia		*Rediviva*/Melittidae	Africa	Hilliard and Burtt, 1984; Vogel, 1984; Steiner, 1990; Steiner and Whitehead, 1988; 1990; 1991; Whitehead *et al*., 1984.
Hemimeris		*Rediviva*/Melittidae	Africa	Steiner, 1993
Ixianthes		*Rediviva*/Melittidae	Africa	Steiner and Whitehead, 1996

Monttea		*Centris*/Apidae	Argentine, Chile	Simpson *et al.,* 1990; Sérsic and Cocucci, 1999
SOLANACEAE	**Trichomatic**			
Nierembergia		*Centris*/Apidae	Argentine	Cocucci, 1991
		Lanthanomelissa/ Apidae	Argentine	Cocucci, 1984; 1991
		Tapinotaspis/ Apidae	Argentine	Cocucci, 1984; 1991; Simpson and Neff, 1981
		Paratetrapedia/ Apidae	Argentine	Cocucci, 1991

Table 2 – Records on visitation of oil-collecting bee species (in alphabetical order) and the host plants in Brazil.

Bee species	**Plant species**	**References**
Arhysoceble dichroopoda	15, 26	Faria-Mucci *et al*., 2003
Augochloropsis aff. crassigena	16	Rego and Albuquerque, 1989
Centris aenea	3, 12, 19, 21, 23, 30, 33, 35, 38, 44, 50	Vogel and Machado, 1991; Freitas *et al*., 1999; Teixeira and Machado, 2000; Gimenes *et al*., 2002; Ramalho and Silva, 2002; Gaglianone, 2003; Aguiar *et al*., 2003; Bezerra 2004
C. analis	10, 16, 23, 21, 29, 43	Albuquerque and Rego, 1989; Rego and Albuquerque, 1989; Sazima and Sazima, 1989; Teixeira and Machado, 2000; Ramalho and Silva, 2002; Gaglianone, 2003
C. bicolor	5, 43	Gaglianone, 2003
C. burgdorfii	15, 17, 22, 24, 27, 43	Barros, 1992; Gaglianone, 2003
C. byrsonimae	16	Albuquerque and Rego, 1989; Rego and Albuquerque, 1989
C. caxiensis	14, 16, 19, 44	Albuquerque and Rego, 1989; Rego and Albuquerque, 1989; Albuquerque and Mendonça, 1996; Bezerra, 2004
C. cockerelli	17, 20, 22, 24, 27, 28	Barros, 1992
C. collaris	10, 11	Sazima and Sazima, 1989; Gaglianone, 2003
C. discolor	15, 17, 22, 24, 27, 28	Gottsberger, 1986; Barros, 1992
C. dorsata	5, 8, 11	Gottsberger, 1986; Gaglianone, 2003
C. flavifrons	16, 17, 19, 21, 23, 33, 34, 36, 44	Albuquerque and Rego, 1989; Rego and Albuquerque, 1989; Magalhães and Oliveira, 1997; Teixeira and Machado, 2000; Ramalho and Silva, 2002; Aguiar *et al*., 2003; Gaglianone, 2003; Bezerra, 2004
C. frontalis	23	Teixeira and Machado, 2000;
C. fuscata	4, 5, 12, 16, 19, 23, 30, 33, 34, 38, 41, 44, 47, 50	Vogel, 1974; Albuquerque and Rego, 1989; Rego and Albuquerque, 1989; Vogel and Machado, 1991; Machado *et al*., 1997: Teixeira and Machado, 2000; Ramalho and Silva, 2002; Gaglianone, 2003; Aguiar *et al*.,

C. hyptidis	49, 53	Vogel and Machado, 1991; Machado *et al*., 2002; Aguiar *et al*., 2003
C. iheringi	15, 17, 20, 22, 24, 28	Barros, 1992
C. lanipes	36	Magalhães and Oliveira, 1997
C. leprieuri	3, 19, 23, 44, 49	Teixeira and Machado, 2000; Gimenes *et al*., 2002; Ramalho and Silva, 2002; Bezerra, 2004
C. longimana	16, 32, 45	Albuquerque and Rego, 1989; Rego and Albuquerque, 1989; Gaglianone, 2003
C. aff. lutea	9, 21, 39	Gaglianone, 2003
C. maculata	23	Teixeira and Machado, 2000;
C. minuta	23	Teixeira and Machado, 2000;
C. mocsaryi	5, 17, 21, 43	Gottsberger, 1986; Gaglianone, 2003
C. moerens	42	Aguiar, 2003; Aguiar *et al*., 2003
C. nitens	6, 9, 12, 17, 19, 21, 23, 37, 43	Gottsberger, 1986; Ramalho and Silva, 2002; Gaglianone, 2003; Bezerra, 2004
C. obsoleta	33, 50	Machado *et al*., 2002; Aguiar *et al*., 2003
C. pectoralis	11	Gaglianone, 2003
C. ptilotopus	23	Teixeira and Machado, 2000;
C. pulcra sp.n.	3	Gimenes *et al*., 2002
C. scopipes	9, 11, 15, 21, 22, 27, 28, 39, 43	Barros, 1992; Gaglianone, 2003
C. similis	10, 39, 43	Sazima and Sazima, 1989; Gaglianone, 2003
C. spilopoda	16, 17, 21, 23, 42	Albuquerque and Rego, 1989; Rego and Albuquerque, 1989; Teixeira and Machado, 2000; Ramalho and Silva, 2002; Aguiar, 2003; Aguiar *et al*., 2003; Gaglianone, 2003
C. sponsa	5, 9, 11, 17, 23, 33, 43	Ramalho and Silva, 2002; Aguiar *et al*., 2003; Gaglianone, 2003
C. tarsata	3, 4, 6, 12, 16, 19, 21, 23, 30, 33, 34, 35, 42, 43, 44, 45, 50, 52	Albuquerque and Rego, 1989; Rego and Albuquerque, 1989; Vogel and Machado, 1991; Machado *et al*., 1997: Freitas *et al*., 1999; Gimenes *et al*., 2002; Ramalho and Silva, 2002; Gaglianone, 2003; Aguiar *et al*., 2003; Bezerra, 2004
C. trigonoides	3, 11, 16, 23, 33, 34, 47,	Vogel, 1974; Albuquerque and

C. varia	21, 23	Teixeira and Machado, 2000; Gaglianone, 2003
C. versicolor	36	Magalhães and Oliveira, 1997
C. violacea	30	Gaglianone, 2003
C. vittata	33	Aguiar *et al.*, 2003
C. xanthomelaena	33, 48, 50, 51	Vogel and Machado, 1991; Aguiar *et al.*, 2003
Epicharis albofasciata	21	Gaglianone, 2003
E. affinis	5, 7, 9, 10, 15, 17, 21, 22, 24, 28, 43	Sazima and Sazima, 1989; Barros, 1992; Gaglianone, 2003
E. analis	5, 9, 11, 15, 20, 21, 22, 24, 27, 28, 31, 43	Barros, 1992; Gaglianone, 2003
E. bicolor	6, 9, 12, 13, 15, 16, 17, 18, 21, 22, 23, 24, 27, 37	Gottsberger, 1986; Albuquerque and Rego, 1989; Rego and Albuquerque, 1989; Teixeira and Machado, 2000; Neves and Viana, 2001; Ramalho and Silva, 2002; Gaglianone, 2003
E. cockerelli	17, 21	Gottsberger, 1986; Gaglianone, 2003
E. fasciata	21, 23	Teixeira and Machado, 2000; Gaglianone, 2003
E. flava	5, 9, 11, 16, 21, 23, 31, 43,	Albuquerque and Rego, 1989; Rego and Albuquerque,1989; Teixeira and Machado, 2000; Ramalho and Silva, 2002; Gaglianone, 2003
E. grandior	5, 9, 11, 21, 22, 24, 28, 43	Barros, 1992; Gaglianone, 2003
E. iheringi	9, 15, 21, 22, 24, 27, 28, 39, 43	Barros, 1992; Gaglianone, 2003
E. ligulata	23	Teixeira and Machado, 2000;
E. maculata	23	Teixeira and Machado, 2000;
E. minima	21	Gaglianone, 2003
E. nigrita	21, 23	Teixeira and Machado, 2000; Ramalho and Silva, 2002; Gaglianone, 2003
E. obscura	10, 29	Sazima and Sazima, 1989
E. rustica	10, 15, 17, 22, 24, 27, 28, 46	Gottsberger, 1985; Sazima and Sazima, 1989; Barros, 1992
E. schrottkyi	5, 10, 21, 43	Sazima and Sazima, 1989; Gaglianone, 2003
Lanthanomelissa discrepans	1, 2	Schilindwein, 1995; Cocucci and Vogel, 2001
L. mageliae	2	Cocucci and Vogel, 2001
Monoeca pluricincta	40	Faria-Mucci *et al.*, 2003
Paratetrapedia	14	Albuquerque and Mendonça,

Paratetrapedia tarsalis	16	Albuquerque and Rego, 1989; Rego and Albuquerque, 1989
Paratetrapedia aff. tricolor	26	Faria-Mucci *et al*., 2003
Paratetrapedia testacea	16	Albuquerque and Rego, 1989; Rego and Albuquerque, 1989
P. xantopoda	15, 17, 20, 22, 24, 27, 28	Barros,1992
Paratetrapedia huberi	48, 50, 51, 53	Vogel and Machado, 1991
P. maculata	29	Sazima and Sazima, 1989
P. pygmaea	29	Sazima and Sazima, 1989
Tapinotaspis sabularum	1	Schilindwein, 1995
Tapinotaspis sp. nov	49	Machado *et al*., 2002
Tetrapedia aff. diversipes	16	Albuquerque and Rego, 1989; Rego and Albuquerque, 1989
T. cf. rugulosa	10, 15, 17, 20, 22, 24, 27, 28, 29, 48, 50, 51, 53	Sazima and Sazima, 1989; Vogel and Machado, 1991; Barros, 1992
Trigona fulviventris	16	Rego and Albuquerque, 1989
T. pallens	16	Rego and Albuquerque, 1989

Table 3 – List of plants visited by oil-collecting bees in different ecosystems in Brazil.

Nº	**Family/Plant species**	**Vegetation**	**References**
	Iridaceae		
1	*Sisyrinchium micranthum*		Schlindwein, 1995
2	*S. setaceaum*		Cocucci and Vogel, 2001
	Krameriaceae		
3	*Krameria bahiana*	Restinga	Gimenes *et al*., 2002
4	*K. tomentosa*	Caatinga	Machado *et al*., 1997
	Malpighiaceae		
5	*Banisteriospsis adenopoda*	Savanna	Gaglianone, 2003
6	*B. anisandra*	Savanna	Gaglianone, 2003
7	*B. argyrophylla*	Savanna	Gaglianone, 2003
8	*B. latifolia*	Savanna	Gottsberger, 1986
9	*B. malifolia*	Savanna	Gaglianone, 2003
10	*B. muricata*	Semi-deciduos forest	Sazima and Sazima, 1989
11	*B. oxyclada*	Savanna	Gaglianone, 2003
12	*B. pubipetala*	Savanna	Gaglianone, 2003
13	*B. stellaris*	Savanna	Gaglianone, 2003
14	*Byrsonima amoena*	Savanna	Albuquerque and Mendonça, 1996
15	*B. crassa*	Savanna; Campo rupestre	Barros, 1992; Faria-Mucci *et al*., 2003
16	*B. crassifolia*	Evergreen forest	Albuquerque and Rego, 1989
17	*B. coccolobifolia*	Savanna	Gottsberger 1986; Barros, 1992; Aguiar *et al*., 2003
18	*B. correifolia*	Caatinga	Neves and Viana, 2001
19	*B. gardneriana*	Caatinga/Campo rupestre	Bezerra, 2004
20	*B. guilleminiana*	Savanna	Barros, 1992
21	*B. intermedia*	Savanna	Gottsberger, 1986; Gaglianone, 2003
22	*B. laxiflora*	Savanna	Barros, 1992
23	*B. sericea*	Atlantic forest; Restinga	Teixeira and Machado, 2000; Gimenes *et al*., 2002; Ramalho and Silva, 2002
24	*B. subterranea*	Savanna	Barros, 1992
25	*B. vaccinifolia*	Savanna	Gottsberger, 1986
26	*B. variabilis*	Campo rupestre	Faria-Mucci *et al*., 2003
27	*B. verbascifolia*	Savanna	Gottsberger, 1986; Barros, 1992
28	*B. umbellata*	Savanna	Barros, 1992
29	*Heteropteris aceroides*	Semi-deciduos forest	Sazima and Sazima, 1989
30	*H. byrsonimifolia*	Savanna	Gaglianone, 2003

31	*H. pteropetala*	Savanna	Gaglianone, 2003
32	*Lophanthera lactescens*	Savanna	Gaglianone, 2003
33	*Macvaughia bahiensis*	Caatinga	Aguiar *et al*., 2003
34	*Malpighia glabra*	Caatinga	Aguiar *et al*., 2003
35	*M. emarginata*	Crop	Freitas *et al*., 1999
36	*M. punicifolia*	Crop	Magalhães and Oliveira, 1997
37	*Mascagnia cordifolia*	Savanna	Gaglianone, 2003
38	*M. rigida*	Caatinga	Aguiar *et al*., 2003
39	*Peixotoa reticulata*	Savanna	Gaglianone, 2003
40	*P. tomentosa*	Campo rupestre	Faria-Mucci *et al*., 2003
41	*Ptilochaeta bahiensis*	Caatinga	Aguiar *et al*., 2003
42	*Stigmaphyllon. auriculatum*	Caatinga	Aguiar, 2003; Aguiar *et al*., 2003
43	*S. lalandianum*	Savanna	Gaglianone, 2003
44	*S. paralias*	Caatinga/campo rupestre/Restinga	Bezerra, 2004
45	*S. tomentosum*	Savanna	Gaglianone, 2003
46	*Tetrapterys ramiflora*	Savanna	Gottsberger, 1986
	Scrophulariaceae		
47	*Angelonia biflora*	Crop	Vogel, 1974
48	*A. bisaccata*	Caatinga	Vogel and Machado, 1991
49	*A. cornigera*	Caatinga; Restinga	Machado *et al*., 2002; Gimenes *et al*., 2002
50	*A. hirta*	Caatinga	Vogel and Machado, 1991
51	*A. hookeriana*	Caatinga	Vogel and Machado, 1991
52	*A. interregima*	Caatinga	Aguiar *et al*., 2003
53	*A. pubescens*	Caatinga	Vogel and Machado, 1991; Aguiar *et al*., 2003

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LIST OF CONTRIBUTORS

Adam G. Drucker
International Livestock Research Institute
(ILRI), Addis Ababa, Ethiopia
a.drucker@cgiar.org

Adriaan van Doorn
Koppert Biological Systems
POBox 155, 2650 AD
Berkel en Rodenrijs, NL
avdoorn@koppert.nl

Anthony Raw
Departamento de Ciências Biológicas
Universidade Estadual de Santa Cruz
Ilhéus, Bahia, Brazil. 45650-000
tonyraw@uesc.br
anthony_raw@yahoo.com.br

Antonio Felicioli
Department of Anatomy, Biochemistry and
Veterinary Physiology
Viale delle Piagge n° 2, 56100
Pisa University, Pisa, Italy.
Antonio.Felicioli@vet.unipi.it
Antonio_Felicioli@yahoo.it

Barbara Gemmill
Environment Liaison Center International
P.O. Box 72461
Nairobi, Kenya
barbarag@elci.org

Braulio Ferreira Souza Dias
Brazilian Ministry of Environment

Breno M. Freitas,
Universidade Federal do Ceara
Departamento de Zootecnia
C.P. 12168 Campus do Pici, 60.021-970
Fortaleza-CE, Brazil.
freitas@ufc.br

Carlos Alberto Garófalo
Depto. Biologia, FFCLRP-USP.
Universidade de São Paulo
Av Bandeirantes. Ribeirão Preto/SP
14040-901

Celso Feitosa Martins
Depto. Sistemática e Ecologia
Universidade Federal da Paraíba, UFPB.
João Pessoa, PB 58059-900

Claire Kremen
Princeton University
Department of Ecology
Evolutionary Biology
Guyot Hall, Washington Road
Princeton, NJ 08544 USA
1-609-258-7313 (phone)
1-609-258-7892 (fax)
ckremen@princeton.edu

Claudia Mohra
Zoological Institute
University of Tübingen
Auf der Morgenstelle 28, D-72076
Tübingen, Germany

Clemens Schlindwein
Departamento de Botânica
Universidade Federal de Pernambuco
Av. Prof. Moraes Rêgo, s/n
Cidade Universitária, 50670-901
Recife, PE, Brazil
schlindw@ufpe.br

Connal Eardley
Agricultural Research Council
Plant Protection Research Institute
Private Bag x134
Queenswood, Pretoria, 0121
South Africa
vrehcde@plant5.agric.za

Christian Westerkamp
Departamento de Biologia,
Universidade Federal do Ceará
Campus do Pici
Cx. P. 6009, 60451970
Fortaleza-Ceará, Brazil
chrisbio@ufc.br

David W. Roubik
Smithsonian Tropical Research Institute
Unit 0948, APO AA 34002-0948, USA

Fernando A. Silveira.
Departamento de Zoologia
Instituto de Ciências Biológicas
Universidade Federal de Minas Gerais,
Minas Gerais, Brazil.

Food and Agriculture Organization of the United Nations
Viale delle Terme di Caracalla,
00100 Rome, Italy

Gordon W. Frankie
University of California,
Berkeley, California

Hayo H.W. Velthuis
Klemit 1, 5325 KG Wellseind, NL
hhwv@xs4all.nl

Isabel Alves-dos-Santos
Diretoria de Pós Graduação, UNESC
Universidade do Extremo Sul Catarinense,
CP 3167. Criciúma, SC 88806-000

Isabel Cristina Machado
Departamento de Botânica
Universidade Federal de Pernambuco.
imachado@ufpe.br

Jordi Bosch
Unitat d'Ecologia
Universitat Autònoma de Barcelona, Spain
Biology Department
Utah State University, Logan, Utah, USA

Julio Otavio P. Pereira
Universidade Federal do Ceara
Departamento de Zootecnia
C.P. 12168 Campus do Pici, 60.021-970
Fortaleza-CE, Brazil.
otavioportela@hotmail.com

Katja Hogendoorn
School of Agriculture
Wine Waite Campus
The University of Adelaide SA 5005
Phone: +61 08 8303 6555,
Fax: +61 08 8303 7109
Katja.Hogendoorn@adelaide.edu.au

Laurie Adams
Coevolution Institute
San Francisco, California, USA

Márcia Motta Maués
Embrapa Amazônia Oriental
CP 48, Belém, PA, 66.017-970 Brasil
Universidade de Brasília
Doutorado em Ecologia
marcia@cpatu.embrapa.br

Martin Fellendorf
Zoological Institute
University of Tübingen
Auf der Morgenstelle 28, D-72076
Tübingen, Germany

Mauro Pinzauti
Department of CDSL.
Sect Agricultural Entomology, via S. Michele
degli Scalzi n°2, 56100, Pisa, Italy
mpinzaut@agr.unipi.it

Michael Ruggiero
Integrated Taxonomic Information System,
Washington, DC, USA

Milene Silva de Souza
Museu Paraense Emílio Goeldi
Mestrado em Botânica

Miloje Krunic
Institute of Zoology
Faculty of Science
16 Studenteski Trg 11000
University of Belgrade, Yugoslavia
Krunicm@eunet.yu

Milton Kanashiro
Embrapa Amazônia Oriental
CP 48, Belém, PA, 66.017-970 Brasil

Peter Kwapong
Department of Zoology
University of Cape Coast
University Post Office, Cpe Coast, Ghana
pkwapong@yahoo.com

Robert J. Paxton
Zoological Institute
University of Tübingen
Auf der Morgenstelle 28, D-72076
Tübingen, Germany
School of Biology and Biochemistry
Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK

Rogel Villanueva-Gutiérrez
El Colegio de la Frontera Sur
Av. Centenario km5.5, Apdo. postal 424
Chetumal, Quintana Roo
C.P. 77900 Mexico.
rogel@ecosur-qroo.mx

S. Bradleigh Vinson
Texas A&M University
College Station, Texas

Simon G. Potts
Centre for Agri-Environmental Research (CAER), School of Agriculture
University of Reading, PO Box 237,
Reading, RG6 6AR, UK
s.g.potts@reading.ac.uk

Stephen L. Buchmann
The Bee Works, 1870 W.
Prince Rd., Ste. 16
Departments of Entomology
Ecology and Evolutionary Biology,
The University of Arizona, Tucson, AZ U.S.A.
steve@thebeeworks.com

Uma Partap
International Centre for Integrated
Mountain Development (ICIMOD)
PO Box 3226, Kathmandu Nepal
upartap@icimod.org.np

Vera Lucia Imperatriz Fonseca
Ecology Department, Biosciences Institute,
University of Sao Paulo

Wanja Kinuthia
Department of Invertebrate Zoology
National Museums Of Kenya
Box 40658,00100;Nairobi, Kenya,
eafrinet@africaonline.co.ke
William P. Kemp
Bee Biology and Systematics Laboratory,
USDA-ARS, Logan, Utah, USA