

São Paulo Declaration on Pollinators plus 5 Forum

Group 2. Assessment of Pollinator - Mediated Gene Flow

Protocol

Aim: to propose standard methodologies for the assessment of pollen transfer in crops and natural areas



EXPECTED PRODUCTS:

- 1) list of methodologies (including rapid assessment protocols)
- 2) recommendations for the gene flow assessment and management of plant species

This working group will focus on the assessment of pollinator-mediated gene flow of economically important plant species.

Discussion Session 1 - October 29th 2003 Morning

Monitoring of Flower Visitor Behaviour

Target: database containing comparative behaviour of visitors in different plant species within a given area

Products of workshop to accomplish target: list of RAPs, examples with case studies, some lab methods

Methods (just a "quick and dirty" checklist, open for discussion)

- Observations of bee behaviour on the flower/inflorescence: pollinator or visitor? (frequency and kind of stigmatic contact),
- Amount and availability of pollen carried: where in the body, how tightly packed?
- Bee flight among plants: do individuals follow nearest neighbour pollination rules (optimal foraging)? Is there along-row behaviour? Is there flower constancy? How to estimate carryover?
- Energy economics of foraging: measure temperature, wind, RH
- Creation of user-friendly protocols for bee behaviour assessment and comparison
- Application of simple statistics to determine pollen shadow

Questions:

- How to adapt the observational methods proposed to different plant habits and morphologies, and landscapes (ex: trees vs. shrubs; natural vs. agricultural systems)?
- Should these methods be made widespread and user-friendly?
- Should we encourage their use by laymen in order to increase amount of areas and situations?
- If so, how to standardize the use and application of the RAP with a minimum of mistakes?
- Will it be feasible to recommend pollen containment measures based on this information?

Discussion Session 2 – Oct 29th 2003 Afternoon-

Pollination Requirements

Target: list of important plant species and their pollinator requirements (list should include their breeding system, pollination syndrome, resource offer)

Products of workshop to accomplish target: experimental design for testing breeding systems, examples with case studies, fruit/seed set monitoring methods

Methods

- Standard methodology to test for breeding system (includes bagging, hand pollination and emasculation, followed by fruit/seed set), adapted to each case
- Creation of user-friendly protocols for breeding system assessment
- Application of simple statistics to analyse results

Questions:

- How to adapt the tests proposed to different plant habits and morphologies, and landscapes (ex: trees vs. shrubs; natural vs. agricultural systems)?
- Should these methods be made widespread and user-friendly?
- Should we encourage their use by laymen in order to increase amount of areas and situations?
- If so, how to standardize the use and application of the RAP with a minimum of mistakes?

Discussion Session 2 - October 29th 2003 afternoon

Applied Issues in Pollinator-Mediated Gene Flow

Target: to increase awareness/knowledge of mechanisms for containment measures or enhanced productivity for selected plant species

Expected product: list of recommendations for containment measures or enhanced productivity for selected plant species

Methods

- Estimates of gene flow through genetic markers compared with estimates of pollen transport (see above)
- Estimates of pollen shadow with dyes
- Comparison of crop productivity under different pollinator 'regimes'
- Selection and discussion of case studies (e.g. cotton)

Questions:

- How to adapt the tests proposed to different plant habits and morphologies, and landscapes (ex: trees vs. shrubs; natural vs. agricultural systems)?
- How to determine the need to assess gene escape (ex. in the case of unwanted hybridization between crop varieties and wild relatives, or related contamination problems concerning GMOs)?
- Should recommendations be made on a case-by-case basis, or could we generalize part of them?

REPORT OF ACTIVITIES AND DISCUSSIONS RESULTS

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ABSTRACT

This group discussed the interaction between plant and pollinator emphasizing the importance not only of pollination, but of good agricultural, forest management, and conservation practices for sustainable development. The group was comprised of researchers whose areas of expertise encompass floral biology, plant breeding systems, plant population genetics, and pollination of tropical plants. The recommendations below intend to contribute for future discussions regarding the Brazilian Pollinator Initiative (BPI), although some of them are general enough to be considered more broadly. Emphasis was given to recommendations other than methods, since there is a vast literature available (including FAO publications) on the abovementioned subjects.

Pollen-mediated gene flow in plants is effected by abiotic agents, such as wind, and a number of biotic agents, of which the single most important pollinator group worldwide is that of bees. Also of great importance for many native fruit trees in the tropics are bats, beetles and flies. The production of fruits, seeds, and more individuals of the species depend directly on these agents in the majority of plants, and very often in commercially important ones. Exceptions are self-fertilizing plants, but even these frequently benefit from cross-pollination provided by these agents. Traditional selective breeding of plants, habitat fragmentation and overexploitation of natural stands are currently narrowing the genetic base, and leading to genetic erosion of economically important plants. In addition, genetically modified varieties resistant to herbicides or pesticides could create potential "superweeds" through pollen-mediated gene flow.

Among the methods proposed to help perform the recommendations, rapid assessment protocols (RAP) are suggested, for the collection of data on bee behaviour, pollination syndromes and landscape diagnosis. These protocols allow data collection in the field by people with little or no formal biological training. Other methods, including statistical and genetic analyses, require both expertise and specific facilities, and will be usually performed by researchers and trained personnel.

POLLINATOR-MEDIATED GENE FLOW

Pollen-mediated gene flow in plants is effected by abiotic agents, such as wind, and biotic agents, such as bees, butterflies and moths, beetles, flies, bats, birds, and other less frequent agents like rodents, marsupials, and thrips (Proctor *et al.*, 1996). Many pollinator populations are probably suffering nowadays from some kind of stress derived from habitat loss, parasites, insecticides, and misunderstanding by the general public. Meanwhile, the need for their services in natural, agricultural and agroforestry systems is growing day by day. In the tropics, pollination is effected not only by bees (natives and introduced *Apis*), but also by less known animals, whose service is poorly understood by the general public. Among these, bats are particularly important because they pollinate several fruit trees, while beetles and flies are responsible for high yields in palms. With such a variety of pollinator species and of plants that need animal pollination, it is important that the characteristics that effect gene flow are well understood for each pollinator and likewise the plants they pollinate.

The pollinator requirements of a plant species depend on its breeding system. Standard methods to test for self-fertility and self-pollination are among the first features assessed when evaluating the need for pollinators. Other usual procedures allow to evaluate if a flower visitor is an effective pollinator. When focusing on gene flow, several techniques have been tested to assess how far can pollen go from a focal

plant of group of plants. This last parameter involves knowledge of the amount of pollen harvested from a flower, flight range, and resource distribution, and is directly related to the pollinator morphology, the flower morphology, and whether the resource is a crop or grows wild. Evaluating the extent of pollinator-mediated gene flow also requires knowledge of the pollinator's fidelity to a given plant species, and of the type of resource it is being visited for. Finally, commercial parameters that increase the crop value are usually taken into account to compare differences in yield among different pollination regimes.

The understanding of pollinator behaviour with respect to each plant species is vital not only to establish adequate agricultural actions that increase yield, but to adopt measures that lead to sustainability. Among these are actions to reduce genetic loss in forest species due to isolation, to determine levels of isolation or contamination of crops, to prevent invasiveness of exotic plants and improve conservation of native genetic diversity, and to enhance awareness of pollinators' services to humanity.

At present, given the enormous variety of pollinators and commercially important tropical plant species (Roubik, 1995), only a small percentage of plants, be they wild or cultivated, have undergone comprehensive studies, and most of the gene flow data which are needed to support conservation and management initiatives are still lacking.

IMPORTANCE OF POLLINATOR-MEDIATED GENE FLOW IN CROP MANAGEMENT AND SILVICULTURE

Pollinator services have been traditionally considered expendable in many crops. However, case studies have shown that yield is significantly improved when pollinators introduced. Such is the case of coffee (Roubik, 2002), and crops in Brazil (cited in a recent survey by Couto, 2002): passion fruit, red pepper, strawberry and orange. Possibly, many others such as sunflower and soybean will also show increased yield with insect-mediated pollination. Although pollinators are not essential for fruit or seed set in many species, on account of their self-fertility, it is clear that cross-pollination usually enhances crop performance, when evaluated by commercial parameters. For example, cashew (*Anacardium occidentale*), which is partially self-fertile, requires a high rate of visitation to obtain good nut yields, since most of the fruits derive from cross-pollination (Holanda-Neto *et al.*, 2002). The introduction of bee management to increase yield in crops should be carefully evaluated case by case, since it has additional costs involved.

On the other hand, alleged low productivity of some cultivated plants may simply be the result of incorrect agricultural practices that have led to low genetic diversity of crop or forest stands. Poor fruit and seed production can be the result of inbreeding, not lack of pollinators. The common practice of plantations based on seeds from very few trees or even clonal orchards should be analyzed carefully since this may lead to genetic erosion, low productivity and inbreeding depression. It is possible that premature fruit drop in cashew is also related to genetic causes.

Current practices of natural stand exploitation for timber, pharmaceuticals, rubber, fruit, seeds, and dyes may be reducing genetic variability and gene flow to levels that permanently affect the viability of populations. Some studies from Brazilian economically important species are available. Recent data (Peres *et al.*, in press) from 23 populations throughout the Brazilian, Bolivian and Peruvian Amazon have shown that the historical levels of exploitation of Brazil nuts (*Bertholletia excelsa*, Lecythidaceae) have a major impact on recruitment into natural populations. Populations subjected to moderate and high levels of harvest over many decades lack juvenile trees; in contrast, only populations with a history of light, recent or no exploitation contain large numbers of juvenile trees.

At present, there are still few laboratories in Brazil (governmental or private) that are involved in genetic studies of native plant populations. We are hopeful that in future years this situation is solved, since many laboratories are nowadays well equipped and their personnel trained for this task.

HABITAT FRAGMENTATION AND REDUCTION OF GENETIC DIVERSITY

Some Brazilian biomes have undergone extensive clearing, notably for agricultural purposes, in the past decades. This has resulted in the inclusion of the *cerrado* (savanna) in the latest list of “hotspots” (threatened world regions that should be given priority in biodiversity programs). This biome is home to several commercially important plant species that are intensively used by the locals.

A typical case of plant populations already suffering from habitat fragmentation is that of *Caryocar* (Caryocaraceae) species. The piqui (*C. brasiliense*) from the Central Brazil *cerrado* vegetation, and the piquiá (*C. villosum*) from the Amazonian forest yield fruits and seeds which are an excellent source of edible oil. Pollination studies in both species (Gribel and Hay, 1993; Martins, 2002) showed a moderate degree of self-compatibility, and pollination by bats and sphingid moths. Habitat and roost disturbance may affect the populations of these sensitive pollinators. Genetic data for *C. brasiliense* from 10 microsatellite *loci* indicated a high level of biparental inbreeding, which could be attributed to the limited flight range of its pollinators and restricted seed dispersal. Habitat fragmentation would isolate populations and their pollinators, aggravating the scenario of fruit overexploitation (Collevatti *et al.*, 2001).

In the case of mahogany (*Swietenia macrophylla*, Meliaceae), the most valuable neotropical timber species, habitat degradation caused by selective logging and, most importantly, by conversion of forest into soybean plantations and cattle ranch pastures with recurrent use of fire have clearly reduced local population sizes and led many populations to local extinction (Grogan, 2001). Recent studies using polymorphic microsatellite markers (Lemes *et al.*, 2003) suggest that the small, isolated, remnant populations may not constitute viable units in the long term owing to the loss of genetic variation caused by genetic drift and inbreeding.

Brazilian legislation has contemplated the habitat loss problem by establishing, among other protection measures, that a percentage of uncultivated land be maintained in private properties (known as *reserva legal*) in Brazilian biomes such as the Amazon forest and *cerrado*. These areas are important both to sustain ecological services such as pollination, and to maintain gene flow and diversity of native plant species by behaving as corridors between fragments. There is, however, intense debate nowadays concerning the reduction of those percentages. Attempts to change the law decreasing the proportion of these reserves should be contested using scientific and economic arguments. In addition to habitat preservation, habitat rehabilitation programs might benefit from the introduction and management of pollen and seed dispersers. This action is a cheap alternative that could increase gene flow among fragments and accelerate rehabilitation.

POLLINATOR-MEDIATED GENETIC CONTAMINATION

It is known that crops easily hybridize with wild relatives (Ellstrand *et al.*, 1999). The visiting patterns of pollinator forage can create crop-to-wild and crop-to-crop pollen exchange. If genetically modified varieties (GMO) are involved, there is the risk that non-target plants (wild relatives or conventional crop varieties) acquire the characteristics of resistance to herbicides or pesticides through pollen-mediated gene flow and turn into unmanageable weeds (the so-called “superweeds”). The occurrence of agricultural weeds from GMO crop releases has already been reported. In Canada,

the presence of unwanted herbicide-resistant canola (*Brassica napus*, bee pollinated) is becoming an agricultural nuisance (Simard *et al.*, 2002).

Other genetically engineered varieties (modified to resist attack by insects), such as sunflower (*Helianthus annuus*, bee pollinated), and papaya (*Carica papaya*, pollinated by bees, birds and moths) have also shown enhanced fitness. They are less attacked by insects (moths) and in turn produce more seeds that are themselves resistant to insect attack. This suggests that non-managed populations may in turn accelerate development of resistant insect pests. It is evident that to maintain the utility of herbicides and pesticides in agriculture (i.e. to reduce the risk of hastening the development of superweeds) agricultural practices which include herbicide management (such as rotation and combination with other actions) are mandatory.

Existing containment measures are mostly designed to assure seed purity levels, and may not be adequate for preventing or, more realistically, reducing gene escape from GMO crops (Kareiva *et al.*, 1994). Physical barriers such as bare land or non-GMO crops around the target variety have been used to prevent pollen contamination, but have been inefficient in many cases, partly because of lack of knowledge on the dynamics of pollen flow in each case.

If reducing the risk of contamination is a main concern, then the choice of managed pollinators of a given crop (most notably if GMO) should weigh not only commercial aspects but environmental safety as well. The risk of gene escape to non-target species is related to behaviour of the pollinator, such as flight range and its effectiveness as pollinator, which vary according to aspects associated both to the pollinator and crop characteristics. Purity standards should be stricter in cases of gene escape risk than in cases of seed purity, more so in centres of diversity such as the tropics, where traditional varieties, including progenitors, may disappear.

In addition, environmental monitoring actions focusing on gene escape should give priority to high-risk crops. These are those with little domestication (that is, that are still ecologically and reproductively similar to wild relatives), that grow sympatrically with wild relatives or cross-compatible domesticated species, which can turn into weeds themselves, and those whose commercialization requires that the crop blooms or sets fruits/seeds. In Brazil, cotton would be one of the best candidates.

RECOMMENDATIONS

CROP AND SILVICULTURE MANAGEMENT

1. Commonly accepted (“common wisdom”) practices of crop pollination should be re-evaluated to extend the knowledge of the mechanisms of pollination, and of pollinator role and benefits.

Case studies have shown that fruit yield is improved with pollinator service in crops where pollinators had traditionally been considered expendable.

PROPOSED METHODS:

- standard breeding system tests and exclusion experiments
- pollination syndrome
- pollinator behaviour in flower and among plants

- statistical comparison of productivity parameters between traditional methods and hand cross-pollination experiments
2. Traditional genetic improvement methods such as by phenotype selection of tree crops should be re-evaluated.
- Alleged low productivity of some plantations may be due to inbreeding depression, not lack of pollinators.

PROPOSED METHODS:

- molecular techniques (microsatellite, allozymes) to evaluate genetic diversity and dynamics: inbreeding depression, levels of outcrossing, pollen flow, cross-compatibility between varieties of cultivars and clones
 - pollinator behaviour in flower and among plants
 - statistical comparison of productivity parameters between cultivars
3. Traditional methods of exploitation of timber and NTFP (non-timber forest products) should be re-evaluated
- Natural stand exploitation practices may be reducing genetic variability and gene flow.

PROPOSED METHODS:

- molecular techniques (microsatellites) to evaluate genetic diversity, mating systems, and gene flow
 - observation of flight patterns of each pollinator species
4. GMO crops with geographically close wild relatives should receive priority in environmental impact assessment actions.
- Crops such as cotton and corn may hybridize with wild relatives and these may become “superweeds”.

PROPOSED METHODS:

- paternity analysis (microsatellites)
 - investigate time (phenology) or biological barriers (common pollinators)
 - observation of flight patterns of each pollinator species
5. Containment measures should be tested and proposed in the case of any crop that has a risk of gene escape or has to maintain its purity.
- Existing containment measures are still under development and have shown to be inefficient in many cases.

PROPOSED METHODS:

- test and adoption of physical barriers that discourage pollinator flight
- observation of flight patterns of each pollinator species
- paternity analysis (microsatellites)
- production of gene flow curves

POLLINATOR CONSERVATION

1. The percentage of legally determined uncultivated land in private properties (known as “reserva legal”) in Brazilian biomes such as the Amazon forest and “cerrado” (savanna) should be maintained and enforced by law.

These areas are important both to sustain ecological services such as pollination and to maintain gene flow and diversity of native plant species.

PROPOSED METHODS:

- molecular techniques (microsatellite, allozymes) to evaluate genetic diversity and gene flow
 - statistical comparison of reproductive parameters of native plants in isolated versus connected forest fragments
 - legal actions
2. Exotic plants or pollinators should be priority in long-term, in-depth monitoring programs, to detect overall impact in the ecosystem.

The invasiveness of an exotic plant may be benefited by a native pollinator, or the exotic pollinator may outcompete native species for resources and reduce productivity, or regeneration patterns of native plants.

PROPOSED METHODS:

- observation of pollinator behaviour in flower and among plants
 - survey of pollinators in the area
 - monitoring of the invasive plant spread
3. Pollen and seed dispersal by animals should be encouraged in habitat rehabilitation programs

Their action enhance seed set and accelerates the rehabilitation process, besides being a cheap alternative.

PROPOSED METHODS:

- introduction of meliponiculture in rehabilitation areas
- introduction of nesting and roosting places

4. Native species cultivation in urban areas, instead of exotics, should be preferred and encouraged, as well as urban ecological interactions among native plants and their pollen and seed dispersers.

Their use in public parks will preserve native plant-pollinator interactions, aid in the conservation of genetic diversity, and promote awareness of native species by the public.

PROPOSED METHODS:

- promote urban ecology awareness programs
- distribute material (specimens, seeds) and know-how among the authorities responsible for urban green areas
- introduce nesting and roosting places, and artificial nests for bees

OTHER RECOMMENDATIONS

1. National programs should be promoted to increase public awareness on the need for pollination studies and conservation.

Efforts should be made to create awareness of the services to humanity performed by pollinators, to change the perception of a variety of animals whose important service as pollinators is currently unknown to the public, so as to reduce the pressure on this group caused by pesticides, deforestation and others.

PROPOSED METHODS:

- list flagship plant species and explain their need for pollinators
- stress the beneficial role of non-charismatic animals, such as bats
- invent popular, appealing names for pollinators
- promote courses on the use of urban green areas
- produce and distribute user-friendly, informative material

2. Leading laboratories should be encouraged to participate in the mapping of genetic diversity and structure of native plant populations of economic importance.

Currently many laboratories throughout Brazil are well equipped for the task, but few of them are involved in studying these organisms.

PROPOSED METHODS:

- cooperative training courses
- government funding through public calls
- propose charismatic national or local species to obtain private or public funds

3. Government agencies should produce, support and made widely available user-friendly material (printed, electronic, training courses) such that the above recommendations are put to practice by final users.

There are currently many comprehensive studies of economically important plants and pollinators whose results are unknown to the final user because of restrict (academic, technical) circulation. It is important that the initiative of producing this material be officially sponsored and supervised by scientists so as to gain credibility.

PROPOSED METHODS:

- books could be transformed into PDF with the author's consent and be made available at official sites linked to agriculture, such as FAO, and EMBRAPA (Brazil)
- production and distribution of user-friendly, informative material such as leaflets or booklets
- training courses

METHODS

Explanations of some recommended techniques follow, with emphasis on examples of Brazilian study cases. Among the methods proposed to help perform the recommendations, several rapid assessment protocols (RAP) could be developed, grouped broadly into two: botanical (phenology, flower density, pollination syndromes, plant breeding systems) and pollinator (bee behaviour in the flower and among plants, flight range) RAPs. There is a vast literature on the parameters to be considered within each of these two, and how to measure them. These protocols will allow data collection in the field by people with little or no formal biological training. Other methods, including statistical analyses, require both expertise and specific facilities, and will be usually performed by researchers and trained personnel.

GENETIC DIVERSITY

Isozyme electrophoresis is a technique for measuring the rate and direction of movement of organic molecules (in this case, enzymes) in response to an electric field (Alfenas *et al.*, 1998; Pinto *et al.*, 2001). The rate and direction of enzyme movement in a starch or and agar gel will depend on the enzyme's net surface charge, size and shape. Enzymes can then be stained, resulting in a series of bands in the gel. Those enzymes that migrate to the same place in a slab of agar or starch gel and yield similar banding patterns when stained are considered to represent homologous enzymes (isozymes). When the isozymes are controlled by alleles of one gene, they are called allozymes. The banding patterns of specific types of enzymes in the gel may vary from plant to plant. Allozyme electrophoresis is most useful to analyze genetic diversity and outcrossing rate (the proportion of the progeny generated from cross-pollination) of populations within species. As a codominant marker, allozymes may directly identify heterozygous genotypes. Heterozygosity indices may be easily calculated for populations or samples of plants.

The visualization of electrophoretical patterns of isozymes requires simple procedures, since isozyme bands are obtained through the reaction that identifies the enzyme. The technique of isozyme electrophoresis is simple to learn, cheaper and involves faster procedures than DNA markers.

This method has been used in a number of studies on the genetic diversity and population structure of Brazilian species, such as the commercially important palmheart (*Euterpe edulis*, Conte *et al.*, 2003), rubber (*Hevea brasiliensis*, Yeang & Chevallier, 1999), and “cagaita” (*Eugenia dysenterica*, Telles *et al.*, 2001). However, some native and most cultivated plants have shown either very low or no isozyme variability. In this case, molecular techniques can provide higher number of markers than isozymes.

DNA markers, on the other hand, show a higher number of alleles per locus and can be more useful and accurate than isozymes. Higher number of markers can give more accurate genetic diversity indices and paternity analysis. However, most markers, such as random amplified polymorphic DNA (RADP) and amplified fragment length polymorphism (AFLP), are dominant and cannot show heterozygous genotypes. In some case, however, the high sensitivity of these techniques may limit the detection of the same markers among individuals genetically divergent. Molecular markers requires specialized training and more sophisticated and expensive equipment, since their protocols are more elaborated. Those techniques are also more expensive than isozymes, but their prices are dropping (Ferreira & Grattapaglia, 1996).

Microsatellites are stretches of DNA that consist of tandem repeats of a simple sequence of nucleotides. These repeats can be easily amplified using PCR (polymerase chain reaction). The number of repeat units that an individual has at a given locus can be easily resolved using polyacrylamide gel electrophoresis. From the gels, we can see two genetic marks for most individuals; each individual inherits one length of nucleotide repeats from its mother and one from its father (individuals with one band received the same band from both their mother and their father). Primers to the microsatellite flanking regions can be labelled with fluorescent dyes, allowing the amplified products to be separated in a polyacrylamide gel electrophoresis on an automated DNA Sequencer.

In the last decade, microsatellites or simple sequence repeats (SSR) markers have become an attractive tool for population genetic studies in plants due to their co-dominant inheritance, high allelic diversity and their abundance in plant genomes. The variability observed at SSR loci allows the accurate discrimination of individuals in natural populations and the estimation of genetic parameters such as levels of inbreeding, heterozygosity, gene flow and mating system, relevant aspects for the genetic conservation and management of tropical trees under intensive human pressure. Microsatellite markers have recently been developed for a number of tropical tree species such as the edible piqui (Collevatti *et al.* 1999) and palmheart (Gaiotto *et al.*, 2001), and the timber species mahogany (*Swietenia macrophylla*, Lemes *et al.*, 2002, 2003), “anani” (*Symphonia globulifera*, Aldrich *et al.* 1998), “andioba” (*Carapa guianensis*, Dayanandan *et al.* 1999), and “angelim-vermelho” (*Dinizia excelsa*, Dick & Hamilton 1999).

PLANT REPRODUCTIVE BIOLOGY AND POLLINATOR BEHAVIOUR

The importance of pollinator visits to a plant species depends on its breeding system. Two aspects are usually evaluated through experimental manipulation: self-compatibility and self-pollination.

The first evaluates if a flower receiving pollen from the same plant is capable of producing viable seeds, and to which degree. If the species is self-incompatible, then it will need to be visited by pollinators that bring along pollen from a second plant to effect cross-pollination. Dioecious plants are obligate outcrossers. To test for self-compatibility, manual self- and cross-pollination experiments are performed on flowers, and the results (usually fruit and seed set) are then compared to control (unmanipulated) flowers. The difference in fruit or seed set also indicates if natural pollination is deficient in a population. If this is the case, further observations should

follow to see if low fruit or seed set is caused by reduced number of visits or by their quality. Poor quality visits are related to visitors who do not perform pollination (thieves, for example), or who deposit the wrong kind of pollen (self-pollen if the plant is self-incompatible, or pollen from other species if the pollinator carries pollen from other plant species). If the plant is self-compatible, then it might not need the help of pollinators to set seeds. This is usually checked by bagging buds to exclude visitors and then verifying fruit and seed set. These procedures are standard and well explained in a number of books (Dafni, 1992; Kearns & Inouye 1993; Proctor *et al.*, 1996). In addition, data on commercially important parameters may be measured and compared among treatments, such as colour, weight, shape, size and nutrient contents of the fruits.

Different pollinators respond to resource landscapes and this in turn has consequences on the extent of pollen dispersal (Bronstein 1995). Foraging flights may vary according to the homogeneity of resources (crop vs. natural environments), plant spacing (Manasse, 1992; Morris *et al.*, 1994; Morris 1993), and their flight range (Jacobi, 2000, Turchin 1998), among others. Several statistical and mathematical methods have been used to compare flight behaviour of pollinators, particularly insects. They rely on field data that involve tracking techniques such as telemetry for vertebrates, the use of dyes and the direct observation and mapping of flight trajectories in some cases (Turchin 1998). These flight path analyses are sometimes compared with gene flow curves using marked pollen or, if available, genetically marked seeds (Kareiva *et al.*, 1994). All the above procedures are the basis for pollen flow estimation, and are useful for establishing actions concerning gene escape, contamination risk, and plant population isolation.

References

- ALDRICH P.R., HAMRICK J.L., CHAVARRIAGA P., KOCHERT G. 1998. Microsatellite analysis of demographic genetic structure in fragmented populations of the tropical tree *Symphonia globulifera*. *Molecular Ecology* 7:933-944.
- ALFENAS, A. C.; PETERS, I.; BRUNE, W.; PASSADOR, G. C. 1998. Eletroforese de proteínas e isoenzimas de fungos e essências florestais. Universidade Federal de Viçosa.
- BRONSTEIN, J.L., 1995. The plant-pollinator landscape. In: L. Hansson, L. Fahrig e G. Merriam (eds.). *Mosaic landscapes and ecological processes*. Chapman and Hall, London.
- COLLEVATTI R.G., BRONDANI R.V., GRATTAPAGLIA D. 1999. Development and characterization of microsatellite markers for genetic analysis of a Brazilian endangered tree species *Caryocar brasiliense*. *Heredity* 83:748-756.
- COLLEVATTI, R.G.; GRATTAPAGLIA, D.; HAY, J.D. 2001. High resolution microsatellite based analysis of the mating system allows the detection of significant biparental inbreeding in *Caryocar brasiliense*, an endangered tropical tree species. *Heredity* 86:60-67.
- CONTE, R.; NODARI, R.O; VENCOVSKY, R.; DOS REIS, M.S. 2003. Genetic diversity and recruitment of the tropical palm, *Euterpe edulis* Mart., in a natural population from the Brazilian Atlantic Forest. *Heredity* 91:401-406.

- COUTO, R.H.N. 2002. Plantas e abelhas, uma parceria em crise? IN: Anais do V Encontro sobre Abelhas, pg. 87-94.
- DAFNI, A. 1992. Pollination ecology. A practical approach. Oxford University, New York, 250 pp.
- DAYANANDAN S., DOLE J., BAWA K., KESSELI R. 1999. Population structure delineated with microsatellite markers in fragmented populations of a tropical tree, *Carapa guianensis* (Meliaceae). *Molecular Ecology* 10:1585-1592.
- DICK C.W., HAMILTON M.B. 1999. Microsatellites from the Amazonian tree *Dinizia excelsa* (Fabaceae). *Molecular Ecology* 8:1753-1768.
- ELLSTRAND, N.C.; PRENTICE, H.C.; HANCOCK, J.F. 1999. Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics* 30:539-563.
- FERREIRA, M. E. & D. GRATTAPAGLIA 1996. Introdução ao uso de marcadores moleculares em análise genética. Perkin Elmer, S. Paulo.
- GAIOTTO F.A.; BRONDANI R.P.V.; GRATTAPAGLIA D. 2001. Microsatellite markers for Heart of Palm - *Euterpe edulis* and *E. oleracea* Mart. (Arecaceae). *Molecular Ecology Notes* 1/2:86-88.
- GRIBEL, R. & HAY, J. D. 1993. Pollination ecology of *Caryocar brasiliense* (Caryocaraceae) in Central Brazil cerrado vegetation. *Journal of Tropical Ecology* 9:199-211.
- GROGAN, J.E. 2001. Bigleaf mahogany (*Swietenia macrophylla* King) in southeast Pará, Brazil: a life history study with management guidelines for sustained production from natural forests. Ph.D. thesis. Yale University School of Forestry & Environmental Studies, New Haven, CT, USA.
- HOLANDA-NETO, J.P.; FREITAS, B.M.; BUENO, D.M.; ARAUJO, Z.B. 2002. Low seed/nut productivity in cashew (*Anacardium occidentale*): effects of self-incompatibility and honey bee (*Apis mellifera*) foraging behaviour. *Journal of Horticultural Science and Biotechnology* 77:226-231.
- JACOBI, C.M. 2000. Modelamento de rotas de visitação. IN: Ecologia e Comportamento de Insetos, R.P. Martins, T.M. Lewinsohn e M.S. Barbeitos (eds.), *Oecologia Brasiliensis*, v. VII, p. 39-58, PPGE-UFRJ, Rio de Janeiro.
- KAREIVA, P.M.; MORRIS, W.F.; JACOBI, C.M. 1994. Studying and managing the risk of cross-fertilization between transgenic crops and wild relatives. *Molecular Ecology* 3:15-21.
- KEARNS C. A. & INOUE D. W. 1993. Techniques for pollination biologists. Colorado University Press, Niewot.
- LEMES M.R.; BRONDANI R.P.V.; GRATTAPAGLIA D. 2002. Multiplexed systems of microsatellite markers for genetic analysis in mahogany, *Swietenia macrophylla* King (Meliaceae), a threatened Neotropical timber species. *Journal of Heredity* 93:287-290.
- LEMES, M. R., GRIBEL R., PROCTOR J. & GRATTAPAGLIA D. 2003. Population genetic structure of mahogany (*Swietenia macrophylla* King, Meliaceae) across the Brazilian Amazon, based on variation at microsatellite loci: implications for conservation. *Molecular Ecology* 12: 2875- 2883.

- MANASSE, R. 1992. Ecological risks of transgenic plants: effects of spatial dispersion on gene flow. *Ecological Applications* 2:431-438.
- MARTINS, R. L. 2002. Polinização, Sistema de Cruzamento e Fluxo Gênico em *Caryocar villosum* (Aubl.) Pers. (Caryocaraceae), uma Árvore Emergente da Floresta Amazônica. Dissertação de Mestrado, Curso de Ecologia, Programa de Pós-graduação em Biologia Tropical e Recursos Naturais Renováveis, INPA-UA, 63 pp.
- MORRIS, W.F. 1993. Predicting the consequences of plant spacing and biased movement for pollen dispersal by honey bees. *Ecology* 74:493-500.
- MORRIS, W.; KAREIVA, P. & RAYMER, P. 1994. Do barren zones and pollen traps reduce gene escape from transgenic crops? *Ecological Applications* 4:157-165.
- PERES, C. A., BAIDER, C., ZUIDEMA, P. A., WADT, L. H. O., KAINER, K. A., GOMES-SILVA, D. A. P., SALOMÃO, R., SIMÕES, L. L., FRANCIOSI, E. R. N., VALVERDE, F. C., GRIBEL, R., SHEPARD JR., G., KANASHIRO, M., CONVENTRY, P., YU, D. W. WATKINSON, A. R. & FRECKLETON, R. P. (in press). Demographic threats to the sustainability of Brazil nut exploitation. *Science*.
- PINTO, L. R.; VIEIRA, M. L. C.; SOUZA, A. P.; JUNIOR, C. L. S. 2001. Isoenzimas e microssatélites em plantas. *Biotecnologia, Ciência & Desenvolvimento* 20: 16-19.
- PROCTOR, M.; YEO, P.; LACK, A. 1996. The natural history of pollination. HarperCollins, London.
- ROUBIK, D.W. (ed.) 1995. Pollination of cultivated plants in the tropics. *FAO Agricultural Services Bulletin* 118, Rome.
- ROUBIK, D.W. 2002. The value of bees to the coffee harvest. *Nature*: 417:708.
- SIMARD, M.J.; LEGERE, A.; PAGEAU, D.; LAJEUNESSE, J.; WARWICK, S. 2002. The frequency and persistence of volunteer canola (*Brassica napus*) in Quebec cropping systems. *Weed Technology* 16:433-439.
- TELLES, M.P.D.; SILVA, R.S.M.; CHAVES, L.J.; COELHO, A.S.G.; DINIZ, J.A.F. 2001. Divergence among local populations of *Eugenia dysenterica* in response to edaphic patterns and spatial distribution. *Pesquisa Agropecuária Brasileira* 36:1387-1394.
- TURCHIN, P. 1998. Quantitative analysis of insect movement. Sinauer, Massachusetts.
- YEANG, H. Y.; CHEVALLIER, H. M. 1999. Range of *Hevea brasiliensis* pollen dispersal estimated by esterase isoenzyme markers. *Annals of Botany* 84: 681-68.