European commission DG RESEARCH
INCO-DEV, International role

Sustainable management of Neo -Tropical Tree Genetic Resources: Combining molecular and modelling methods to understand the structure and dynamics of gene diversity

GENEO-TROPECO

Final Scientific Report February 2002 – January 2006

	Major target	Additional target
Research programme	Systems research on natural capital and the human environment	Tools for sustainable development
Key action	Strategies for rural productivity; ecosystem management for sustainability (b.i)	Technologies for sustainable crop and animal production: building blocks for improvement. Cash crops and forestry (c.ii-1)
Region code	LAM	
Contract Number	Project number: ICA4-CT-2001-10101	
Project homepage	http://www.edinburgh.ceh.ac.uk/geneo	

Table of Contents

Project Identification

Title:	Sustainable management of Neo-Tropical Tree Genetic Resources: Combining molecular and modelling methods to understand the structure and dynamics of gene diversity
Acronym:	GENEO-TROPECO
Contract type:	Shared cost RTD
Contract Number:	ICA4-CT-2001-10101
Project cost:	1 332 183 € (EC contribution 900 000 €)
Duration:	48 months
Period:	01.02.02 – 31.01.06
Key words:	Genetic-resources, forest, management, molecular, modelling
Project website:	http://www.edinburgh.ceh.ac.uk/geneo

Consortium

Coordinator
NERC Centre for Ecology and Hydrology, CEH-Edinburgh, Bush Estate, Penicuik, Midlothian EH26 0QB, UK

Stephen Cavers
Tel: (+44) (0) 131 445 4343.
Fax: (+44) (0) 131 445 3943.
Email: scav@ceh.ac.uk

Partner 2 (Contractor):
Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Turrialba, 7170, Costa Rica.

Carlos Navarro
Tel: (+506) 556 6431.
Fax: (+506) 556 1533.
Email: cnavarro@catie.ac.cr

Partner 3 (Contractor):
INRA Laboratoire de Genetique et d'Amelioration des Arbres Forestières (French Guiana), PO Box 45, Gazinet Cestas, France.

Henri Caron
Tel: (+33) 5 57 97 90 00.
Fax: (+33) 5 57 97 90 88.
Email: henri.caron@pierroton.inra.fr

Partner 4 (Contractor):
Instituto Nacional de Pesquisas da Amazônia (INPA), Avenida André Araújo, 2936, 69083-300 Manaus, Brazil.

Maristerra Lemes
Tel/Fax: (+55) 92 6433112.
Email: mlemes@inpa.gov.br

Partner 5 (Contractor):
Universidade Federal do Rio de Janeiro (UFRJ) Laboratorio de Genetica Molecular Vegetal, CCS - Ilha do Fundao - Instituto de Biologia, CEP 21944-270 - Rio de Janeiro – Brasil.

Rogerio Margis
Tel and Fax: (+55) 21 590 01 11.
Email: rogerio.margis@ufrgs.br

Partner 6 (Contractor):
Institute for Plant Biotechnology for Developing Countries (IPBO), Department of Molecular Genetics, Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium.

Godelieve Gheysen
Tel: (+32) 9 264 87 27.
Fax: (+32) 9 264 87 95.
Email: Godelieve.Gheysen@rug.ac.be

Sustainable management of Neo-Tropical Tree Genetic Resources: Combining molecular and modelling methods to understand the structure and dynamics of gene diversity (ICA4-CT-2001-10101)

Final Scientific Report

Authors

Coordinator: S Cavers, K Walker, RC Munro, S Davies, AJ Lowe.
NERC Centre for Ecology and Hydrology, UK.

Partner 2 (Contractor): C Navarro, B Finegan, G Hernandez, C Cascante, L Coto, P Madriz, V Herra.
Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Costa Rica.

Partner 3 (Contractor): H Caron, A Kremer, B Degen, E Bandou
Institut National de Recherches Agronomique (INRA) France.

Partner 4 (Contractor): M Lemes, R Gribel, T Andre, T Martiniano, A Queiroz, M da Gloria P Assis.
Instituto Nacional de Pesquisas da Amazônia (INPA), Brazil.

Partner 5 (Contractor): R Margis, M Margis, F Salgueiro, M Ferreiro, F Scarano
Universidade Federal do Rio de Janeiro (UFRJ) Brasil.

Partner 6 (Contractor): G Gheysen, N Colpaert, T Kyndt, D Broucke
Institute for Plant Biotechnology for Developing Countries (IPBO), Belgium.

Speakers, InBio workshop, San Jose, Costa Rica, Oct 2005. L-R: G. Gheysen, T Kyndt, R. Gribel, C. Cascante, M. Lemes, K. Walker, H Caron, S Davies, A Lowe, G Hernandez, S Cavers, C Navarro, B Finegan

Abstract

Original Objectives:

- Examine the structure and dynamics of genetic variation for a range of economically and ecologically important Central and South American tree species within natural ecosystems and identify the main factors that are responsible for partitioning of variation within species
- Examine the impact of identified extraction methods/habitat degradation (i.e. fragmentation, logging, forest clearance and domestication) on selected economically important species
- Produce a model tailored to the individual study species that will integrate field observations and DNA-based technologies to provide realistic simulations of the impact of differing land-use strategies and extraction regimes on the genetic resource base of impacted species
- Improve capacity to execute sound natural forest management by improving awareness of genetic implications of natural forest management and implementation of a modelling approach to setting sustainability strategies

Results and Milestones:

Development of molecular techniques

Several new microsatellite primers were developed in a range of species; DNA extraction methods for target species were optimised; AFLP protocols were optimised and applied across the range of target species. Practical measures to maximise comparability of AFLP datasets between labs were designed, and standardised scoring and analysis techniques developed for the analysis stages. Several aspects of the methodological development were published in peer-reviewed journals.

Identifying biological determinants of genetic diversity

For a list of 50 target species collections and information on biological characteristics were prepared, following protocols from WP1. All target species were analysed using AFLPs and a meta-analysis conducted. Within the timescale of the project only preliminary analysis was possible but this indicated significant effects of pollination and seed dispersal mechanism on population differentiation and within-population genetic diversity respectively. Geographic distribution was not found to have any effect.

Effect of human-mediated processes on genetic diversity

Substantial case studies were carried out examining the effects of human-mediated processes on genetic diversity in *Pinus oocarpa*, *Araucaria angustifolia*, *Swietenia macrophylla* (Brazil and Belize), *Vochysia ferruginea, Symphonia globulifera* and *Theobroma grandiflorum.* Important outcomes of the case studies in general showed the impact of population modification on tree mating systems (and hence genetic diversity maintenance), the scale of real gene movement within tree populations, the significance of population densities for gene flow patterns and highlighted potential forest management strategies to mitigate the genetic diversity impacts of harvesting.

Simulation modelling of population genetic dynamics

The model, ECO-GENE, was adapted for use with the genetic datasets derived from case studies. In particular, a new module was designed for the model allowing sensitivity analysis to be performed. Simulations using empirical data from studies of *Symphonia globulifera* and *Swietenia macrophylla* were conducted. These studies highlighted the necessity for preparation of site-specific plans, and the importance of population density and the interaction between demography and growth and gene dispersal capability for capture and maintenance of genetic diversity in populations.

Designing Management strategies to maximize diversity

Both scientific and non-technical communications were achieved during the project lifetime. Amongst other individual peer-reviewed papers, special issues of the journals *Heredity* (Nature Publishing Group) and *Silvae Genetica* were produced. Further paper writing and publication is ongoing. Additional dissemination of project outputs was achieved through preparation of a board game for schools and colleges and a high-level public workshop for the biodiversity conservation sector (in San Jose, Costa Rica), including attendees from the government's Biodiversity Conservation Commission as well as a range of other public and private bodies with interests in the field.

Final Scientific Report: Summary

Overall objectives

Tropical forests are complex ecosystems, and their management often involves the sustainable exploitation of a range of resources, including non-timber products (e.g. fruits and nuts, medicines etc). Genetic diversity represents an essential component promoting population level adaptation ensuring the continued proliferation of individual species within tropical systems. Reduced genetic diversity can lead to loss of adaptive variation and inbreeding depression, both of which can threaten the long-term survival of isolated populations. Many tropical species are currently extracted at unsustainable levels or their habitats are being degraded, threatening the long-term survival of species within this ecosystem. Whether harvested from natural or managed landscapes, there is a need to develop a practical, operational system concerned with the management of genetic sustainability.

In this proposal, we present a programme of research aimed at measuring key genetic indicators of sustainability in tropical forest ecosystems. Our aim is to evaluate the level and dynamics of genetic diversity in natural forest populations. The sustainability of current management practices will be assessed in selected extracted species using computer simulation of field genetic data. Specific sustainable extraction/management strategies arising from this process will be promoted to forestry stake holders, who will be made more aware of the genetic implications of management. To aid this process it is necessary to establish criteria and indicators. An important criterion already identified for genetic diversity is conservation of the processes that maintain genetic variation (Namkoong *et al*., 1996), for which four indicators have been identified: 1. levels of genetic variation, 2. mating system processes, 3. directional change in gene or genotype frequencies and 4. gene migration between populations. However there are three additional issues that need to be considered for genetic diversity in tropical ecosystems. 1. The formulation of genetic criteria and indicators depend on understanding of the processes we are proposing to research. 2.These criteria and indicators are many, complex and interacting, and that therefore a modelling approach is appropriate. 3. There are still many knowledge gaps that need to be filled before a model produces realistic information, these can be examined further through integrated field and laboratory work.

The project had the following general objectives:

- to examine the structure and dynamics of genetic variation for a range of species within natural ecosystems and identify the main factors that are responsible for the partitioning of variation within a range Central and South American forest tree species
- to examine the impact of identified extraction methods/habitat degradation on selected economically important species
- to produce a model that will integrate field observations and DNA-based technologies to provide realistic simulations of the impact of differing land-use strategies and extraction regimes on the genetic resource base of impacted species.
- to improve capacity to execute sound natural forest management by improving awareness of genetic implications of natural forest management and implementation of a modelling approach to setting sustainability strategies.

Work Package 1 - Development of DNA techniques

Objectives

- Optimise collection of field material and DNA extraction for all species
- Optimise AFLP procedure for all species involved in WP2
- Develop new SSR loci for *Araucaria angustifolia*
- Optimise existing SSR loci and develop new ones for *Swietenia macrophylla*, *Vochysia ferruginea*, *Symphonia globulifera* and *Theobroma* species
- Optimise molecular methodology for *Pinus* species
- Develop methods of analysis for all partners and make analysis software available

Activities

- Project website established http://www.nbu.ac.uk/geneo/
- Collection and DNA extraction protocols tested and optimised
- AFLP protocols tested, optimised and standardised across labs
- Existing SSR loci optimised and new SSRs developed for 5 species

Results & conclusions

- Sampling (plot layout, tissue sampling) and DNA extraction procedures optimised
- AFLP analysis procedure optimised for target species
- New SSR loci developed and published for:
    - *Araucaria angustifolia*
    - *Swietenia macrophylla*
    - *Vochysia ferruginea*
- Existing SSR loci optimised for :
    - *Theobroma grandiflorum*
    - *Symphonia globulifera*
- Five peer-reviewed publications produced detailing methods and markers

Work Package 2 - Identifying biological determinants of genetic diversity

Objectives

- Determine correlation between life-history and ecological characteristics of tree species and patterns of structure of genetic variation amongst populations
- Classify tree species according to their predicted vulnerability to population declines in logged and fragmented forests

Activities

- 50 target species selected for AFLP meta-analysis
- Collections for all species prepared using standardised sampling design from WP1
- Additional data (life history, distribution, genome size etc) gathered for all species
- Production of AFLP marker datasets for all species
- Meta-analysis of AFLP datasets

Results & conclusions

- For preliminary meta-analysis of 20 datasets:
    - No significant effect of geographic range on genetic diversity or differentiation
    - Genetic diversity significantly correlated with seed dispersal mechanism
    - Population differentiation correlated with pollination syndrome

Work Package 3 - Effect of human-mediated process on genetic diversity

Objectives

- Examine the impact that human-mediated disturbance and fragmentation processes have on the dynamics of genetic diversity through specific case studies.
- Identify the best management strategies for each individual case study to maximise the genetic resource base of the species within an exploited/disturbed environment

Activities

- *Pinus oocarpa*: analysis of impact of fragmentation on mating system completed using morphological markers
- *Swietenia macrophylla*:
    - Brazil - analysis of the impact of logging on genetic diversity and mating system using SSR markers
    - Belize - analysis of impact of logging and variable density on mating system and pollination distances using SSR markers

- *Araucaria angustifolia* : analysis of the impact of fragmentation on genetic diversity and gene flow using SSR markers
- *Symphonia globulifera* : analysis of spatial genetic structure, gene flow and mating system with respect to logging vulnerability using SSR markers
- *Vochysia ferruginea* : analysis of colonisation and regeneration dynamics of a pioneer tree species using SSR markers
- *Theobroma grandiflorum* : comparison of genetic diversity in wild and cultivated populations and analysis of the impact of domestication using SSR markers.

Results & conclusions

- *Pinus oocarpa*: the isolation by distance caused by forest management and deforestation, increases levels of self-fertilization and therefore inbreeding depression
- *Swietenia macrophylla*:
    - Brazil:
        - Genetic diversity significantly lower in seedlings following logging
        - Population showed no spatial genetic structure
        - Pollination distances substantial (mean of 1610 m) potentially due to reduced density caused by logging
    - Belize:
        - Genetic diversity levels similar, but slightly lower than South American populations
        - Populations showed strong spatial genetic structure
        - Logged plots showed higher levels of correlated mating than unlogged plots
        - Local density of conspecifics correlated with number of pollen donors
        - Mean pollen dispersal distance across plots was 371.15 m
- *Araucaria angustifolia* :
    - Genetic diversity higher within population than among populations
    - Despite fragmentation, the species still presents an intermediate level of genetic diversity
    - Differentiation and genetic structure were found among populations from South and Southeastern of Brazil
    - Genetic structure found in two out of three populations analyzed at the local level: São Francisco de Paula e Itatiaia. It was associated to the reproductive biology of the specie and the different ecological characteristics of each population.

- *Symphonia globulifera* :
  - High genetic diversity observed in study population in French Guiana
  - Although predominantly outcrossed, significant biparental inbreeding was detected
  - Weak but significant spatial genetic structure was detected (up to 150m)
  - Pollen flow more limited than seed dispersal, due to pollinator behaviour
- *Vochysia ferruginea* :
  - Genetic diversity estimates generally higher in primary than secondary stands
  - High diversity found in seed lots suggesting significant pollen flow
  - Mean pollen dispersal distance greater in primary forest than secondary
  - Mating system is mixed but largely outcrossed, due to long distance dispersal
  - No observed fitness effect due to biparental inbreeding
- *Theobroma grandiflorum* :
  - Higher levels of genetic diversity found in natural population
  - Higher levels of inbreeding in cultivated population
  - Diversity reduction likely reflects natural populations bottlenecks or artificial selection during plantation
  - Introduction of pollinators benefits fruit production in plantations

Work Package 4 - Simulation modelling of population genetic dynamics

Objectives

- Develop existing simulation model ECO-GENE to work with more complex tropical ecosystems rather than temperate systems for which it was originally designed
- Simulate the temporal and spatial dynamic of genetic structures of populations that are subject to extraction/land-use change pressures
- Interpret results for inclusion in species management strategies

Activities

- ECOGENE model developed for use with tropical systems
- Case studies, based on empirical data derived from WP3 and applying simulations of multiple potential logging strategies, conducted for:
  - *Symphonia globulifera*
  - *Swietenia macrophylla*
- Management recommendations derived

Results & conclusions

- *Symphonia globulifera:*
  - Population reasonably robust to logging scenarios, recuperating on a 30 yr rotation
  - Most sensitive parameters were demographic factors (basal area, population size) and fixation index
  - Among genetic parameters, most sensitive were 'number of genotypes' and 'genetic distance'
  - *S. globulifera* least sensitive to logging of all species tested so far from F. Guiana
  - Slow growing species stabilise genetic diversity through potential for contributions to many reproductive events
- *Swietenia macrophylla*:
  - Density of populations had significant effects on susceptibility to logging
  - Belize populations high density and relatively robust but 'recruitment rate' important
  - Brazilian populations lower density and therefore more sensitive
  - Individual sites require unique assessment for management planning
    - Density must be characterised
    - Recruitment must be promoted (probably through gap creation)

Work Package 5 - Designing management strategies to maximize diversity

Objectives

- Disseminate results of this proposal to the forestry community of Latin America
- Communicate specific recommendations for changes in forest management practices to those concerned
- Increase general awareness of the genetic aspects of silviculture
- Increase sustainability of forest management practices through circulation of techniques to maximise the genetic resource base of forest trees

Activities

- Disseminate results of this proposal to the forestry community of Latin America
- Communicate specific recommendations for changes in forest management practices to those concerned
- Increase general awareness of the genetic aspects of silviculture
- Increase sustainability of forest management practices through circulation of techniques to maximise the genetic resource base of forest trees

Results & conclusions

- Peer-reviewed publications
    - >40 individual peer-reviewed primary research papers published
    - Special Issue of *Heredity*
    - Special Issue of *Silvae Genetica*
- High-level workshop for policymakers, scientists and end-users held in San Jose, CR
- Production of Spanish language 'Forest Genetic Diversity' board game for schools and colleges

Final Scientific Report: Consolidated Report

Overall objectives

Tropical forests are complex ecosystems, and their management often involves the sustainable exploitation of a range of resources, including timber and non-timber products. Genetic diversity represents an essential natural resource, promoting population level adaptation ensuring the continued proliferation of individual species within tropical systems. Reduced genetic diversity can lead to loss of adaptive variation and inbreeding depression, both of which can threaten the long-term survival of isolated populations. Many tropical species are currently extracted at unsustainable levels or their habitats are being degraded, threatening the long-term survival of species within these fragile ecosystems. Whether harvested from natural or managed landscapes, there is a need to develop a practical, operational system concerned with the management of genetic sustainability.

In this proposal, we present a programme of research aimed at measuring key genetic indicators of sustainability in tropical forest ecosystems. Our aim is to evaluate the level and dynamics of genetic diversity in natural forest populations. The sustainability of current management practices will be assessed in selected extracted species using computer simulation of field-gathered genetic data. Specific sustainable extraction/management strategies arising from this process will be promoted to forestry stake holders, who will be made more aware of the genetic implications of management.

The project had the following objectives*:*

- Examine the structure and dynamics of genetic variation for a range of Central and South American tree species within natural ecosystems and identify the main factors that are responsible for partitioning of variation within species
- Examine the impact of identified extraction methods/habitat degradation on selected economically important species
- Produce a model tailored to the individual study species that will integrate field observations and DNA-based technologies to provide realistic simulations of the impact of differing land-use strategies and extraction regimes on the genetic resource base of impacted species
- Improve capacity to execute sound natural forest management by improving awareness of genetic implications of natural forest management and implementation of a modelling approach to setting sustainability strategies

Work package 1: Development of molecular techniques

Workpackage number:	1					
Start date:	Month 1					
End date: Planned / Actual	Month 36 / Month 36					
Participant number	1	2	3	4	5	6
Person-months per participant	2	3	1	8	30	12
Person-months delivered	2	3	1	8	30	12

Objectives

- Optimise collection of field material and DNA extraction for all species
- Optimise AFLP procedure for all species involved in WP2
- Develop new SSR loci for *Araucaria angustifolia* (for WP3)
- Optimise existing SSR loci and develop new ones for *Swietenia macrophylla*, *Vochysia ferruginea*, *Symphonia globulifera* and *Theobroma* species (for WP 3)
- Optimise SSR methodology for *Pinus* species (WP 5)
- Develop methods of analysis for all partners and make analysis software available (P3, all work packages)

Plant material from the field will be collected as either leaf or cambium tissue, which needs to be dried or stored in preservative solution respectively. Both techniques will be tried and the best selected before full-scale collection of samples proceeds. Once good quality DNA is extracted, the process will be scaled up to facilitate the analysis of large numbers of samples. For each species, the labs undertaking the work will optimise the AFLP method, but initial optimisation of techniques will be done by partner 6.

Existing SSR primers will be synthesised and conditions optimised. New SSR primers will be designed for *Araucaria angustifolia* species from sequence data obtained from genomic libraries enriched for microsatellite repeats (P6). PCR conditions will be optimised and new primers will be screened against a sample range of species DNA to select the most polymorphic loci. Molecular analysis of pine megagametophytic tissue will be optimised (P2)

All partners will be made aware of standard analytical techniques to be used for analysis. These will be discussed and agreed during the opening workshop and revised during the progress of the project. Existing computer software will be further developed for use by all partners. A project website will be established to disseminate details of standard protocols to the consortium (P1). Specific software designed by P3 for diversity assessment (WP2) and gene flow studies (WP3) will be distributed to partners or data will be submitted for analysis through the website by P1.

Changes to original workplan

- RAPD analysis of Pinus dropped (in proposal), changed to SSR analysis (using markers transferred from Pinus radiata) - also dropped due to technical difficulties. Finally AFLP analysis was carried out as part of WP2, plus analysis of human impacts on regeneration based on quantitative trait analysis
- Repeat of cross-lab standardisation of AFLP analysis, initial testing suggested technical problems with comparisons; process was repeated and allowed identification of standard analysis approaches for all labs carrying out AFLP analysis.
- Specific analysis software was not designed, as detailed in proposal, as sufficient existing programs were available

Deliverables & Milestones

Deliverables				
No	Name	Due date	Delivery date	Outputs
1	New SSR primers made available to partners	Month 12	Month 12	Delivered
2	Developed molecular methods and software made available to all partners through website	Month 18	Month 18	Delivered
3	Developed software available to all partners through website and journals	Month 36	Month 24	Delivered
Milestones				
No	Name	Due date	Delivery date	Delivered
1	Optimisation of DNA extraction	Month 3	Month 6	Delivered
2	Optimisation of AFLP, SSR and isozyme techniques	Month 12	Month 12	Delivered
3	Development of new SSR loci for 3 species	Month 12	Month 18	Delivered
4	Development and availability of computer analysis software to all partners	Month 18	Month 24	Delivered

Activities & Results

- The project website was set up following the first coordination meeting and is available at http://www.nbu.ac.uk/geneo/. The website has open-access areas for publicising the project and restricted areas for sharing information between partners such as lab methods, data analysis techniques and results.

- Collection and DNA extraction protocols optimised for all target species. Cambium sampling and collection protocol was optimised and published as Colpaert et al (2005). (appendix 1)

- AFLP procedure for all species involved in WP2 optimised. Pre-samples (2-5 samples of cambium and leaf tissue) were obtained for all target species, to allow testing of DNA extraction and subsequent AFLP amplification. Species for which successful protocols were available were retained on the target species list for collection.

- AFLP analysis standardisation procedure: An inter-lab comparison of AFLP analysis was been completed incorporating contributions from CEH, IPBO and INRA (process repeated twice). The principal conclusion was that diversity level comparisons are valid given that certain restrictions are placed on scoring and analysis procedures (score all bands, score only those between 60-600bp, analyse loci within 0.05-0.95 frequency limits). Diversity estimates obtained between sites and between researchers scoring the same gels were highly consistent. The main discrepancy between estimates was due to site differences: i.e. system (protocol, machine, chemical) differences. The recommendations derived from the standardisation procedure were applied in all downstream AFLP analyses.

- Existing SSR loci optimised and new markers developed for *Araucaria, Swietenia macrophylla*, *Vochysia ferruginea*, *Symphonia globulifera* and *Theobroma* species (for WP 3). SSRs optimised (one from Aldrich *et al* 1998, two new loci) for Symphonia (Degen et al 2004), Vochysia (Lowe et al 2002), Swietenia (Lemes et al 2002), Araucaria (Salgueiro et al 2005). SSR for *T. cacao* were successfully optimised for use in *T. grandiflorum*. Pinus SSR transfer from *P radiata* to *P.oocarpa* failed but analysis was undertaken using AFLPs.

Results

Colpaert N, Cavers S, Bandou E, Caron H, Gheysen G, Lowe AJ (2005) Sampling tissue for DNA analysis of trees: trunk cambium as an alternative to canopy leaves, *Silvae Genetica.* 54(6): 265-269.

Degen B, Bandou E, Caron H (2004) Limited pollen dispersal and biparental inbreeding in *Symphonia globulifera* in French Guiana. *Heredity*, **93**(6): 585-591.

Lowe AJ, Goodall-Copestake WP, Caron H, Kremer A, Decroocq S (2002) A set of polymorphic microsatellites for *Vochysia ferruginea*, a promising tree for land reclamation in the Neotropics. *Molecular Ecology Notes* 2:209-210.

Lemes, Brondani, Grattapaglia (2002) Multiplexed systems of microsatellite markers for genetic analysis of mahogany, Swietenia macrophylla king (Meliaceae), a threatened Neotropical timber species. *Journal of Heredity* 93: 287-291.

Salgueiro F, Caron H, De Souza MIF, Kremer A and Margis R (2005) Characterization of nuclear microsatellite loci in South American Araucariaceae species. *Molecular Ecology Notes* 5(2): 256-258.

Problems encountered

- RAPD analysis of Pinus dropped (in proposal), changed to SSR analysis (using markers transferred from Pinus radiata) - also dropped due to technical difficulties. Finally AFLP analysis was carried out as part of WP2, plus analysis of human impacts on regeneration based on quantitative trait analysis
- Repeat of cross-lab standardisation of AFLP analysis, initial testing suggested technical problems with comparisons; process was repeated and allowed identification of standard analysis approaches for all labs carrying out AFLP analysis.

Specific analysis software was not designed, as detailed in proposal, as sufficient existing programs were available.

Work Package 2: Identifying biological determinants of genetic diversity

Workpackage number:	2					
Start date:						
End date						
Participant number	1	2	3	4	5	6
Person-months per participant	12	15	19	0	60	11.5
Person-months delivered	12	23	19	15	60	20

Objectives

- Determine correlation between life-history and ecological characteristics of tree species and patterns of structure of genetic variation amongst populations
- Classify tree species according to their predicted vulnerability to population declines in logged and fragmented forests

In this work package 50 species will be selected, on the basis of their relative ecological and economic importance, for analysis in forest blocks across Costa Rica, French Guyane and Brazil. Species will be selected to test the importance of particular life history and ecological factors on the partitioning of diversity and vulnerability to disturbance and. For example, two groups of long-lived pioneers could be contrasted, with similarly mobile pollen vectors but with quite different seed vectors. The 50 species will be split into a number of uniform subgroups along these lines to obtain maximum contrast of characters. Trees need to be classified into the following categories pioneer/climax, middle storey/canopy, local endemic/widely dispersed, high population density/low density, pollination system, seed dispersal mechanism, taxonomic group, geographic location, sexual system, and dry/wet forest inhabitants.

Species to be considered for analysis include those that are distributed throughout the Neo-tropics and are fast growing, long-lived pioneers; *Schefflera* (*Didymopanax*) *morototoni*, *Simarouba amara*, *Laetia procera*, *Apeiba* spp., *Rollinia* spp. *Stryphnodendron* spp.; and those that are predominantly canopy or middle storey species and are slow growing and shade tolerant, e.g. members of the family Sapotaceae (*Pouteria*, *Micropholis*) and Chrysobalanaceae (*Couepia*, *Hirtella*, *Maranthes*). Many of the species that are ecologically important have no commercial value and in many cases these are the ones with the specialised seed dispersal mechanisms involving vertebrates. Typical, fairly speciose middle storey genera such as *Guarea* and *Protium* will also be considered. Examining several species within a family or genera limits other inherent differences and difficulties of species comparisons. The genus *Anacardium* will also be considered for analysis as a whole for this reason. *Anacardium* constitutes 10 species, three of them closely related. Trees of the genus exhibit several major growth forms and range from narrow endemics to wide ranging species. The exact species to be analysed will be confirmed at the first coordination meeting and this choice will be facilitated by the existence of inventorised and mapped permanent plots that respective partners have access to.

Once the species and forest block choice has been finalised collection will proceed. For each species 50 individuals will be located, mapped and sampled within 2 to 4 populations (by partners 2, 3, 4 and 5). Using this number of individuals per population will allow an accurate estimation of spatial distribution of variation within populations. Such samples will allow estimates to be compared across the sampled populations and used to estimate other population statistics and partitioning of variation at the amongst population level. The AFLP analysis will be split between three partners (P1, P3 and P4) who will be responsible for the analysis of material from their region. Thus each partner undertaking AFLP analysis will process approximately 2000 samples, a realistic expectation using automated DNA extraction and genotyping procedures. Data will be analysed according to standard procedures agreed during WP1. Data will remain the property of each partner for publication but processed measures of spatial structure and population subdivision will be made available by all partners for WP 4 and 5. In addition, AFLP data sets already accumulated for *Vochysia ferruginea*, *Swietenia macrophylla, Cedrela odorata* and *Eugenia uniflora* during a previous INCO-DC funded project will be combined in a final full analysis of the data and incorporated into the outputs of WP 4 and 5.

Changes to original workplan

None

Deliverables and Milestones

Deliverables				
No	Name	Due date	Delivery date	Outputs
4	Full list of species and locations of forests to be analysed to be placed on website	Month 18	Month 18	Delivered
5	Results made available in a useful form for comparative regression analysis and processing in WP 4 and 5	Month 30	Month 24	Delivered
6	AFLP data interpreted and published to highlight case studies of specific interest. Partners to publish analysis of combined data sets jointly	Month 42	Month 48	Delivered
				Delivered
Milestones				
No	Name	Due date	Delivery date	Delivered
5	Selection of species and populations	Month 6	Month 12	Delivered
6	Sampling of species and populations	Month 24	Month 36	Delivered
7	AFLP analysis of samples	Month 36	Month 48	Delivered
8	Analysis and interpretation of data	Month 48	Month 48	Delivered

Activities

Species selection.

As devised in the original proposal, a list of 50 target species was selected and optimised according to amenability to molecular analysis (via testing in WP1). The final list was targeted for collection as detailed below. Species were assigned to individual partners for various stages of the processing: sampling, DNA extraction, AFLP analysis and data analysis. All samples were collected according to an agreed common sampling design (detailed in Results below) and all analysis followed the protocols devised under WP 2.

Additional data regarding species life history characteristics, genome sizes (via flow cytometry) and distribution were gathered as part of the meta-analysis and synthesised from literature, partner knowledge and observations.

Table WP2.1: Final species list and some basic data gathered for target species analysed in the AFLP meta analysis undertaken in WP2.

Species	family	Phylog. class	Distn	mating	outcross	pollination	p. dist	seed	seed dist
Araucaria angustifolia	araucariaceae		narrow			wind		wind	
Bagassa guianensis	moraceae		wide range			insect		animal (?)	
Bocoa prouacensis	ceasalpiniaceae		narrow			insect		animals	
Brosimum guianense	moraceae		wide range			insect		birds	
Calophyllum brasiliense	clusiaceae		wide range			insect		bats (Mx), birds, rodents, fish	
Carapa guianensis	meliaceae		wide range			insect		rodents	
Caryocar glabrum	caryocaraceae		wide range			bats		animals	
Cecropia sciadophylla	moraceae		wide range			wind		bats	
Cedrela odorata	meliaceae		wide range			insect		wind	
Ceiba pentandra	bombacaceae		wide range			bats		wind	
Chrysophyllum sanguinolentum	sapotaceae		wide range			insect		monkeys	
Dicorynia guianensis	ceasalpiniaceae		narrow			insect		wind	
Eperua falcata	ceasalpiniaceae		narrow			bats		unassisted	
Eperua grandiflora	ceasalpiniaceae		narrow			insect		unassisted	
Eschweilera costaricensis	lecythidaceae		narrow			insect		rodents	
Eugenia uniflora	myrtaceae		narrow			insect		birds	
Goethalsia meiantha	tiliaceae		narrow			insect		wind	
Goupia glabra	celastraceae		wide range			insect		birds	
Hyeronima alchorneoides	euphorbiaceae		wide range			insect		birds	
Jacaranda copaia	bignonaceae		wide range			insect		wind	
Laetia procera	flacourtiaceae		wide range			insect		birds	
Lecythis ampla	lecythidaceae		wide range			insect		bats/rodents	
Lonchocarpus costaricensis	papilonaceae		narrow			insect		wind	
Manilkara huberi	sapotaceae		narrow			insect		animal	
Maranthes panamensis	chrysobalanaceae		narrow			insect		rodents	
Minquartia guianensis	olacaceae		wide range						
Moronobea coccinea	clusiaceae		narrow			birds?		rodents	
Pinus oocarpa	pinaceae		narrow			wind		wind	
Pseudobombax munguba	bombacaceae		narrow			bats		wind	
Schefflera morototoni	araliaceae		wide range			insect		birds	
Sextonia rubra	lauraceae		narrow			insect		unassisted	
Sideroxylon capiri	sapotaceae		narrow						
Simarouba amara	simaroubaceae		wide range			insect		birds/animals	
Simarouba glauca	simaroubaceae		narrow			bees			
Swietenia macrophylla	meliaceae		wide range			insect		wind	
Symphonia globulifera	clusiaceae		wide range			birds		animals	
Tabebuia cassionoides	bignonaceae		narrow			insect		wind	
Tapirira guianensis	anacardiaceae		wide range			insect		bird (Br) / animals (Pn)	
Tetragastris panamensis	burseraceae		wide range			insect		birds	
Virola michelii	myristicaceae		narrow			insect		monkeys	
Voschysia ferruginea	voschysiaceae		narrow			birds		wind	
Vouacapoua americana	ceasalpiniaceae		narrow			insect		rodents	

Results

Meta-analysis

The overall aim of WP2 was to see if it was possible to identify some key biological or ecological characteristics linked to the level and structuring of genetic variation within natural populations. Derivation of such biological/ecological surrogates may allow precautionary management plans for genetic resources to be identified without the need for expensive molecular marker work.

To date several workers and studies have attempted to undertake this type of correlation between species' life history traits and genetic diversity and differentiation. Perhaps the largest scale study has been by Hamrick and coworkers, who have synthesised a large body of isozyme studies (over 1000), but other syntheses have been undertaken by Nybom and Bartish and others. Several of these studies indicate that habit (trees vs herbs), mating system and pollen dispersal agent are the best predictors of variability within genetic diversity and differentiation within natural populations. However for all studies a large proportion of variation remained unexplained (>50%).

A recent study by Duminil and coworkers (Duminil J, Fineschi S, Hampe A, Jordano P, Salvini D, Vendramin GG and Petit RJ, 2007, Can Population Genetic Structure Be Predicted from Life-History Traits? American Naturalist 169: 662-672) has also identified other issues with the previously conducted correlation studies. Based on meta-analyses of 164 studies the authors classified each study species for a range of life history characteristics, including;

- Growth form: herbaceous, shrub,
- Perenniality: annual, biennial, short-lived perennial, long-lived perennial
- Pollination mode: anemophilous, zoophilous
- Reproductive type: sexual and veg., sexual only
- Mating system: selfed, mixed, outcrossed
- Breeding system: monoecious, gynodioecious, dioecious
- Geographic range: endemic, narrow, regional, widespread
- Seed dispersal: 5 categories

These life history characteristics were then combined within a multiple regression to see which had highest predictive capacity for measures of nuclear and chloroplast genetic differentiation. The study found that whilst a number of these characters were highly correlated with genetic differentiation (e.g. growth form, mating system pollination mode, reproductive type), most were not significantly correlated once a correction had been applied for phylogenetic similarity. This is because a number of these characters are highly autocorrelated (trees generally have outcrossing mating systems with wind or highly motive pollen vectors, whereas herbs more often have mixed mating systems with more restricted pollination syndromes). Thus this new study actually questions whether it is possible gain a suite of life history characters that can be used to predict genetic differentiation.

One of the main problems with this new study and that of the previous authors is that the data sets are based on a met-analysis of existing data and therefore combine data with very different sampling strategies, varying over sample number, spatial scale and even genetic loci. Based on simulation modeling of spatial genetic within populations (Cavers S, Degen B, Caron H, Hardy O, Lemes M, Gribel R, Margis R, Salgueiro F, Lowe AJ, 2005, Optimal sampling strategy for estimation of spatial genetic structure in tree populations. *Heredity* **95**: 281-289), such sampling variation can have a huge influence on the detection of significant patterns. Thus a study employing standardized sampling strategies, considering phylogenetically independent contrast analysis and controlling for correlated character expression is required to truly answer the question; is there correlation between life history traits and genetic diversity/differentiation?

Study design

We chose to examine genetic diversity and differentiation within a subgroup of plants, i.e. trees, which allows for some control over habit/mating system/pollination syndrome autocorrelation. For this study 50 neotropical tree species were selected to represent a range of ecological and genome characteristics, and classified for the following characteristics, which include some newly assessed genomic traits:

- **Ecology**: Distribution, succession, growth, density, longevity
- **Breeding + dispersal**: mating system, pollination agent, pollination dispersal class, seed dispersal agent, seed dispersal class
- **Genome**: chromosome no., genome size

For each species a standardized sampling design was agreed, including a single large 'focal' population, comprising of 80 mapped individuals sampled randomly over an area of approximately 5 x 5 km. Two additional populations of 40 individuals were also sampled at either local (within 50-100 km of the focal site) or distant scales (>500 km), depending on the overall species distribution.

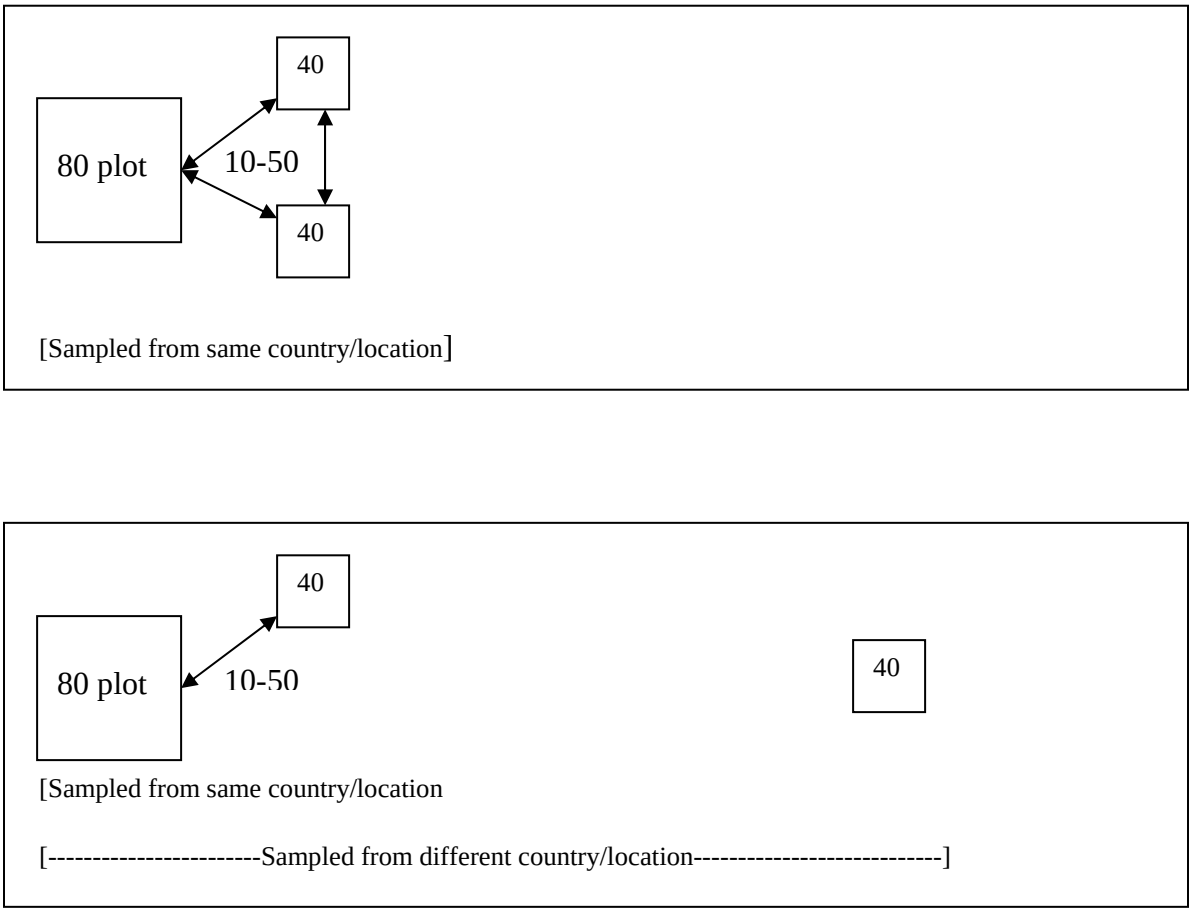


Figure WP2.1: Sample design for AFLP studies

All individuals were genotyped for >200 AFLP loci and the following genetic parameters calculated:

- He – expected heterozygosity, a measure of average within population genetic diversity
- Ht – overall heterozygosity, a measure of total diversity across the range of a species
- % polymorphism, a measure of genetic diversity
- Fst, genetic differentiation, averaged and between local and distant sites
- Spatial Genetic Structuring and , correlation of relatedness within the focal site

The final analysis will perform a regression of all life history and genome characters against all measures of genetic diversity/differentiation and apply phylogenetic corrections. Compilation of the final data set is still in progress, however we present here a preliminary analysis on a subset of data including 20 species and the following life history traits and genetic diversity measures: distribution range, pollination mode, seed dispersal mode: He, Ht, Fst.

Preliminary results

For geographic range (Geographic range: 1 = wide, 2 = narrow) no significant difference was found between taxa that were widely or narrowly distributed for any of the measured of genetic diversity or differentiation tested.

variable	*df*	*F*	Sig.	*R*2	*P*
HE	*1*	*2.7*	*no*	*0.113*	*>0.05*
Ht	*1*	*2.66*	*no*	*0.129*	*>0.05*
FST	*1*	*0.71*	*no*	*0.038*	*> 0.05*

For seed dispersal agent (1 = animal, 2 = bird/bat, 3 = wind, 4 = unassisted, 5 = combination terrestrial animal and bird), the two measures of genetic diversity were significantly correlated.

variable	*df*	*F*	Sig.	*R*2	*P*
HE	1	6.22	yes	0.257	<0.05
Ht	1	3.92	close	0.179	~0.05
FST	1	0.077	no	0.004	> 0.05

For pollination syndrome (1 = bird/bat, 2 = insect, 3 = wind), there was an observable correlation with genetic differentiation, but not with either measure of genetic diversity.

variable	*df*	*F*	Sig.	*R*2	*P*
HE	1	1.05	no	0.055	>0.05
Ht	1	0.04	no	0.002	>0.05
FST	1	4.76	yes	0.209	< 0.05

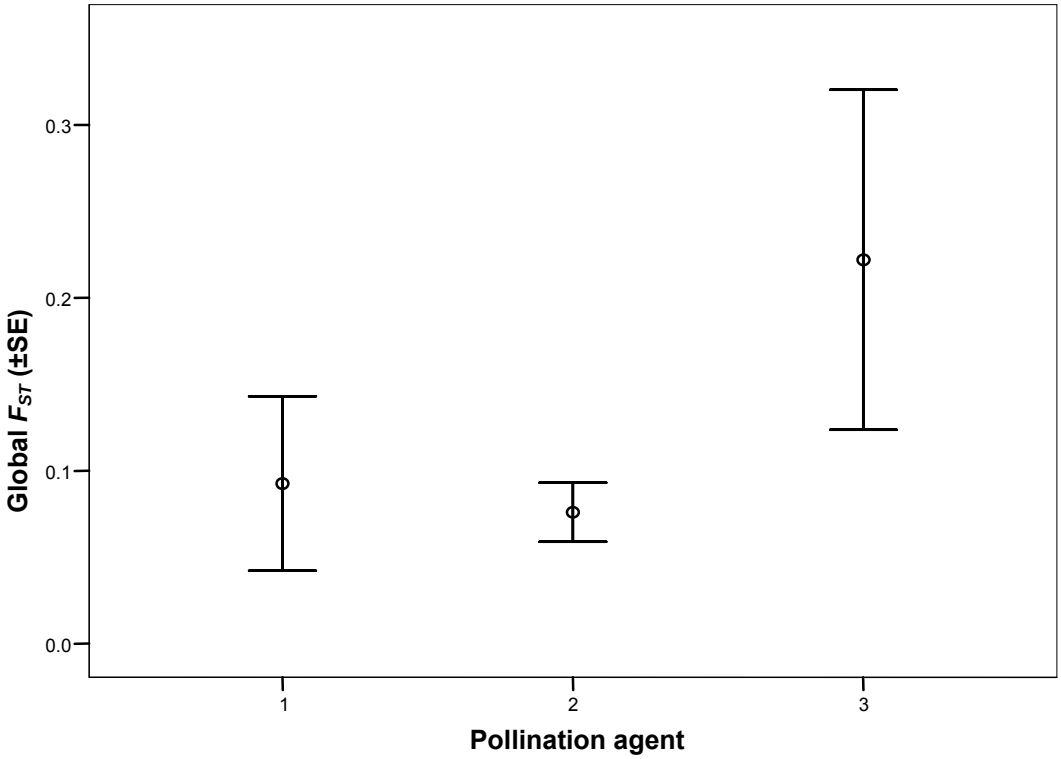


Figure WP2.2: showing range of Fst estimation with pollination syndrome, where: 1 = bird/bat, 2 = insect, 3 = wind

Conclusions

The analysis is yet to be completed and a number of key data sets are still be generated, however the preliminary results, even based only on a limited data set indicates a good possibility of identifying life history : genetic correlations based on the standardized sampling methodologies promoted by this study. In addition several novel character correlations will be possible for the first as part of this analysis, including genome size (see table below) and spatial genetic structure measures.

Table WP2.2. Average and standard deviations for genome size estimations for several neotropical tree taxa. Sizes are given in comparison to *Arabidopsis thaliana* (=1).

species	average	stdev	number of measurements
Cedrela odorata	0.572	0.03063	5
Carapa guianensis	0.398	0.05578	2
Hevea guianensis	1.912	0.08208	2
Vouacapoua americana	0.904	0.05518	3
Eperua grandiflora	1.583	0.00753	2
Ceiba pentandra	1.786	0.24658	2
Tabebuia heterophylla	0.586	0.03568	6
Swietenia macrophylla	0.257		1
Callophylum brasiliense	0.632	0.02907	6
Bagassa guianensis	0.277	0.01190	3
Bocoa prouacensis	0.824	0.02891	2
Chrysophyllum sanguinolentum	0.309	0.05469	2
Dicorynia guianensis	0.650	0.04287	2
Goupia glabra	0.361	0.02225	3
Laetia procera	0.353	0.01874	3
Moronobea coccinea	0.168	0.06430	2
Sextonia rubra	1.090	0.03406	2
Simarouba amara	0.918		1
Symphonia globulifera	1.612	0.09634	3
Virola michelii	0.882	0.05415	3
Virola surinamensis	0.809	0.09520	2

The final multiple regression and GLM model comparisons on raw classes, and after phylogenetic correction for the complete data set, will allow us to more readily assess the power of life history and genome characteristics to predict genetic diversity and differentiation measures within natural populations and therefore their potential for use as genetic resource surrogates.

Problems encountered

The large number of AFLP analyses undertaken in the project presented significant technical challenges as the system itself is highly sensitive and requires considerable skill to apply consistently successfully to different species. The time delays encountered in gathering collections meant that, in many cases, a single population was not collected within sufficient timescale to allow simultaneous analysis with the other samples gathered: the lengthy storage periods that resulted for many samples meant that it was difficult to complete all AFLP analyses to the standard targeted at the outset. Therefore several cases arose where datasets contained fewer than optimal marker numbers and two as opposed to three populations: however, as the initial targets had been set high, the resulting datasets still provided useful information.

Work Package 3: Effect of human-mediated processes on genetic diversity

Workpackage number:	3					
Start date:						
End date						
Participant number	1	2	3	4	5	6
Person-months per participant	20	46	23	64	48	0
Person-months delivered	20	58	23	64	70.8	7

Objectives

- Examine the impact that human-mediated disturbance and fragmentation processes have on the dynamics of genetic diversity through specific case studies.
- Identify the best management strategies for each individual case study to maximise the genetic resource base of the species within an exploited/disturbed environment

3.1 Impact of fragmentation and isolated population management

Pine -Six stands will be selected in Honduras: three post-regeneration felling and three unmanaged or pre- regeneration felling. In each of the former, 10-20 cones will be collected from 10-15 seed trees. In the unmanaged stands, seed will be collected from 10-15 comparable trees. Spatial and reproductive data will be collected. Seed will be extracted in Honduras and exported to Costa Rica. Twenty to thirty seed of each seed-tree will be subjected to RAPDs analysis of haploid (megagametophyte) and diploid tissue. The following population genetic parameters will be estimated: multilocus outcrossing rate of each tree and population; observed and expected heterozygosities for the progeny generation, by stand; percentage polymorphic loci, effective number of alleles, by stand. Interpretation will be based on comparison of estimated parameters in managed and unmanaged stands, both on individual trees and on a whole-stand basis.

3.2 Logging

Mahogany - Initial plans in the proposal had targeted natural populations of Swietenia macrophylla/ However during initial project planning, it became apparent that analysis of populations under significant human pressure was more appropriate, desirable and relevant to the species current status. Therefore populations of *S. macrophylla* trees within south/central Brazil and Belize were selected for analysis using SSRs. Within each site, approx. 100-200 mapped trees, approx. 1000 seeds from 20 mother trees and approx. 200 seedlings in each population will be sampled and used to calculate outcrossing rates, pollen and seed dispersal distances. Fragmented and non-fragmented blocks will be compared for mature tree and progeny genetic and quantitative variation and gene flow parameters.

Araucaria - Six different *Araucaria angustifolia* populations will be studied. Three virgin and undisturbed populations will be studied in Rio Grande do Sul, Santa Catarina and Parana States. Another three populations with different logging histories will be analysed from Rio Grande do Sul, Minas Gerais and Parana States. An average of 30 to 60 individuals will be mapped and sampled from each population together with a sample of associated seeds and seedlings. Plant tissue will be subject to microsatellite and cpDNA analysis to estimate three major parameters; level of diversity in the respective sites, out crossing rate and seed dispersal.

Symphonia -Symphonia will be sampled from three intensively studied plots (ISP), one in French Guiana and two in Brazil. The analysis of genetic data for the adult trees will lead to information about spatial genetic structure and level of inbreeding in the adult population. Moreover, the comparison of genetic structures in different diameter classes will provide some information about the dynamics of genetic processes during the past. In the ISPs the situation before and after logging will be analysed at the two Brazilian ISPs whereas the ISP in French Guiana serves as a reference for genetic processes in undisturbed natural tropical forest. Different logging systems will be applied. For the study of gene flow, microsatellites will be applied to single tree progenies collected at “trap trees” in the ISPs. Those trees will be permanently marked and not felled during

the logging operations. The study will be repeated two years before and two years after logging to get an idea about the inter-annual variation of the estimated gene flow and mating system. The application of different intensive logging systems will lead to a reduction in densities of potential reproducing trees. The interesting question is whether the pollen dispersal is strong enough to avoid reduction of genetic variation in the seed set and where the critical thresholds are. For each sampled seed, paternity analysis will be used to find pollen donors within the plot and to estimate the frequencies of pollen coming from outside the plot. The distribution of physical distances between seed tree and identified pollen donor will lead to an estimation of pollen dispersal (e.g. Streiff *et al*. 1999). Within the same studies the relative reproductive success of different adult trees and the amount of inbreeding due to self-fertilisation will be determined. Moreover, the combined effect of pollen and seed dispersal will be studied by determining the two parents of seedlings in the stand. Again microsatellites and paternity analysis will be used to find the two parents and to determine the amount of seeds coming from outside the plot (e.g. Aldrich and Hamrick 1998; Aldrich *et al*. 1998; Dow and Ashley 1996).

3.3 Secondary regeneration

Vochysia -As part of the previous INCO-DC project two populations of *V. ferruginea* in northern Costa Rica were sampled (approx. 50 trees in each), mapped and analysed for SSR variation. One is primary (Tirimbina) and the other secondary forest (Florencia) and they are separated by a distance of approximately 60 km, As part of this previous analysis approximately 500 seed were collected from 10 mother trees per population and analysed for SSR variation from which outcrossing rates were calculated. The present proposal aims to extend the previous work by 1. sampling mature trees in primary forest adjacent to the secondary forest already sampled (Florencia site) which functioned as the seed source for the establishment of the latter, 2. sampling mature trees in secondary forest adjacent to the primary forest already sampled (Tirimbina site) 3., sampling seedling populations in primary forest at both sites and 4., sampling isolated trees and additional secondary forest patches within 5 km of the primary and secondary forest at both sites. Sample sizes will be approximately 50 mature trees and approx 200 seeds will be collected from 10 mother trees. For all natural regeneration techniques in secondary forest, approximately 150 established seedlings will be sampled. SSR data will be analysed to estimate outcrossing rates, pollen and seed dispersal distances and to make a comparison of variation within and between mature and progeny cohorts, and to make initial estimates of metapopulation structure.

3.4 Domestication

Theobroma -In the current proposal we intend to transfer microsatellites developed for *T. cacao* (Lanaud *et al*., 1999) to *T. grandiflorum,* in order to have a set of highly polymorphic genetic markers available to investigate the breeding and mating system of this economically valuable species. Application of SSR markers will be used to understand the ecological and genetic factors affecting fruit and seed production in orchards of cupuaçu in the central Amazon. The following studies are planned: (a) paternity analysis of seeds, (b) measurement of the pollen flow distance, and (c) quantify the level of individual self-compatibility and cross-compatibility among cultivars.

Changes to orginal workplan

- Alteration of Pinus study to non-molecular due to lack of success with SSR transfer
- Reorientation of Swietenia studies to logging focus
- Change of sites for Vochysia
- Change of Symphonia study: completed FG work then compared with data from pre / post logging work to Dendrogene ? focussed effort on simulation study (ask BD)
- Additional work on Theobroma grandiflorum, exploring practical measures to increase fruit yields based on ecological and genetic data

Deliverables and Milestones

Deliverables				
No	Name	Due date	Delivery date	Outputs
7	Location and description of species and forests analysed to be placed on website	Month 21	Month 48	Delivered
8	Description of the way that human-mediated processes affect the structure and dynamics of gene diversity for each case study	Month 36	Month 48	Delivered
9	Suggested strategies to reduce impact of human-mediated process and maximise genetic diversity	Month 42	Month 48	Delivered
Milestones				
No	Name	Due date	Delivery date	Delivered
9	Selection and sampling mature trees, seeds and seedlings where appropriate	Month 24	Month 36	Delivered
10	Finish SSR/RAPD/cpDNA analysis of material	Month 36	Month 48	Delivered
11	Complete field observations including dbh measurements and pollination observations	Month 36	Month 36	Delivered
12	Finish analysis of molecular data	Month 48	Month 48	Delivered
13	Interpret data for publication and process into simulation modelling and management strategies.	Month 48	Month 48	Delivered

Activity Report

3.1 Impact of fragmentation and isolated population management: Pinus oocarpa

Pinus oocarpa var. *oocarpa* Schiede ex- Schlechtendal has the largest natural distribution of any conifer in the neotropics, and is predominantly outcrossing with wind pollination. There is evidence in conifers that isolation by distance increases the rates of self-fertilization, and consequent inbreeding depression. The extended self-compatibility in the conifers means thy carry high and known genetic loads, making possible the use of morphological markers for a range of recessive alleles that are lethal and deleterious in homozygous condition. In the first stages of the life cycle, dominance exerts a directional effect on the phenotypic values and the additive genetic variance. Smith *et al* (1988) found a positive and highly significant correlation (*P* >0.01), between the frequency of full-seeds and a gradient of demographic density in *P. contorta*; also Sorensen (2001) compared three populations of *P. contorta* var*. murrayana* with frequencies in mixed forest of 0.08, 0.49 and 0.81 and found significant evidence for an increase of inbreeding depression in seedlings. The latter was attributed to the increasing self-fertilization induced by the demographic isolation. Both *P. contorta* and *P. oocarpa* belong to the group of closed cone pines of Mesoamerica.

In the Lajas Unit of Forest Management (Comayagua, Honduras) three stands were analyzed. The results showed that demographic density is reduced in trees/ha: R3 = 190, R2 = 102 (continuous stands) and R1 = 7 (grassland with trees). The site was used to test the hypothesis that the demographic reduction, and consequent isolation by distance, increases the natural selfing rate in *P. oocarpa*. For this, morphological markers, which have been widely reported for others pine species, were used. It was observed that inbreeding depression was significantly greater in the early stages of development, than observed in non-cotyledonous seedlings (up to 193 days). Generally it is accepted that inbreeding depression diminishes with age. A greater incidence of lethal and deleterious genes was observed in R1, an explanation for why there were no significant differences in the small contrast R2xR3, but there were in the bigger contrast R1xR3. The following were used as morphological markers to indicate inbreeding: 1) low proportion of full-seeds by cone (P_{189gl} <0,0001), 2) on one hand, the reduction of seeds of low weight and, on the other, a significant increase of the heavy seeds (P_{gl}. <0,0001, asymmetric tails +*g*1), 3) greater weight of full-seeds by cone (P_{175gl} = 0,0468), 4) delay in emergence (P_{1gl} <0,0001), 5) high proportion of nonemergents (P_{1gl} = 0.0228), and excessive number of cotyledons (P_{1gl} = 0.0032). The number of empty seeds also are a genetic indicator of inbreeding; Bishir and Namkoong (1987) proposed that the variations in the proportion of empty seeds, imply that the genetic load varies in different localities in the forest, while Sorensen (2001) studied the extreme values in noncontinuous traits and associated them with products of inbreeding. We observed in less dense xv stands the CVphenotypic was increased: this was attributed to extreme values, the number of cones by stand with at least one empty seed was greater in R2 and R1 that in R3.

The nonsignificant increases in the genetic component of additive variance (CVA or CVfamily(stand)), of eight quantitative variables in non-cotyledonous seedlings, provided evidence of the effect of small deleterious mutations, at least up to 192 days after the beginning of the test. The CVA in R1 was slightly greater in 6/8 variables, whereas in the densest stands R2 and R3 was only in 4/8 and 3/8 respectively. The small increases of the genetic variances between the families of R1 were attributed to some few extreme values, like those due to low vigour of some individuals. This could suggest slightly unbalanced contributions to reproduction between the families demonstrating some degree of inbreeding. We concluded that the isolation by distance caused by forest management and deforestation, increases levels of self-fertilization and therefore inbreeding depression.

Analysis of *Pinus oocarpa* for WP3 formed the research project for completion of an M.Sc. by Pablo Madriz (MADRIZ, JP. 2004. Genetic changes in the natural regeneration of *Pinus oocarpa* var. *oocarpa* Schiede ex- Schlechtendal, caused by forest management and deforestation, Comayagua Honduras, Central America - an observational study, M.Sc. Thesis, CATIE.) The above report is adapted from the summary of this study.

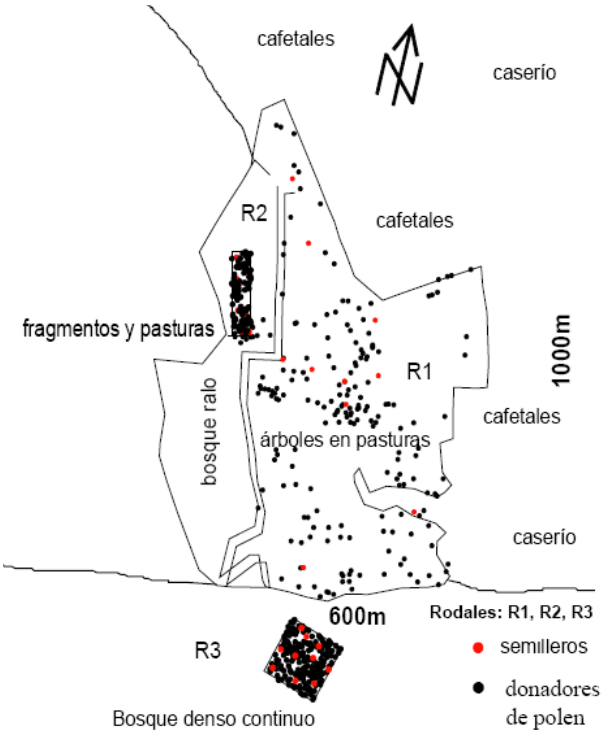


Figure WP3.1: Map showing location of stands of *Pinus oocarpa* at Lajas, Comayagua, Honduras. (from Madriz, JP 2004). Stands R1, R2 and R3 are indicated.

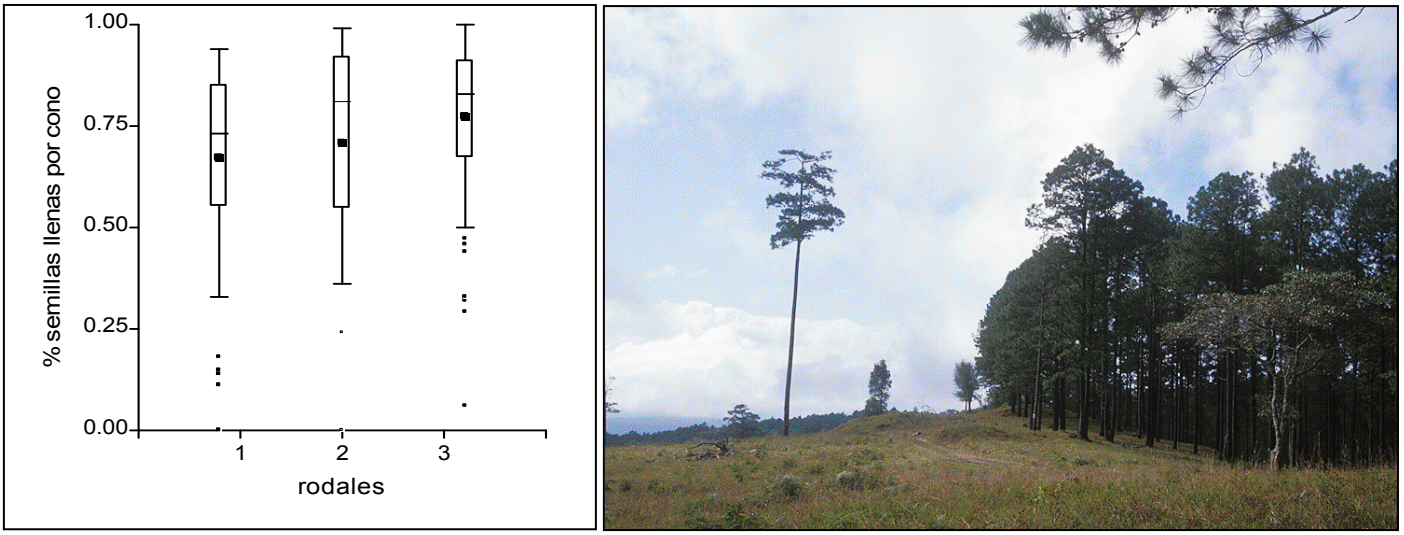


Figure WP3.2: Left – Chart shows variation between stands R1,R2,R3 for % of filled seeds per cone. Right: Photo shows different isolation categories at Lajas – background: continuous forest, right: forest fragment and left: isolated tree (photo – P Madriz).

3.2 Logging

3.2.1 Swietenia macrophylla: Brazil

Big Leaf Mahogany (*Swietenia macrophylla*, Meliaceae) is naturally distributed from Southern Mexico to the Amazon region of South America, in humid zones. It is usually evergreen and reaches heights of up to 30-35 m. The tree is monoecious, and has unisexual flowers which are pollinated by insects. Development from flower to mature fruit takes 9-12 months, flowering and fruiting occur annually from 10 to 15 years of age. Flowering usually takes place when trees are leafless or just coming into new leaf shortly before the rainy season, but fruit set can be low due to lack of pollinators. Seed is wind-dispersed, up to a maximum of around 100m from the maternal tree. Due to its value, *S. macrophylla* has been heavily overexploited throughout its range, and is now protected under Appendix II of CITES. Baseline assessments of remaining genetic diversity and guidelines for the conservation of genetic resources in the species are urgently needed. To address the lack of empirical data available for individual populations of *Swietenia macrophylla* and analyse the impact of human-mediated disturbance via logging on genetic diversity two studies were initiated, representing the extremes of the species range: southern Amazonia, Brazil and Belize, Central America.

3.2.1.1 Swietenia macrophylla: Brazil

Field work was conducted in the Marajoara Management Project (*ca.* 07^{o} 50'S, 50^{o} 16'W), south Pará, Brazil. The management area of the project has around 4,100 ha (13 km long by 3.15 km wide) of selectively logged forest sub-divided into 13 tracks with approximately 340 ha (1.08 km by 3.15 km each). Genetic samples were collected from trees in tracks 1 to 6. The western half of the project's area was logged for mahogany in 1985, at an unknown intensity. Tracks 1, 2, and 3, were selectively logged between 1992-1994 by the SEMASA logging company, which harvested 268 mahogany stems, leaving at least 108 standing stems as seed trees (Grogan, 2001). Therefore, overall stand density in these three tracks was reduced from approximately one tree per 2.7 ha (0.37/ha) to one tree per 9.4 ha (0.11/ha). At the Marajoara area, however, the mahogany trees are not randomly distributed. The majority of trees are located in aggregations along the seasonal streambeds, at densities of 0.1 to 3 trees/ha (Grogan 2001).

A total of 220 adult trees (dbh > 10 cm) and 51 seedlings) were sampled. A subset of 154 of the adult trees was used for analysis. The trees sampled were considered as potential pollen donors, and represented approximately 70% of the total number of adult trees in the area. Total genomic DNA was extracted following standard CTAB procedure (Doyle & Doyle, 1987). DNA quantification was performed by comparison with standard concentrations (Lambda DNA) in ethidium bromide-stained 1% agarose gels.

PCR amplification of eight highly polymorphic loci (Lemes *et al*. 2002: *sm01, sm22, sm31, sm32, sm40, sm46, sm47, sm51*) was carried out for all samples. PCRs were performed in 25 µl for multiplex reactions or 10 µl for single reactions (one primer). Each reaction had 1.25 – 2.0 µM of primer, 1 unit of Taq DNA polimerase, 200 µM of each nucleotide (dNTP), PCR buffer 1X (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), BSA (2.5 mg/ml), and 5.0 ng of DNA. PCR amplifications were performed as follows: 1) 94º C for 5 minutes; 2) 30 cycles of: 94º C for 1 minute + 56º C for 1 minute (all primers) + 72º C for 1 minute; 3) final extension at 72º C for 45 minutes. Following amplification, PCR products were diluted, added to internal size standard (GeneScan 500 TAMRA, ABI), and electrophoresed in 5% denaturing polyacrylamide gel on an ABI 377XL sequencer. GeneScan and Genotyper (ABI) softwares were used for data collection and allele size estimation.

All individuals were genotyped and the number of alleles (A), expected and observed heterozygosities (He and Ho), the number of distinct multilocus genotypes (Go) and the inbreeding coefficient (f) for the three generations were estimated. Genetic parameters were estimated using GDA program (Lewis & Zaykin, 2001). Significance was tested for differences between generations using Wilcoxon test (Sokal & Rohlf, 1995).

The correlation between relatedness and spatial distance was evaluated pair to pair for the adult, juvenile and seedling generations, considering all individuals, using the SPAGEDI program (Hardy & Vekemans, 2002). The relatedness index (R) was estimated as described in Queller & Goodnight (1989). A Mantel test was used to determine the statistical significance of the correlation between genetic and spatial distances (Sokal & Rohlf 1995).

The parentage analysis was based on the allele frequencies of eight microsatellite loci, considering all adult trees as potential parents. We used the CERVUS 2.0 program (Marshall *et al.* 1998) to estimate the probabilities of parentage exclusion as well as the critical values of (Δ) statistics. The probability of parentage exclusion for the eight microsatellite combined was 0,995755 for the first parent and 0,999811 for the second one. Based on the parentage analysis, pollen flow was reconstructed considering 19 identified matings.

Table WP3.1: Genetic diversity and inbreeding coefficient for different cohorts in a managed population of mahogany in Eastern Amazonia.

	Locus	A	He	Ho	Go**	f
Seedlings	sm01	12	0,840	0,813	8	0,033
	sm22	12	0,782	0,583	3	0,256
	sm31	13	0,893	0,739	14	0,174
	sm32	12	0,888	0,783	8	0,120
	sm40	8	0,761	0,680	1	0,107
	sm46	5	0,685	0,477	1	0,306
	sm47	3	0,407	0,412	0	-0,012
	sm51	7	0,669	0,683	1	-0,021
		9*	**0,741**	**0,646***	**36**	**0,129***
Juveniles	sm01	14	0,873	0,761	13	0,130
	sm22	9	0,800	0,619	3	0,228
	sm31	15	0,882	0,833	11	0,056
	sm32	13	0,887	0,809	8	0,089
	sm40	10	0,808	0,723	7	0,106
	sm46	7	0,750	0,723	1	0,035
	sm47	5	0,427	0,375	3	0,124
	sm51	10	0,713	0,708	5	0,006
		10,38	**0,767**	**0,694**	**51**	**0,097**
Adults	sm01	16	0,875	0,891	15	-0,018
	sm22	13	0,793	0,691	10	0,130
	sm31	16	0,910	0,833	16	0,085
	sm32	13	0,846	0,815	7	0,038
	sm40	10	0,752	0,636	4	0,155
	sm46	7	0,778	0,611	5	0,216
	sm47	5	0,514	0,556	3	-0,081
	sm51	9	0,685	0,745	1	-0,089
		11,13*	**0,769**	**0,722***	**61**	**0,062***

* = Significance level using Wilcoxon test (A, p=0.011; Ho, p=0.025; f, p=0.017).
** = Go in bold represents the sum of values per locus (according to Aldrich *et al.* 1998).

In general, the genetic diversity was significantly lower for seedlings compared to adults, and juveniles presented intermediate values (Table WP3.1). The comparison between adults and seedlings showed a significant reduction in the number of alleles (p = 0,011), observed heterozygosity (p = 0,025), and a decrease of 40% in the number of distinct multilocus genotypes (Go). The inbreeding coefficient (f) was much higher in the seedling cohort compared to the adults (p = 0,017). The conjunction of these parameters suggests a severe loss of genetic diversity from the pre- to the post-logging generations.

The adult trees are remnants of a previous larger, denser and more continuous population in the region, while the seedlings resulted from matings among remaining trees of the logging activity carried out 12-14 years ago. The juvenile generation was likely established 20-30 years ago, when the deforestation and forest fragmentation process was initiated in the whole region. Selective logging likely contributed to increase the proportion of homozygotes (Ho) in the population in two different ways: by the elimination of individuals, and consequently, alleles from the population, and by increasing selfing, since the density of flowering individuals in a population is expected to be positively correlated with the outcrossing rate (Murawski & Hamrick, 1991, 1992; Nason & Hamrick, 1997).

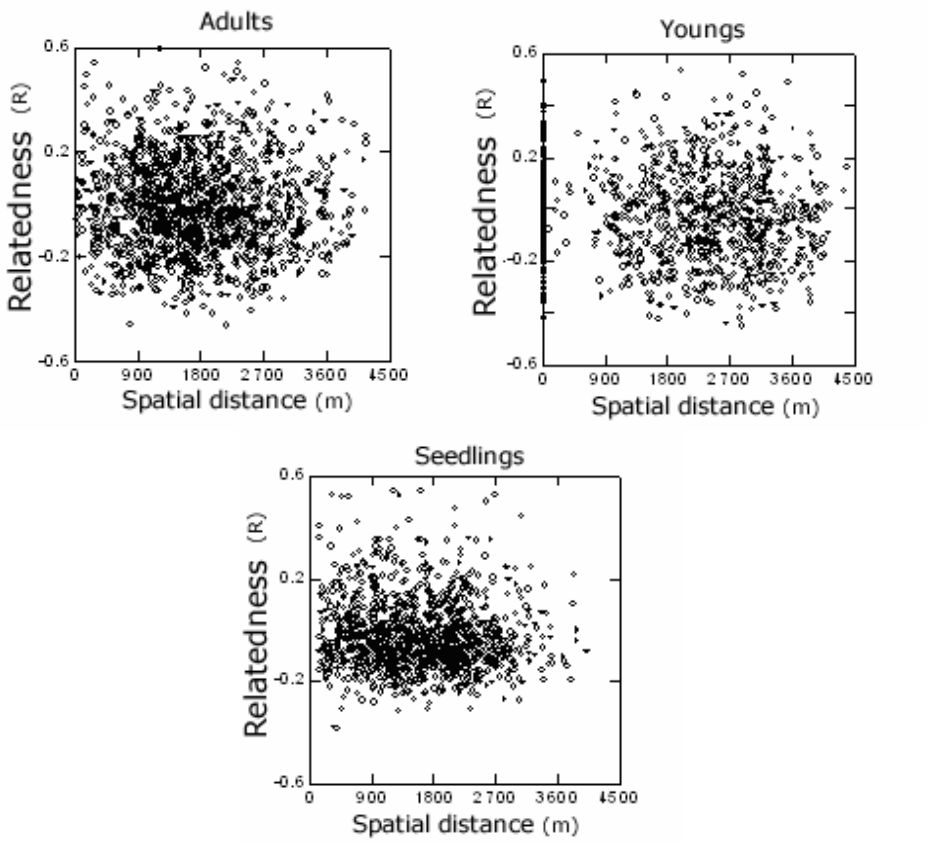


Figure WP3.3: Relationship between pairwise relatedness and spatial distance for adults (n = 55), juveniles (n = 48), and seedlings (n = 51). Mantel correlation test non-significant for the three generations.

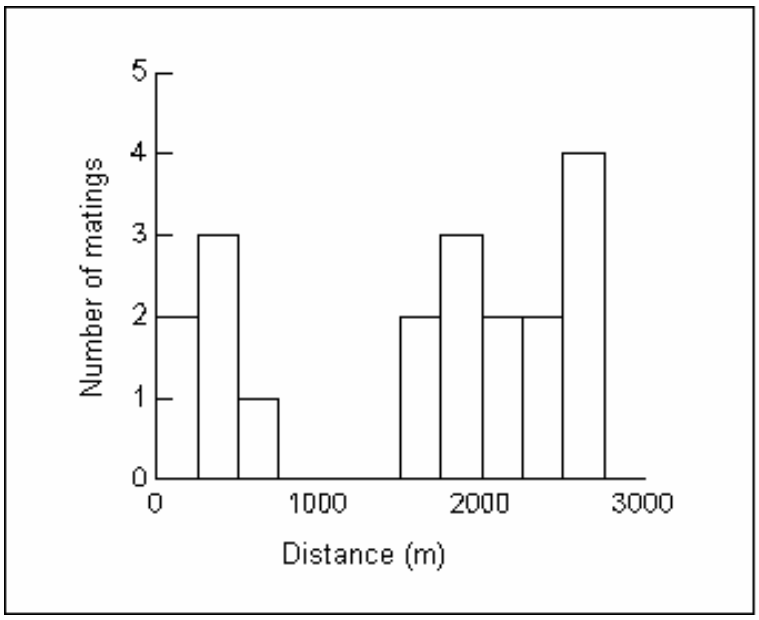


Figure WP3.4 - Number of matings correlated with distance between parents based on microsatellite parentage analysis of seedlings in a logged population of *Swietenia macrophylla* in Eastern Amazonia.

Another possible explanation for the higher diversity of the adults compared to the seedlings is related to the putative occurrence of stronger selection against inbred progeny, which might favour heterozygotes during the different post-germination life-cycle phases. However this alternative does not explain the loss of alleles and the reduction in the number of distinct multilocus genotypes observed in the seedling cohort.

The Mantel test showed no significant correlation between spatial distance and genetic relatedness for the adult, juvenile and seedling cohorts (Figure WP3.3). The results suggest the occurrence of long distance gene flow or historical factors such as multiple colonization events within the aggregations, resulting in a non-structured population.

In parentage analysis, the minimum distance detected between two parents for the observed matings was 69.1 m and the greatest pollen flow distance was 2,736.1 m (Figure WP3.4). The mean pollen flow distance in the Marajoara population was 1,610 m (SD = 963,7). For the total identified matings (considering the 51 seedlings analysed) 40% showed no incongruencies between the genotypes of the seedlings and the parents, the remaining 60% showed no more than two incongruencies and weren't considered for the pollen flow analysis. The results showed an extensive pollen flow (mean 1,610 m) for *S. macrophylla* in the Marajoara population. Our results corroborated the data found for *Swietenia humilis* (White *et al*. 2002), for which minimum and maximum distances of pollen flow between mahogany trees in fragmented areas, in Central America, were 300 and 4500 m respectively. Selective logging in Marajoara population caused the decrease of population density of mahogany trees. It may have contributed to the extensive gene flow observed in this study since the pollinators probably had to move long distances to find flowering individuals in the area.

See 'publications arising' in Theobroma section

3.2.2 Swietenia macrophylla: Belize

As part of a regional effort to protect forest habitat, the NGO 'Programme for Belize' has sectioned 18,000 ha of its Rio Bravo Conservation and Management Area (RBCMA) in Belize, CA for sustainable logging of key commercial timber species, of which *S. macrophylla* is one. However, the sustainable logging plan was devised using forestry principles and does not take account of genetic diversity. To assess the likely impact of the logging plan on genetic diversity and identify ways in which it can be modified to include management of genetic resources, four *S. macrophylla* populations (two as-yet unlogged, two logged) were selected and analysed to determine patterns of genetic structure and levels of genetic diversity & gene flow.

Samples were collected from four sites, Punta Gorda (PG), East Botes (EB), West Botes (WB) and West Marimba (WM). At each site cambium tissue was collected from an exhaustive sample of approx. 200 trees. In addition, in each plot approx. 20 mother trees, distributed randomly across the plot, were selected and 20-30 seeds collected from each tree (Figure WP3.5). Sites EB and WM have been unlogged since 1985, whereas WB and PG were both selectively logged in 1998. Cambium was collected using a hammer and punch; samples were dried on silica gel. Seed was collected from the ground around mother trees and stored in paper bags.

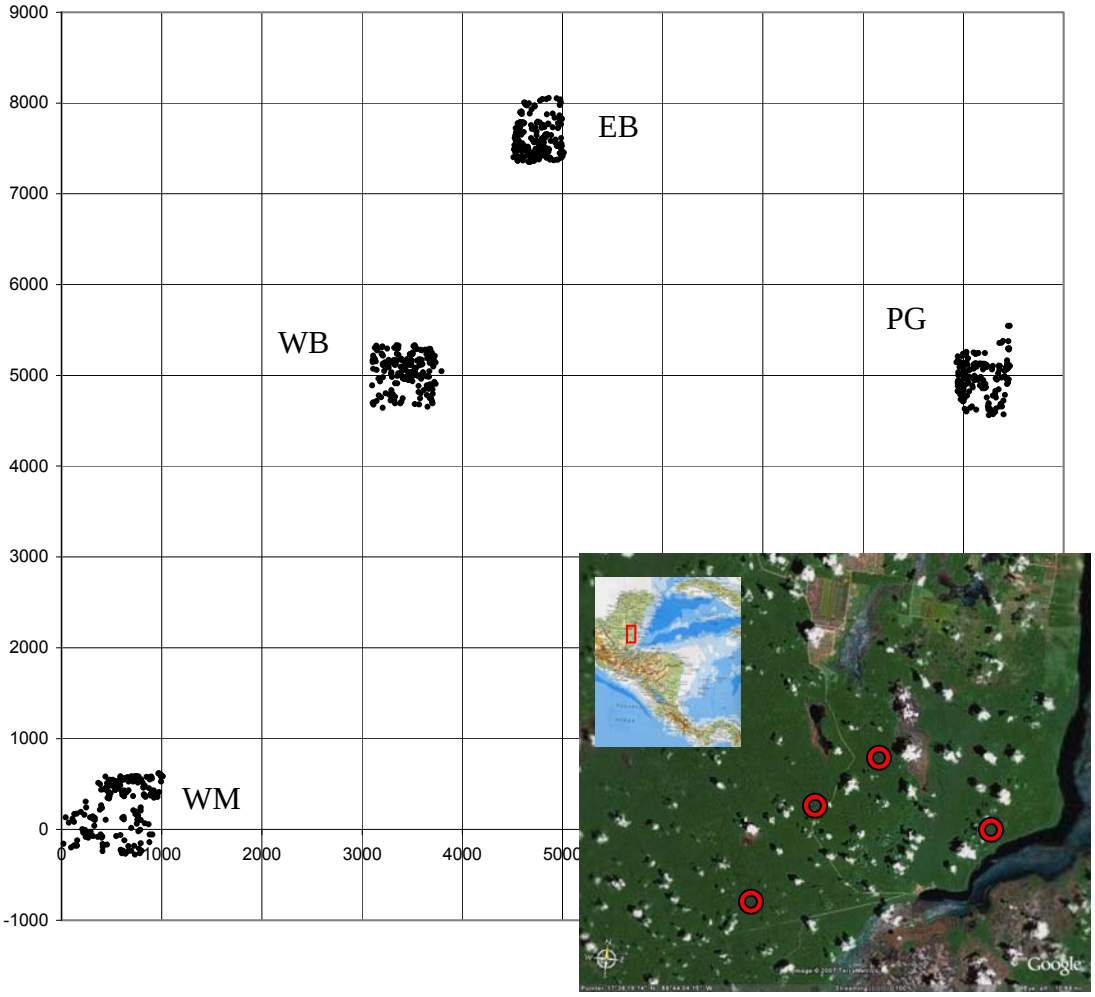


Figure WP3.5: Map of plots of *Swietenia macrophylla* sampled in Belize. Inset: location of Belize, and plots on aerial photo of Hill Bank Station.

The DNA was extracted using standard CTAB procedure (Doyle & Doyle 1987) for all samples except the WB seeds which were extracted using the Qiagen DNeasy kit. For the WM seed collection, the extraction method was modified by an initial treatment of samples with Proteinase K for 1 hour. DNA extracts were quantified by electrophoresis on 1% agarose gel stained with ethidium bromide, visualised under UV light with known standards.

Microsatellite analysis of 795 adult individual samples and 1229 seed samples, representing the four populations, were carried out using seven microsatellite marker loci (sm31, sm22, sm46, sm01, sm32, sm40 and sm51) developed for *S. macrophylla* (Lemes et al. 2002). PCR amplification reactions were carried out in a total volume of 25µl containing 200µM of each dNTP; 1 unit of Taq polymerase (New England Biolabs); 2µl 10x buffer (supplied with the enzyme); 1.25-2.0µM of each primer; BSA (2.5mg/ml), 5ng of DNA template, and the reaction mixture was made up to 25µl with sterilized dH_2O. PCR conditions were initial denaturation of 1 min at 94ºC, 40 cycles of 92ºC for 30 s, 55ºC for 30 s and 72ºC for 1 min, a final 5 min step at 72ºC to ensure full extension of all products. PCR and acrylamide gel electrophoresis on LI COR DNA sequencer 4200 were done at IPBO Belgium for PG and EB for loci sm31, sm22, sm01 and sm46. The rest (all loci for WB and WM; sm51, sm40 and sm32 for PG and EB) were carried out, using the same instrumentation, at CEH Edinburgh. All scoring was carried out by one person at CEH Edinburgh using LICOR SAGA software.

In order to ensure consistency in the scoring, several controls were used from the PG/EB data and run at the Edinburgh site. Allele frequency distributions for the three loci analysed in different labs, by different researchers were compared for each population to check for bias. The dataset was tested for departure from Hardy-Weinberg equilibrium (GENEPOP v3.3, Raymond & Rousset 1995) and numbers of alleles per locus (A), observed (Ho) and expected heterozygosity (He) and fixation index (f) were estimated. Fine-scale spatial genetic structure (SGS) in each population was analysed using 20 even distance classes (using Spagedi v1.2, Hardy & Vekemans, 2005). Mating system analysis was carried out, with estimates of multi (tm), and single (ts) locus outcrossing rates and correlation of paternity determined from progeny arrays, including maternal genotypes (MLTR v2.4 Ritland 2002).

All individuals were genotyped and the number of alleles (A), expected and observed heterozygosities (He and Ho), the number of distinct multilocus genotypes (Go) and the inbreeding coefficient (f) for the three generations were estimated. Genetic parameters were estimated using Genepop. Tests for spatial genetic structure were carried out using SPAGEDI (Hardy & Vekemans, 2002). Parentage analysis was carried out for each of the four plots in isolation, using CERVUS v3.2 (Marshall *et al.* 1998). Initial simulations were conducted using 10,000 simulated offspring, assuming no inbreeding and then applying various levels of inbreeding via selfing and mating between close relatives to estimate stability of the LOD score and delta thresholds and the likely effect of violations of the assumption of strict outcrossing. Using conservative threshold values it was possible to identify 30 matings to high levels of probability.

Levels of genetic diversity were similar though variable across plots (Ho = 0.517-0.626) and all plots showed some degree of heterozygote deficit (mean f = 0.032 - 0.137). Diversity levels were comparable to those obtained for the Marajoara populations, although consistently slightly lower, but consistent with those obtained in other Central American populations (Novick et al, 2003, mean Ho = 0.559). Mean levels of diversity were lower in plots which had experienced selective logging.

Patterns of spatial genetic structure (Figure WP3.6) were markedly similar amongst plots, apart from West Botes. Here, the spatial scale of structuring was similar but the degree of relatedness (and at greater spatial scales, lack of relatedness) was distinctly larger than for other plots. The reasons for this are unclear at this stage.

Table WP3.2: Genetic diversity and inbreeding coefficients for four populations of mahogany from Hill Bank, Belize.

Pop	Locus	N	Na	Ne	Ho	He	f
WM	sm31	177	17.000	8.778	0.893	0.886	-0.007
	sm22	172	7.000	3.013	0.517	0.668	0.225
	sm46	190	12.000	2.384	0.389	0.581	0.329
	sm01						
	sm32	170	5.000	2.228	0.494	0.551	0.103
	sm51	178	10.000	4.645	0.798	0.785	-0.017
	sm40	167	10.000	4.840	0.665	0.793	0.162
					0.626	**0.711**	**0.133**
WB	sm31	161	14.000	2.846	0.484	0.649	0.253
	sm22	169	3.000	1.462	0.331	0.316	-0.049
	sm46	161	12.000	2.273	0.199	0.560	0.645
	sm01						
	sm32	165	4.000	2.008	0.576	0.502	-0.147
	sm51	166	11.000	5.101	0.783	0.804	0.026
	sm40	171	8.000	5.121	0.731	0.805	0.092
					0.517	**0.606**	**0.137**
PG	sm31	141	14.000	6.174	0.738	0.838	0.120
	sm22	171	8.000	1.945	0.515	0.486	-0.059
	sm46	80	5.000	1.263	0.162	0.209	0.221
	sm01	106	16.000	4.736	0.755	0.789	0.043
	sm32	122	5.000	2.168	0.443	0.539	0.178
	sm51	82	11.000	6.761	0.756	0.852	0.113
	sm40	103	11.000	5.666	0.699	0.823	0.151
					0.581	**0.648**	**0.110**
EB	sm31	159	12.000	6.986	0.899	0.857	-0.050
	sm22	164	8.000	1.880	0.451	0.468	0.036
	sm46	145	6.000	1.212	0.172	0.175	0.015
	sm01	147	16.000	5.073	0.837	0.803	-0.042
	sm32	179	5.000	2.145	0.385	0.534	0.278
	sm51	191	9.000	5.476	0.843	0.817	-0.031
	sm40	122	11.000	4.525	0.762	0.779	0.021
					0.621	**0.633**	**0.032**
ALL pops	**sm31**	**638**	**18.000**	**7.037**	**0.757**	**0.858**	**0.118**
	sm22	**676**	**9.000**	**2.018**	**0.454**	**0.505**	**0.100**
	sm46	**576**	**13.000**	**1.860**	**0.250**	**0.462**	**0.459**
	sm01	**253**	**17.000**	**4.993**	**0.802**	**0.800**	**-0.003**
	sm32	**636**	**6.000**	**2.140**	**0.475**	**0.533**	**0.109**
	sm51	**617**	**13.000**	**5.631**	**0.802**	**0.822**	**0.025**
	sm40	**563**	**13.000**	**5.192**	**0.712**	**0.807**	**0.118**
					0.607	**0.684**	**0.133**

In all plots, progeny arrays showed complete outcrossing with scant evidence for biparental inbreeding (tm-ts = 0.011 - 0.050) or populations substructuring (rpm-rps = 0.001-0.041) and no evidence for consistent differences among logged and unlogged populations. In all plots there was evidence for a degree of true selfing (r_t = 0.100-0.243) with the maximum limit being found in the most spatially dispersed population, West Marimba. Levels of correlated mating (rpm = 0.032-0.137) were variable across plots but showed a consistent difference, with logged plots in both cases having lower levels than unlogged plots (and consequently lower estimates of mean numbers of pollen donors per mother tree, 1/rpm).

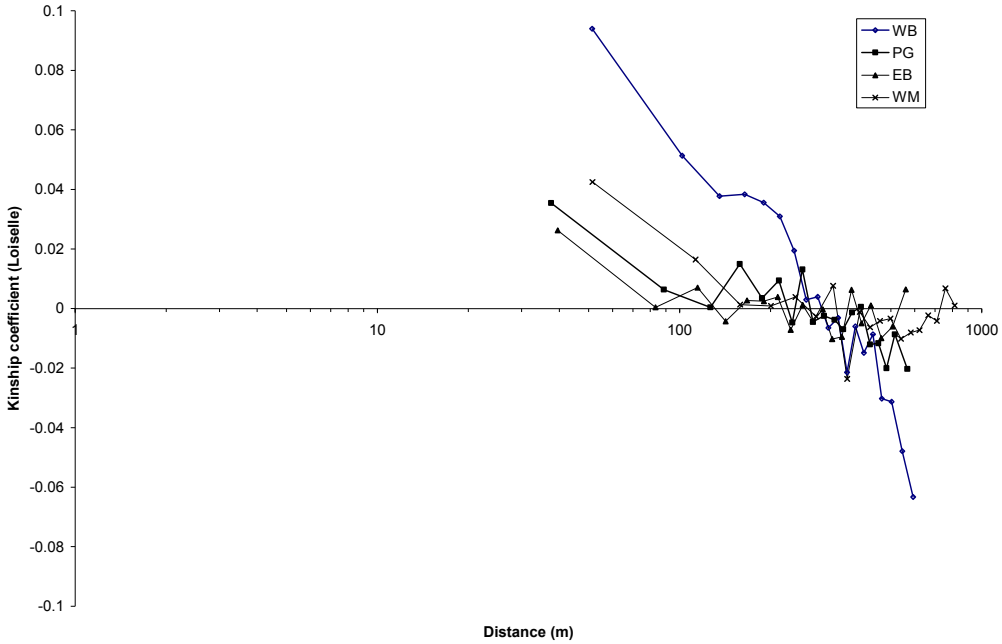


Figure WP3.6: Patterns of spatial genetic structure for four plots of *S. macrophylla* from Belize, based on the Loiselle kinship coefficient (analysis conducted using Spagedi v1.2, Hardy & Vekemans 2002). Distance scale is logarithmic. Populations are Punta Gorda (PG), West Botes (WB), West Marimba (WM), East Botes (EB).

In individual based analysis of mating system per mother tree, a clear trend was detectable across all plots (Figure WP3.), with mothers having greater numbers of conspecific trees within 100 m showing increased numbers of pollen donors, as would be expected. Absolute densities of all trees surrounding mothers are currently being generated to investigate the effect of total forest density on mating system.

Table WP3.3: Mating system variation for progeny arrays from four plots of *S. macrophylla* from Belize. Populations are Punta Gorda (PG), West Botes (WB), West Marimba (WM), East Botes (EB). PG and WB have been logged, using a selective logging strategy, during the late 1990's. Analysis was carried out using mltr v3.2 (Ritland, 2002).

Plot	Plot treatment	F	tm	ts	tm-ts	rt	rpm	rps	rpm-rps	1/rpm
PG	LOGGED	0.069	0.991	0.980	0.011	0.100	0.032	0.073	0.041	31.25
EB	UNLOGGED	0.000	1.000	0.982	0.018	0.111	0.091	0.092	0.001	10.99
WB	LOGGED	0.056	0.987	0.936	0.050	0.117	0.099	0.115	0.017	10.10
WM	UNLOGGED	0.023	0.982	0.952	0.030	0.243	0.137	0.156	0.020	7.30

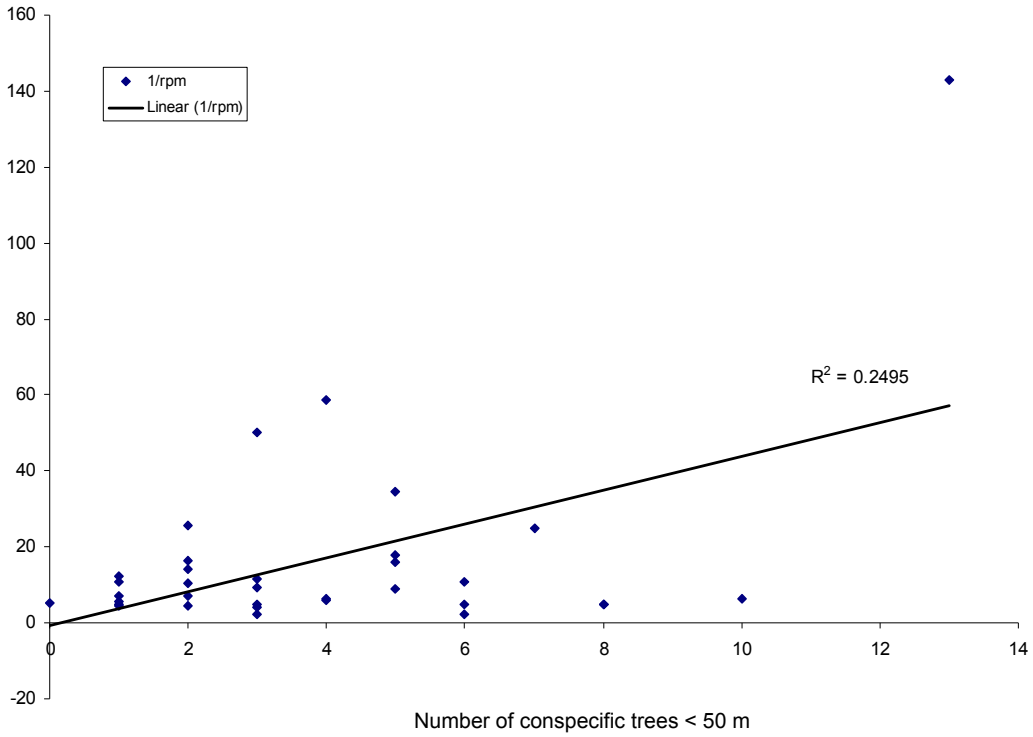


Figure WP3.7: Plot of mean numbers of pollen donors per mother tree (inferred from rpm estimates generated by individual family based mating system analysis, using mltr v3.2 (Ritland 2002)) versus local density of conspecific trees, in this case figures are numbers of conspecific trees within 50 m of the mother tree.

In paternity analysis, stability of LOD scores was obtained when some degree of biparental inbreeding allowed for, an expectation deriving from the clear spatial genetic structure detected in all plots, although not clearly signalled by mating system analysis. Selfing, which was observed in mating system analysis, was also allowed to be a possibility. LOD score thresholds were tested 10 times for each simulation to determine at what point repeatable results were being obtained and then these limits were used for paternity assignment. Outputs from the simulations are given in Table WP3.4. Using this approach (which provided highly conservative confidence limits) it was possible to reconstruct a total of 33 matings from the complete dataset. A high proportion (>70%) of matings appear to have arisen outside the sampled plot, as would be expected within an area of continuous forest. On the basis of the mating events detected, the minimum distance detected between two parents for the observed matings was 38.47 m and the greatest pollen flow distance was 843.93 m. The mean pollen flow distance was 371.15 m (SD = 219.03m). Reconstructed mating events are shown, for the West Marimba plot, in Figures WP3.8 and 3.9. Interestingly the majority of matings appear to take place in a plane, although what influences this pattern is not clear and is currently being evaluated.

Table WP3.4: Results of paternity analysis simulations carried out using CERVUS 3.2 (Marshall et al, 1998). Simulations used individual plot genotype data to generate allele frequencies, and a simulated sample of 10,000 progeny. Populations are Punta Gorda (PG), West Botes (WB), West Marimba (WM), East Botes (EB).

Identity of most likely candidate father, given known mother	WM	WB	EB	PG
True Father	0.13	0.12	0.16	0.14
Non-father (true father sampled)	0.18	0.17	0.13	0.15
Non-father (true father unsampled)	0.69	0.71	0.71	0.71

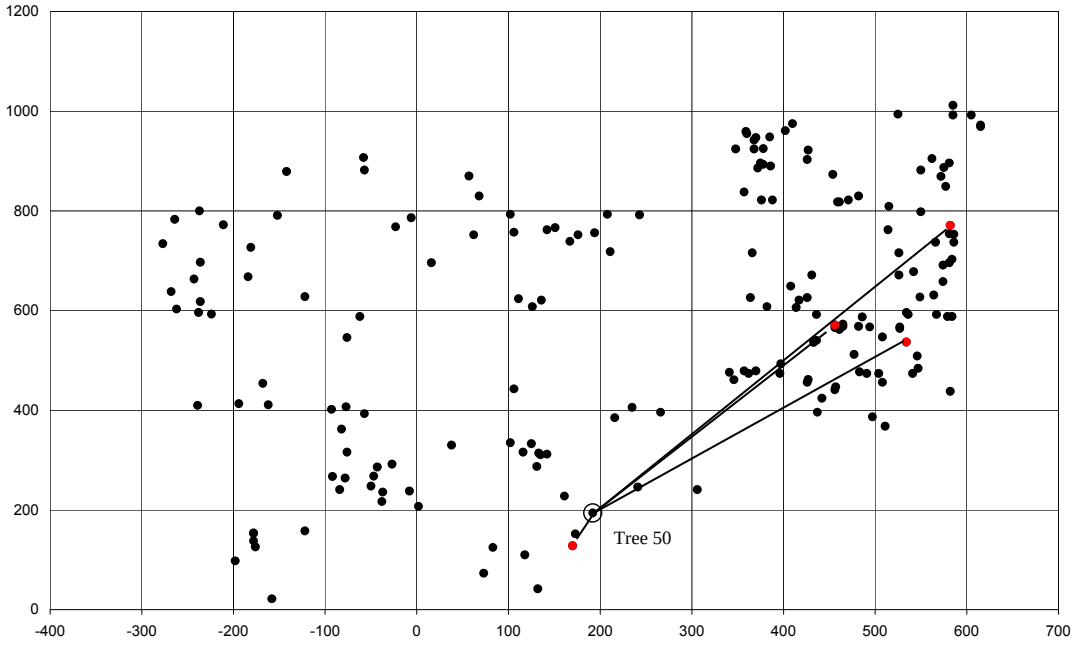


Figure WP3.8: Reconstructed mating events for mother Tree #50 in plot West Marimba. Paternity analysis was conducted using CERVUS 3.2 (Marshall et al, 1998). Simulations used individual plot genotype data to generate allele frequencies, and a simulated sample of 10,000 progeny.

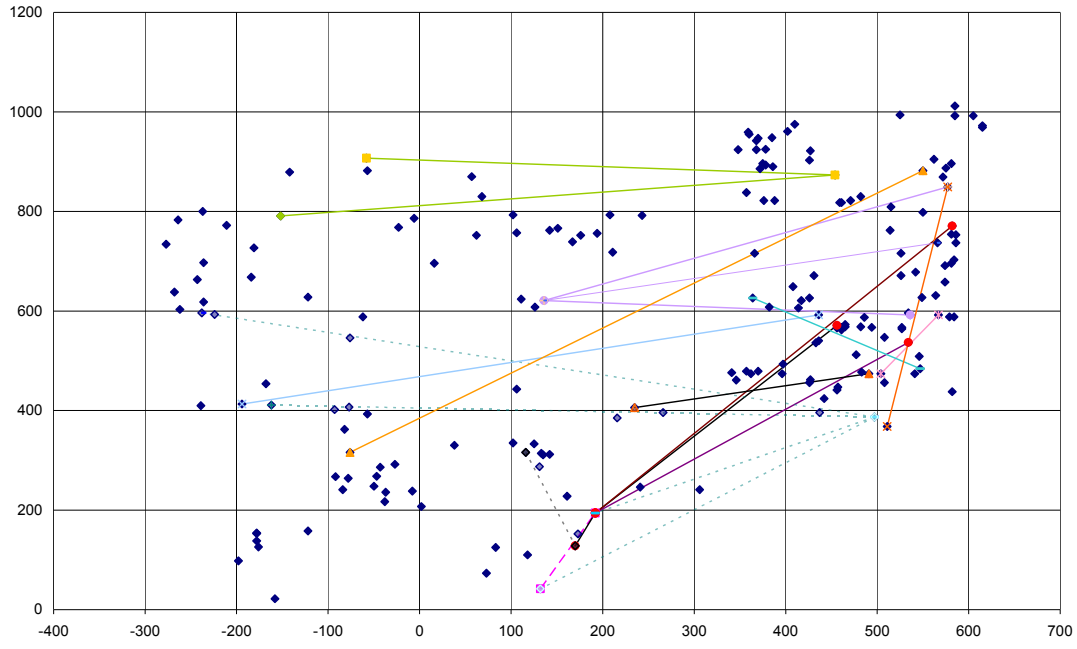


Figure WP3.9: Reconstructed mating events for all detectable matings in plot West Marimba. Paternity analysis was conducted using CERVUS 3.2 (Marshall et al, 1998). Simulations used individual plot genotype data to generate allele frequencies, and a simulated sample of 10,000 progeny.

3.2.3 Araucaria angustifolia

Araucaria angustifolia (or Brazilian pine) occurs mainly in southern Brazil, with some small extant populations in southeastern Brazil, northeastern Argentina and eastern Paraguay, growing in low mountains at altitudes of 500-1800 meters. The main distribution of *A. araucana* or monkey-puzzle tree is in the Andean region at the frontiers between Chile and Argentina, with two disjunct populations in the Coastal Range of Chile. It is an evergreen tree growing to 40 m tall and 1 m trunk diameter. The leaves are thick, tough and scale like, triangular, 3-6 cm long, 5-10 mm broad at the base, and with razor-sharp edges and tip. They persist for 10-15 years, so cover most of the tree except for the trunk and older branches. It is usually dioecious, with the male and female cones on separate trees. The male (pollen) cones are oblong, 4 cm long at first, expanding to 10-18 cm long by 15-25 mm broad at pollen release. Like all conifers it is wind pollinated. The female (seed) cones, which mature in autumn about 18 months after pollination, are globose, large, 18-25 cm diameter, and hold about 100-150 seeds. The cones disintegrate at maturity to release the 5 cm long nut-like seeds, which are then dispersed by the Azure Jay *Cyanocorax caeruleus*. It is closely related to *Araucaria araucana* from further southwest in South America, differing most conspicuously in the narrower leaves.

Araucaria angustifolia prefers well drained, slightly acidic soil but will tolerate almost any soil type provided drainage is good. It requires a subtropical climate with abundant rainfall, tolerating occasional frosts down to about −5 °C to −8 °C. It is a popular garden tree in subtropical areas, planted for its unusual effect of the thick, 'reptilian' branches with a very symmetrical appearance. The seeds, similar to large pine nuts, are edible, and are extensively harvested in Brazil, particularly by Native American people. The seeds, called *pinhão* are popular as a winter snack.

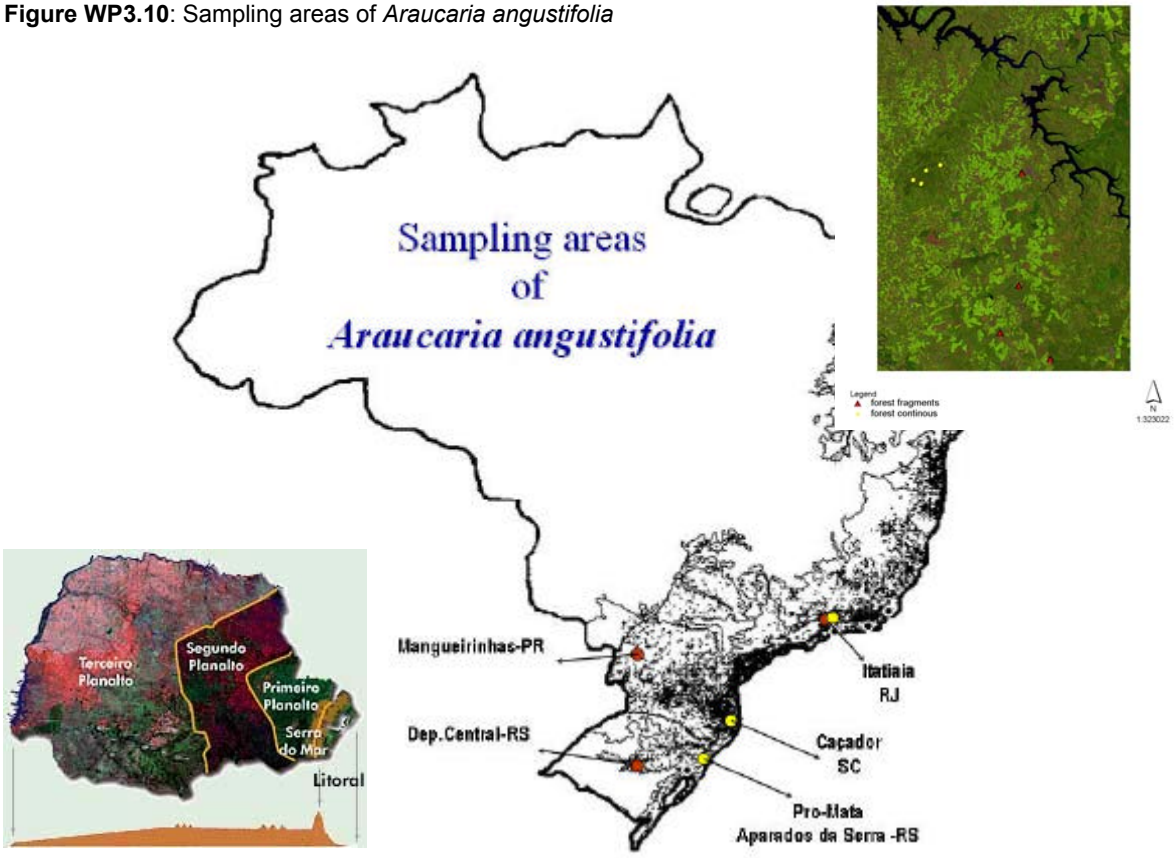
Logging has resulted in an extreme reduction in population size over the course of the 20th century. It is estimated that a reduction of 97% of the species area of occupancy has taken place due to logging between the beginning of the 20th century and the early 1980's (Enright and Hill, 1995) - a forest reduction of over 97% within three tree generations. Plantation forestry with *Pinus* and *Eucalyptus* as well as other land use have made restoration unlikely in much of the area; on the other hand plantation of Araucarias reached 90,000 ha in the mid 1990s. The species has been placed on the IUCN Red List as Critically endangered (CR A1cd).

Table WP3.5 : Characteristics of the eight *Araucaria angustifolia* sites sampled.

Plot (Owner)	Area (ha) of forest	Disturbance history	N_i
1 RIM	4000	Continuous	58
2 RIM	4000	Continuous	56
3 RIM	4000	Continuous	26
4 RIM	4000	Continuous	26
5 (Claudio Calgaro)	22	Fragmented/isolated/selectively logged /regeneration	91
6 (Cassemiro Sene)	5	Fragmented/selectively logged /regeneration/grazing cattle	29
7 (Edersio Reis)	18	Fragmented/selectively logged/grazing cattle	66
8 (Marcelo Reis)	10	Fragmented/selectively logged /regeneration	60

N_i – number of individuals sampled in each area inside of 1 ha plot.

Figure WP3.10: Sampling areas of *Araucaria angustifolia*



For the study, two sites were selected: one in continuous forest and the other in fragmented forest. The "Reserva Indigena de Mangueirinha - RIM" was chosen as the continuous forest site, and belongs to FUNAI (National Foundation for Indians). RIM is the largest remnant of Araucaria forest in Paraná state. Forest fragments were selected from Mangueirinha municipality where Araucaria angustifolia was located in forest patches and the landowner permitted access to the site. Eight subpopulations were selected: 4 in continuous forest and 4 in the fragmented sites (Figure WP3.10). All the subpopulations of the study area are located on the third plateau of Parana State (Figure WP3.10), 535 km from the Atlantic coast. The entire area is within the Iguaçu catchment (Figure WP3.10). The mean maximum temperature is 20.3 C and the mean minimum is 12.2 C. Severe frost occurs during the winter.

The natural vegetation of the region was Araucaria angustifolia forest and associated broadleaved species. After fragmentation the landscape comprises a mosaic of patches of agricultural land, pasture, urban areas and forest. The plots were established following a standardised design in the continuous and fragmented areas: all subplots were characterized by homogeneous conditions. Homogeneity of the environmental conditions means minimal human impact, layers of forest easily recognized, similar conditions of soil and slope. The spatial distribution of Araucaria angustifolia in the field was similar in each of the study plots. Table WP3.5 shows the number of trees per plot of one hectare in the continuous forest and fragmented plots.

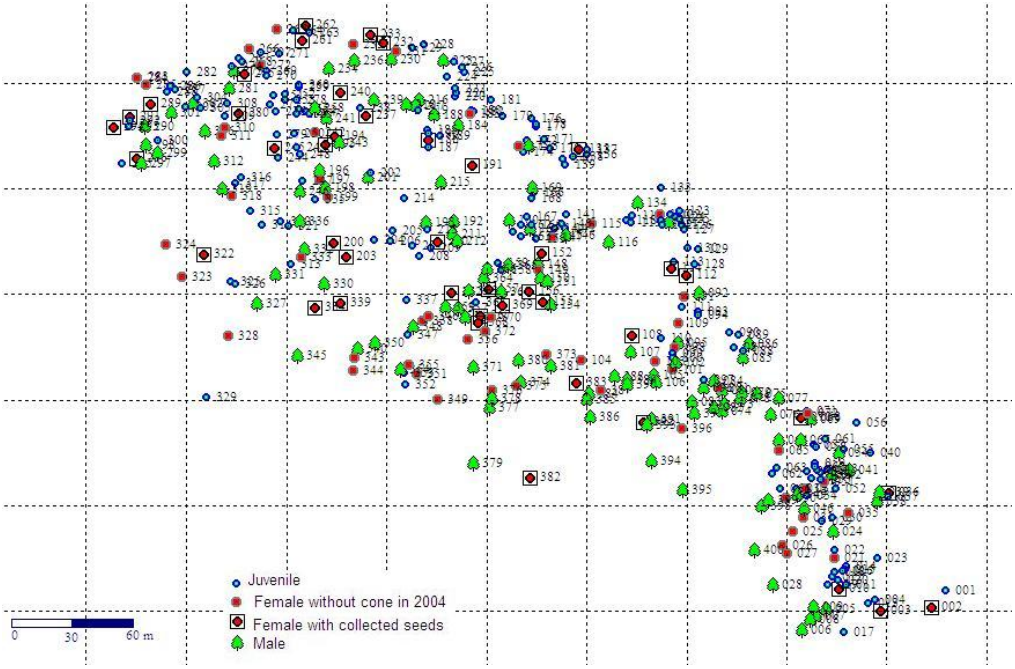


Figure WP3.11: Intensive study site for *Araucaria angustifolia*

Cambium material was collected from each mapped tree. The cambium material was preserved in eppendorf tubes with a solution of CTAB buffer (1/3) and ethanol (2/3). On the return to the laboratory the material was stored at –20° C before DNA extraction. Total genomic DNA was extracted from cambium using a modification of the small-scale method of Doyle and Doyle (1987). Genetic diversity was assessed for 8 microsatellite loci. Polymerase chain reaction (PCR) conditions used were establish by Master Mix Qiagen (1022830) protocol and fluorescent dye labeling. The choice of the fluorescent dye label for each microsatellite primer was based on its observed allelic size range. Loci with overlapping allele sizes were multiplexed by labeling them with different dyes. Two multiplexed systems of microsatellites were developed with the 8 primers. Following PCR, the reactions were diluted according to the intensity checked in agarose gel. Then this dilution was run in ABI sequencer 3100 according the apparatus specification. Gene Scan and Genotyper software were used for data collection and analysis.

To understand the dynamics of genes and seed dispersion of *Araucaria angustifolia*, the tree collection was carried in plot 6 (Figure WP3.11). Seed collections were taken from all the trees in plot 6 that produced seed in 2004 (38 mother trees). Seed from the same number of mother trees was collected in the continuous forest to compare the gene flow process with the fragments. Seed from the continuous forest was collected around plot 3; this place shows a very good forest structure (less disturbance). In 2004, seeds of 38 mothers were collected. All these seeds were frozen without DNA extraction. The adult trees collection comprised, males, females, juveniles and regeneration (seedling): relative proportions are given in Figure WP3.12. Juvenile and seedling are individuals without sex distinguished. Cambium tissue was samples for juveniles; leaf was collected for seedlings.

Figure WP3.12: Relative proportions of *A. angustifolia* individuals collected in different classes.

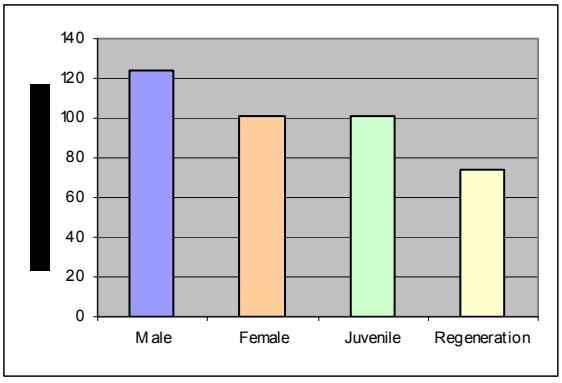


Table WP3.6:

Test of Hardy-Weinberg equilibrium for the four SSRs loci.

Loci	P	SD
AS25	1,0000	0
AS90	0,051*	0,003
CRCAc1	1,0000	0
CRCAc2	0,0624*	0,0038

Results obtained using the TFPGA (Tool for Population Genetics Analysis) software with the procedure proposed by Guo and Thompson (1992). P= probability to be in equilibrium; SD = Standard deviation; *when more than two alleles were present, p = probability of exclusion of the existance of equilibrium.

Clumps of trees in agriculture or pastureland are common in the state of Paraná. In the conservation of the species clumps of trees can act as stepping-stones or even seed sources. To cover these aspects tissue samples from the trees in the clumps were collected between plots 5 and 6 (which are separated by 40 km). In total, samples were collected from 4 clumps; each one differs in the number of trees, sex ratio and seed production. So when seeds were found, these were collected. This activity will also provide information about gene flow. The size of each island was not determined by the spatial area (hectare); it was according of the existence of single *Araucaria angustifolia* trees in a no forest patch and not physically connected with araucaria forest. Places with this characteristic along the motorway between fragment 6 and 5 could be found four islands with single *Araucaria angustifolia* trees. The number of trees in each island was different. In the island only adult trees were found, as is always the case in agriculture land. DNA was extracted from the single tree, from trees 512, 523, 528 and 533 and from seeds (20 embryo and their megagametophytes). From female trees found with seeds in the islands seeds were collected and DNA extracted. At least 40 seeds per female trees producing cones in 2004 were collected.

Publications arising:

Salgueiro F., H. Caron, M.I.F. de Souza, A. Kremer, R. Margis (2005) Characterization of nuclear microsatellite loci in South American Araucariaceae species. *Molecular Ecology Notes*, **5**, 256-258.

Salgueiro F (2005) Organization and dynamic of the genetic diversity in two species from the Brazilian Atlnatic Rain Forest: *Araucaria angustifolia* [Bert.] O. Kuntze and *Eugenia uniflora* L. Ph.D. Thesis of , Department of Genetics, Federal University of Rio de Janeiro, July 2005.

de Souza MIF (2006) Analyses of genetic diversity of *Araucaria angustifolia* [Bert.] O. Kuntze using AFLP markers". Master Degree Thesis, Department of Genetics, Federal University of Rio de Janeiro, March 2006.

3.2.4 Symphonia globulifera

Symphonia globulifera L. f. (Clusiaceae) is an hermaphroditic species with an exceptionally large geographic distribution, occurring from Mexico south to Rio de Janeiro, and including tropical West Africa. Dick et al (2003) studied the rangewide phylogeography of *S. globulifera* and identified likely colonisation of Central and South-America through marine dispersal from Africa during the mid Miocene (ca. 15 million years ago). The density of *S. globulifera* is extremely variable among populations. Counting trees with diameter at breast height (d.b.h.) ≥10 cm, densities of 122 N/ha (Quakal swamp forest) and 65 N/ha (Manicole swamp forest) have been reported in Guyana (Andel, 2003). At the other extreme, the population of *S. globulifera* on Barro Colorado Island (Panama) has a density of only 0.5 trees/ha (Center for Tropical Forest Sciences, 2000 forest census). There are also contrasting reports on the community of pollinators of *S. globulifera* at different places. In Costa Rica, Pascarella (1992) observed Lepidoptera as the most important pollinators; in central French Guiana, Gill et al (1998) identified perching birds as the principal pollinators, whereas Bittrich and Amaral (1996) and Maues (2001) suggested hummingbirds as the pollinators in the Central Amazon.

Three microsatellite loci were used (two newly developed for this study under WP1, one as available from Aldrich et al, 1998) to study gene flow and mating system of *S. globulifera* at the experimental site 'Paracou' in French Guiana. This site is characterised by a relatively high density of this species. The following specific questions were addressed: Do we also find long distance pollen dispersal for this abundant tropical tree species? Do we observe a spatial genetic autocorrelation in the adult population and, if yes, does it fit to expectations given by the measured gene flow and mating system? Is the mating system of high-density animal-pollinated tree species different from observations of low-density species? The answers to these questions will help to address whether abundant species are more sensitive to logging operations than rare species.

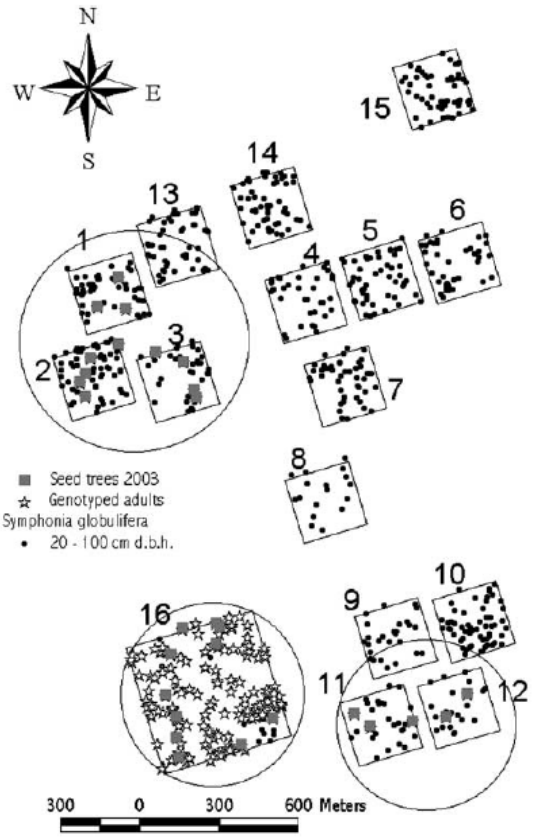


Figure WP3.12: Distribution of *S. globulifera* with d.b.h.≥20cm in Paracou. Most of the trees sampled for genotyping were located in plot 16 (stars). We sampled 560 seeds from 28 mother trees (squares) in three different parts of Paracou (circles) (from Degen et al 2004).

Cambium was collected from 164 trees, including 147 trees from plot 16, (500x500m) and the 17 sampled seed trees outside plot 16. In February 2003, we collected 560 seeds from 28 mother trees (20 seed each) distributed in three clusters over the experimental site. This provided samples at different spatial scales, because we had no *a priori* information whether the average pollination distance would be in the 30 or 500m range. Genetic variation and heterozygosity: For each locus, the number of different alleles (A), observed heterozygosity (H_o), expected heterozygosity (H_e), the effective number of alleles (A_e) and the Fixation index (F) were calculated as described by Weir (1996). Spatial genetic structure: Moran's Index I_q was computed with the program SGS (Degen et al, 2001b) for multilocus genotypes of the adults (Sokal and Oden, 1978a). Mating system: Using the mixed mating model (Ritland and Jain, 1981), we estimated the single- and multilocus outcrossing rates. The outcrossing rates were calculated with the program MLTR version 2.3 (Ritland, 2002) by maximum likelihood, fitting the observed proportions of genotypes descended from a known maternal genotype to the proportions expected under the mixed mating model. TwoGener: Following Smouse et al (2001) we made a Twogener analysis on the progeny arrays. The principle of this method is to estimate Φ_{FT}, the differentiation of allelic frequencies among the pollen pools sampled by several mother trees in the population. This provides an estimate of the pollen dispersal distance (delta) assuming a given dispersal curve and a density of reproducing adults (d) in the landscape.

Table WP3.7: Sample size (N), number of alleles (A), effective number of alleles (Ae), observed and expected frequencies of heterozygotes (Ho, He), Fixation index (F) and probability for departure from Hardy–Weinberg Heterozygosity (P) for adults and seeds.

Locus	*Adults (N = 164)*						*Seeds (N = 534)*					
	A	A_e	H_o	H_e	F	P	A	A_e	H_o	H_e	F	P
Sg03	26	9.38	0.59	0.89	0.33	0.000	20	8.46	0.70	0.88	0.20	0.000
SgC4	30	14.52	0.93	0.93	0.00	0.500	26	14.02	0.90	0.92	0.02	0.026
Sg18	13	5.03	0.66	0.80	0.17	0.000	17	7.25	0.74	0.86	0.13	0.000
Mean	*24*	*8.02*	*0.73*	*0.87*	*0.16*	*0.000*	*21*	*9.16*	*0.78*	*0.89*	*0.12*	*0.000*

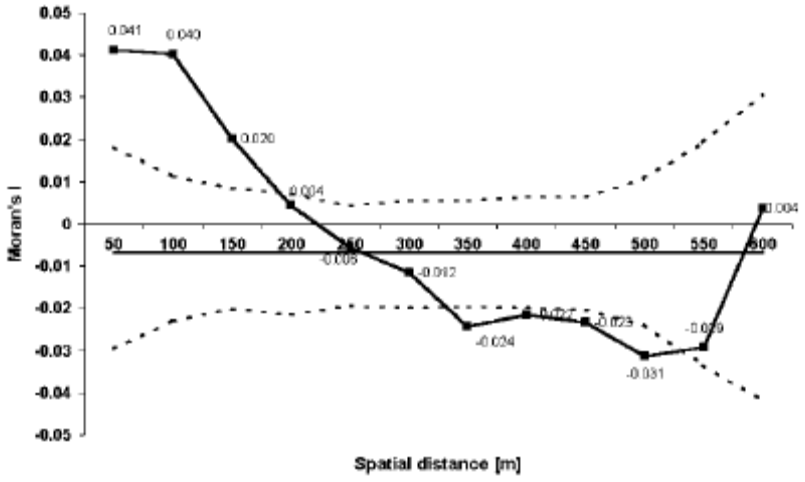


Figure WP3.13: Mean Moran's Index among trees in different spatial distance classes (line with squares), 95% confidence interval as drawn from 1000 permutations (dotted line) and expected Moran's Index for absence of spatial genetic structure (black central line).

Table WP3.8: Density of reproductive trees and mean pollen dispersal distance (delta) estimated for the normal and exponential dispersal model. The error is a quadratic criterion for the fit between expected and observed values for pairwise Fij estimates (Austerlitz and Smouse, 2002)

Dispersal function	*Density constraint*	*Density of reproductive trees (N/ha)*	*Delta (m)*	*Error*
Normal	Fixed	4.0	27.4	1.70
Normal	Estimated	1.6	42.9	1.69
Exponential	Fixed	4.0	30.9	1.69
Exponential	Estimated	1.3	53.1	1.68

With mean values of Ae=8.02 and 9.16, for adults and progeny respectively, we observed relatively high values for the effective number of alleles. Seeds were sampled in three different parts of the Paracou sites, whereas the adults are mostly from one area (plot 16), which might explain the higher variation in the seed array. Our values were slightly higher than the mean value observed by Aldrich et al (1998) for *S. globulifera* in Costa Rica (Ae=7.02). Most other neotropical tree species had lower genetic diversity. In French Guiana, Latouche-Halle et al (2003) observed a mean value of Ae=3.23 for the tree species *Dicorynia guianensis*, and in the same region Dutech et al (2002) found values between 1.69 and 2.08 for *Voucapoua americana.* Dick et al (2003) measured a mean value of Ae=3.50 for *Dinizia excelsa* in Manaus (Brazil). The high level of diversity in *S. globulifera* is probably linked to its large geographic distribution, fitting the pattern seen for many tropical tree species using allozymes (Loveless, 1992).

For both the seeds (F=0.12) and the adults (F=0.16), we observed a significant excess of homozygotes compared to the expected Hardy–Weinberg proportions. This may be explained by the presence of null alleles, selfing and/or biparental inbreeding. Using information on the selfing rate (s), mean pollen dispersal (delta) and spatial genetic structure of adults, Fenster et al (2003) provided a formula to estimate an expected Fixation index (Fexp) due to selfing and biparental inbreeding. Using this, we calculated an expected value of Fexp=0.065. Hence inbreeding would explain at least part of the observed excess of homozygotes. There seems to be no strong selection against inbred progeny because the adult sample also had an excess of homozygotes. In other studies, a decrease of F-values has been reported with increasing age of the analysed ontogenetic stages (Morgante et al, 1993).

The estimated multilocus outcrossing rate (tm) was 0.920, confirming that *S. globulifera* is a predominantly outcrossing species. We observed a significant level of biparental inbreeding in Paracou (tm-ts=0.156), which may be explained by limited pollen dispersal within the range of family structures. We found significant positive spatial autocorrelation of genotypes for the adults up to 150m (Figure WP3.13). The high biparental inbreeding can be explained, if we compare the estimated range of mean pollen dispersal of 27–53m with the scale of the observed spatial structure. It is quite clear that in Paracou most of the pollen flow is within a range of significant spatial structure. Another striking result was the high proportion of full-sibs (rp=0.47). This fits with the high level of biparental inbreeding. In Paracou, the trees are pollinated by a rather limited number of trees close to the mother tree. The population in Paracou has a high density of adult trees. Hence, we expected to have more different pollen donors represented in the offspring in Paracou. This contradictory result might be explained by unsynchronised flowering phenology in Paracou and by the composition and behaviour of the pollinators. As seen elsewhere (Franceschinelli and Bawa, 2000), the trees in Paracou might be pollinated by rather territorial pollinators. The TwoGener approach assumes homogeneous tree densities and independent pollen dispersal events following an isotropic distribution. Using Clark and Evan's index (R), it could be shown that S. globulifera trees have a random spatial distribution in Paracou (Degen et al, 2001a). If pollinator behaviour causes strong preferential mating among particular adults, the mean dispersal distance (delta) should be underestimated. Similarly, variation in flowering

intensities or phenology among adults causes delta to be underestimated when the density is fixed. Nevertheless, these effects should be minimised by the joint estimation. By use of the TwoGener approach we estimated, for the population in Paracou, mean pollen dispersal distances (delta, Table WP3.8) between 27 and 53 m. The values differed according to the dispersal model used (normal vs exponential model) and the estimation method (only delta estimation vs joint estimation of delta and density). The joint estimation calculated an effective density of 1.6 reproductive trees/ha for the normal model and 1.3 reproductive trees/ha for the exponential model. This would imply that effectively about 13% of all trees ≥10 cm d.b.h. contributed to reproduction. In comparison to other results (e.g. Dick et al 2003, Sork et al 2002) , the pollen dispersal of *S. globulifera* is short. This raises the possibility that birds, as the suspected main pollinators, might be less efficient than bees or wind. In general, pollen dispersal seems to be negatively correlated with the tree density: the high tree density in Paracou led to short pollen dispersal. In contrast to studies with RAPDs (Degen et al, 2001a) we found a weak but significant positive spatial genetic autocorrelation up to 150m (maximum of Moran's I=0.041) and a significant negative autocorrelation from 300 to 500m (minimum of Moran's I=-0.031). This means close individuals are genetically more similar and pairs of individuals in a distance between 300 and 500m are more different than expected for a random distribution. This is a pattern expected for clinal variation due to limited gene flow (Sokal and Oden, 1978b). The range of Moran's I is small compared to other tree species studied at the same site. Latouche-Halle et al (2003) observed for the rather aggregated tree species *Dicorynia guianensis* maximum values for Moran's I at microsatellite loci of between 0.1 and 0.25 in the first distance class up to 50 m. Hence, of the two species, *S. globulifera* has a weaker but larger spatial genetic structure. This can be explained by long distance seed dispersal (Hardy and Vekemans, 1999) and an overlapping of seed shadows. Seed dispersal is bat mediated in *S. globulifera*, suggesting more limited pollen dispersal than seed dispersal, in contrast to other species, due to its pollinator behaviour.

Publications arising:

Degen B, Bandou E, Caron H (2004) Limited pollen dispersal and biparental inbreeding in *Symphonia globulifera* in French Guiana. *Heredity*, **93**(6) 585-591.

3.3 Secondary Regeneration: Vochysia ferruginea

Vochysia ferruginea is a pioneer tree of Central America, it is a widespread dominant of secondary forests and occasionally found as a canopy tree in primary forest. It is able to tolerate low nutrients, high concentrations of soil toxic elements and high levels of disturbance. These characteristics make it of interest to forestry as a crop species and as a tree able to regenerate degraded land. Three sites in Costa Rica have been selected, each of which contain a stand of dense *Vochysia* dominated secondary forest adjacent to a stand of primary forest containing fewer source *Vochysia* trees. At each site a continuous block of 100 adult trees has been sampled from primary and secondary forest and at two sites a seed collection has been taken from 20 adult trees in both primary and secondary forest for paternity analysis. For all sites CATIE have long term ecological data making these populations particularly useful to investigate genetic parameters along with ecological factors.

Figure WP3.14: La Ladrillera sample site for *Vochysia ferruginea* in Costa Rica. Total sample 407 adults, 100 seedlings

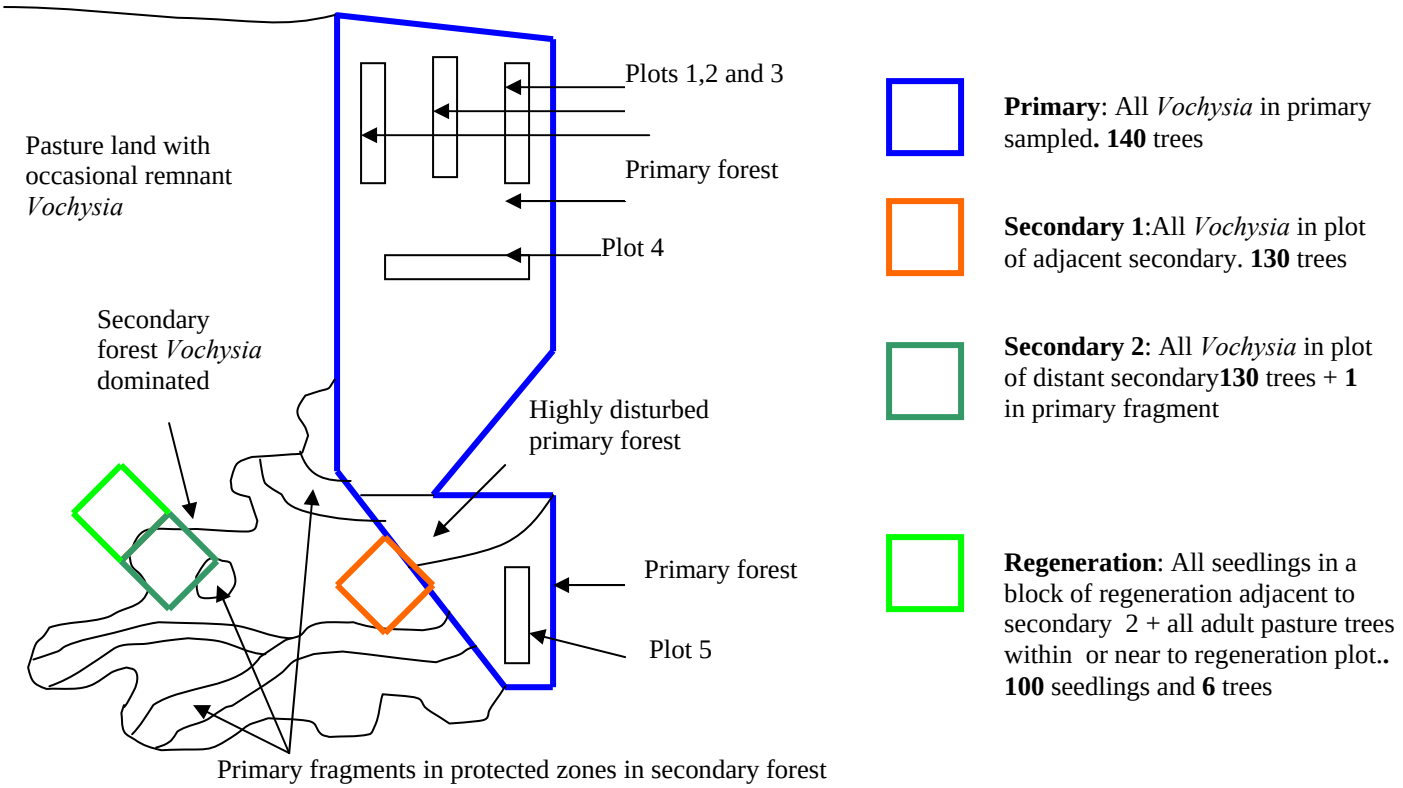
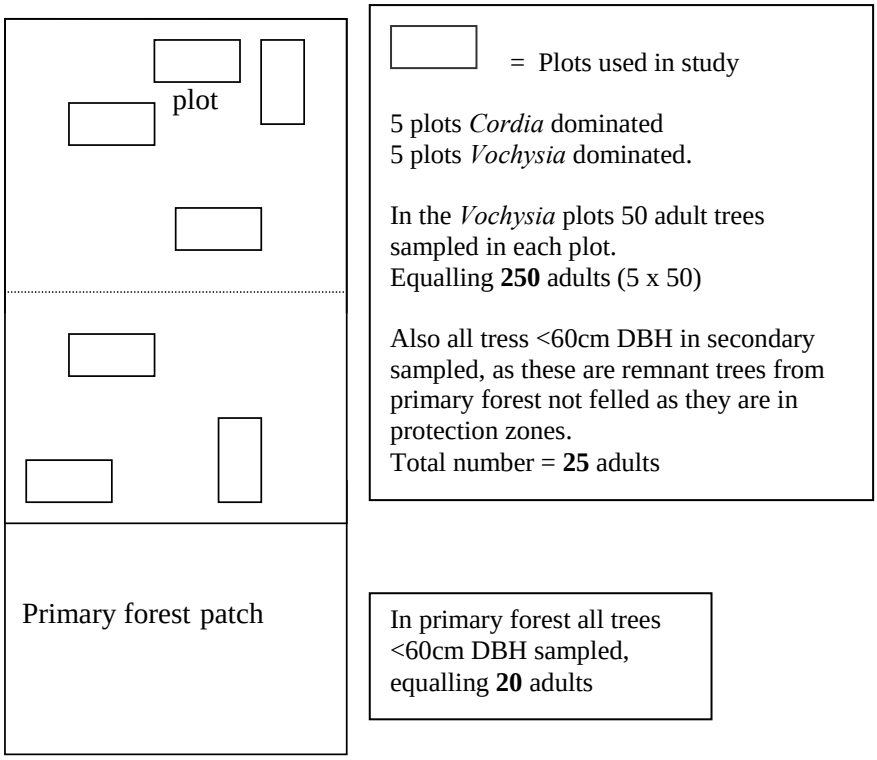


Figure WP3.15: Florencia sample site for *Vochysia ferruginea* in Costa Rica. Total sample 295 adults



Methods.
DNA was extracted from leaf material using a standard CTAB protocol (Doyle & Doyle 1987) and DNA was extracted from cambium using a commercial extraction kit (DNeasy 96 Plant Kit, UK QIAGEN LTD 2003 – 2005). Microsatellite analyses were made using 5 marker loci, developed and optimised for *V. ferruginea* (Lowe *et al.* 2003). Microsatellite locus amplification was performed using a touchdown PCR protocol. PCR products were visualised on polyacrylamide gels using Licor 4800 IR2 automated genotyper and allele size was determined by manual scoring using SAGA software.

Statistical analysis
Gene diversity was calculated as $H_{S,}$ a measure of within sample gene diversity, and H_T, a measure of overall gene diversity. Hardy-Weinberg equilibrium over all samples was tested with 1000 permutations, using the program GENEPOP 3.4. The level of genetic variation within populations (quantified by assessing allelic richness and genetic diversity for each of the five microsatellite loci and averaged over all loci) and level of inbreeding (F_{IS}) were estimated using FSTAT 2.9.3 and tested using bootstrap sampling with 1000 simulations. Estimates of gene diversity per locus and over all loci use an unbiased estimator. Genetic differentiation (F_{ST}) between all pairs of populations was estimated by multilocus weighted analysis of variance using the program GENEPOP 3.4. This analysis was tested using bootstrap sampling with 1000 simulations.

Mating system parameters were estimated using maximum likelihood procedures based on the mixed mating model proposed by Ritland & Jain (1981) and using the multilocus mating system analysis program MLTR. The parameters estimated from the progeny array data were the multilocus outcrossing rate (tm), the average singlelocus outcrossing rate (ts), the biparental inbreeding rate (tm-ts) and the correlation of paternity (rp).

Results

Locus diversity and Hardy-Weinberg equilibrium

All five microsatellite loci were highly polymorphic, the mean number of alleles per locus was 16.2, ranging from 13 to 24 (see Table WP3.9). The observed proportion of heterozygosity ranged from 0.568 to 0.8, loci with higher levels of polymorphism did not necessarily show higher levels of heterozygosity. Gene diversities values within samples were similar to the overall gene diversity. Diversity within samples ranged from 0.583 to 0.79 and overall diversity ranged from 0.719 to 0.838 (Table WP3.9). Loci differed greatly in tests for Hardy-Weinberg equilibrium with *P* ranging from 0.000 to 0.998.

Within populations, the mean sample size per locus was significantly smaller than the population size, in most cases mean sample size was halved (see Table WP3.10). This was a consequence of difficulties in successful amplification of loci, particularly A1-5 (see Table WP3.9). Table WP3.10 also shows mean number of alleles per locus for each population; however, due to the differing sample sizes allelic richness is a better measure of the number of alleles per population. Populations differed significantly regarding deviation from Hardy-Weinberg equilibrium, with *P* values ranging from 0 to 1.

Genetic diversity

The two sites showed similar patterns in the changes of allelic richness between different populations but varied in the way diversity changed across populations. At Ladrillera, diversity and allelic richness decreased moving from primary to secondary 1, then to secondary 2, then to seedlings. In Tirimbina, across the same gradient, diversity stayed constant, whilst allelic richness alone dropped from primary forest through to secondary forest then to seedlings. See Table WP3.11 for values of genetic diversity (Nei 1987) and allelic richness.

At Tirimbina the greatest diversity was found in the primary fragments (H_E =0.77), although this small population showed very low allelic richness (1.82). The primary forest population at Tirimbina had both greater diversity (H_E =0.74) and allelic richness (8.86) than secondary forest (0.72 and 7.95 respectively), although the reduction is small. There was no significant difference in diversity between both the secondary and primary forest populations and the seedling population (seedling population diversity = 0.74); however, there were significantly fewer alleles (allelic richness = 4.76). There was a high level of diversity and allelic richness found in seeds taken from mother trees in both the primary and secondary populations, with the number of alleles found in the progeny arrays exceeding that found in the adult populations. Seeds taken from trees in primary forest had a diversity of 0.75 and an allelic richness of 9.08, secondary forest had a diversity of 0.78 and an allelic richness of 9.90. Therefore, at the Tirimbina site genetic diversity was similar across all populations but allelic richness was greatest in primary forest, decreases in secondary forest and decreases further in seedlings.

A more complex pattern is observed in Ladrillera populations, here primary forest showed a lower level of diversity and allelic richness to that found in Tirimbina (diversity is 0.68 and allelic richness is 5.71). The two secondary forest populations showed very different patterns of diversity. The secondary forest population adjacent to primary forest had greater diversity than the primary forest population (0.74) and showed higher allelic richness (8.2). The other secondary forest population, further from the primary forest, showed a lower diversity and allelic richness than both the first secondary population and that of the primary forest (diversity = 0.6 and allelic richness = 4.5). The seedling population at Ladrillera, adjacent to this lower diversity secondary forest, exhibited a further decrease in diversity (0.47) and again a loss of alleles (allelic richness = 3.48). As in the Tirimbina populations, diversity and allelic richness was high in the progeny arrays with seeds collected from both primary and secondary forest showing a diversity equivalent to that of primary forest (0.7 in progeny from secondary forest and 0.67 in progeny from primary). Progeny from both secondary and primary forest populations also had a greater number of alleles present than in the adult populations. At Ladrillera, seeds taken from trees in primary forest had a diversity of 0.67 and an allelic richness of 9.44, secondary forest had a diversity of 0.7 and an allelic richness of 10.07.

Deficit of heterozygotes (F_{IS})
Most populations had low or negative levels of F_{IS} (see Table WP3.11).There was a small excess of homozygotes found in the seedling population at Tirimbina (F_{IS} = 0.11) but this was not seen in seedlings at Ladrillera. There was a small excess of heterozygotes found in the secondary forest population in Tirimbina (F_{IS} = -0.136) and also the low diversity secondary population at Ladrillera (F_{IS} = -0.157). However, the largest deficit of heterozygotes was found in Ladrillera secondary forest adjacent to primary forest block (F_{IS} of 0.175).

Mating system
Results from all populations show that *V. ferruginea* was largely outcrossing in both primary and secondary forest populations and also when found as isolated remnant trees in the abandoned plantation (outcrossing rate ranged from 0.85 to 1, see Table WP3.12). The approximate measure of uniparental selfing (the correlation of selfing among loci, rs) showed that there was very little selfing in most of the populations, (rs ranges from 0.001 to 0.217).

In Tirimbina primary forest populations, seeds were completely outcrossed (tm = 1) with very little biparental inbreeding found (tm – ts = 0.036). The multilocus correlation of paternity was estimated as 0.281, with an estimated 2.74 pollen donors contributing to the progeny array. In the secondary forest population, the seeds sampled were predominantly outcrossed with an increase in the amount of biparental inbreeding (tm – ts = 0.101) and a higher proportion of siblings sharing the same father (rp(m) = 0.425). The progeny from the two remnant trees in abandoned plantation at the Tirimbina site showed complete outcrossing (tm = 1.143) and a high degree of biparental inbreeding (tm – ts = 0.196). An outcrossing rate above 1 may be a consequence of small population size leading to high standard deviations. Most siblings did not share the same father (rp (m) = 0.206) and these trees had a higher number of pollen donors contributing to the progeny arrays (an estimated 4.854 donors) than all other populations.

A similar pattern was found in the Ladrillera populations. In the primary forest *V. ferruginea* was highly outcrossing (tm = 0.930) with little biparental inbreeding (tm – ts = 0.085) and a small proportion of siblings shared the same father (rp(m) = 0.299). However, there was a higher correlation of selfing in this population suggesting that where there was inbreeding found in the progeny array it is, compared to the other populations, more likely to be from uniparental inbreeding than biparental inbreeding. Progeny from the secondary forest also showed an increased level of selfing compared to Tirimbina (tm = 0.853) and a higher difference between multi and single locus measures of outcrossing (tm – ts = 0.109) also suggesting a large component of biparental inbreeding. The proportion of siblings sharing the same father (rp(m) = 0.365) was larger than in the primary forest but not as much as in the secondary forest at Tirimbina.

Table WP3.9: Characterisation of the five microsatellite loci employed using data from all populations. The SSR locus name; *N*, number of individuals; *A*, total number of alleles H_O, observed heterozygosity H_S, within sample gene diversity H_T, overall gene diversity; *P*, departure from Hardy-Weinberg among all populations using the Markov chain method

Locus	*N*	*A*	H_O	H_S	H_T	*P*
A1-5	884	23	0.758	0.79	0.838	0.000 (0.000)
A1-10	1455	13	0.8	0.746	0.838	0.998 (0.002)
A1-15	1495	14	0.649	0.585	0.751	0.023 (0.005)
A1-20	1236	16	0.568	0.673	0.719	0.000 (0.000)
A1-35	1010	15	0.772	0.717	0.784	0.013 (0.004)
Mean over all loci		16.2	0.709	0.702	0.786	0.004

Table WP3.10: Sample size, number of alleles and departure from Hardy-Weinberg in all populations averaged over loci. *N*, population size; *S*, mean sample size per locus; *A*, mean number of alleles per locus; *P*, departure from Hardy-Weinberg

Site	Population	*N*	*S*	*A*	*P*
Tirimbina	Progeny primary	15 x 20 = 300	163.8	10.2	0.485 (0.031)
	Progeny secondary	12 x 20 = 240	180.8	9.6	
	Seedlings	132	20	5.8	0.204 (0.012)
	Adult secondary	120	78	8.6	0.980 (0.006)
	Adult primary	100	60.4	9	0.000 (0.000)
	Primary fragments	12	7.6	4.8	0.118 (0.006)
Ladrillera	Progeny primary	20 x 20 = 400	289	10.2	0.000 (0.000)
	Progeny secondary	20 x 20 = 400	223.2	9.8	
	Seedlings	100	49.2	3.4	1.000 (0.000)
	Secondary 1	130	49.6	6.8	0.001 (0.000)
	Secondary 2	136	37.4	6.4	0.647 (0.019)
	Primary	140	57	7.6	0.081 (0.011)

Table WP3.11: Genetic diversity, allelic richness and F_{IS} in adult, seedling and progeny arrays where at the Ladrillera site secondary 1 is adjacent to primary and secondary 2 is adjacent to seedlings. *N*, population size; H_E, average genetic diversity over all loci according to Nei (1987); R_T, allelic richness; F_{IS}, deficit of heterozygosity

Site	Population		*N*	H_E	R_T	F_{IS}
Tirimbina	Progeny	In secondary	300	0.78	9.90	-0.01
		In primary	240	0.75	9.08	-0.007
	Seedlings		132	0.74	4.76	0.11
	Adult secondary		120	0.72	7.95	-0.136
	Adult primary		100	0.75	8.86	-0.026
	Primary fragments		12	0.77	1.82	0.016
Ladrillera	Progeny	In secondary2	400	0.70	10.07	0.067
		In primary	400	0.67	9.44	0.042
	Seedlings		100	0.47	3.48	-0.082
	Secondary1		130	0.74	8.20	0.175
	Secondary2		136	0.60	4.50	-0.157
	Primary		140	0.68	5.71	-0.069

Table WP3.12: Mating system. t = outcrossing rate; tm = the multilocus population outcrossing rate, ts = the (minimum variance) singlelocus population outcrossing rate; rp = the correlation of paternity (fraction of siblings that share the same father), rp(s) = the singlelocus correlation of paternity, rp(m) = the multilocus correlation of paternity ,1/rp = estimated number of pollen donors and rs = the correlation of selfing among families. Standard deviation in brackets.

	Tirimbina primary	Tirimbina secondary	Tirimbina remnant	Ladrillera primary	Ladrillera secondary
tm estimate	1.000 (0.014)	0.951 (0.030)	1.143 (0.424)	0.930 (0.027)	0.853 (0.057)
ts estimate	0.964 (0.034)	0.850 (0.050)	0.947 (0.347)	0.845 (0.043)	0.744 (0.058)
Difference tm-ts	0.036 (0.032)	0.101 (0.028)	0.196 (0.096)	0.085 (0.026)	0.109 (0.031)
Rp(m) estimate	0.281 (0.165)	0.425 (0.208)	0.206 (0.057)	0.299 (0.081)	0.365 (0.127)
Rp(s) estimate	0.286 (0.148)	0.339 (0.211)	0.238 (0.063)	0.201 (0.088)	0.175 (0.088)
1/rp	3.559	2.353	4.854	3.344	2.74
Difference [rp(s)-rp(m)]	-0.005 (0.044)	0.087 (0.090)	-0.032 (0.006)	0.099 (0.039)	0.190 (0.064)
rs among loci	0.001 (0.312)	0.042 (0.024)	0.104 (0.212)	0.217 (0.085)	0.001 (0.040)

Contemporary pollen flow was estimated using TwoGener (Austerlitz and Smouse 2001, 2002; Smouse *et al.* 2001) and KINDIST (Robledo-Arnuncio *et al.* 2007) models. For both analyses a normal distribution and the two-parameter, exponential-power distribution (Clark 1998) were used to estimate pollen flow.

Pollen dispersal estimates: TwoGener analysis

At Tirimbina, differences in successional stage translated into differences in density of mother trees, with an average distance between mothers of 215.01 m in primary and 74.7 m in secondary forest. The differentiation among pollen pools was greater in primary forest with the estimate of Φ_{FT} 0.105 in primary forest and 0.085 in secondary forest (see Table WP3.10). Mean pollen dispersal in primary forest was estimated as 28.88 m, using a normal distribution, and as 2431.29 m, assuming an exponential-power distribution. In secondary forest mean pollen dispersal using a normal distribution was 12.85 m. In both secondary forest populations, values for Φ_{FT} did not increase with distance, therefore it was not possible to estimate pollen dispersal assuming an exponential-power distribution. At Ladrillera there was also a large difference in density, such that the average distance between mothers was 421.37 m in primary and 67.56 m in secondary forest. Differentiation among pollen pools was similar in both primary and secondary forest (Φ_{FT} 0.075 in primary and 0.081 in secondary forest). Under a normal distribution, mean pollen dispersal in primary forest was greater than in Tirimbina, estimated as 51.71 m, and rose to 608.95 m assuming an exponential-power distribution. In the secondary forest at Ladrillera mean pollen dispersal using a normal distribution was estimated as 5.29 m.

Pollen dispersal estimates: KINDIST analysis

There was a decrease in among-sibship correlated paternity with distance in both primary forest populations and to a lesser extent in Ladrillera secondary forest population. In Tirimbina secondary forest, among-sibship correlated paternity increased with distance, showing that there

was no pollen-pool structure within the spatial scale of this population. Therefore this population was not included in further analysis.

As in the TwoGener analysis, the exponential-power model provided a better fit of the data with the least square residual reduced in all populations (see Table WP3.11). For both distributions, the least square residual was still high for Ladrillera secondary forest and analysis from this population may be less accurate as there was less pollen pool structure within this compact population. In primary forest, the mean estimated pollen dispersal was low assuming a normal distribution, 16.97m at Tirimbina and 21.07 m at Ladrillera. Mean pollen dispersal increased extensively assuming an exponential-power distribution, to 728.27 m at Tirimbina and 622.01 m at Ladrillera. In secondary forest, mean estimated pollen dispersal was low for both a normal and exponential-power distribution (1.46 m and 3.81 m respectively). The kurtosis excess was high in both primary populations (289.75 and 333.93 respectively) and lower in the secondary forest population (10.07) and all estimated values of *b* (the shape parameter) were less than one and were very low in both primary forest populations (0.18 and 0.19 respectively, see Table WP3.11)

Table WP3.13: Estimates of gene flow from KINDIST analysis using a normal and exponential-power dispersal function, where *Tm* is the defined reference threshold distance of unrelated pollen pools; *a* is the scale parameter; *b* is the shape parameter, *σ* is the estimated mean pollen dispersal distance; *SRAV* is the sq-root of the axial variance of pollen dispersal; *k* is the two-dimensional kurtosis and *LSR* is the least square residual.

Population	*Tm*	*a*	*b*	*σ*	*k*	*LSR*
Normal distribution						
Tirimbina primary	220	19.15	-	16.97	2	5.76
Ladrillera primary	220	23.77	-	21.07	2	36.68
Ladrillera secondary	47	1.64	-	1.46	2	64.27
Exponential-power distribution						
Tirimbina primary	220	0.00	0.18	728.27	333.93	5.04
Ladrillera primary	220	0.00	0.19	622.01	289.75	31.74
Ladrillera secondary	47	0.16	0.48	3.81	10.07	63.82

Heterozygosity and growth rate

Level of heterozygosity in individual trees was compared to ecological data on growth rate within the Tirimbina primary and secondary forest plots using data from a long-term study undertaken by B. Finegan (Centro Agronómico Tropical de Investigación y Enseñanza, unpublished data). Tree condition and mortality did not vary in the sample sets and so were ignored. Individuals were given a score based on the level of heterozygosity found at up to 5 microsatellite loci. At each locus a score of +1 was given if the alleles differed and -1 if the alleles were the same. The sample was split into two groups; trees with high heterozygosity (a score of +3 or greater) and those with low heterozygosity (a score of +2 or less). Sample means of these two groups were tested for a significant difference in growth rate. For secondary forest, growth rate was compared over 16 years. For primary forest, the most complete data was between years 1998 and 2003 so total growth over this period was compared. Other ecological factors that could influence growth rate (light incidence and initial DBH) were tested for their effect on growth rate and whether there was any difference in the effect of these ecological factors between the two groups of high and low heterozygosity trees.

The average DBH increment increase between 1998 and 2003 in Tirimbina primary forest population was 79.1 mm (standard deviation 28.8) for trees in the sample with low heterozygosity and 64.6 cm (36.3) for trees in the high heterozygosity sample. A statistical test comparing these

means showed that the difference was insignificant. In secondary forest, trees with high heterozygosity had higher growth in all years except between years 1 and 2, with the difference in growth rate increasing from year 2 to year 8 (Figure WP3.17). However, even the most significant result (that for years 8-9) showed that the difference between the means was not statistically significant. Thus, although primary and secondary forest display different trends for growth rate against heterozygosity there is no significant evidence to suggest that heterozygosity influences growth rate, at least at the life stage at which adults were analysed in this study.

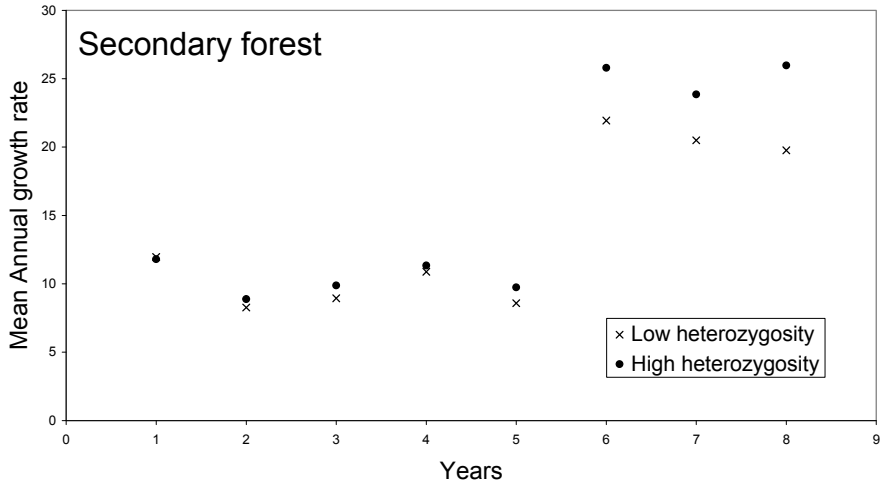


Figure WP3.17: Mean growth rate for trees with low and high levels of heterozygosity in secondary forest over eight years.

Tests were undertaken to see if the results were biased by other environmental factors, in particular, initial DBH and crown illumination. In both primary and secondary forest there was no significant difference in initial DBH between individuals with low and high levels of heterozygosity. No effect of initial DBH on growth rate was found for primary forest although a positive effect was noted for the secondary forest population. In the secondary forest there was minimal variation in crown illumination between individuals and no correlation between crown illumination and growth rate.

Conclusions

Estimated mean pollen dispersal differed between primary and secondary forest with the mean pollen dispersal distance consistently greater in primary forest. The difference infers that, within populations, pollinators may respond differently to *V. ferruginea* occurring at different densities in secondary and primary forest. Assuming a normal distribution for pollen dispersal, mean dispersal distances were low in all populations, ranging from 1.46 to 12 m in secondary forest and between 16.97 to 51 m in primary forest. With a two component dispersal parameter, estimated pollen flow distances in primary forest increased greatly, up to 2431.4 m suggesting highly leptokurtic pollen dispersal in primary forest.

Mating system analysis showed that *V. ferruginea* populations had a mixed mating system and that this self-compatible species (Bawa *et al.* 1985b) was largely outcrossed. This pattern is likely to be a consequence of fat-tailed, long distance pollen dispersal, so that even isolated trees can be pollinated. However, most pollen flow was observed between near neighbours, and in dense secondary forest, pollen dispersal was largely limited to a few meters. Restricted pollen dispersal in secondary forest meant that even though there were many potential pollen donors, fewer fathers contributed to progeny arrays compared to primary forest. Restricted pollen dispersal

within a population of family clusters increases the potential for matings between sibs and biparental inbreeding was higher in secondary compared to primary forest (mean tm-ts in primary forest was 0.065 and 0.105 in secondary forest).

There was no statistically significant evidence for a fitness effect due to increased biparental inbreeding. It is possible that, as this species may have undergone a series of genetic bottlenecking events through repeated colonisation, it has purged its genetic load. As a species naturally adapted to rapid colonisation it may be resilient to genetic effects and so tolerate low heterozygosity caused by increased biparental inbreeding. However, there was some evidence that, over time, trees with high heterozygosity may outperform trees with lower heterozygosity. The effect of inbreeding on fitness either as reproductive output or growth is very relevant to forestry so it would be valuable to investigate this further in the future with more ecological and genetic investigations.

Publications arising:

Davies S.J, (2006) The population genetic consequences of gene flow during colonisation and regeneration of forest trees. Ph.D. Thesis. Heriot-Watt University.

Davies S.J, Finegan B, Cavers S, Lowe A.J (*In prep*) The impact of colonisation dynamics on mating system and pollen-mediated gene flow for a pioneer tree, *Vochysia ferruginea* Mart.

Davies S.J, Lowe A.J, Cavers S, Finegan B (*In prep*) Population structure and the consequences for genetic diversity and differentiation in regenerated populations of a neotropical pioneer tree, *Vochysia ferruginea* Mart.

3.4 Domestication: Theobroma grandiflorum

Cupuaçu (*Theobroma grandiflorum*), is a tropical rainforest tree related to Cacao (*Theobroma cacao*). Common throughout the Amazon basin, it is widely cultivated in the north of Brazil, with the largest production in Pará. Cupuaçu trees usually range from 5 to 15 meters (16 to 50 feet) in height, though some can reach 20 meters (65 feet). As trees mature, their leaves change from pink-tinted to green, and eventually they begin bearing fruit. Cupuaçu fruits are oblong, brown, and fuzzy, 20 cm long, 1–2 kg in weight, and covered with a thick (4–7 mm), hard exocarp.The white pulp of the cupuaçu is uniquely fragrant, and it contains theacrine instead of the xanthines (caffeine, theobromine, and theophylline) found in cacao. Cupuaçu can replace cocoa in many day-to-day foods, such as chocolate milk. It is frequently used in desserts and sweets. Cupuaçu seeds can be made into cupulate, which looks and tastes just like chocolate but is cheaper and more heat resistant. The wood is also commonly used for timber.

The goals of this research are: (1) to quantify the genetic diversity in wild and cultivated populations of *Theobroma grandiflorum* in an East-West transect along of the Amazon basin, and (2) to test the effect of domestication in the genetic diversity of this species. Here we show the results of a microsatellite analysis carried out in one remnant wild population of *T. grandiflorum* located at Eastern Amazonia and another cultivated population located on the eastern border of the species distribution also in the Brazilian Amazonia.

Leaves were collected from 89 adult trees of *T. grandiflorum* in a natural population located at the Tucuruí region, Para State, Brazil and 64 adult trees from orchards located at the Amazonia Maranhense region (Figure WP3.18). Leaves were collected for DNA extraction and preserved in silica gel at –20 ^{o}C. DNA extraction followed a standard CTAB protocol (Doyle & Doyle, 1987). DNA quantification was performed by comparison with standard concentrations (Lambda DNA) in ethidium bromide-stained 1% agarose gels.

We used eight microsatellite primers, which were developed for *T. cacao* (Lanaud *et. al*., 1999) and successfully amplified polymorphic loci in *T. grandiflorum*. PCR amplification was carried out in a final reaction volume of 13 µl containing 0.9 µM of each primer, 1 unit Taq DNA polymerase, 200 µM of each dNTP, 1X reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), BSA (Bovine Serum Albumine – 2.5 mg/ml), 7.5 ng of template DNA, and ultrapure water. Amplifications were performed using a MJ Research PTC-200 thermal controller using the following program: an initial denaturation at 94^{o}C for 5 min followed by 30 cycles of 94^{o}C for 1 min, annealing temperature of each primer (^{o}C) for 1 min and 72^{o}C for 1 min; and a final elongation step at 72^{o}C for 7 min. The PCR products were visualised in 3.5% agarose gel containing 0.1 µg/ml of ethidium bromide in 1X TBE buffer (89 mM Tris-borate, 2mM EDTA pH 8.3) and sized with a 1Kb DNA ladder (Gibco, MD). The genotyping was then performed on 4% PAGE stained with silver nitrate (Creste *et al*., 2001) and sized by comparison to a 10 bp DNA ladder (Gibco, MD).

The following genetic parameters were estimated for loci and populations: number of alleles per locus, allelic frequency, expected and observed heterozygosities for each locus and averaged over all loci, using the software GDA (Lewis & Zaykin, 2001). Genetic differentiation between populations was estimated by θ (Weir & Cockerham, 1984) and R_{ST} indexes (Goodman 1997). We also estimated the inbreeding coefficient (f) using FSTAT program (Goudet 2000). Statistical significance of **θ** was tested, by bootstrapping over loci with a 95% nominal confidence interval.

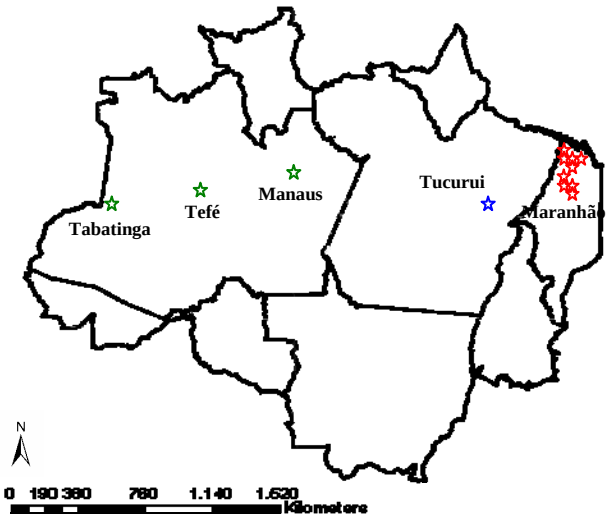


Figure WP3.18: Locations of sampled populations of *T. grandiflorum* in the Brazilian Amazon.

Manaus, Tefé, Tabatinga Amazonia Maranhense Tucurui-PA

We found high allelic variation for the eight amplified microsatellite loci in the analysed population (Table WP3.13). The number of alleles per locus varied from 5 to 16. Expected heterozygosity for the eight loci ranged from 0.42 to 0.89 (Table WP3.13). For all loci, except the locus mTcCIR22, observed heterozygosity levels were lower than expected heterozygosity. The results indicate a high level of genetic variability on a local scale.

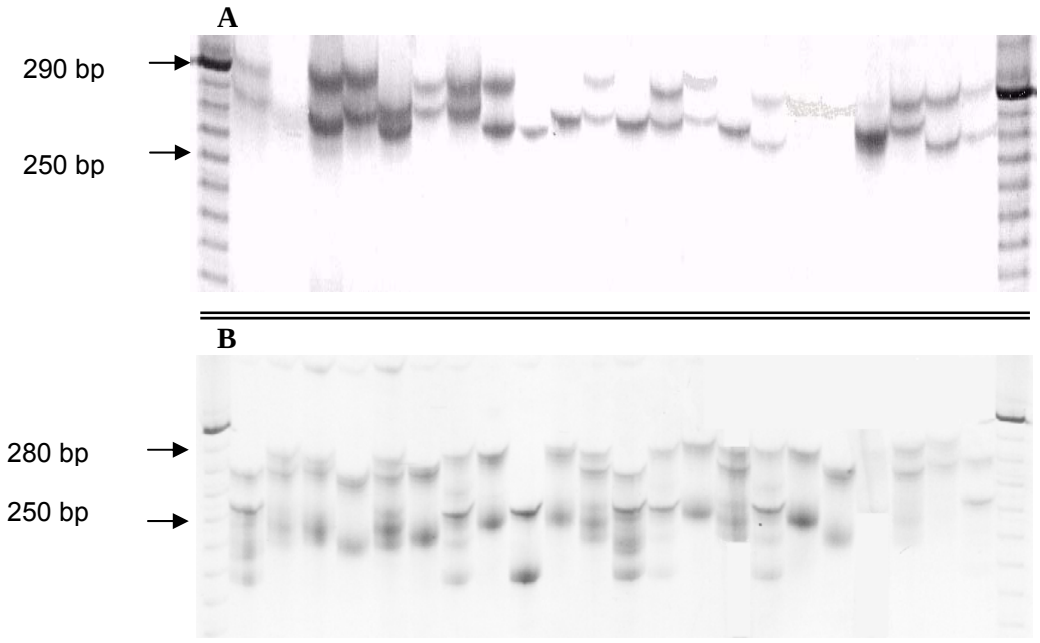


Figure WP3.19: Allelic variation for two microsatellite loci in *T. grandiflorum*, in Tucuruí population, PA, Brazil: (A) mTcCIR 02 and (B) mTcCIR 17. First and last columns correspond to ladder 10 bp.

Table WP3.13 Microsatellite diversity in two populations of *T. grandiflorum* from Eastern Amazon; T_a (^oC) - primer annealing temperature, fragment size range in base pairs, N – number of individuals, A - mean number of alleles; H_E - mean expected heterozygosity and H_o - mean observed heterozigosity.

Locus	**T_a (^oC)**	**Allele size (bp)**	N	A	H_E	**Ho**
mTcCIR 02	51	250 - 292	152	16	0.89	0.62
mTcCIR 03	46	176 - 188	151	7	0.65	0.48
mTcCIR 04	51	240 - 270	152	7	0.74	0.61
mTcCIR 17	51	250 - 280	152	8	0.65	0.49
mTcCIR 19	51	358 - 380	152	8	0.74	0.48
mTcCIR 22	49	268 - 310	147	7	0.42	0.49
mTcCIR 25	57	130 - 138	152	5	0.68	0.48
mTcCIR 26	46	248 - 286	153	7	0.62	0.43
Mean	-		151.3	8.1	0.67	0.51

The natural population from Tucuruí, PA showed much higher levels of genetic diversity compared to the cultivated population sampled in the Amazonia Maranhense (Table WP3.14). The inbreeding coefficient (0.19) found for Amazonia maranhense population was more than twice the f (0.08) observed for the Tucuruí population. The genetic differentiation indexes **θ** and R_{ST} between the two populations were 0.253 and 0.281, respectively, indicating high differentiation between them. The values are statically significant by bootstrapping over loci with a 95% nominal confidence interval (0.126 a 0.357).

The greater diversity observed for the Tucuruí population may be related to the fact that this is a native population located in the central area of the species distribution in the Eastern Amazonia. In contrary the Amazonia Maranhense population, despite sampling has been done from distinctive locations, is a population constituted of progenies collected from orchards or native populations situated in the boundary of the species' distribution. The genetic results showed a loss of allelic richness from the Central region of occurrence to the boundary of the species' distribution. The significant reduction on genetic diversity for Amazonia maranhense population may be reflecting two distinct events: (1) population bottlenecks during the natural colonization of sites located Eastern of the natural distribution area of the species, and (2) Artificial selection promoted by human action in planted populations.

Table WP3.14: Diversity, genetic differentiation and inbreeding coefficient for two populations of *T. grandiflorum* based on variation at eight microsatellite loci. N – number of individuals, A - mean number of alleles; H_E - mean expected heterozygosity and H_o - mean observed heterozigosity, f – inbreeding coefficient.

Population	N	A	He	Ho	*f*
Tucuruí-PA	88	7	0.66	0.61	0.083
Amazônia Maranhense	63	4	0.46	0.37	0.187
Mean	75.5	5.4	0.56	0.49	

θ = 0.253 (bootstrapping - 95% confidence interval – Weir & Cockerham, 1984). R_{ST} = 0,281 (Goodman, 1997).

Pollinator management in the self-incompatible fruit tree Theobroma grandiflorum

This study is part of a wider research on the Amazonian fruit tree *Theobroma grandiflorum,* which aims to understand how ecological and genetic data on the breeding system can be used to increase fruit yield in this valuable species. Previous results have showed that *T. grandiflorum* is a self-incompatible species and that the cupuaçu's fruit and seed-set depend entirely on cross-pollination promoted by very small stingless bees (*Plebeia* and *Aparatrigona spp*.). In Central Amazonia, where commercial plantations of *T. grandiflorum* started 25 years ago, there is evidence that cupuaçu's fruit yield is limited by deficiency of pollinators. So one may ask: How to manage the cupuaçu's pollinators in order to have a higher yield? This study aims to develop management techniques by rearing the pollinator bee species of *T. grandiflorum,* in order to increase the population sizes of these pollinators in the plantation areas during the flowering period, aiming to increase the pollination rate and fruit yield.

Figure WP3.20: Different wood substrates used to rear colonies of *Plebeia sp* and *Aparatrigona sp*, the main pollinators of *T. grandiflorum* in Central Amazonia.

We performed an experiment by introducing *Plebeia* and *Aparatrigona* hives in two cupuacu areas around Manaus, AM, in order to assess the effect of reared hives placed in the plantations on the fruit output. The first part of the experiment consisted in the localization of natural nests of *Plebeia* and *Aparatrigona spp*. in orchards located in the Manaus region and transferability of the hives to different wood boxes in order to test the best artificial substrate for capture, transferability and division of *Plebeia* and *Aparatrigona sp*p. colonies (Figure WP3.20). During the experiment the bees were maintained with artificial food (honey bee) until adaptation to the substrates. After the multiplication of the bee colonies in the artificial substrates the hives within the wood boxes were transferred to two plantations of cupuaçu located at INPA's Fruticulture Station about 45 Km from Manaus, AM.

The transferability of the colonies was done during the cupuaçu flowering period (September to November) in 2005.Observations were made regarding to the visit of the bee's species on the *Theobroma grandiflorum* flowers. Bees of each species were collected when they returned to the hives to analyse the pollen load of *T. grandiflorum* in their bodies. The collected pollen will be analysed for identification following the technique described by Beattie (1971). Pollen samples were also collected from honey samples within the hives, in order to determine the proportion of *T. grandiflorum* pollen on it. The fruit production was quantified by marking flowers (open-pollinated) with plastic tags, in the days the hives were introduced into the plantations. The marked flowers were monitored from the day of the anthesis until 60 days after to observe fruit development. The rate of fruit yield during the experiment period was compared with periods without introduced hives in the plantation (control).

The figure WP3.21 showed the structure of the hives within wood boxes constructed for *Plebeia sp*.(A) and *Aparatrigona sp*.(B), the main pollinators of *T. grandiflorum* in the Manaus region, AM.

A **B**

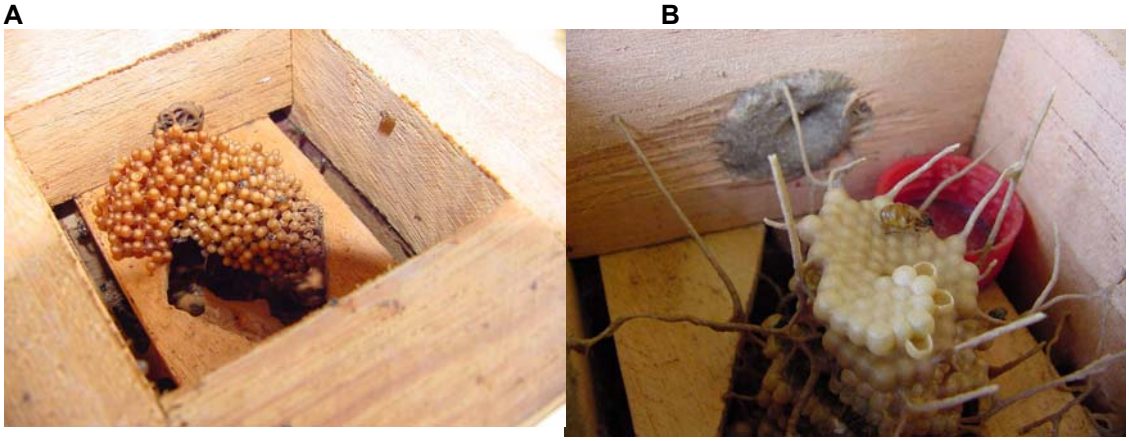


Figure WP3.21: Hives of *Plebeia sp.(*A) and *Aparatrigona sp*.(B) in wood boxes.

The preliminary data showed that the fruit production of *T. grandiflorum* was higher in the treatment were hives were introduced into the plantations, compared to the control treatment (without hives), for the two sampled areas (Figure WP3.22).

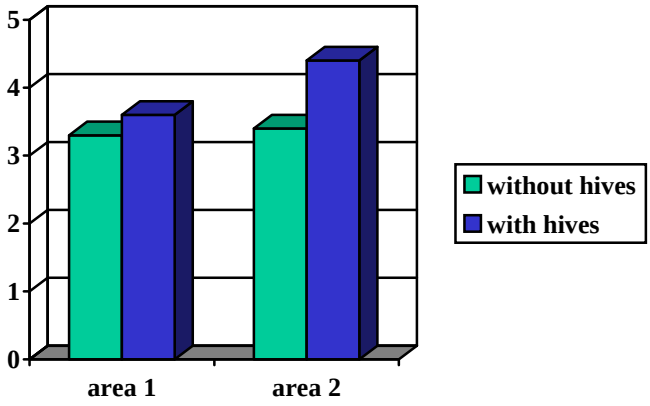


Figure WP3.22: Percentage of developing fruits of *Theobroma grandiflorum* 20 days after flower anthesis in two plantations located in Manaus region, AM, with and without introduction of hives of *Plebeia sp* and *Aparatrigonna sp.* (Area 1 – 48 individuals, Area 2 – 26 trees).

The data regarding to the pollen load present in the bees bodies and honey samples collected within the hives were not analysed yet.

Recommendations for Pollination Management of *T. grandiflorum*:

- Leave undisturbed areas around the crops.
- Ensure adequate food plants when crops (cupuaçu) are not flowering.
- Provide natural and artificial nesting supports (fences, rural building etc)
- Minimise the use of pesticides.
- Rear colonies in proper wood**.**

Publications arising:

André TJC (2005). Fluxo gênico e diversidade genética em uma população manejada de mogno (*Swietenia macrophylla* King – Meliaceae) na Amazônia Oriental. M.Sc. thesis. Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brazil.

Lemes MR, Reis VM, Martiniano TM, Faria CP & Gribel R (2007 In press) Cross-amplification and characterization of microsatellite loci for three species of *Theobroma* from the Brazilian Amazon.*Genetic Resources and Crop Evolution*. 54 (8): 1653-1657

André T, Lemes MR, Grogan J & Gribel R Post-logging loss of genetic diversity in a mahogany Swietenia macrophylla King, Meliaceae) population in Brazilian Amazonia. (submitted to Forest Ecology and Management)

Lemes MR, Grattapaglia D, Grogan J, Proctor J, Gribel R (2007) Flexible mating system in a logged population of *Swietenia macrophylla* King (Meliaceae): implications for the management of a threatened neotropical tree species. Plant Ecology. 192: 169-179.

WP3: Problems encountered

No significant problems were encountered.

Work Package 4: Simulation modelling of population genetic dynamics

Workpackage number:	4					
Start date:						
End date						
Participant number	1	2	3	4	5	6
Person-months per participant	1	0	15	2	24	0
Person-months delivered	1	4	15	0	0	0

Objectives

- Develop existing simulation model ECO-GENE to work with more complex tropical ecosystems rather than temperate systems for which it was originally designed
- Simulate the temporal and spatial dynamic of genetic structures of populations that are subject to extraction/land-use change pressures
- Interpret results for inclusion in species management strategies

The simulation model ECO-GENE was originally developed to allow a comprehensive evaluation of human influences on the genetic system of tree populations within temperate systems. The model combines population genetic and population dynamic processes with elements of forest growth models. It is an individual based, distance dependent model that includes stochastic and deterministic processes. The dynamics of genetic structures and demographic processes can be simulated. Spatial and temporal genetic dynamics with respect to several population genetic processes are included in the model, and overlapping or separated generations can be created. Different modes of forest management systems can be implemented (e.g. frequency and intensities of logging, spatial distribution of buffer areas, different types of natural regeneration or different modes of seed harvesting as basis for artificial regeneration). The model can be run with empirical and fictitious input data.

Under this workpackage, the ECO-GENE model will be adapted to simulate the more complex situation of tropical forests. Specifically the following further developments are planned:

- Integration of modules to simulate dispersal of pollen and seeds by animals
- Enlargement of the scale from stand level (1-50 ha) to forest district level (1000-5000ha)
- Integration of long distance gene flow, extinction and recolonisation events for natural forests
- Integration of data into DENDROBASE (data base on genetic system of tropical tree species) as basis to define the range of model parameters and as a basis to generate complete data sets
- Development of an advanced data generating engine to create complete input data sets in line with the results of sampled inventories

The indicators from earlier work packages will be used to develop criteria for the management of tropical forest ecosystems using the ECO-GENE simulation modelling procedures.

In particular, data from intensively studied plots (ISPs) where the position, diameter and genotypes of all reproductive trees have been recorded are suitable for the following application aspects of the model:

- Importance of natural or artificial (logging) disturbances to maintain diversity
- Impact of different scenarios of short and long distance pollen and seed dispersal on genetic variation and spatial genetic structure
- Impact of spatial species distribution on level and spatial organisation of genetic diversity within species

The recovery of exploited species can be aided through techniques that maintain genetic diversity and adaptive variation within species, facilitating natural regeneration. This can be achieved within degraded habitats by farmers inhabiting the ecosystem and foresters from the American tropics. The ECO-GENE model will also be applied to estimate the impact of alternative forest utilisation strategies on genetic diversity for individual species in tropical forest stands. For this purpose data sets of different stands will be used for model initialisation and parameterisation. In particular the following silvicultural practices will be addressed:

- different logging intensities and frequencies
- different spatial distributions of clear cuts that lead to different forest fragmentation
- different spatial distribution and size of unlogged buffer areas

Within the project the application of the model will be done using (1) experimental data and (2) generated data for the initialisation, parameterisation and validation of the simulations. The experimental data will be collected in the form of genetic, ecological and forestry inventories in field plots. There will be a gap of necessary data for a broad application of ECO-GENE. In order to overcome this restriction the development of a data generation machine is planned. This software will use aggregated parameters of the data base DENDROBASE to generate artificial but realistic data sets of forest stands and genetic structures of trees that can be used for simulations.

Changes to original workplan

- The simulation study of Symphonia globulifera was expanded to include data on three additional species gathered at the same site.

Deliverables and Milestones

Deliverables				
No	Name	Due date	Delivery date	Outputs
10	ECO-GENE model adapted for use in more complex tropical systems	Month 24	Month 24	Delivered
11	Simulation models for exploited ecosystems and single species cases will be produced. In each case the best management strategy for maintaining the genetic resource base through maximising gene flow will be identified.	Month 36	Month 48	Delivered
12	Best case management and land use strategies as predicted by the ECO-GENE will be prepared for publication and dissemination to the forestry community (WP5)	Month 42	Month 48	Delivered
Milestones				
No	Name	Due date	Delivery date	Delivered
14	Completion of adapted ECO-GENE model	Month 24	Month 24	Delivered
15	Complete individual species simulations	Month 36	Month 48	Delivered
16	Interpret data for publication and management strategies	Month 48	Month 48	Delivered

Activities

Introduction.

The simulation model Eco-Gene (Degen et al., 1996; Degen and Roubik, 2004) was used to examine the genetic consequences associated with logging in the species *Symphonia globulifera* and *Swietenia macrophylla*, parameterising the model using data obtained in this project under WP3 and comparing this with datasets derived elsewhere. The objectives were to use the model for (a) comparative analysis of the impact of selective logging on the genetic diversity of different timber species and (b) to identify, by use of sensitivity analysis, those parameters that have a significant impact on genetic and demographic dynamics of logged tropical forests.

The model simulates the temporal and spatial dynamics of allele and genotype frequencies, tree growth, and demographic processes (Degen et al., 1996; Degen and Roubik, 2004). At the beginning of the simulation, the model was initiated by reading the input data and by setting parameters. The dynamics of tree populations were simulated over timescales appropriate to species life history. Each simulated year included the steps ‘‘tree growth’’, ‘‘logging’’ if appropriate, ‘‘reproduction’’ if appropriate, and ‘‘mortality’’. The spatial position, diameter and genotype of each tree from a stand served as input for each run. For inclusion in the model, each species was assigned a growth function derived from empirical measurements. The growth function specifies the following information: (a) mean diameter growth rate (cm/yr); (b) the standard deviation of the mean diameter growth rate (cm/ yr); (c) temporal autocorrelation (the correlation between the growth rate of the previous year to the current year’s growth rate); and (d) maximum diameter (the maximum possible diameter in the model representing the size at which individuals die in the model). To simulate the applied logging system we included in the model the: ‘‘Rotation of treatments’’ (logging cycles in years), the first and last year of treatment, the minimum dbh of extracted trees (cutting diameter) and the proportion of remaining trees with a dbh greater than the cutting diameter (voluntarily left seed trees, hollow trees, etc.). Another set of parameters characterised the post-logging mortality. For each species and for three different diameter classes, specific diameter limits and mortalities were estimated from data at hand. In the simulations, we assumed a post-logging mortality of between 10% and 20%. The simulated reproduction includes parameters controlling the flowering phenology, differences in male and female fertilities of the trees, pollen and seed dispersal. Population and individual level phenology, fertilities and seeds, pollen dispersal, seed dispersal and mortality were all derived from either empirical data or estimates from indirect studies.

Case Study 1: Symphonia globulifera

The simulation model Eco-Gene (Degen et al., 1996; Degen and Roubik, 2004) was used to examine the genetic consequences associated with logging in the species *Symphonia globulifera,* parameterising the model using data obtained from the experimental plot at Paracou in French Guiana. Results were compared with those obtained for other timber species from French Guiana. The objectives were to use the model for (a) comparative analysis of the impact of selective logging on the genetic diversity of different timber species in French Guiana, and (b) to identify, by use of sensitivity analysis, those parameters that have a significant impact on genetic and demographic dynamics of logged tropical forests. The model was parameterised using data on genetics, demography, growth, phenology, and pollen and seed dispersal of four well-studied timber species: *Dicorynia guianensis* (Dg), *Sextonia rubra* (Sr), *Symphonia globulifera* (Sg) and *Vouacapoua americana* (Va). These four species include the three most important timber species in French Guiana (Dg, Va, Sr) and *Symphonia globulifera* as a model for a species with low logging pressure.

The simulation model Eco-Gene was used to evaluate disturbance and human influence on the demography and genetics. The model simulates the temporal and spatial dynamics of allele and genotype frequencies, tree growth, and demographic processes (Degen et al., 1996; Degen and Roubik, 2004). At the beginning of the simulation, the model was initiated by reading the input data and by setting parameters. The dynamics of tree populations were simulated over 215 and 460 years. Each simulated year included the steps ''tree growth'', ''logging'' if appropriate, ''reproduction'' if appropriate, and ''mortality''. The spatial position, diameter and genotype of each tree from a 100 ha stand served as input for each run. For inclusion in the model, each species was assigned a growth function derived from measurements in the unlogged plots of Paracou. The growth function specifies the following information: (a) mean diameter growth rate (cm/yr); (b) the standard deviation of the mean diameter growth rate (cm/ yr); (c) temporal autocorrelation (the correlation between the growth rate of the previous year to the current year's growth rate); and (d) maximum diameter (the maximum possible diameter in the model representing the size at which individuals die in the model). To simulate the applied logging system we included in the model the: ''Rotation of treatments'' (logging cycles in years), the first and last year of treatment, the minimum dbh of extracted trees (cutting diameter) and the proportion of remaining trees with a dbh greater than the cutting diameter (voluntarily left seed trees, hollow trees, etc.). Another set of parameters characterised the post-logging mortality. For each species and for three different diameter classes, specific diameter limits and mortalities were estimated from data at hand. In the simulations, we assumed a post-logging mortality of between 10% and 20%. The simulated reproduction includes parameters controlling the flowering phenology, differences in male and female fertilities of the trees, pollen and seed dispersal. Population and individual level phenology, fertilities and seeds, pollen dispersal, seed dispersal and mortality were all derived from data obtained directly from the Paracou plot or from studies conducted on the populations there.

For each species two control scenarios without logging (C1 and C2) and two scenarios with logging representing the moderate logging system in French Guiana (L1) and the much more intensive logging practice in the Brazilian Amazon (L2) were run. The applied logging scenarios used in French Guiana have much longer cutting cycles and higher cutting diameters compared to those in Brazil. In both logging scenarios we applied seven cutting cycles but changed the number of simulated years. The comparison of the two control and logging scenarios allowed the effect of pure genetic drift (C1 versus C2) and the additional effect of different logging systems (L1 versus L2) to be distinguished. The simulations were repeated 50 times in order to estimate the stochastic variation of results for the same parameter configuration. The following parameters were calculated for each simulation as output: N = number of individuals, BA = basal area in m2, A =mean number of alleles, Ae = mean effective number of alleles, Ha =mean observed heterozygosity, F =mean fixation index, Dis = genetic distance between the initial population and the population at the end of the simulations, NG = number of single locus genotypes at all loci. We also carried out sensitivity analyses: at the end of each simulation seven output parameters were computed for the population including all trees at year 215: mean number of alleles (A), effective number of alleles (Ae), number of genotypes (NG), fixation index (F), genetic distance to origin (Dis), basal area (BA) and number individuals (N).

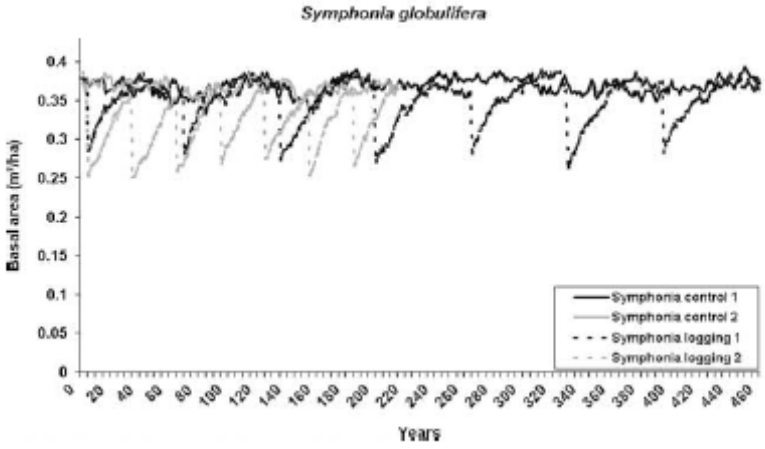


Figure WP4.1: Example of the dynamics of the total basal area in the two control and two logging scenarios for *Symphonia globulifera* during 215 years and 460 years of simulation.

At the end of the simulations, the values for the genetic distance between the initial genetic composition and the genetic structure at the end were higher in all four scenarios for *Symphonia globulifera* than for the other species. All species had a slight excess of homozygotes (positive F-values) indicating the impact of inbreeding. *Symphonia globulifera* had a higher number of individuals and the same basal area at the end of the logging scenario. Thus, logging did change the diameter distribution towards smaller trees, but the total stock recuperated each time on a 30 year logging rotation.

Table WP4.1: Results of sensitivity analysis

Fixation index (F)						
	Data set *Dicorynia guianensis* F = 0.107 ± 0.022			Data set *Vouacapoua americana* F = 0.045 ± 0.014		
Step	Parameter	β	R^2	Parameter	β	R^2
1	Percentage of flowering adults	0.350	0.127	Percentage of flowering adults	0.394	0.151
2	Distance of random pollination	−0.303	0.218	Distance of random pollination	−0.303	0.239
3	Maximum flight distance pollinator	−0.257	0.281	Maximum flight distance pollinator	−0.281	0.321
4	Temporal autocorrelation growth	0.196	0.317	Cutting diameter r	0.133	0.343
5	Mean growth rate	0.190	0.352	Mean growth rate	0.115	0.356
6	Attractor effect for pollinator	0.122	0.369	Exponent for seed dispersal	0.099	0.366
7	Exponent for seed dispersal	0.086	0.376	Density diameter class 10–20 cm	0.091	0.375
8	Density diameter class 30–40 cm	0.049	0.378	Density diameter class 30–40 cm	0.073	0.380
9				Temporal autocorrelation growth	0.070	0.385
10				Attractor effect for pollinator	0.049	0.387
Basal area (BA)						
	Data set *Dicorynia guianensis* BA = 0.240 ± 0.065			Data set *Vouacapoua americana* BA = 0.244 ± 0.066		
Step	Parameter	β	R^2	Parameter	β	R^2
1	Cutting diameter	0.595	0.329	Cutting diameter	0.626	0.450
2	Density diameter class 30–40 cm	0.589	0.642	Density diameter class 30–40 cm	0.545	0.758
3	Temporal autocorrelation growth	0.317	0.754	Maximum diameter	0.178	0.791
4	Mean growth rate	0.282	0.836	Mean growth rate	0.159	0.820
5	Maximum diameter	0.171	0.866	Temporal autocorrelation growth	0.116	0.833
6	Proportion remaining trees	0.102	0.876	Proportion remaining trees	0.119	0.846
7	Density diameter class 20–30 cm	−0.033	0.878	Density diameter class 10–20 cm	0.105	0.857

Stepwise multiple regression of fixation index (F) and basal area (BA) against the main input parameters (Table 1) using the two tree data sets *Dicorynia guianensis* and *Vouacapoua americana*.
R^2 is the fraction of the variance accounted for by the model, adjusted for the number of independent variables; β is the standardized regression coefficient (all significant).

The results of the sensitivity analysis showed the highest R^2 values were for the demographic parameters (BA, N) and the fixation index (F). Among the output parameters for genetic variation and genetic differentiation, the number of genotypes (NG) and the genetic distance (Dis) were most sensitive. Among the input parameters, the number of genotypes (NG) was largely determined by growth and demographic parameters as well as the cutting diameter. The genetic distance (Dis) was negatively correlated with the cutting diameter and densities in different diameter classes were among the parameters with significant impact. The highest number of significant input parameters was found for the fixation index (F).The parameter ‘‘percentage of flowering adults’’ had the strongest positive correlation with the fixation index (F), otherwise parameters controlling the pollen dispersal were next most significant (positive correlation) The parameter ‘‘cutting diameter’’ had the strongest correlation with the basal area (BA).

The simulations indicated that, even for the moderate logging system applied in French Guiana (L1), *V. americana* and *Sextonia rubra* were unable to recover their initial volume at the end of the rotation period. In the stronger logging scenarios *D. guianensis* was also unable to recuperate the original basal area. Only the demography of *Symphonia globulifera* gave no indication of a negative impact of logging. Although the differences among the genetic parameters were small we saw a general tendency of increased genetic distances and a reduced number of genotypes due to logging. No clear tendencies were observed for the other genetic parameters: A, Ae, Ha, and F. The comparison of the genetic distances between the two control scenarios (C1 versus C2) and the two logging scenarios (L1 versus L2) showed that the impact of genetic drift as a function of time was even higher than the impact of a stronger logging. From the demographic and genetic differences of the simulations, we conclude the following ranking from sensitive to less sensitive species: *V. americana* > *Sextonia rubra* > *D. guianensis* > *Symphonia globulifera.*

The demographic structure of a tree species, the possibility of recruitment after logging and the genetic similarity among different demes of a population are the important points for predicting the sensitivity of a tree species to genetic erosion due to logging (Jennings et al., 2001). Higher risks for genetic diversity due to logging are expected for fast growing pioneer species with deficits in tree abundance in lower diameter classes and weak overlapping of generations (Jennings et al., 2001). Thus, for most slow growing climax tree species selective logging per se is not expected to have a strong negative impact on the genetic diversity. More critical for the genetic conservation of tree species are changes at a higher spatial scale due to forest fragmentation (Aldrich and Hamrick, 1998). The mating systems of most tropical tree species are adapted to low densities of reproductive trees (dominance of animal pollination, mechanisms to avoid selfing). Therefore, the risk of gene erosion by logging is expected to be low as long as the neighbourhood of a logged forest has sufficient reproductive trees.

Most sensitive parameters were the fixation index (F), number of genotypes (NG) and the genetic distance (Dis). The fixation index measures the excess or deficit of homozygotes compared to Hardy–Weinberg proportions. Values >0 indicate an excess which might be caused by inbreeding. Flowering phenology and pollination distance were the most important input parameters influencing F-values. Against expectations, a higher percentage of flowering trees was positively correlated with the fixation index. This might be explained by reinforced bi-parental inbreeding. If more trees are flowering and if the pollinators prefer short flight distances, than the risk of pollination among nearby trees (relatives) increases (Degen et al., 2004). The number of genotypes (NG) is the sum of genotypes at all loci. Thus, it is determined by the number of alleles and their combination. This multilocus approach of the output parameter NG explains its higher sensitivity to impact. The genetic distance is more sensitive than just the number of alleles because it considers both changes in the number of alleles and their relative frequencies (Gregorius, 1978). When experimental studies on logging do not find proof for negative impact of logging this may be because (a) there was really no negative impact, or (b) the genetic parameters used were not sufficiently sensitive. The contrasting responses of the genetic output parameters used in our simulation indicate that future studies should also include multilocus measures because of their higher sensitivity. As shown by the sensitivity analysis, the output parameters genetic diversity and genetic distance from the original population

were largely determined by the logging system, demographic and growth parameters and only to a small extent to pollen and seed dispersal and phenology. Rapid growth combined with small maximum diameters and low densities in the diameter classes characterised the most sensitive tree species. This is typical for species with low population size and weak overlapping of generations. The main advantage of slow growing species is that they allow many reproductive events which stabilize genetic diversity.

Publications arising:

Degen B., Blanc L., Caron H., Maggia L., Kremer A. and S. Gourlet-Fleury (2006) Impact of selective logging on genetic composition and demographic structure of four tropical tree species. *Biological Conservation.* 131: 386-431.

Case study 2: Swietenia macrophylla

In Case Study 2 the species *Swietenia macrophylla* was examined, parameterising the model using two datasets: one obtained from plots sampled at Hill Bank, Belize, the other from plots at Marajoara, Para, Brazil. The dynamics of tree populations were simulated over 285 years, comprising sufficient time, where logging simulations were applied, for seven logging cycles on a 40 year rotation, with the first cut after 5 years. The model growth function was modified for Swietenia macrophylla based on published data, to allow non-linear variation of growth rate with age. Then two density / mortality scenarios were defined, to allow investigation of the effects of the interaction of the gap-colonisation habit of S. macrophylla and variable disturbance regimes. One scenario limited densities in small diameter classes mimicking the effect of the low recruitment that would be expected for this species under undisturbed, closed forest conditions; the second permitted much higher densities of small diameter trees, such as might be created by regular gap creation, artificial or otherwise. Belizean and Brazilian density functions differed in that Brazilian densities were set to be significantly lower than for Belize, as observed for both sites, but in both cases high and low recruitment scenarios were tested as controls. Simulated reproduction parameters controlling population and individual flowering phenology, differences in male and female fertilities of the trees, pollen and seed dispersal were all derived from either empirical data, estimated from indirect studies, or estimated. For scenarios testing Brazilian datasets, maximum pollination distances were permitted to be longer to account for reduced density populations. Imposed on these underlying conditions were set of scenarios simulating likely logging strategies: on a 40 year rotation, all combinations of 40, 50, 60 cm DBH minimum cutting diameter and 70, 90, 100% removal of trees above minimum DBH. In each case, simulations were repeated 50 times in order to estimate the stochastic variation of results for the same parameter configuration.

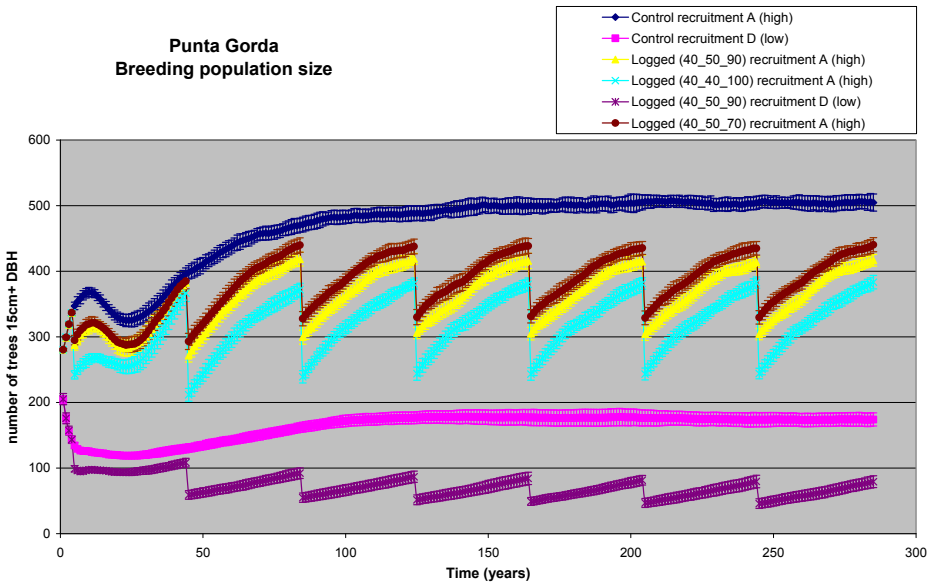


Figure WP4.2: Simulations for Punta Gorda plot, Belize. Variation in populations breeding size for four logging strategies (Logging strategies are summarised by: Cycle length in years_minimum cutting DBH in cm_percentage of trees above minimum DBH cut in each cycle).

The following parameters were calculated for each simulation as output: N = number of individuals, BA = basal area in m2, A =mean number of alleles, Ae = mean effective number of alleles, Ha =mean observed heterozygosity, F =mean fixation index, Dis = genetic distance between the initial population and the population at the end of the simulations. A sensitivity analysis (random testing, within specified ranges, of correlations between input and output parameters) was carried out to identify parameters having greatest impact on the simulations. The parameters investigated are shown in Table WP4.2.

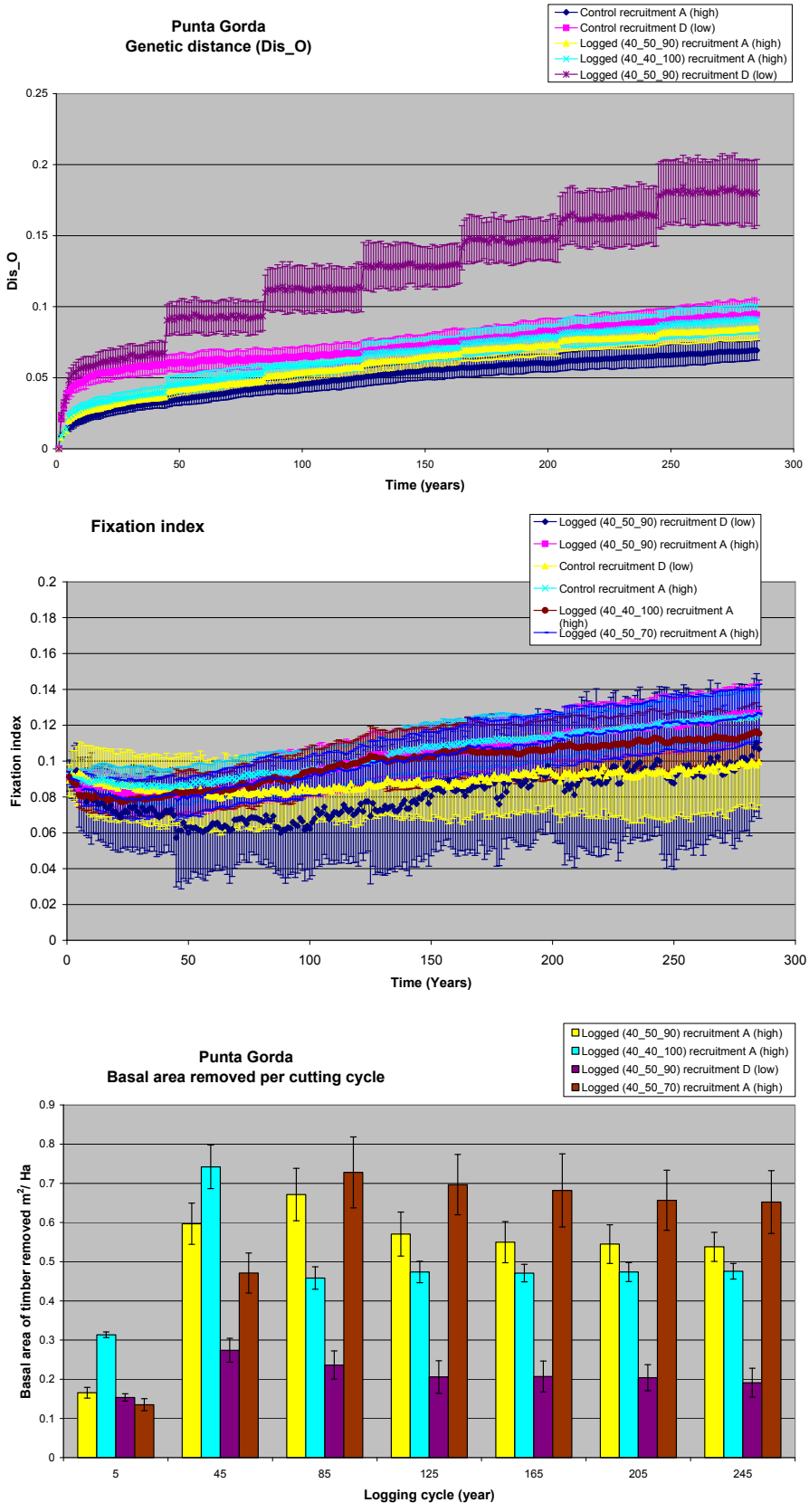
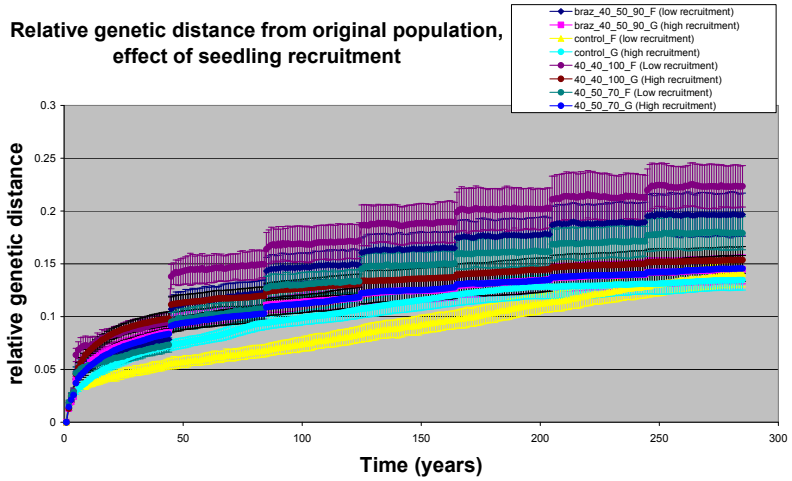
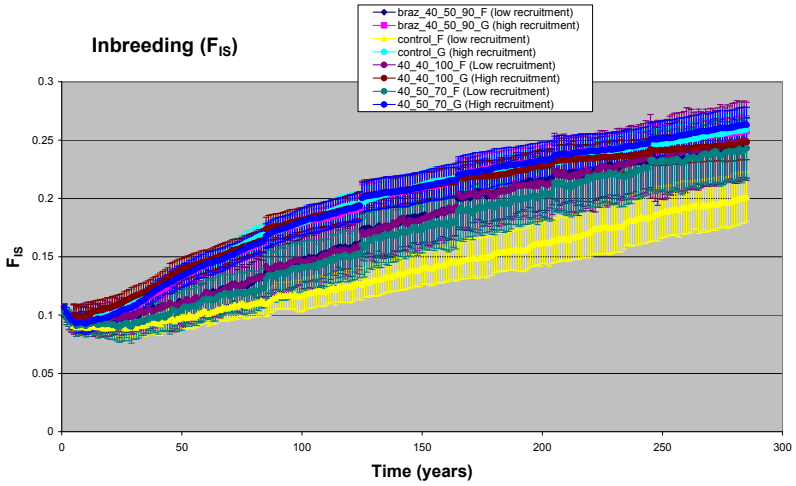


Figure WP4.3: Outputs for Punta Gorda plot, Belize for four logging strategies (Logging strategies are summarised by: Cycle length in years_minimum cutting DBH in cm_percentage of trees above minimum DBH cut in each cycle). Top - Genetic distance between initial and final populations; middle - level of inbreeding, bottom - total basal area lost per logging cycle





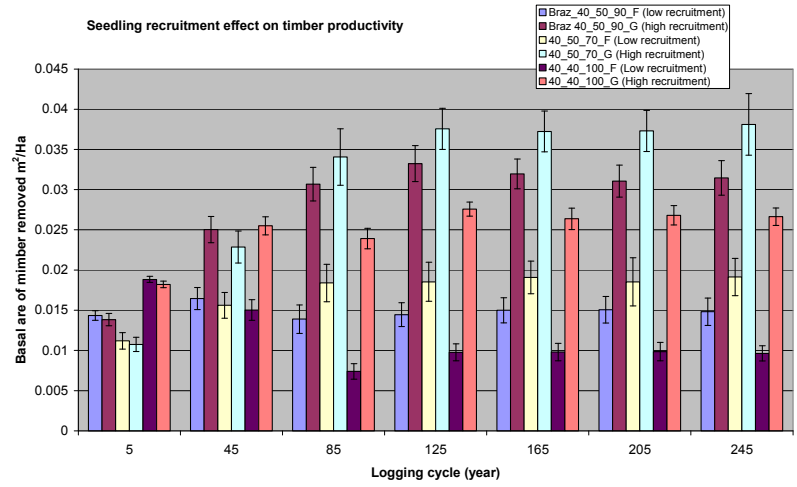


Figure WP4.4: Ouputs for Marajoara plot, Brazil for different logging strategies (Strategies are summarised by: Cycle length in years_minimum cutting DBH in cm_percentage of trees above minimum DBH cut in each cycle). Top - Genetic distance between initial and final populations; middle - level of inbreeding, bottom - total basal area lost per logging cycle

By incorporating data from Belize from four plot samples and from Brazil, it was possible to explore the relative effects of varying tree density (trees / ha) as well as the various scenarios described above. Therefore, for each control and logging scenario, outputs also examine: low versus high density populations and low versus high recruitment rates.

Genetic Distance. In terms of genetic distance between original and final populations across the timescale of the simulation,

- In high density populations (Belize), in the absence of logging, a small amount of drift occurs (likely a model effect related to small population size). However:
  - with low recruitment rates, any logging strategy has a significant impact on drift such that logged populations drift much more.
  - with high recruitment rates, all logging strategies have much less impact, but small differences in strategy can make a difference such that higher DBH limits and greater numbers of trees left after logging result in lower drift.
- In low density populations (Brazil), impacts are generally much greater, with all populations much more prone to drift. Recruitment rates and logging scenarios here have proportionately less effect as all have strong impacts.
  - small changes to strategy have large effects
  - increased DBH limits and greater numbers of trees remaining post-logging decrease drift

Inbreeding. Patterns of change in levels of inbreeding were markedly different between the low density populations in Belize and those in Brazil.

- In high density populations (Belize), a small amount of inbreeding was consistently present but changed little on the timescale of the simulation. However
  - lower density populations showed slightly increased levels of inbreeding
  - a non-significant tendency was apparent for populations with higher recruitment rates to show higher inbreeding levels
- In low density populations (Brazil), as for genetic distance, impacts are generally much greater and all scenarios showed increasing levels of inbreeding over the simulation lifetime.
  - low recruitment rate scenarios (control and logging) showed lower rates of increase of inbreeding

Heterozygosity. Again, patterns of change were markedly different between the low density populations in Belize and those in Brazil.

- In high density populations (Belize), little detectable impact on heterozygosity levels was apparent, with no significant difference in levels at the end of the simulation, for any of the strategies.
- In low density populations (Brazil), all scenarios showed a loss of heterozygosity over time.
  - the low recruitment rate control showed least decline in heterozygosity levels

Basal Area. In terms of timber volumes yielded by different scenarios, although as would be expected the lower density populations in Brazil yielded lower volumes per hectare, the patterns of response to different logging strategies were similar:

- scenarios with higher recruitment rates yielded higher timber volumes than scenarios with low recruitment rate
- in high recruitment scenarios,
  - lower DBH cutting limits and reduced numbers of post-logging trees remaining yielded initially higher timber volumes over first rotation
  - increased DBH cutting limits and reduced numbers of post-logging trees remaining resulted in much higher volumes in later rotations

Table WP4.2 Parameters investigated in sensitivity analysis

Parameter name	Minimum	Maximum
Pollination - Maximum flight distance Mean (m)	200	5000
Pollination - Attractor effect Remaining *	0	1
Phenology - Minimum diameter for flowering (cm)	10	35
Phenology - Percentage flowering	20	100
Seed dispersal Wind - Exponent Δ	0.1	10
Logging - Cycle (years)	15	60
Logging - Cutting diameter (cm)	30	70
Logging - Proportion of remaining trees above cutting diameter	0	1
Growth - Mean rate	0.2	0.8
Demography Density Class1 (0-5cm DBH)◊	0.5	5
Demography Density Class2 (5-10cm DBH) ◊	0.5	5
Demography Density Class3 (10-20cmDBH) ◊	0.5	5
Demography Density Class4 (20-30cm DBH) ◊	0.5	5

*Attractor effect remaining is the probability of an individual pollinator staying on a tree to visit other flowers. Δ Seed dispersal Wind – exponent is the function that determines the distance seed is carried by wind from the mother tree. ◊The four Demography Density classes specified represent the target densities of trees per hectare for each of the smallest tree size classes.

Sensitivity analysis showed an informative contrast between Belize and Brazil simulations. In Belize, the highest R^2 values in Belize simulations were for minimum percentage of flowering trees, seed dispersal exponent, growth rate and density of young trees (0-5cm DBH). For these input parameters, the output parameters showing strongest correlation are shown in Table WP4.3. Factors measuring the size of the reproductive population (effective population size (+)) and spatial extent of mating (pollen dispersal distance (-)) correlate with min percentage of flowering trees; those sensitive to spatial structuring of gene flow (fixation index (+), probably resulting from biparental inbreeding) and spatial genetic structure (-) correlate with input parameter 'seed dispersal exponent'. As well as a simple demographic response (Total population size (+)) input parameters determining the rate at which genetic diversity could be captured and retained (growth rate and density of young trees) in the population were correlated with measures of genetic diversity (number of observed alleles, number of genotypes). Growth rate correlations with genetic distance (+) and number of alleles (-), reflects the rate at which of trees progress through a 'window of reproductive maturity' - a faster rate of progress means a faster rate of sampling of the available (limited) gene pool, hence stochastic loss of alleles and a faster rate of genetic drift.

In contrast, broadly speaking, simulations based on Brazilian data showed the same directionality but reduced strength of correlations with parameters important in Belize, but increased strength of other correlations. Generally, it was possible to interpret results for Brazilian simulations as consequences of lower density of populations and increased pollen dispersal distances. As for Belize, the minimum percentage of flowering trees was also important but in Brazilian case it was the more spatially influenced parameters which were affected: inbreeding (+) and spatial genetic structure (+). In other words, in the case of Brazil, as the number of flowering trees increases, due to the reduced density and spatial capability of dispersal the probability of mating between related trees increases and inbreeding increases, but simultaneously the probability of longer distance mating increases (as pollination distance is large) hence spatial genetic structure is reduced. Parameters with smaller spatial influence (seed dispersal) have less effect than for Belize. Growth rate not as significant as in Belize, but density of young trees also very important, showing impacts for the number of genotypes, total population size and genetic distance.

In general, comparing outputs for both sites, it is possible to say that for the purposes of maintenance of genetic diversity in *Swietenia macrophylla*, individual sites will require unique assessment. Density of populations should be characterised, with lower density populations treated with lower impact strategies. In both cases, it will be necessary to encourage recruitment.

However, growth rates are also an important consideration and determine, to a large extent, the rate at which extant diversity is captured and retained in the population.

Table WP4.3: Sensitivity analysis - output parameters showing strongest correlations with input parameters (with r^2 values indicating proportion of variance explained).

Input parameter	**Parameter showing strong correlation ***	**+/-**	**r^2 value**	
			Belize	**Brazil**
Minimum percentage of trees flowering	Effective population size	+	0.473	0.128
	Pollen dispersal distance	-	0.319	0.073
	Fixation Index	+	0.161	0.333
	Spatial genetic structure	-	0.072	0.288
Seed dispersal exponent	Fixation index	+	0.394	0.031
	Spatial genetic structure	-	0.551	0.014
Growth rate	Genetic distance	+	0.424	0.108
	Number of observed alleles	-	0.264	0.662
Density of young trees in size class 0-5cm DBH	Number of different single locus genotypes	+	0.301	0.095
	Total population size	+	0.511	0.331
	Genetic distance	-	0.002	0.331

Publications arising:

Cavers S, Home Robertson P, Lemes M, Grogan J, Gribel R, Andre T, Sabido W, Walker, K, Degen B (In Prep) Impact of logging on genetic diversity and structure of *Swietenia macrophylla*.

Work Package 5: Designing management strategies to maximize diversity

Workpackage number:	5					
Start date:						
End date						
Participant number	1	2	3	4	5	6
Person-months per participant	3	8	1	4	28	0.5
Person-months delivered	3	22	1	4	5	4

Objectives

- Disseminate results of this proposal to the forestry community of Latin America
- Communicate specific recommendations for changes in forest management practices to those concerned
- Increase general awareness of the genetic aspects of silviculture
- Increase sustainability of forest management practices through circulation of techniques to maximise the genetic resource base of forest trees

The outputs and indicators from work packages 2-4 provide the basis for development of criteria for the management of genetic diversity in tropical systems, and the sustainable extraction and management of intensively harvested single species landscapes. The simulation scenarios used in ECO-GENE were developed in close co-operation with relevant forest service and forest planning institutions. Materials and events explaining how genetic diversity considerations can be incorporated into management strategies will be produced in local languages for circulation in the countries of Latin American partners and relevant neighbouring countries. Scientific outputs from the project are expected to be of very high quality and will be disseminated to the academic community through papers in referred journals, conference presentations and the project website.

An accompanying measure was applied for, to fund a professionally organized symposium to which community and national foresters, forestry policy makers and stake holders will be invited where outputs and suggested management strategies will be explained. The accompanying measure will also fund the publication of booklets in local languages detailing the results and recommendations of the project and the proceedings of the symposium.

Changes to original workplan

Alterations to the media and content of outputs were made as the final outcomes of the science work packages became clear. Rather than a leaflet, a board game was produced (principally through the efforts of Dr Navarro at CATIE) as the medium was more original and unusual, and therefore provided a more effective means to disseminate project messages.

Deliverables and Milestones

Deliverables				
No	Name	Due date	Delivery date	Outputs
1	Leaflets produced in local languages to explain practical outputs of the project, management strategies and the importance of managing forest genetic resources	Month 42	Month 42	Delivered
2	Papers drafted for publication in high quality, refereed, international scientific journals	Month 45	Month 45	Delivered

3	Hold small scale workshop for Forestry workers in Central America. If accompanying measure application is successful organise international conference to cover the whole of Latin America and interested parties.	Month 48	Month 48	Delivered
Milestones				
No	Name	Due date	Delivery date	Delivered
17	Assimilation of data from workpackages 2-4 to form realistic and practical management strategies	Month 40	Month 40	Delivered
18	Planning and completion of workshop	Month 48	Month 48	Delivered
19	Completion of forest literature and its dissemination	Month 48	Month 48	Delivered
20	Submission of individual and integrated scientific papers on work of the project	Month 48	Month 48	Delivered

Activities

Dissemination Meeting, INBio, San Jose, Costa Rica.
As the final day of activities during the fourth coordination meeting, a public dissemination meeting was held at INBio, the National Biodiversity Institute, in the Costa Rican capital San Jose. The meeting was held as an open session with attendees invited from the Costa Rican National Biodiversity Committee, Ministry of the Environment, CATIE, Technological Institute of Costa Rica amongst others. The meeting was well supported and senior figures from the invited bodies were present. Simultaneous translation between Spanish and English was provided.

Presentations were made by each of the partners in the GENEO-TROPECO project, giving specific case studies featuring work carried out during the project lifetime. Andrew Lowe also made an initial introductory speech presenting the project, putting it in context, outlining European support for international collaborative research and stressing the need to ensure communication of the outputs of such project to policymaking figures such as those present in the room. He also chaired a final question and answer session, drawing together the results presented and addressing the specific concerns raised by the audience – to facilitate this a questionnaire had been circulated during the lunch interval.

Dr Lowe presents the opening speech at the INBio meeting

The meeting was very well received by the audience and specific comments were made expressing their appreciation that the scientific community had taken the time to present primary research in a public forum and requesting that the level of effort shown to communicate the project outputs be maintained, as it plays a vital role in connecting the research and policy communities.

Genetic Diversity Board game for schools and colleges

Through substantial efforts by Dr Carlos Navarro at CATIE, a genetic diversity game was prepared for education in primary and secondary schools. Preliminary designs were presented and discussed at the final annual meeting in Costa Rica with subsequent refinement and production undertaken by Dr Navarro.

The final product consisted of a double sided board on a 'snakes and ladders' design featuring two levels of questions suitable for either school or college age players. Questions dealt with aspects of genetic diversity maintenance such as sourcing and planting of seed for tree cultivation, maintenance of genetic diversity and effects of fragmentation. Sets of bonus cards provided

Efforts are underway to secure funding to produce several hundreds of copies of the game for distribution across Mesoamerica and Mexico. Ultimately the game may also be translated into Portugese.

Accompanying measures application to FP6 INCO SSA call
As an agreed action of the project, to promote dissemination and communication of project results, CEH prepared and submitted a proposal to the FP6 INCO SSA call. This would be designed to hold a workshop in Latin America uniting collaborators in GENEO-TROPECO with those from other major projects in the field (e.g. DENDROGENE) and the principal researchers. It would also allow for preparation and translation of dissemination materials and focussing of plans for future projects under FP7.

The bid passed all of the threshold criteria and was placed on the reserve list but was ultimately not funded. The proposal was subsequently revised following the feedback from reviewers and was resubmitted in early 2006. Unfortunately the revised bid met with the same outcome and funding was not secured. It was not therefore possible to hold a dedicated international symposium within the project lifetime, although the consortium partners have all expressed interest in pursuing the concept.

Scientific publications
A significant number of peer-reviewed scientific publications were produced based on results from the project. These ranged in scope from methodological publications resulting from the optimisations and development tasks undertaken in WP1 to overarching reviews summarising broader aspect of project outcomes in the context of previous work and existing published literature. A full publications list is given below. The major contributions to the scientific literature were two special issues:

1. Population Genetics of Neotropical trees, *Heredity* Special Issue.

2. Population genetic studies of tree populations in the Neotropics, *Silvae Genetica* Special Issue.

Problems encountered

Due to the failure of the bid for a supporting action, which targeted funding for a dedicated conference plus professional production of dissemination materials, it was not possible to achieve the hoped-for level of awareness of project outcomes. However, as the supporting action bid is a stand-alone action to synthesise the state-of-the-art in knowledge for genetic diversity conservation in Neotropical forest management, efforts will continue beyond the project lifetime.

Final Scientific Report: Role of participants

Coordinator: CEH

The NERC Centre for Ecology and Hydrology (CEH) was responsible for the overall scientific and financial coordination of the project. In WP1, CEH coordinated standardisation activities for the sampling, and AFLP analysis methodologies and developed new microsatellite markers for *Vochysia ferruginea*. In WP2 CEH, as part of the meta-analysis, undertook AFLP analysis of *Cedrela odorata*, *Pinus oocarpa*, *Swietenia macrophylla*, *Vochysia ferruginea*, *Tetragastris panamensis, Carapa guianensis, Laetia procera*. In WP3 CEH collected and analysed samples of *Swietenia macrophylla* from Belize, using previously developed SSRs - (some SSR analysis undertaken by IPBO in collaboration), collected (in collaboration with CATIE) and analysed (using newly developed SSRs) samples of *Vochysia ferruginea;* analysis of data was undertaken in collaboration with CATIE. In WP4, CEH undertook simulations of forest management scenarios based on empirical data derived from SSR analysis of *Swietenia macrophylla* from Belize and (in collaboration with INPA) Brazil. In WP5 CEH coordinated preparation and submission of the supporting action bid and commissioning and editing of the Heredity Special Issue (A. Lowe).

Partner 1: CATIE

CATIE coordinated sampling activities for all species from Central America, mapping and sampling populations species including *Swietenia macrophylla, Lonchocarpus costaricensis, Tetragastris panamensis, Cedrela odorata, Tapirira guianensis, Vochysia ferruginea, Pinus oocarpa, Maranthes panamensis, Eschweilera costaricensis, Lecythis ampla, Goethalsia meiantha, Laetia procera, Vochsia allenii* and 'pre-sample' collections as well as full collections. Through the exchange visit of one researcher to CEH, CATIE contributed the AFLP dataset for *Pinus oocarpa* to WP2 and collated life history and distribution data for species. In WP3, CATIE was responsible for the case study of *Pinus oocarpa*, ultimately conducted using morphological characters and (in collaboration with CEH) the analysis of populations of *Vochysia ferruginea* built on mapped collections from their extensive long-term forest plots, from which SSR data was integrated with ecological data from these plots for analysis of inbreeding effects on performance. CATIE was instrumental in organising outputs in WP5, including organisation of the closing workshop held at InBio in San Jose Costa Rica and the design and production of the Genetic Diversity Board game.

Partner 2: INRA

INRA participated in standardisation of protocols and sampling through presample collection and testing of extraction methods and AFLP analysis and development of SSR markers for *Symphonia globulifera*. IN WP2, INRA prepared collections from F. Guiana of *Schefflera morototoni, Jacaranda copaia, Goupia glabra, Symphonia globulifera, Cecropia sciadophylla, Brosimum guianense, Simarouba amara, Caryocar glabrum, Chrysophyllum sanguinolentum, Dicorynia guianensis, Vouacapoua americana, Virola michelii, Eperua grandiflora, Bocoa prouacensis, Sextonia rubra, Eperua falcata, Moronobea coccinea* and conducted AFLP analysis of most of these. IN WP3, INRA was responsible for the case study of *Symphonia globulifera* using SSRs and, in WP4, the integration of this empirical data with other existing datasets to conduct simulations of forest management strategies using ECOGENE. INRA also devised and developed the ECOGENE model and adapted it for use with the datasets from this project. In WP5 INRA coordinated and edited the Special Issue of the journal *Silvae Genetica* focused on population genetics of Neotropical trees.

Partner 3: INPA

In WP1, INPA undertook development of new SSR markers for *Swietenia macrophylla* and transfer and optimisation of existing SSRs for *Theobroma grandiflorum*. In WP2, INPA coordinated sampling of species from Brazil, including *Swietenia macrophylla, Theobroma grandiflorum, Calophyllum brasiliense, Chrysophyllum sanguinolentum, Cecropia sciadophylla, Carapa guianensis, Goupia glabra, Jacaranda copaia, Minquartia guianensis, Pseudobombax munguba, Simarouba amara, Symphonia globulifera, Tetragastris panamensis, Tapirira guianensis*. In addition, INPA coordinated the long and complex process of securing Brazilian export permission for all of these samples. In WP3, INPA was responsible for case studies of *Swietenia macrophylla* in Brazil and of *Theobroma grandiflorum.* In WP4 (in collaboration with CEH) INPA was involved in simulation of forest management strategies for Brazil. In WP5, INPA produced several peer-reviewed publications, including contribution to the *Heredity* special issue.

Partner 4: UFRJ

In WP1, UFRJ undertook development of new SSR markers for the species *Araucaria angustifolia*, and participated in the standardisation testing for the AFLP protocol development. In WP2, UFRJ conducted sampling for *Araucaria angustifolia, Calophyllum brasiliense* and *Tabebuia cassinoides.* AFLP analyses of *A. angustifolia* and *Calophyllum brasiliense* were completed and contribute to the meta-analysis. In WP3, UFRJ was responsible for the case study of fragmentation effects on *Araucaria angustifolia* undertaken using SSRs and AFLPs. In WP5, UFRJ produced several peer-reviewed publications, including contribution to the *Heredity* special issue.

Partner 5: IPBO

IPBO played a major role in the development and testing of sampling, extraction and analysis protocols during WP1, including presample analysis and participation in the standardisation testing for AFLP analysis. In WP2, IPBO undertook AFLP analysis of *Goupia glabra, Maranthes panamensis, Goethalsia meiantha, Lecythis ampla, Tapirira guianensis, Pseudobombax munguba* for which the datasets were incorporated in the meta-analysis. In addition, IPBO undertook new flow cytometric analyses of several species to provide genome size estimates as a factor for the meta-analysis. In WP3, IPBO collaborated with CEH to produce the SSR dataset for the case study of *Swietenia macrophylla* in Belize, analysing four SSR markers for two of the four populations.

Final Scientific Report: Project Management

Management Summary

Broadly speaking the project achieved the objectives originally set out in the project proposal, with a number of minor technical alterations to individual work packages. In most cases the outputs of the work packages significantly exceed the initial expectations, as demonstrated by the quantity and notably high quality of the scientific output – which continues beyond the project lifetime, with a number of major publications remaining to be completed. Some delays were experienced due to the requirement for a large number of rangewide collections to be completed before AFLP analysis could be initiated, which was held back by a change of rules combined with a change of administration in Brazil that stopped export of tissue samples for most of the project lifetime. However, in most cases, it was still possible to complete analysis and indeed, partners involved in AFLP analysis contributing to the WP2 meta-analysis continued to produce data right up to the project end and beyond. For this reason, the analysis presented in this report is given as 'preliminary' and a full analysis is in the process of being completed this year, a process that will result in a high-level scientific publication.

From a management point of view, the consortium partners worked extremely well together: all partners attended annual meetings, collaborated directly on shared project work, including the sharing of data, human resources and ideas, and maintained an atmosphere of positive interaction, with regular communication throughout the project lifetime. Annual meetings were held in rotation amongst partners, alternately in Europe and Latin America :

Coordination meeting 1 – 25-27 March	2002,	Bordeaux,	France
Coordination Meeting 2 – 14-16 June	2003,	Manaus,	Brazil
Coordination meeting 3 – 5-8th July	2004,	Edinburgh,	UK
Coordination meeting 4 – 3-7 October	2005,	Turrialba,	Costa Rica

Integration amongst consortium partners was maintained by email and the means of the project website, where annual reports, the minutes of meetings and shared protocols, outputs and progress reports were regularly posted.

Project progress was monitored throughout the project by checking actual project outputs against planned deliverables from the proposal. A summary of project delivery rate is provided below:

Timetable of Activities: planned vs actual

WP	Year	Year 1				Year 2				Year 3				Year 4			
	Quarter	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
WP1	Planned				D1		D2						D3				
	Actual				D1		D2		D3								
WP2	Planned						D4				D5				D6		
	Actual						D4		D5								D6
WP3	Planned							D7					D8		D9		
	Actual																D7,8,9
Wp4	Planned								D10				D11		D12		
	Actual								D10								D11,12
WP5	Planned														D13	D14	D15
	Actual														D13	D14	D15

Grey – planned schedule, Green – actual matches planned, Red – actual exceeds planned.

List of deliverables

Del. No.	WP	Deliverable title	Planned delivery date
1	1	Optimised DNA extraction and AFLP / SSR protocols to be placed on website	Month 12
2	1	New SSR primers made available to all partners through website and journals	Month 18
3	1	Developed software available to all partners through website and journals	Month 36
4	2	Full list of species and locations of forests to be analysed to be placed on website	Month 18
5	2	Results made available in a useful form for comparative regression analysis and processing in WP 4 and 5	Month 30
6	2	AFLP data interpreted and published to highlight case studies of specific interest. Partners to publish analysis of combined data sets jointly	Month 42
7	3	Location and description of forests analysed to be placed on website	Month 21
8	3	Description of the way that human-mediated processes affect the structure and dynamics of gene diversity for each case study	Month 36
9	3	Suggested strategies to reduce impact of human-mediated process and maximise genetic diversity	Month 42
10	4	ECO-GENE model adapted for use in more complex tropical systems, ECO-GENE (NEO-TROP)	Month 24
11	4	Simulation models for exploited ecosystems and single species cases will be produced. In each case the best management strategy for maintaining the genetic resource base through maximising gene flow will be identified.	Month 36
12	4	Best case management and land use strategies as predicted by the ECO-GENE (NEO-TROP) will be prepared for publication and dissemination to the forestry community (WP5)	Month 42
13	5	Leaflets produced in local language to explain practical outputs of the project, management strategies and the importance of managing forest genetic resources	Month 42
14	5	Papers drafted for publication in high quality, refereed, international scientific journals	Month 45
15	5	Hold small scale workshop for Forestry workers in Central America. If accompanying measure application is successful organise international conference to cover the whole of Latin America and interested parties	Month 48

List of milestones

Milestone No.	WP	Milestone title
1	1	Optimisation of DNA extraction
2	1	Optimisation of AFLP, SSR and isozyme techniques
3	1	Development of new SSR loci for 3 species
4	1	Development and availability of computer analysis software to all partners
5	2	Selection of species and populations
6	2	Sampling of species and populations
7	2	AFLP analysis of samples
8	2	Analysis and interpretation of data
9	3	Selection and sampling mature trees, seeds and seedlings where appropriate
10	3	Finish SSR/RAPD/cpDNA analysis of material
11	3	Complete field observations including dbh measurements and pollination observations
12	3	Finish analysis of molecular data
13	3	Interpret data for publication and processing into simulation modelling and management strategies.
14	4	Completion of adapted ECO-GENE model
15	4	Complete individual species simulations
16	4	Interpret data for publication and management strategies
17	5	Assimilation of data from workpackages 2-9 to form realistic and practical management strategies
18	5	Planning and completion of workshop
19	5	Completion of forest literature and its dissemination
20	5	Submission of individual and integrated scientific papers on work of the project

Final Scientific Report: Exploitation and dissemination activities

Publications 2002-2006 derived all or in part from project-generated data

2002

1. Lemes, Brondani, Grattapaglia (2002) Multiplexed systems of microsatellite markers for genetic analysis of mahogany, Swietenia macrophylla king (Meliaceae), a threatened neotropical timber species. *Journal of Heredity* 93: 287-291.

2. Lowe AJ, Goodall-Copestake WP, Caron H, Kremer A, Decroocq S (2002) A set of polymorphic microsatellites for *Vochysia ferruginea*, a promising tree for land reclamation in the Neotropics. *Molecular Ecology Notes* 2:209-210.

2003

3. Brondani, RPV Gaiotto, FA Missiaggia, AA Kirst, M Gribel, R Grattapaglia, D (2003) Microsatellite markers for *Ceiba pentandra* (Bombacaceae), an endangered tree species of the Amazon forest. *Molecular Ecology Notes*, **3**: 177-179.

4. Cavers S, Navarro C, Lowe AJ (2003) A combination of molecular markers (cpDNA PCR-RFLP, AFLP) identifies evolutionarily significant units in *Cedrela odorata* L. (Meliaceae) in Costa Rica. *Conservation Genetics* **4**:571-580.

5. Degen B, Bandou E, Caron H (2004) Limited pollen dispersal and biparental inbreeding in *Symphonia globulifera* in French Guiana. *Heredity*, 93(6) 585-591.

6. Squirrell, J Hollingsworth, PM Woodhead, M Russell, J Lowe, AJ Gibby, M Powell, W (2003) How much effort is required to isolate nuclear microsatellites from plants? *Molecular Ecology* **12**:1339-1348.

2004

7. Cavers S, Navarro C, Lowe AJ (2004) Targeting genetic resource conservation in widespread species: a case study of *Cedrela odorata* L. *Forest Ecology and Management*. 197: 285-294.

8. Degen B, Roubik D (2004) Effects of animal pollination on pollen dispersal, self-pollination and effective population size of tropical trees: a simulation study. *Biotropica* 36 (2): 165-179.

9. Latouche-Halle C, Ramboer A, Bandou E, Caron H , Kremer A (2004) Long-distance pollen flow and tolerance to selfing in a neotropical tree species . *Molecular Ecology* 13 (5): 1055-1064.

10. Phillips PD, de Azevedo CP, Degen B, Thompson IS , Silva JNM , van Gardingen PR (2004) An individual-based spatially explicit simulation model for strategic forest management planning in the eastern Amazon . *Ecological Modelling* 173 (4): 335-354.

11. Phillips PD, Thompson IS, Silva JNM, van Gardingen PR , Degen B (2004) Scaling up models of tree competition for tropical forest population genetics simulation . *Ecological Modelling* 180: 419-434.

12. Salgueiro F, Felix D, Caldas JF, Margis-Pinheiro M , Margis R (2004) Even population differentiation for maternal and biparental gene markers in Eugenia uniflora, a widely distributed species from the Brazilian coastal Atlantic rain forest . *Diversity and Distributions* 10 (3): 201-210.

2005

13. Salgueiro F., H. Caron, M.I.F. de Souza, A. Kremer, R. Margis (2005) Characterization of nuclear microsatellite loci in South American Araucariaceae species. *Molecular Ecology Notes*, 5, 256-258.

***Heredity Special Issue*:**

14. Lowe AJ (2005) Population Genetics of Neotropical trees focus issue. *Heredity.* 95(4): 243-245

15. Cavers S, Degen B, Caron H, Hardy O, Lemes M, Gribel R, Margis R, Salgueiro F, Lowe AJ (2005) Optimal sampling strategy for estimation of spatial genetic structure in tree populations. *Heredity*. 95(4):281-289

16. Kremer A, Caron H, Cavers S, Colpaert N, Gheysen L, Gribel R, Lemes M, Lowe A, Margis R, Navarro C, Salgueiro F (2005) Monitoring genetic diversity in tropical trees with multilocus dominant markers. *Heredity*. 95(4):274-280

17. Lowe AJ, Boshier D, Ward M, Bacles CFE, Navarro C (2005) Genetic resource loss following habitat fragmentation and degradation; reconciling predicted theory and empirical evidence. *Heredity*. 95(4):255-273

18. Ward M, Dick CW, Gribel R, Lemes M, Caron H, Lowe AJ (2005) To self or not to self. A review of outcrossing and pollen-mediated gene flow in neotropical trees. *Heredity*. 95(4):246-254.

Silvae Genetica Special Issue

19. Degen B. (2005) Population genetic studies of tree populations in the Neotropics, Silvae Genetica. 54(6): 257.

20. Cavers S, Navarro C, Hopkins P, Lowe AJ (2005) Genetic diversity and population structure of *Vochysia ferruginea* Mart. in Costa Rica, assessed using cpDNA and AFLP markers. *Silvae Genetica* 54(6): 258-264.

21. Colpaert N, Cavers S, Bandou E, Caron H, Gheysen G, Lowe AJ (2005) Sampling tissue for DNA analysis of trees: trunk cambium as an alternative to canopy leaves. *Silvae Genetica*. 54(6): 265-269.

22. Veron V., Caron H. and Degen B. (2005) Gene flow and mating system of the tropical tree *Sextonia rubra*. *Silvae Genetica* 54 (6), 275-280

23. Navarro C, S Cavers, A Pappinen, P Tigerstedt, J Merilä, A Lowe (2005) Ecotypic differentiation and variability at both quantitative traits and neutral markers in Mesoamerican *Cedrela odorata*. *Silvae Genetica* 54(6): 281-292.

24. Navarro C, Cavers S, Breyne P, Colpaert N, Lowe AJ (2005) High genetic diversity and differentiation are maintained in remnant populations of the Costa Rican endemic tree, *Lonchocarpus costaricensis. Silvae Genetica* 54(6): 293-300.

2006

25. Degen B, Blanc L, Caron H , Maggia L, Kremer A, Gourlet-Fleury S. (2006) Impact of selective logging on genetic composition and demographic structure of four tropical tree species. *Biological Conservation*. 131: 386-431.

2007

26. Lemes MR, Grattapaglia D, Grogan J, Proctor J, Gribel R (2007) Flexible mating system in a logged population of *Swietenia macrophylla* King (Meliaceae): implications for the management of a threatened neotropical tree species. Plant Ecology. 192: 169-179.

27. Lemes, M. R., Reis, V. M., Martiniano, T. M., Faria, C. P. & Gribel, R. (2007) Cross-amplification and characterization of microsatellite loci for three species of *Theobroma* from the Brazilian Amazon. *Genetic Resources and Crop Evolution* 54 (8): 1653-1657

Books

28. Caron H., Dutech C., Bandou E. (2004) Reproductive phenology and mating system of six tree species in Paracou stand pp 137-147 *in* Ecology and Management of a neotropical Rainforest, Gourlay Fleury S, Laroussinie O , Guehl JM (eds), Elsevier France.

29. Caron H., Bandou E., Kremer A. (2004). Multilocus assessment of levels of genetic diversity in tropical trees in Paracou stands pp 148-159 *in* Ecology and Management of a neotropical Rainforest, Gourlay Fleury S, Laroussinie O , Guehl JM (eds), Elsevier France.

30. Lowe AJ, Harris SA, Ashton P (2004). Ecological Genetics: Design, Analysis and Application. Blackwells, Oxford. 326 pp.

31. Lowe AJ (2004) Why use spatially explicit and direct gene flow approaches in conservation genetics? In M.F. Fay, J. Sutcliffe, B.Jones, I. Taylor (eds). proceedings of a Conservation Genetics Workshop, Royal Botanic Gardens, Kew. pp. 28-29. English Nature Research Reports, No. 607. Peterborough, UK.

Submitted

In prep.

32. André T, Lemes MR, Grogan J & Gribel R (*submitted)* Post-logging loss of genetic diversity in a mahogany *Swietenia macrophylla* King, Meliaceae) population in Brazilian Amazonia. (submitted to Forest Ecology and Management)

33. Bottino MC, D.B.Felix, F. Salgueiro, F. Scarano, M. Alves-Ferreira and R. Margis (*In Prep*) Assessment of genetic diversity in populations of *Calophyllum brasiliense* Camb using AFLP markers. *Genetics and Molecular Biology*.

34. Cavers S, Home Robertson P, Lemes M, Grogan J, Gribel R, Andre T, Sabido W, Walker, K, Degen B (*In Prep*) Impact of logging on genetic diversity and structure of *Swietenia macrophylla*

35. de Souza, M.I.F. , F. Salgueiro, D.B.Felix, J.V.M. Bitencourt, F. Scarano, M. Alves-Ferreira and R. Margis (*In Prep*) Genetic diversity of *Araucaria angustifolia* [Bert.] O. Kuntze using AFLP markers *Biodiversity and Conservation*.

36. Davies S.J, Finegan B, Cavers S, Lowe A.J (*In prep*) The impact of colonisation dynamics on mating system and pollen-mediated gene flow for a pioneer tree, *Vochysia ferruginea* Mart.

37. Davies S.J, Lowe A.J, Cavers S, Finegan B (*In prep*) Population structure and the consequences for genetic diversity and differentiation in regenerated populations of a neotropical pioneer tree, *Vochysia ferruginea* Mart.

38. Davies S.J., Cavers S, Lowe A.J, (*Submitted*) The genetic diversity consequences of reforestation and habitat restoration. (sub to Genes, Genomes and Genomics)

39. Cavers S, Sabido W, Walker, K, Colpaert N, Lowe AJ (*In Prep*) Genetic diversity, gene flow and mating system in *Swietenia macrophylla* in Belize, and the practice of sustainable logging.

40. Lowe AJ, Cavers S, Walker K, Brown C, Heller T, Navarro C, Finegan B, Hernandez G, Villalobos H, Gheysen G, Colpaert N, Lemes MR, Gribel R, Kyndt T, Margis R, Margis M, Bottino MC, de Souza MIF, Caron H, Petit RJ, Kremer A (*In prep)* Ecological predictors of genetic diversity and spatial genetic structure in Neotropical trees.

Poster presentations

Cavers S, Lowe AJ, Davies S, Walker K, Kremer A, Caron H, Petit R, Degen B, Bandou E , Gheysen G, Colpaert N, Finegan B, Navarro C, Hernandez G, Margis R, Margis-Pinheiro M, Felix D, Salgueiro F, Gribel R, Lemes M (2005) From lab to legislation: international collaboration for conservation of genetic resources. *Sustainable management of genetic resources in tropical forests: combining molecular analysis and computer simulation to understand the structure and dynamics of diversity*. Biodiversity Science and Governance, Paris, January 2005.

André, T., Lemes, M. R. & Gribel, R. (2005) No genetic structure indicates high gene flow in a mahogany (*Swietenia macrophylla*, MELIACEAE) logged population in Eastern Amazônia. XIX Annual Meeting of the Society for Conservation Biology. Universidade de Brasília, Brasília, DF – Brazil.

Theses

Ph.D.

1. Salgueiro F (2005) Organization and dynamic of the genetic diversity in two species from the Brazilian Atlantic Rain Forest: *Araucaria angustifolia* [Bert.] O. Kuntze and *Eugenia uniflora* L. Ph.D. Thesis of , Department of Genetics, Federal University of Rio de Janeiro, July 2005.
2. Davies S.J, (2006) The population genetic consequences of gene flow during colonisation and regeneration of forest trees. Ph.D. Thesis. Heriot-Watt University.
3. *M.Sc.*
4. Heller T. (2003) Population genetic structure in two neotropical tree species, *Swietenia macrophylla* king. and *Tetragastris panamensis* (engl.) kuntze.: the influence of life-history traits. MSc Thesis, University of Edinburgh
5. Brown C. (2003) The impact of sustainable logging practices on gene flow dynamics of Mahogany (*Swietenia macrophylla*) in a continuous forest. MSc Thesis, University of Edinburgh
6. Aubrey C. (2004) The impact of sustainable logging practices on gene flow dynamics of Mahogany (*Swietenia macrophylla*) in a continuous forest. MSc Thesis, University of Edinburgh
7. André, T. J. C. (2005). Fluxo gênico e diversidade genética em uma população manejada de mogno (*Swietenia macrophylla* King – Meliaceae) na Amazônia Oriental. Master thesis. Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brazil. Xpp. Supervisor: Rogério Gribel.
8. Madriz J.P. (2005) Cambios genéticos en la regeneración natural de *Pinus oocarpa* var. *oocarpa* Schiede ex Schlechtendal, causado por el manejo forestal y la deforestación. Comayagua Honduras, América Central. M.Sc. Thesis, CATIE.
9. Home Robertson P (2006) Do 'sustainable logging' practices maintain genetic diversity? An assessment of a management strategy for Mahogany (*Swietenia macrophylla* King) using simulation modelling. MSc Thesis, University of Edinburgh
10. de Souza MIF (2006) Analyses of genetic diversity of *Araucaria angustifolia* [Bert.] O. Kuntze using AFLP markers". Master Degree Thesis of , Department of Genetics, Federal University of Rio de Janeiro, March 2006.
11. Bottino MC (2006) Analyses of genetic diversity in populations of *Calophyllum brasiliense* Camb, present in Brazil and Costa Rica, using AFLP markers". Master Degree Thesis of Mariana Carnavale Bottino, Department of Genetics, Federal University of Rio de Janeiro, March 2006.

Exchanges / Personnel movements

1. July 2003 - **Nathalie Colpaert** and **Lieve Gheysen** went to CEH for a training in data analysis.

2. February - July 2003 **Ms Francimary Carneiro da Silva**, forest engineer, came to the genetic lab of INRA in Kourou in French Guiana. Co-financed by the Dendrogene project, she worked on the genetic inventories of *Symphonia globulifera* as part of her master thesis at EMBRAPA in Belém.

3. July 2003 - **Dr B. Degen** visited INRA in Bordeaux to discuss programme, progress and results of the project with Dr A Kremer, Dr H Caron and Dr C Dick.

4. 2003 - **Fabiano Salgueiro**, a PhD student (P6), was welcomed in Bordeaux in order to develop additional SSR markers for *Araucaria augustifolia* and to learn using softwares of data analysis.

5. November 2003 - **Dr R. Margis** (P6), visited INRA to discuss training program of F. Salgueiro

6. **Gustavo Hernandez** (P2, CATIE) visited CEH (P1) May-July 2004 for training in molecular techniques

7. April to October 2004 **Ms Francimary Carneiro da Silva**, forest engineer, came to the genetic lab of INRA in Kourou for the second time. She worked on the genetic inventories of *Symphonia globulifera*.

8. **Dr Degen** left INRA in june 2004 but he is going on with WP4. He has been replaced by **Dr Ivan Scotti** in February 2005.

9. **Fabiano Salgueiro** worked in Bordeaux until August 2004.

10. **Dr H. Caron** went to Rio in April 2005.

11. **Dr R. Margis** (P6) visited INRA in November 2005.

12. **Heidy Villalobos** secured funding for research visit to lab of Dr A Lowe at University of Queensland, results to be incorporated into meta-analysis

Final Scientific Report: Ethical and safety provisions

There are no special ethical considerations associated with this project and all activities adhere to national health and safety guidelines. All partners implemented equal opportunities employment policies for the purposes of staff and student recruitment.

Final Scientific Report: Appendix 1

Sample publications

Sampling Tissue for DNA Analysis of Trees: Trunk Cambium as an Alternative to Canopy Leaves

By N. Colpaert[1], S. Cavers[2,6], E. Bandou[3], H. Caron[3], G. Gheysen[1,4] and A. J. Lowe[2,5]

(Received 31th May 2005)

Abstract

The number of studies of tropical tree species that use molecular tools is increasing, most of which collect leaf tissue for genomic DNA extraction. In tropical trees the canopy is not only frequently inaccessible, but also, once reached, the leaf tissue is often heavily defended against herbivory by high concentrations of anti-predation compounds, which may inhibit downstream applications, particularly PCR. Cambium tissue, accessed directly from the tree trunk at ground level, offers a readily accessible resource that is less hampered by the presence of defensive chemicals than leaf tissue. Here we describe a simple method for obtaining tissue from the cambial zone for DNA extraction and test the applicability of the method in a range of tropical tree species. The method was used successfully to extract DNA from 11 species in nine families. A subset of the DNA extracts was tested in more detail and proved to be highly suitable for AFLP analysis.

Key words: DNA extraction, trees, tropical, cambium, AFLP, sample preservation.

Introduction

Tropical tree populations are increasingly being studied using molecular methods. Such studies require fresh or, more commonly given the often remote field sites, dried tissue samples for DNA extraction (Schierenbeck, 1994). In addition, the increasingly ambitious scale of population genetic analyses of tropical tree species means that samples are frequently required in substantial numbers.

The most common tissue sampled for genetic studies of tree populations has been leaf material. However, both collection and use of tropical tree leaf samples for molecular analysis are problematic. Firstly, tropical tree species commonly reach heights of greater than 40 metres and leaf tissue is inaccessible. Sampling of tropical tree populations has been conducted using climbing equipment (Dick, 2001) or employing tree climbers (Lowe *et al.*, 2003), but this is time consuming, labour intensive and relatively hazardous. In addition the damage caused to the trees by the spikes used for climbing can be extensive (G. Hernandez, *pers comm.*) and, although the potential effects are un-evaluated, there may be long term consequences for the individuals sampled. Secondly, tropical tree leaf tissue is subject to high rates of herbivory and infection (Coley and Barone, 1996) and consequently is frequently heavily defended through high concentrations of anti-predation agents including alkaloids, cyanide, polyphenols and terpenes (Turner, 2001). Such compounds may all inhibit downstream PCR applications. Furthermore, although it is usually recommended that young leaves be sampled for successful DNA extraction, in tropical species young tissue is commonly more heavily protected against herbivory than older leaves, with higher concentrations of tannins and other polyphenols (Coley, 1983; Turner, 1995). Finally, leaf material frequently contains microorganisms and small insects which are not always visible. By sampling cambium tissue contamination of target DNA by foreign DNA can be avoided.

Here we describe an efficient technique for sampling tissue suitable for traditional DNA extraction procedures that accesses a resource at ground level. The method was tested on a range of tropical tree species of which 6 were tested in more detail by spectrophotometric analysis and reproducibility in AFLP analysis.

Methods

The procedure for sampling of cambial zone tissue used an ethanol-cleaned 1cm diameter leather punch (*Fig 1.*), hammered into the bark of the target tree until stopped by the wood. The resultant plug of bark was removed with the cambial zone found on the inner surface. A very thin disc of tissue was sliced off using a clean scalpel, which was continually sterilised using ethanol between trees. The bark plug was replaced in the tree trunk and hammered tight. The 1 cm disc of cambium tissue was then preserved either by placing directly on silica gel in an 'O'-ring sealed 1.5 ml screw-cap plastic tube (Anachem Ltd.) or by suspension in a 1.5 ml plastic tube containing transport buffer consist-

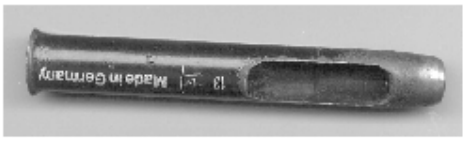


Figure 1. – Leather punch used for removing bark plug from tree trunk, from which cambium tissue can be sliced.

[1]) Institute for Plant Biotechnology for Developing Countries (IPBO), Ghent University, KL Ledeganckstraat 35, B-9000, Ghent, Belgium.

[2]) Centre for Ecology and Hydrology-Edinburgh, Bush Estate, Penicuik, Midlothian EH26 0QB, Scotland, UK.

[3]) Institut National de la Recherche Agronomique, UMR BIOGECO, 69 Route d'Arcachon, 33612 Cestas cedex, France.

[4]) Department of Molecular Biotechnology, Ghent University, Coupure links 653, B-9000, Ghent, Belgium.

[5]) School of Life Sciences, University of Queensland, St Lucia, Brisbane, Queensland 4170, Australia.

[6]) To whom correspondence should be addressed: email: scav@ceh.ac.uk

Heredity (2005) 95, 243–245

www.nature.com/hdy

EDITORIAL

Population genetics of neotropical trees focus issue

Heredity (2005) **95**, 243–245. doi:10.1038/sj.hdy.6800755

Few can fail to be impressed by the amazing biodiversity and complexity of tropical forest ecosystems. Decades of work from biologists in a range of disciplines, including ecological, reproductive, behavioural, dispersal and evolutionary biology, have provided insight into the processes and functioning of these systems. Perhaps the most thoroughly investigated tropical ecosystems are the forests of the neotropics, and in particular the components of biodiversity and ecological interactions of their tree species (eg pollen and seed dispersal mechanisms and vectors). Over the last decade, the application of molecular markers in particular has led to a dramatic improvement in our knowledge of the historical and contemporary processes that contribute to the level, structure and functioning of biodiversity in this globally important bioregion.

In many cases, the scientific questions posed within tropical forests are unique to those ecosystems. For example, what has driven the large-scale diversification within the tropics, and how do so many low-density species retain genetic connectivity? What is the nature of the mutualistic relationships between plants and their pollinators and seed dispersers, how did these relationships evolve, and what are the resultant gene dispersal implications of these mechanisms? Finally, with the widespread and increasing rates of deforestation and logging within neotropical countries, new concerns are arising. For example, how do species cope with life in small isolated remnants that result from loss of habitat?

Many researchers and scientific groups from around the globe have contributed to the development of knowledge and scientific study in the neotropics. In addition to scientists working out of national or region research institutes and universities in Latin America (from Mexico to Argentina), many scientists from North America and Europe have been fortunate to receive funding to study some of these amazing systems. Lasting research infrastructures have also been put into place in many regions, by either setting up permanent sample plots or providing roads, buildings and even aerial access facilities to allow better study of forests and canopy interactions. Such infrastructure has contributed significantly to the ability to undertake long-term studies in what can be at times a challenging environment.

In this special focus issue of *Heredity*, we present a group of six papers focusing on aspects of neotropical population genetics, and which represent a product of this scientific investment. Four of the six assembled papers are the culmination of collaborative work between Latin American and European scientists (from Belgium, Brazil, Costa Rica, France, French Guyane, Germany, Panama, United Kingdom) and funded by a series of EU projects in conjunction with national partner grants (through the INCO-DEV programmes in frameworks 3, 4 and 5, and including the project GENEOTROPECO). The other two papers are collaborative outputs of researchers based either in Panama, French Guyane and USA, or Mexico and USA. These six papers take a variety of forms, including two review papers, two papers reporting methodological advancements and two papers reporting primary research results; they represent a range of issues currently being tackled, debated and developed in neotropical systems.

The reviews represent summaries of the contemporary neotropical literature in two important areas. The review by Ward *et al* (2005) offers insight into some of the key mating system and gene flow mechanisms that individual species adopt to maintain genetic connectivity in the notoriously low-density conditions of many rainforests. Taking its starting point from earlier reviews by Murawski (1995) and Loveless (2002), this review examines levels of selfing and gene flow across a range of species case studies. In contrast to very early presumptions that most species must be selfing to survive such low-density conditions, a survey of the contemporary molecular marker literature indicates that most neotropical species are highly outcrossed. Indeed, many have a range of very sophisticated pollination syndromes to overcome their low-density lifestyles. Mixed mating systems are still found in a small proportion of species, but these have so far been confined to a single family, the Malvaceae. Future recommendations of this review include examination of inbreeding depression on observed outcrossing rates, quantification of pollen dispersal for more species, including sampling wider ecological classes, and examination of variation in mating systems due to individual, seasonal, population, ecological and landscape differences.

With deforestation and logging rates in the neotropics being some of the highest in the world, concern over the state of the genetic resources of exploited and forest dependent species is paramount. The review of Lowe *et al* (2005) provides a picture of the level of impact these processes are having on the component genetic diversity of neotropical trees. This review takes its starting point from an earlier synthesis by Young *et al* (1996), which covered mostly temperate case studies but which also identified the lack of research in tropical systems. Since this earlier review, many new studies have been published, and Lowe *et al* highlight some of these recent findings. Most notably, simulation and empirical studies appear to indicate that genetic diversity and differentiation estimates are relatively robust to reductions in population size from habitat fragmentation and logging. Even in heavily exploited populations, the longevity of trees and overlapping generations appear to buffer diversity loss. However, degraded populations suffer significantly from increased inbreeding, reduced reproductive output and decreased fitness (which appears to be due to both inbreeding and ecological effects of tree isolation). Unexpectedly, some studies highlight that extensive networks of gene flow, established at medium spatial scales in fragmented landscapes (up to 10 km),

Population Genetic Studies of Tree Populations in the Neotropics

Bernd Degen

Bundesforschungsanstalt für Forst- und Holzwirtschaft (BFH),
Institut für Forstgenetik und Forstpflanzenzüchtung, Sieker Landstrasse 2, D-22927 Grosshansdorf, Germany,
E-mail: b.degen@holz.uni-hamburg.de

Since the early fifties, forest geneticists have established provenance tests and accumulated data on population differentiation for phenotypic traits in tree populations from around the globe. This information was later complemented by numerous surveys of genetic diversity using gene markers, principally morphological traits and isozymes. A large body of experimental evidence documenting genetic organization has been accumulated for temperate trees. Over the past 20 years, similar efforts have been extended to populations of woody species in the tropics. Most of these studies have been conducted in the neotropics.

In face of rapid deforestation in the tropics a central question is: what is the relative contribution of evolutionary history, logging, forest fragmentation, genetic processes, and demographic phenomena to diversity and genetic structure in the tree populations? In classical population genetics, these issues have been investigated through theoretical work using simplified scenarios. More recently, new techniques and methods have been developed which are now contributing in novel and important ways to our understanding of the evolution of genetic diversity in tropical tree populations. First, computer simulations have been developed and are now routinely used to analyse the dynamics of genetic structure and to test hypotheses about the impact and function of specific processes. Second, new protocols have been developed to enable DNA-extraction for genetic inventories from trunk cambium. Thus rapid sampling of large numbers of trees have become feasible even in high density tropical forests where the access to leaves in the crown is complicated. Third, new genetic markers have become available for many tropical tree species that have opened doors and made it possible to address new and complicated questions within populations. Chloroplast DNA polymorphisms offer a tool for tracking fruit dispersal and for investigating the continuity of maternal lineages in tree populations. AFLP-markers offer the possibility to screen the genetic variation at up to a few hundred loci. Thus former limitations due to small number of sampled loci can be overcome. Microsatellite markers have provided the high levels of polymorphism we need to reconstruct, in detail, mating patterns within a study area. And new techniques and tools are continuously developed, which will offer us even more powerful methods for understanding and for documenting the ways in which elements of evolutionary history, logging and forest fragmentation and ecological processes interact to structure tropical tree populations.

In this special issue of Silvae Genetica, we present a group of six papers focusing on aspects of neotropical population genetics. Five of the six assembled papers are the culmination of collaborative work between Latin American and European scientists (from Belgium, Brazil, Costa Rica, France, French Guiana, Germany, Panama, United Kingdom) and funded by a series of EU projects in conjunction with national partner grants (through the INCO-DEV programmes in frameworks 3, 4 and 5, and including the project GENEO-TROPECO). The other paper is the collaborative output of researchers from Canada and Brazil working on the DENDROGENE-project. The Dendrogene Project was part of the environmental cooperation programme of the Brazilian and British governments with the objective to elaborate recommendations for the "Genetic Conservation within managed Forest in Amazônia".