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

Fourth Annual Scientific Report February 2005 – 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	

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Annex I: Project Progress Summary

Section 1: **PROJECT IDENTIFICATION** Information to be provided for project identification	**NOT CONFIDENTIAL**

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

Acronym of the project: GENEO-TROPECO

Type of contract: Shared cost RTD		**Total project cost** 1 332 183 €
Contract number ICA4-CT-2001-10101	Duration (in months) 48 Months	EU contribution 900 000 €

Commencement date 1st February 2002	**Period covered by the progress report** 01.02.05 – 31.01.06

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Key words
Genetic-resources, forest, management, molecular, modelling

World wide web address
http://www.edinburgh.ceh.ac.uk/geneo

List of participants (see over)

Annex I: Project Progress Summary

Section 1: PROJECT IDENTIFICATION (contd)
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Annex I: Project Progress Summary

Section 2: Project Progress Report **NOT CONFIDENTIAL**

Objectives:

The project has the following 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

Project commitments under WP 1 are now complete. Several new sets of microsatellite primers have been developed in a range of species; DNA extraction methods have been optimised and are in use; AFLP protocols have been optimised and will be applied across the range of target species. Practical measures to maximise comparability of AFLP datasets between labs have been adopted, and standardised scoring and analysis techniques are being developed for the analysis stages. Comparisons of several software packages have been undertaken and recommendations for best practice will be circulated to all partners. Work to gather information on species' chromosome number and genome size for each study species is continuing.

Identifying biological determinants of genetic diversity

A full list of species and locations of forests to be sampled has been placed on the project website, and information on biological characteristics of species (e.g. mating system) are being collated. Sampling of all species has now largely been completed and collections have been circulated to partners with responsibility for AFLP analysis. Export problems in Brazil have been solved, and final sample shipments have taken place. AFLP screening of the remaining species on the project list is complete and analysis is underway.

Annex I: Project Progress Summary

Effect of human-mediated processes on genetic diversity

Field collections have been completed for all of the case study species and detailed information on each site has been collected. Using the newly developed and previously available markers, screening of samples has been completed. Model parameterisation and simulations are beginning and will be presented in the final report. The completed case studies will then be prepared as individual manuscripts for publication.

Simulation modelling of population genetic dynamics

The model, ECO-GENE, has been adapted for use with the project gene dynamic case studies. A new module has been designed for the model allowing sensitivity analysis to be performed. Runs of the revised model have been performed using completed datasets, and those mating system parameters influencing genetic structure of populations are being explored. Datasets from the individual case studies will be made ready for inputting into ECOGENE in during July 2005 and simulations will be completed and interpreted shortly after. Recommendations arising from these runs will be derived for inclusion in the final report.

Designing Management strategies to maximize diversity

Further scientific papers have been published during 2005/2006 in refereed journals. This includes special issues of the journals *Heredity* (Nature Publishing Group) and *Silvae Genetica*. Further paper writing and publication is ongoing and dissemination of results to a wider audience will be achieved via leaflets produced in local languages to explain practical outputs of the project, management strategies and the importance of managing forest genetic resources. Preparations for production of additional dissemination materials, including a game for schools, are underway. A public workshop for Costa Rican biodiversity conservation sector has been held, 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. The Workshop was welcomed as a both a useful update on the state of the art in the field of conservation genetics and a statement of the willingess of the scientific community to engage with policymakers and practiioners.

Annex II: Progress Report

1.OVERVIEW

1.1 PROJECT IDENTIFICATION

<table>
<tr><td colspan="4">Title of the project: Sustainable management of Neo-Tropical Tree Genetic Resources: Combining molecular and modelling methods to understand the structure and dynamics of gene diversity</td></tr>
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Bush Estate, Penicuik, Midlothian, EH26 0QB</td></tr>
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</table>

1.2 LIST OF PARTICIPANTS

Participant number, names and address of the participating organisation:
Partner 1 (Coordinator): Stephen Cavers – Centre for Ecology and Hydrology, CEH-Edinburgh, Bush Estate, Penicuik, Midlothian EH26 0QB, UK. Tel: (+44) (0) 131 445 4343. Fax: (+44) (0) 131 445 3943. Email: scav@ceh.ac.uk
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1.3 OBJECTIVES AND EXPECTED ACHIEVEMENTS

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 and 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 project, we are undertaking 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 is being assessed for selected 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 has 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 by implementation of a modelling approach to setting sustainability strategies

1.4 PROJECT WORKPLAN

1.4.1 Introduction

WP1. *Development of molecular techniques*

The list of target species identified at project initiation has now been addressed through gathering of existing datasets and concerted field collections and, despite severe restrictions due to export problems during the lifetime of the project, complete sample sets have been obtained by most partners. Protocols for molecular analysis were established and explored through testing of presamples and comparative analysis of test sample sets, leading to standardised approaches to lab and data analysis.

WP2. *Identifying biological determinants of genetic diversity*

A final series of collections were exchanged between partners during 2005-6 to enable maximisation of the number of species incorporated in the final meta-analysis. The outputs from the individual species analysis (levels of diversity and differentiation, and fine-scale spatial genetic structure) will now be analysed together to explore the datasets for patterns of variation – the meta-analysis will be presented in the final report. The current report provides final updates on the work undertaken with respect to individual AFLP analyses in 2005-6.

WP3. *Effect of human-mediated processes on genetic diversity*

The case studies of human-mediated impacts on tropical tree populations, designed to examine changes in genetic diversity and gene flow in managed populations, are now nearing completion. These are:

1. Assess the impact of human activities on genetic structure and diversity and gene flow for Mahogany (*Swietenia macrophylla*).
2. Assess the impact of logging and the sustainability of current management regimes on genetic diversity and gene flow for *Symphonia globulifera*, *Araucaria angustifolia*, *Swietenia macrophylla* and *Pinus* species.
3. The value and management of secondary forest blocks of *Vochysia ferruginea* grown for timber and degraded land reclaimation.
4. Domestication and management of seed orchards of cacao (*Theobroma cacao*) and cupuaçu (*T. grandiflorium*).

Each case study has generated new results with significance for the management of the species and six publications will be prepared detailed these data: *Swietenia macrophylla* (2), *Vochysia ferruginea*, *Symphonia globulifera*, *Theobroma* sp., *Araucaria angustifolia*.

WP4. *Simulation modelling of population genetic dynamics*

The simulation model, ECO-GENE, is currently being parameterised with new data derived from the individual case studies in WP3. Different modes of forest management systems will be explored (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) and the implications of management for genetic diversity will be presented in the final

report. Currently, work on *Swietenia macrophylla*, *Symphonia globulifera* and *Vochysia ferruginea* is being completed.

WP5. *Designing management strategies to maximize diversity*

Outputs from simulation modelling will be combined with genetic diversity and dynamic data for each case study species, and used directly to set guidelines for the sustainable exploitation of genetic resources. In this final part of the project these findings will be circulated to the scientific community via publications and conference presentations and to the national and international tropical forestry community through targeted publications and workshops. At the final coordination meeting a workshop was held (coordinated by P2) in Costa Rica to present and publicise project findings – the meeting was well attended, with representatives from government, academia and private bodies with interests in conservation of genetic resources. In addition, partner 2 has designed and produced a genetic diversity board game' targeted for circulation in schools to promote the message of conservation of genetic resources. A prototype version is included with this report (see P2 contribution report).

1.4.2 Project structure, planning and timetable

Work package 1: Development of molecular techniques

Deliverables:	**Status**
D1 New SSR primers will be made available to partners through website, Month 12	**Complete**
D2 Developed molecular methods and software will be available to all partners through website, Month 24	**Complete**
Milestones and expected results:	
Set up project website, Month 3	**Complete**
Optimisation of DNA extraction, Month 12	**Complete**
Development of new SSR loci, Month 12	**Complete**
Optimisation of AFLP, SSR and isozyme techniques, Month 18	**Complete**
Development and availability of computer analysis software to all partners, Month 24	**Complete**
Agreed actions from coordination meeting (see minutes):	
Partner 4 (P4) to contact Brazilian government about obtaining export permits.	**Complete**
P1 to contact EU project officer to draft letter requesting export for project. Once complete P1 and P4 to continue to negotiate for export rights.	**Complete**
Partners have agreed to undertake presample screening to check for DNA extraction and AFLP optimisation from cambium sampled material.	**Complete**
All partners involved in AFLP analysis (i.e. P1, 3, 5, 6) to perform standardisation test on *M. coccinea* samples	**Complete**

circulated by P3 at meeting. P6 to undertake initial analysis to find polymorphic PECs, and to circulate results to all partners. Other partners to undertake AFLP analysis of *M. coccinea* samples as advised by P3 and to send results to P6 and P1 for interpretation and checking of standardisation	
P1 to contact RBGE to negotiate exchange of SSR primers with some population samples of *A. angustifolia* with P5.	**Complete**
All partners to look out chromosome data for their species.	**Complete**
P6 to coordinate with partners to send material for genome size analysis	**Complete**

Work package 2: Identifying biological determinants of genetic diversity

Deliverables:	**Status**
D3 Full list of species and locations of forests to be analysed to be placed on website, Month 18	**Complete**
D4 Analysis of results will be made available in a useful form for comparative regression analysis and processing in WP 4 and 5, Month 30	**Complete**
Milestones and expected results:	
Selection of species and populations, Month 6	**Complete**
Sampling of species and populations, Month 24	**Complete**
AFLP analysis of samples and data interpretation, Month 36	**Complete**
Publication of findings in scientific literature to highlight case studies of specific interest.(Partners agree to publish analysis of combined data sets together when work is completed), Month 48	**Ongoing**
Agreed actions from coordination meeting (see minutes):	
Partners have agreed to undertake sampling according to strategies outlined in minutes, in terms of numbers of samples and species to be sampled.	**Complete**
Partners have agreed to undertake presample screening to check for DNA extraction and AFLP optimisation from cambium sampled material.	**Complete**
Partners have agreed to undertake AFLP analysis according to sample numbers outlined in minutes.	**Complete**

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

Deliverables:	**Status**
D5 Location and description of species and forests analysed to be placed on website, Month 18	**Complete**
D6 Range of growth and genetic diversity and dynamic parameters will be measured for undisturbed and managed	**Complete**

systems for each case study. Data will be in format ready for WP4 and WP5, Month 36	
Milestones and expected results:	
Selection and sampling mature trees, seeds and seedlings where appropriate, Month 24	**Complete**
SSR/RAPD/cpDNA analysis of material, Month 36	**Complete**
Interpret data for individual publication by each partner, Month 48	**Ongoing**
Agreed actions from coordination meeting (see minutes):	
P4 to send P1 details of SSR primer sequences and optimised conditions	**Complete**
P1 to contact PfB about collaboration, particularly sampling for WP2 & 3	**Complete**
Partners have agreed, where possible, to adhere to sampling strategy and volume of analysis described in minutes.	**Complete**

Work package 4: Simulation modelling of population genetic dynamics

Deliverables:	**Status**
D7 ECO-GENE model adapted for use with case studies of WP3, Month 24	**Complete**
D8 Simulation models for exploited ecosystems and single species produced. For each case management strategies that maintain genetic resource base by maximising gene flow will be identified, Month 42	**Ongoing**
Milestones and expected results:	
Completion of adapted ECO-GENE model, Month 24	**Complete**
Complete individual species simulations, Month 42	**Ongoing**
Interpret data for publication and management strategies, Month 48	**Ongoing**
Agreed actions from coordination meeting (see minutes):	
P3 will continue to develop ECOGENE and keep partners aware of what input data is required for model, i.e. should collected as part of WP 2 & 3.	**Complete**

Work package 5: Designing management strategies to maximize diversity

Deliverables:	**Status**
D9 Leaflets produced in local languages to explain practical outputs of the project, management strategies and the	**Ongoing**

importance of managing forest genetic resources, Month 42	
D10 Papers drafted for publication in high quality, refereed, international scientific journals, Month 48	**Ongoing**
D11 Hold workshop for Forestry workers in Central America. If accompanying measure application is successful organise international conference to cover whole of Latin America, Month 48	**Complete**
Milestones and expected results:	
Assimilation of data from work packages 2-4 to form realistic and practical management strategies	**Ongoing**
Planning and completion of workshop	**Complete**
Completion of forest literature and its dissemination	**Ongoing**
Submission of individual and integrated scientific papers	**Ongoing**

Additional agreed action & progress

P3 to make initial contact with DENDROGENE project and P1 will follow up with official visit.	**Complete**
P3 to contact Chris Dick about collaboration .	**Complete**
P1 to contact Federico Albertazzi about collaboration.	**Complete**
P3 will make initial contact with Lyn Loveless over parallels between projects. P1 to follow up.	**Ongoing**

1.4.3 Description of the work packages.

Workpackage 1 :

Workpackage number: Phase: Start date: Completion date: Current status: Partners responsible:	1 – Development of molecular techniques Initiation Feb 2002 Jan 2005 Ongoing P6					
Participants	P1	P2	P3	P4	P5	P6
Person months	2	2	3	6	6	7
Already devoted persons months	2	2	3	6	6	7

The development of molecular techniques for the project has been completed. The previously agreed standard methods for AFLP analysis are currently being implemented by partners. The experiment to ensure standardisation of analysis across labs - essential for the final comparative analysis has been repeated and will be finalised in the final report.

As reported previously, all SSR primers have been synthesised and conditions optimised for *Araucaria angustifolia*, *Theobroma grandiflora, Symphonia globulifera* and *Swietenia macrophylla* and have now been applied in individual case studies (WP3).

All partners have been made aware of standard analytical techniques to be used for analysis. Partners have been made aware of existing computer software through the project website, which has been 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) has been distributed to partners or data will be submitted for analysis through the website by P1.

Workpackage 2 :

Workpackage number: Phase: Start date: Completion date: Current status: Partners responsible:	2 – Identifying biological determinants Core activities Mar 2002 Sept 2005 Ongoing P1					
Participants	P1	P2	P3	P4	P5	P6
Person months	9	20	15	20	70	20
Already devoted persons months	12	23	14	15	60	20

Collection of target species has now been completed: problems with site access and export severely delayed distribution of samples from Brazil to responsible partners during the lifetime of the project, however full sample exchange has now taken place. This delay has meant consequent delays to completion of AFLP analyses and hence the overall meta-analysis and identification of biological determinants of genetic diversity. The individual species analyses are complete and the meta-analysis is being prepared and will be presented in the final report.

To review the target: for each species to be collected, a single large population (80 individuals) was mapped and sampled then, according to species range, additional populations (40 individuals) were collected at local (10-50km apart) and distant (>50km elsewhere in the range - usually several thousand km apart) scales. The sampling strategy aimed to allow analysis of spatial elements of genetic structure at highly localised (within-population) and wider (between-population) geographic scales.

Workpackage 3 :

Workpackage number: Phase: Start date: Completion date: Current status: Partners responsible:	3 – Effect of human-mediated processes Core activities Aug 2002 Sep 2005 Ongoing P3					
Participants	P1	P2	P3	P4	P5	P6
Person months	18	58	20	40	70.8	2
Already devoted persons months	18	58	15	20	70.8	7

Work on the case study species is at an advanced stage. Individual analyses have been completed during and the data prepared for simulations using the ECOGENE model. For all case studies, final analysis will be presnted in the final report, but substantially complete analyses are reported here for *Swietenia macrophylla*, *Vochysia ferruginea* and *Theobroma grandiflorum*.

3.1 Impact of fragmentation

Swietenia macrophylla – Analysis of the collections made in Belize is now complete and final analysis will be presented in the final report. Data is currently being run through simulations in ECOGENE to evaluate various management strategies and the form recommendations for the species.

3.2 Logging and sustainability of management regimes

Araucaria - SSR loci have been successfully optimised and extensive sampling has been completed. Analysis of samples with SSR loci is being finalised.

Symphonia - *Symphonia globulifera* has been sampled. All adult and progeny material has been screened with optimised SSR loci and data will be presented in the final report.

Swietenia – Analysis of adult, juvenile and seedling populations at Marajoara is in progress. Current results indicate a loss of diversity in seedling populations likely resulting from logging activities.

3.3 Secondary regeneration

Vochysia – All populations have now been analysed and complete final results will be presented in the final report. Data is being prepared for ECOGENE simulations.

3.4 Domestication

Theobroma – Analysis of populations using microsatellites is complete, final results indicate high levels of genetic variability on a local scale.

Workpackage 4 :

Workpackage number: Phase: Start date: Completion date: Current status: Partners responsible:	4 – Simulation modelling of popn. dynamics Core activities Aug 2002 Sep 2005 Ongoing P3					
Participants	P1	P2	P3	P4	P5	P6
Person months	-	-	18	-	-	-
Already devoted persons months	-	4	15	-	-	-

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. Under this work package, the ECO-GENE model is being adapted to simulate the more complex situation for each of the tropical case studies (WP3). A new module has been designed for the model allowing sensitivity analysis to be performed. Runs of the revised model have been performed using completed datasets for *Jacaranda* and *Dipteryx*, and those mating system parameters influencing genetic structure of populations are currently being explored. A linkage between ECO-GENE model and the forest growth model Symfor has been has been successfully achieved and demonstrated, resulting in improved realism in simulations of logging scenarios from that achieved using ECO-GENE alone.

The datasets that will be used to parameterise the model: i.e. the detailed stand studies obtained in WP3, are currently being used in simulations. For these data. the ECO-GENE model will be used to estimate the impact of alternative forest utilisation strategies on genetic diversity for individual species in tropical forest stands. In particular the following silvicultural practices will be addressed; 1, different logging intensities and frequencies; 2, different spatial distributions of clear cuts that lead to different forest fragmentation; 3, different spatial distribution and size of unlogged buffer areas.

Workpackage 5 :

Workpackage number: Phase: Start date: Completion date: Current status: Partners responsible:	5 – Designing management strategies Final Jan 2005 Jan 2006 Ongoing P2					
Participants	P1	P2	P3	P4	P5	P6
Person months	4.4	21.4	3.3	14.6	20	5.8
Already devoted persons months	0	22	0	4	5	1

After processing and discussing outputs and indicators from work packages 2-4, criteria for the management of genetic diversity within complex tropical systems, and the sustainable extraction and management of intensively harvested, single species landscapes will be developed and disseminated. The simulation scenarios used in ECO-GENE will be developed in close cooperation with forest service and forest planning institutions.

Leaflets explaining management strategies will be produced in the local language for circulation in the countries of Latin American partners and relevant neighbouring countries. Results will be disseminated to the wider academic community through conference presentations and the project website. As the project matures dissemination measures are being developed that will convert highly academic data into practical information, and circulate it in a variety of languages to a wide range of end-users.

To promote dissemination of results, encourage synthesis of outputs across different projects, build links between the scientific, policy and practicing forestry communities and prepare plans for FP7, an accompanying measure (INCO SSA) has been applied for. This proposal requests funding for a 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 project would also fund the publication of booklets in local languages detailing results and recommendations of the project and the proceedings of the symposium. The proposal passed all criteria and made the reserve list but failed to be funded: it has been substantially revised and re-submitted and the outcome is pending.

We also report here, a public Workshop held at the National Institute of Biology, Costa Rica in conjunction with the project's final coordination meeting. The meeting was attended by a wide variety of interested parties, including the governmental Commission on Biodiversity and formed a very effective forum for publicising project outcomes and the value of international collaboration as well as specific case study data on individual species.

2. ROLE OF PARTICIPANTS

Partner 1: Centre for Ecology and Hydrology (CEH)

Scientific Team: Stephen Cavers (coordinator), Sam Davies (PhD student), Katherine Walker (Scientific Officer), Robert Munro (Higher Scientific Officer), Andrew Lowe (University of Queensland).

Work Package 2: Identifying biological determinants of genetic diversity

AFLP Analysis of *Pinus oocarpa*.

Introduction

Pinus oocarpa is the most widely distributed pine species in Central America, occurring from Mexico to Nicaragua (Barnes & Styles 1983). In Nicaragua, it is the most common pine species, where it occurs as discrete populations located in between 600 1300 m in the North, West and Central regions. All populations are suffering degradation as a consequence of the overexploitation of timber and through forest fires (Diaz et al 2001). Furthermore, the frequency of deliberate fires has greatly increased as people have attempted to create more arable and grazing land. These phenomena have altered the size and genetic structure of the natural populations of the species, and pose a serious threat to the development of mature woodland (Farjon & Styles 1997). Knowledge of the structure and pattern of genetic variation in this species is important to the development of appropriate strategies for *in situ* conservation of natural woods and the regeneration of partially logged forests (Diaz et al 2001).

Methods

Sampling & DNA extraction: Three population samples were collected in Nicaragua by CATIE: Chocolatera (40 mapped indviduals) and Valle Bonito (40 individuals) and Valle Grande (81 individuals). Samples were collected as dried needles. DNA extracts were prepared either at CEH using QIAGEN DNEasy 96-well format extraction kit. AFLP analysis: a suite of eight AFLP primer combinations was used to obtain a total of 383 markers (all Eco+2_MSE+4: AC_ACAA, CC_ACAA, AC_ACAG, CC_ACAG, AC_GACC, CC_GACC, AC_TACC, CC_TACC). Gels were scored using SAGA software and analysed for diversity, population differentiation and spatial genetic structure, using the programs AFLP-SURV and SpageDi.

Results

Diversity levels in all populations were high (Table 1). The mean estimate of Nei's gene diversity within populations is 0.344 (SE=0.015), similar to those estimated in a previous study of the species using AFLP markers (Hj = 0.342, using 392 AFLP loci, Diaz et al, 2001).

Table 1: Diversity levels for each population. Mean number of scored individuals (*n*), number of polymorphic loci with allelic freq within 0.05 to 0.95 (*#loc_P*), percentage of polymorphic loci at the 5% level (*PLP*), expected heterozygosity under HW genotypic proportions or Nei's gene diversity (*Hj*).

Population	n	#loc_P	PLP	Hj	S.E.(Hj)
Grande	35	315	95	0.37151	0.00752
Bonito	36	269	96	0.31904	0.00891
Chocolatera	61	355	93	0.34237	0.00701

Table 2: Population genetic structure. Total gene diversity (Ht), mean gene diversity within populations (Hw), genetic differentiation among populations (Hb), genetic correlation between pairs of genes sampled within a population relative to pairs of genes sampled within the overall set of populations (Fst).

n	Ht	Hw	Hb	Fst
3	0.5066	0.3443	0.1623	0.3203
S.E.		0.015177	0	0
Var		0.00023	0	-0.0015

Table 3: Pairwise Fst between populations

Grande	0	0.3687	0.3471
Bonito	0.3687	0	0.224
Chocolater	0.3471	0.224	0

Population differentiation was 0.186, relatively high compared to previous estimates (G_{ST}= 0.073; Diaz et al, 2001), indicating clear divergence between populations.

Work package 3: Effect of human-mediated processes on genetic diversity Impact of fragmentation: *Swietenia macrophylla*

Previous annual reports have detailed preliminary analyses of the *Swietenia macrophylla* plots. Genotyping has now been completed and the following represents new and updated analysis of the finalised datasets. Further extensive analysis of these data will be presented in the final report.

Introduction

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. 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.

Materials and methods

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 1.4). 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.

Table 1. Sampling: details of numbers of trees / seeds sampled per plot for the four plots now collected from Hill Bank.

Plot	Area	No. adult trees	No. mother trees	No. seeds	Logged/ Unlogged
Punta Gorda 01	35	195	25	141	Logged
East Botes 08	35	215	28	214	Unlogged
West Botes 20	49	196	21	420	Logged
West Marimba 05	90	199	30	500	Unlogged
Totals		811	104	1275	

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.

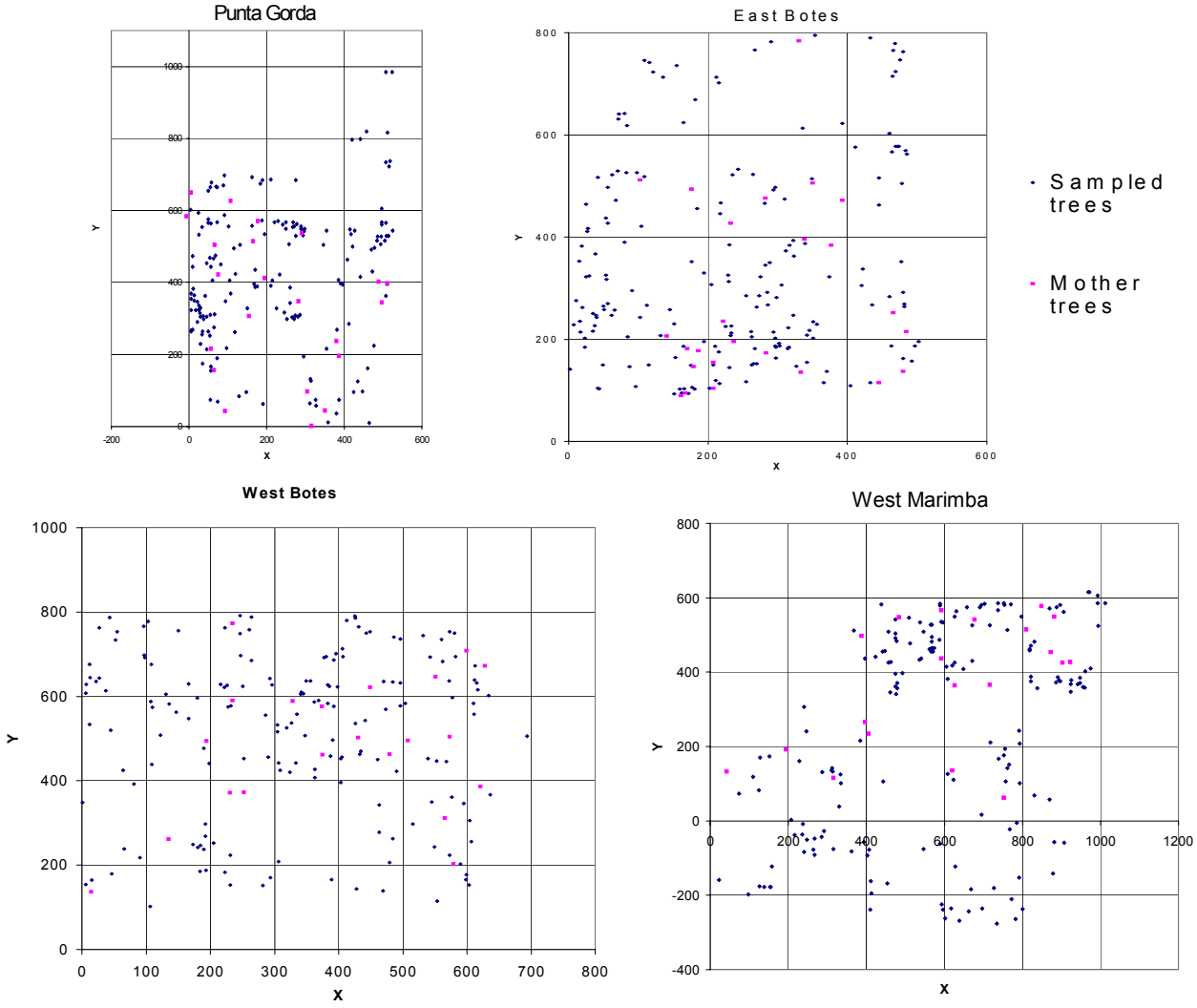

Figure 1. Maps showing positions of trees sampled. Seeds were collected from the around the mother trees. Punta Gorda and West Botes were selectively logged in 1998.

Microsatellite analysis of 795 adult individual samples and 1229 seed samples, representing the four populations (table 1.2), 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 at IPBO Belgium for populations PG and EB 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 LI COR Gene ImagIR 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 was compared for each population to check for bias. The data was run through Micro-Checker (Oosterhout *et al* 2005) to check for null alleles and scoring errors.

Departure from Hardy-Weinberg equilibrium (GENEPOP v3.3, Raymond & Rousset 1995) and numbers of alleles per locus (A), the observed (Ho) and expected heterozygosity (He) and fixation index (f) were estimated (GDA Lewis & Zaykin 1999). Estimates of multi (tm), and single (ts) locus outcrossing rates and correlation of paternity were determined from progeny arrays, including maternal genotypes (MLTR v3.0 Ritland 2004).

Results

Null allele analysis

Tests using micro-checker software (Oosterhout *et al* 2005) show no evidence for null alleles for all loci except for sm32 (table 2). A general excess of homozygotes for most alleles size classes at this locus suggests null alleles may be present.

Table 2: Analysis of null allele frequency

Locus	Null Present	Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
sm31	no	-0.0277	-0.0242	-0.0229	0.3383
sm22	no	0.0156	0.0184	0.0115	0.4179
sm46	no	-0.0078	0.0076	0.0022	0.5453
sm01	no	-0.0296	-0.0206	-0.0187	0.4213
sm32	yes	0.1269	0.1613	0.0967	0.3787
sm51	no	-0.017	-0.0154	-0.0141	0.1819
sm40	no	0.0095	0.0108	0.0094	0.5719

Linkage disequilibrium

Tests were carried out using GENEPOP to check if loci were linked. Out of a possible 84 tests for genotypic linkage disequilibrium, 72 were performed. Of these 2 were significantly different to

the null hypothesis of no linkage between loci, having p-values <0.05. These were sm01/sm40 and sm51/sm40. This result may have been due to high number of dropouts for sm01.

Genetic diversity

All loci were polymorphic. All populations show a slight heterozygote deficiency (table 3) as noted in previous publications (Novick *et al* 2003, Lemes *et al* 2003). Both studies conclude that inbreeding is the most likely cause of the heterozygote deficiency in this species, due principally to spatial clustering of populations although selfing has also been detected. Kin mating is likely to be the cause of the heterozygote deficiency in our Belize populations. This conclusion is supported by the strong family clustering detected by spatial analysis (see figure 2).

Table 3 - Characteristics by locus. Mean sample size (*n*) over all loci, the proportion of samples that were genotyped (scoring rate), the proportion of polymorphic loci (*P*), the mean number of alleles per locus (*A*), the mean number of alleles per polymorphic locus (*Ap*), the expected heterozygosity (*He*), the observed heterozygosity (*Ho*), and an estimate of the fixation index (*f*).

Locus	n	A	Ap	He	Ho	f
Sm31	643	19	19	0.86	0.76	0.12
Sm22	683	9	9	0.51	0.46	0.10
Sm46	577	13	13	0.46	0.25	0.46
Sm01	256	17	17	0.80	0.80	-3E-05
Sm32	641	6	6	0.54	0.48	0.11
Sm51	622	13	13	0.82	0.80	0.02
Sm40	568	13	13	0.81	0.71	0.12
All	570	12.86	12.86	0.69	0.61	0.11

Levels of diversity in the populations (A = 12.86, He = 0.69) were close to previous estimates for Central American populations (A = 13, He = 0.66, Novick et al 2003) and lower than levels estimated in a previous study from the Brazilian Amazon (A = 18, He = 0.79, Lemes et al, 2003). The difference between South and Central American populations is most likely due to the greater reductions in effective population size of Central American Mahogany relative to those experienced by South American populations, due to habitat constriction during the Pleistocene glaciations (Novick et al 2003). The close agreement of our data with that reported previously provides strong evidence for a reduction of diversity in the Central American *S. macrophylla* as a regional characteristic. Between sites at Hill Bank, diversity levels varied little, with only slightly higher diversity found at West Marimba. This site covers a larger area (due to reduced density of trees) and is generally more heterogeneous in habitat: the forest is broken up with low lying swamp – *bajo* – patches. The greater site complexity may be a contributing factor to the raised diversity estimates. However, additional possibilities are being investigated, including population age structure and increased gene flow due to a more fragmented canopy.

Table 4 – Descriptive statistics for each population

Pop	n	A	Ap	He	Ho	f
EB	158	9.6	9.6	0.64	0.62	0.02
PG	118	10	10	0.65	0.58	0.10
WB	167	8. 7	8. 7	0.61	0.51	0.15
WM	176	10.5	10.5	0.71	0.63	0.12
Mean	155	9.7	9.7	0.65	0.59	0.10

Table 5. The aggregation index of Clark and Evans (Ripley 1981). R<1 indicates a clumped and aggregated distribution whereas random distribution is indicated by values of 1. Regular distribution is indicated with values of R>1.

Population	**Aggregation index (R)**
Punta Gorda	0.723
East Botes	0.825
West Botes	0.862
West Marimba	0.703

Mating system analysis

All populations show high outcrossing rates (tm>1). PG has a lower multilocus correlation of paternity (rp)m than the other populations, showing a lower probability of finding siblings that share the same father within a progeny array. EB and WM, both of which unlogged, show the highest correlation of paternity (rp)m indicating that more of their seeds are pollinated by only a few pollen donors. The reduced correlation of paternity estimates for PG and WB (logged) populations may be due to gene flow occurring over a greater distance. EB and WM have negative correlation of outcrossing among progeny arrays (t).

Table 6 - Ritland's multilocus mating system analysis. Multilocus population outcrossing rate (*tm*), the (minimum variance) single locus population outcrossing rate (*ts*), the single locus inbreeding coefficient of maternal parents (*F*), correlation of outcrossing among progeny arrays (t) and correlation of paternity (*rp*).

Pop	F	tm (SD)	ts (SD)	tm-ts (SD)	Corr. of t	rp(m) (SD)	[rp(s)-rp(m)] (SD)	Corr. of t among loci
EB	-0.074 (0.062)	1.073 (0.078)	1.031 (0.050)	0.042 (0.095)	-0.200 (0.302)	0.123 (0.110)	-0.323 (0.110)	0.416 (0.416)
PG	0.036 (0.116)	0.977 (0.062)	1.004 (0.040)	-0.027 (0.048)	0.007 (0.392)	0.019 (0.073)	-0.219 (0.073)	0.001 (0.538)
WB	0.234 (0.189)	0.991 (0.061)	0.951 (0.050)	0.040 (0.056)	0.803 (0.550)	0.096 (0.052)	-0.296 (0.052)	0.443 (0.429)
WM	0.280 (0.073)	1.062 (0.073)	1.049 (0.043)	0.014 (0.074)	-0.200 (0.530)	0.159 (0.042)	-0.359 (0.042)	0.957 (0.402)

In all populations single is lower than multilocus correlated paternity ([rp(s)-rp(m)]) indicating that population substructure has no effect on the male similarity between outcrosses. There is a high correlation of outcrossing rate among loci for all populations except PG.

Secondary regeneration: *Vochysia ferruginea*

Introduction

Vochysia ferruginea is a pioneer tree of Central America, a widespread dominant of secondary forests that is occasionally found as a canopy tree in primary forest. It tolerant of 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 (P2) have long term ecological data making these populations particularly useful to investigate genetic parameters along with ecological factors.

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 1). 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 diversity 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 1). 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 2). This was a consequence of difficulties in successful amplification of loci, particularly A1-5 (see Table 1). Table 2 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 from 0 - 1.

Table 1: 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 2: 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)

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 3 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).

Table 3: 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	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

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, 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 3).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 4). 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).

Table 4: 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)

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.

Further analysis including spatial genetic structure, paternity analysis, correlations of diversity and quantitative measures of fitness are currently being carried out and will be presented in the final report, together with detailed discussion of the results presented here. The work presented forms part of Sam Davies' PhD thesis, due for submission in May 2006.

Partner 2: Centro Agronomico Tropical de Investigación y Enseñanza (CATIE).

Scientific team: Carlos Navarro, Bryan Finegan, Gustavo Hernández, Leonel Coto, Vicente Herra, Pablo Madriz and Carolina Cascante.

SCIENTIFIC REPORT.

Work package 2: Identifying biological determinants of genetic diversity

Leaf and Cambium samples of all the species included in the plan were collected and prepared for analysis, including some additional species that were collected extra, such as: *Vochysia allenii, Goethalsia meiantha* and *Pinus oocarpa*.

Sampling of species and populations

During 2005 all the samples collected were sent for analysis to Australia and Scotland for distribution to other partners. Complete information on all the species sampled is detailed in Tables 1 and 2. In total 20 species have been collected for biodiversity assessment in Costa Rica and one in Honduras (*Pinus oocarpa*).

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

All the collections, maps and works related with this package were completed. Thesis of Pablo Madriz was presented to the committee and approved. One first article is finished and to be submitted.

3.1 Logging and sustainability of management regimes

Pinus oocarpa

A first paper is already prepared for submission resulting from the thesis of Pablo Madriz. The thesis is also in the libraries at CATIE and CEH:

Madriz, P; Navarro, C. (2005) Non-parametric methods to find density-dependent genetic effects in the early natural regeneration of the neotropical pine ***Pinus oocarpa*** var ***oocarpa***: contingency analysis and logistic regression approach.

Secondary regeneration

Vochysia ferruginea

The collection of cambium and fruits *Vochysia ferruginea* was completed for all the sites and samples and permits were taken to Scotland for analysis. DNA analysis of these samples was carried out by G. Hernández in training of laboratory techniques including AFLP

Table 1. Species collected in Geneotropeco for biodiversity assessment in primary forest fragments in Costa Rica and Honduras. See also Table 2.

Species	**Collect 80**	**CATIE Collect 40**
Calophyllum brasiliense	UFRJ	1
Carapa guianensis	**Tapajos**	2
Cedrela odorata	CATIE	2
Ceiba pentandra	Manaus	2
Eschweilera costaricensis	CATIE	2
Goethalsia meiantha	CATIE	2
Hyeronima alchorneoides	UCR	UCR
Laetia procera	CATIE	1
Lecythis ampla	CATIE	2
Lonchocarpus costaricensis	CATIE (HORIZONTES)	2
Maranthes panamensis	CATIE	2
Minquartia guianensis	Tapajos	1
Pinus oocarpa	CATIE	2
Sideroxylon capiri	UCR	UCR
Simarouba amara	INRA	2
Simarouba glauca	UCR	UCR
Swietenia macrophylla	CATIE/INPA	DONE
Tapirira guianensis	CATIE	1
Tetragastris panamensis	CATIE	1
Vochsia allenii	CATIE	2
Vochysia ferruginea	CATIE	2

Table 2. Species sampled in the North and Atlantic Region of Costa Rica for WP 2.

Species sampled	**Initial min dbh**	**Min dbh SampId**	**Site for 80**	**First Site for 40**	**Second site for 40**	**Additional Site**
Tetragastris panamensis	*20 cm*	*20 cm*	Ladrillera1 **(82)**	Selva Verde **(47)**		
Carapa guianensis	*20 cm*	*20 cm*		Ladrillera 1 **(42)**	Corinto **(43)**	
Simarouba amara	*40 cm*	*20 cm*		Lad1+Selva Verde **(45)**	Tosi **(44)**	Corinto (2)
Calophyllum brasiliense	*20 cm*	*20 cm*		Corinto **(40)**		
Tapirira guianensis	*40 cm*	*40 cm*		Tirimbina **(76)**	Corinto **(42)**	
Ceiba pentandra				Turrialba **(42)**	Sarapiquí **(44)**	
Maranthes panamensis	*15 cm*	*15 cm*	Tirimbina **(93)**	Corinto **(44)**	Rojomaca **(41)**	
Eschweilera costaricensis	*8 cm*	*8 cm*	Rojomaca **(87)**	Paniagua **(54)**	Tosi **(42)**	Lad1+Selva Verde (23)
Lecythis ampla	*20 cm*	*10 cm*	Lad1+Selva Verde **(79)**	Corinto **(41)**	Tirimbina **(42)**	Rojo+Paniag (17) + (12)
Goethalsia meiantha	*40 cm*	*40 cm*	Ladrillera 3 **(82)**	Corinto **(41)**	Tosi **(41)**	
Laetia procera	*30 cm*	*30 cm*		Tirimbina **(58)**	Tosi **(43)**	Corinto (11)
Pinus oocarpa	*40*	*40*	Lajas**(80**	Pueblo Nuevo**(40**	Quezalapa **(40)**	
Vochysia allennii	*40*	*40*	Marta1**(80)**	Marta2**(40)**	Grano de Oro**(40)**	
Minquartia guianensis	*15*	*20*		Tirimbina**(40)**		
Lonchocarpus costaricensis	*8*	*8*	Horizontes**(8 0)**	HOrizontes (40)	Palo Verde (40)	

<u>Work package 5</u>: Designing management strategies to maximize diversity

Annual meeting of the partners was celebrated at CATIE, to present results. Also a dissemination workshop celebrated at INBIO was planned with the participation of all the partners programme of the meeting is attached in the annexes. The activity was evaluated with people participating were very well impressed of the results presented. A genetic diversity game (see Fig 1) was also prepared for education in primary and secondary schools, we are looking for funding to produce several hundreds and distribute across Mesoamerica and Mexico.

Deliverables

Two papers for the special series on genetic diversity of tropical trees are accepted and in the process of being published.

A conference on Genetic Diversity of Tropical trees was celebrated in the National Institute of Biodiversity on October with the presence of personnel of Ministry of the Environment. A meeting with Forestry workers and policy markers including deputies at the parliament and the ministry of natural resources will be celebrated in October with the participation of all partners of the project participate as a new law is demanding information for protection and conservation of endangered species.

Figure 1. Preliminary layout of 'genetic diversity' game devised by CATIE for dissemination of conservation of genetic diversity message to primary and secondary schools across Mexico and Central America. Text is being devised and will be presented with the final report.

Partner 3: Institut National de la Recherche Agronomique (INRA).

Scientific team: Henri Caron, Antoine Kremer, Bernd Degen, Eric Bandou

An MSc student, Miss Sylvie Lalleman, was continuing her lab work for two months as a technician with a temporary position. She was in charge of molecular work of AFLP analyses.

Scientific work undertaken and progress at the end of the fourth year

WP/activity	Year 4	Present situation
WP1 - Development of molecular techniques		On target
WP 2 : Identifying biological determinants of genetic diversity		
Sampling of species and populations	An additional species *Minquartia* was collected in Paracou	
AFLP analysis	5 additional species were analysed during the past year. Results available for 10 sp.	3 species still remain to be analysed. Results will be available in June 2006
WP3: Effect of human-mediated processes on genetic diversity		
3.2 Logging and sustainability of management regimes. S.globulifera		On target
WP4 : Simulation modelling of population genetic dynamics		
Completion of adapted ECO-GENE model		On target (month 24). Dataset will be gradually used for model initialisation and parameterisation.

Work package 2: Identifying biological determinants of genetic diversity

Sampling of species and populations

All samples were collected and 40 samples of an additional species *Minquartia,* were collected in Paracou and sent to partner 1 .

AFLP analysis

The molecular analysis of 7 species is completely finished. The analysis is going on in 5 species (molecular work done; data analysis is in progress). The analysis of *C. sciadophylla* must be done.

Table 1: Sampling of studied species. Data have been analysed using software AFLP-surv. For each species genetic parameters, total diversity Ht, mean within population diversity Hw, genetic differentiation Fst were checked. In addition, Nei genetic distance between populations is also calculated by the software.

Species	No of bands	No of populations	No of trees	Remarks
Bocoa prouacensis	**88**	**2**	123	Needs 1 population more
Eperua falcata	**71**	**4**	172	
Vouacapoua americana	**92**	**2**	93	Needs 1 population more 1 PEC's must be read
Dicorynia guianensis	**134**	**3**	92	
Jacaranda copaia	**125**	**3**	92	
Chrisophyllum sanguinolentum	**149**	**3**	121	
Eperua grandiflora	**173**	**3**	113	
Virola melinonii	**241**	**2**	55	Needs 1 population more
Simarouba amara	**157**	**4**	133	
Symphonia globulifera	**184**	**3**	153	
Moronobea coccinea	**-**	**1**	91	Molecular work in progress
Sextonia rubra	**-**	**4**	168	Molecular work in progress
Cecropia sciadophylla	**-**	**4**	160	Work must be done

Table 2: within population genetic diversity Hw in Paracou, total genetic diversity H_t, genetic differentiation F_{st} for the 10 studied species

	H Paracou	H_t	F_{st}
Symphonia globulifera	0.1501	0.2047	0.0389
Virola melinonii	0.2038	0.1912	0.011
Vouacapoua americana	0.218	0.2609	0.015
Eperua falcata	0.2465	0.2752	0.0175

Dicorynia guianensis	0.2468	0.2314	0.011
Bocoa prouacensis	0.2575	0.3251	0.0925
Simarouba amara	0.2652	0.3187	0.076
Jacaranda copaia	0.2832	0.3143	0.11
Eperua grandiflora	0.3002	0.3147	0.041

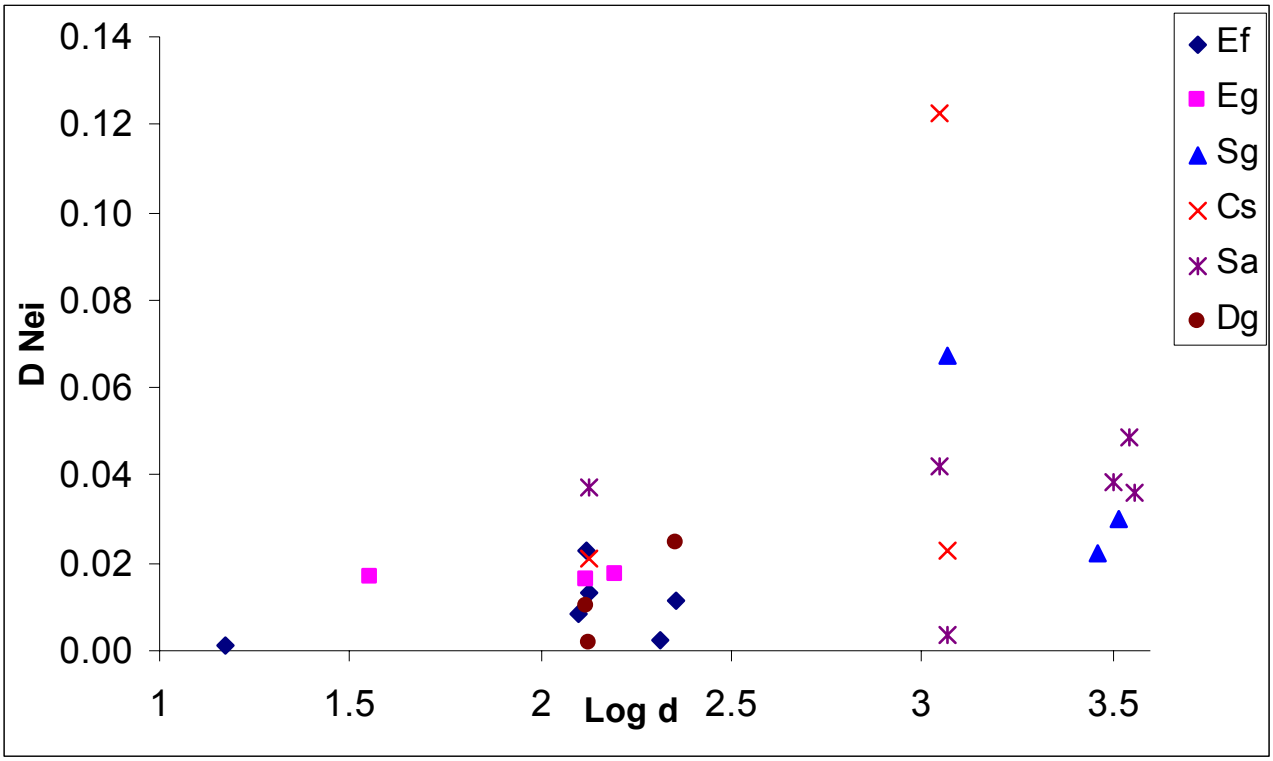

Figure 1 : Genetic distance between populations within each species according to the geographical distance for 6 studied species.

<u>Work package 5</u>: Designing management strategies to maximize diversity

The results of simulation on the impact of selective logging on genetic composition and demographic structure of four tropical tree species are the basis of a publication which was accepted in *Biological Conservation* (Degen B et al. , 2006)

Over-exploitation and fragmentation are serious problems for tropical forests. Most sustainable forest management practices avoid clear-cuts and apply selective logging systems focused on a few commercial species. We applied a simulation model to estimate the impact of such selective logging scenarios on the genetic diversity and demography of four tropical tree species from French Guiana. The simulations used data on genetic and demographic composition, growth,

phenology and pollen and seed dispersal obtained for *Dicorynia guianensis, Sextonia rubra, Symphonia globulifera* and *Vouacapoua americana* at the experimental site in Paracou. Whereas *Symphonia globulifera* serves as a model for a species with low logging pressure, the other three species represent the most exploited tree species in French Guiana. In simulations with moderate logging, typical for French Guiana, with large cutting diameter (>60cm diameter) and long cutting cycles (60 years), the two species *Vouacapoua americana* and *Sextonia rubra* were not able to recover their initial stock at the end of the rotation period, with a large decrease in the number of individuals and in basal area. Under a more intensive logging system (cutting diameter > 45cm diameter, cutting cycles of 30 years) that is common practice in the Brazilian Amazon, only *Symphonia globulifera* showed no negative impact. Generally, the differences between the genetic parameters in the control scenarios without logging and the logging scenarios were surprisingly small. The main reasons for this were the overlapping of generations and the effective dispersal ability of gene vectors in all species, which guarantee relative homogeneity of the genetic structure in different age classes. Nevertheless, decreasing the population size by logging reduced the number of genotypes and caused higher genetic distances between the original population and the population at the end of the logging cycles. Sensitivity analysis showed that genetic changes in the logging scenarios were principally determined by the growth, densities and cutting diameter of each species, and only to a very small extent by the reproductive system including factors such as pollen and seed dispersal and flowering phenology.

Five new peer-reviewed publications were produced:

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

2. 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* (accepted)

3. 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.

4. Kremer A, Caron H, Cavers S *et al* (2005) Monitoring genetic diversity in tropical trees with multilocus dominant markers. *Heredity*, **95**(4) 274-280

5. 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.

Partner 4: Instituto Nacional de Pesquisas da Amazônia – INPA

Scientific Team: Rogério Gribel, Maristerra R. Lemes, Thiago André, Thieme Martiniano, Aldenora L. Queiroz and Maria da Glória P. Assis.

Work Package 2: Identifying biological determinants of genetic diversity

2.1- Collections of Amazonian species for AFLP analysis

We have completed sample collections of the target species in the Brazilian Amazon for the large-scale research on the AFLP. The collections were carried out at INPA´s Reserva Florestal Adolph Ducke, near Manaus, Amazonas State. The cambium and leaves samples were preserved in silica gel and stored at -20C until DNA extraction. Table 1 shows the sampling of target species for AFLP analysis in Reserva Ducke for the period.

Table 1 – Collections of species for AFLP analysis in Reserva Ducke, Manaus, AM (February – September 2005).

Species	Family	Material	Reserva Ducke
Minquartia guianensis	Olacaceae	C	(40) + (40)
Ceiba pentandra	Malvaceae	C, L	80 (mapped)
Pseudobombax munguba	Malvaceae	C	45 (Rio Japurá)
Laetia procera	Flaucortiaceae	C	80
Calophyllum brasiliensis	Clusiaceae	C	40
Cecropia sciadophylla	Moraceae	C	40

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

3.1 – Impact of Selective Logging -_*Swietenia macrophylla*

3.1.1 – Gene Flow Analysis

The main objective of this study was to quantify the pollen flow between trees in a logged population of *S. macrophylla* based on parentage analysis of seedlings using microsatelite markers.

Study site: 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).

METHODS

Sampling – To quantify the reach of pollen flow in Marajoara population, 51 established seedlings and 220 adult trees (dbh > 10 cm) were sampled and leaves collected for genetic analysis. The trees sampled were considered as potential pollen donors, and represented approximately 70% of the total number of adult trees in the area.

DNA extraction – The 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.

Microsatellite analysis – Genetic analyses were based on PCR amplification of eight highly polymorphic loci (*sm01, sm22, sm31, sm32, sm40, sm46, sm47, sm51*) previously isolated and characterized for *S. macrophylla* by Lemes *et al*. (2002). 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.

Data analysis - The parentage analysis was based on the allele frequencies of eight microsatellitye 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.

RESULTS AND DISCUSSION

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 (Figure 01).

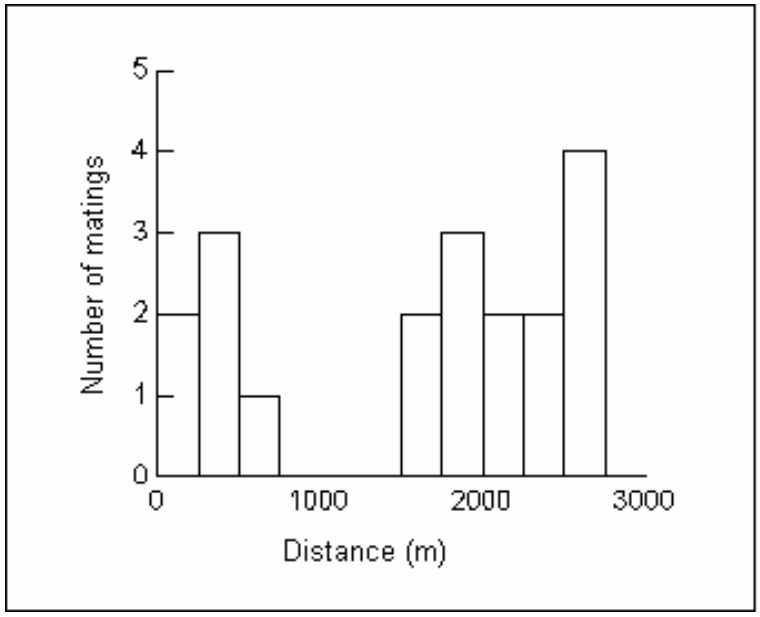

Figure 1 - Number of matings correlated with distance between parents based on microsatellite parentage analysis of seedlings in a logged population of *Swietenia macrophylla* in Eastern Amazonia.

The minimum distance detected between two parents for the observed matings was 69.1 m and the greater distance of pollen flow was 2,736.1 m. The mean distance of pollen flow 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 however they 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), 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 for the extensive gene flow observed in this study since the pollinators probably had to move long distances to find flowered individuals in the area.

3.2 – Effect of Domestication in the Genetic Diversity of *Theobroma grandiflorum*

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 at the East border of the species distribution also in the Brazilian Amazonia.

MATERIALS AND METHODS

Study site and plant collection – 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 02). Leaves were collected for DNA extraction and preserved in silica gel at –20 ^{o}C.

DNA extraction and quantification – DNA extraction followed standard CTAB protocol (Doyle & Doyle, 1987). DNA quantification was performed by comparison with standard concentrations (Lambda DNA) in ethidium bromide-stained 1% agarose gels.

PCR Amplification - 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°C for 5 min followed by 30 cycles of 94°C for 1 min, annealing temperature of each primer (°C) for 1 min and 72°C for 1 min; and a final elongation step at 72°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).

Data Analysis – 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.

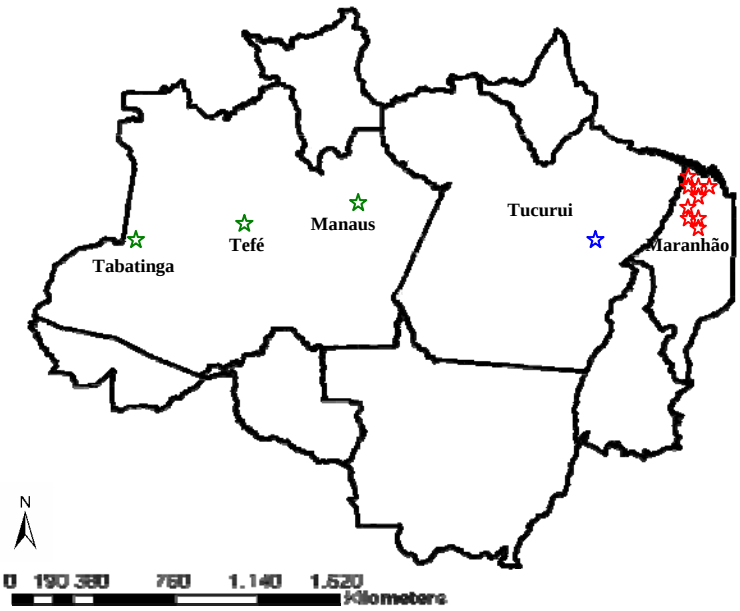

Figure 2 – Locations of sampled populations of *T. grandiflorum* in the Brazilian Amazon.
Manaus, Tefé, Tabatinga Amazonia Maranhense Tucurui-PA

RESULTS AND DISCUSSION

Genetic diversity in *Theobroma grandiflorum* - We found high allelic variation for the eight amplified microsatellite loci in the analysed population (Table 02). The number o alleles per locus varied from 5 to 16. Expected heterozygosity for the eight loci ranged from 0.42 to 0.89 (Table 02). 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.

Table 2 – 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 03). 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's distribution. The genetic results showed a loss of allelic richness from the Central region of occurrence to the boundary of the species's

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 3 – 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).

3.3 – Pollinator management in the self-incompatible fruit tree *Theobroma grandiflorum* (Sterculiaceae)

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 has 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 are evidences 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.

METHODS

We perfomed 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 03). 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 colleted 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).

RESULTS

The figure 4 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. 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 5).The data regarding to the pollen load present in the bees's bodies and honey samples collected within the hives were not analysed yet. Recommendations for Pollination Management of *T. grandiflorum*:

1) To leave undisturbed areas around the crops.
2) Ensure adequate food plants when crops (cupuaçu) are not flowering.
3) Provide natural and artificial nesting supports (fences, rural building etc)
4) Minimise the use of pesticides.
5) Rear colonies in proper wood

Figure 3 – Different wood substrates used to rear colonies of *Plebeia sp* and *Aparatrigona sp*, the main pollinators of *T. grandiflorum* in Central Amazonia.

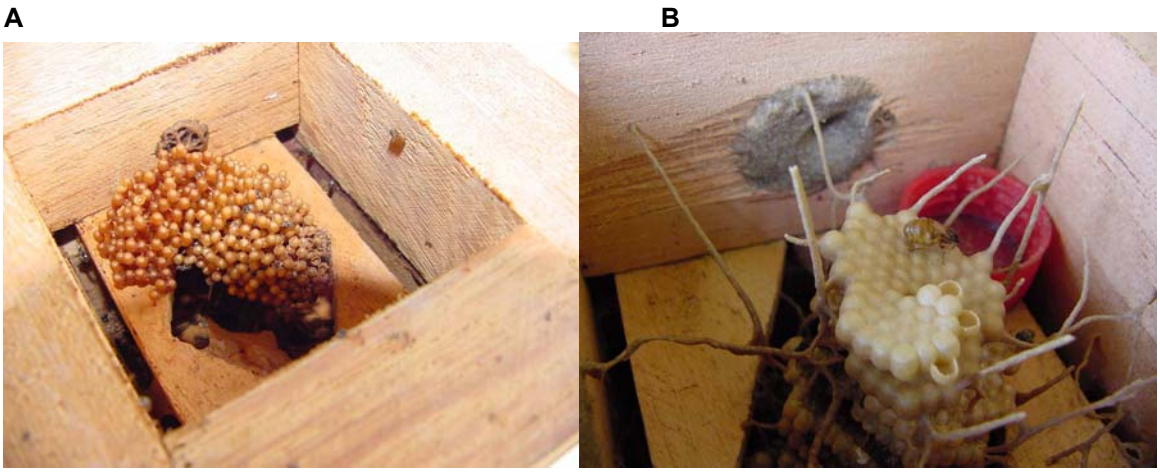

Figure 4 – Hives of *Plebeia sp.(*A) and *Aparatrigona sp*.(B) in wood boxes.

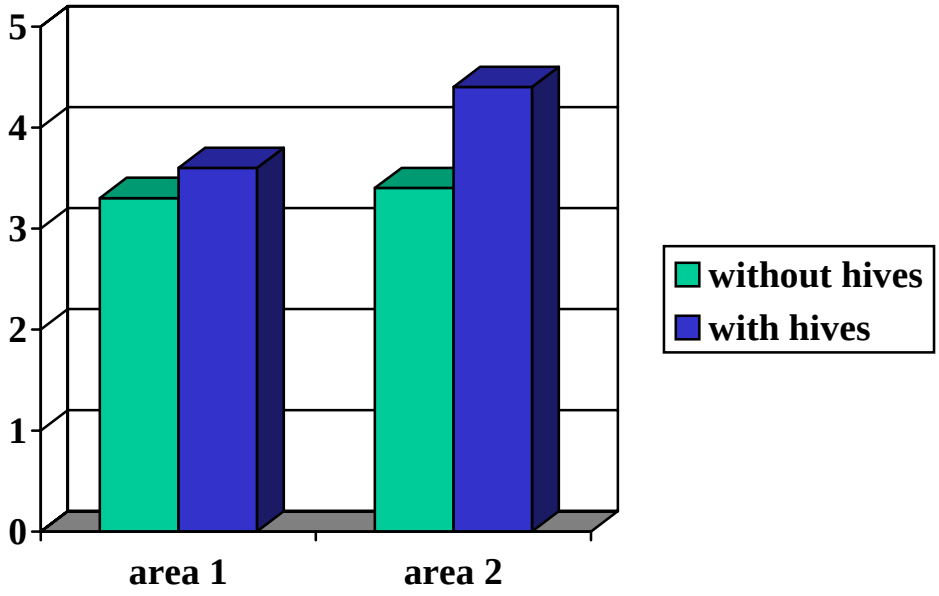

Figure 5 – Percentage of developing fruits of *T. grandiflorum* 20 days after flower anthesis in two plantations located in Manaus region, AM, with and without introduction of hives of *Plebeia sp* and *Aparatrigonna sp.* (Area 1 – 48 individuals, Area 2 – 26 trees).

Partner 5: Universidade Federal do Rio de Janeiro.

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Publications:

1- M.C. Bottino, D.B.Felix, F. Salgueiro, F. Scarano, M. Alves-Ferreira and R. Margis (2006). Assessment of genetic diversity in populations of *Calophyllum brasiliense* Camb using AFLP markers. *Genetics and Molecular Biology* (in preparation).

2- M.I.F. de Souza, F. Salgueiro, D.B.Felix, J.V.M. Bitencourt, F. Scarano, M. Alves-Ferreira and R. Margis (2006). Genetic diversity of *Araucaria angustifolia* [Bert.] O. Kuntze using AFLP markers *Biodiversity and Conservation* (in preparation).

Other outputs:

1- "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 Fabiano Salgueiro, Department of Genetics, Federal University of Rio de Janeiro, July 2005. (pdf file in annex)

2- "Analyses of genetic diversity of *Araucaria angustifolia* [Bert.] O. Kuntze using AFLP markers". Master Degree Thesis of Maria Isabel F. de Souza, Department of Genetics, Federal University of Rio de Janeiro, March 2006.

3- "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.

Results concerning *Araucaria angustifolia* populations using AFLP markers

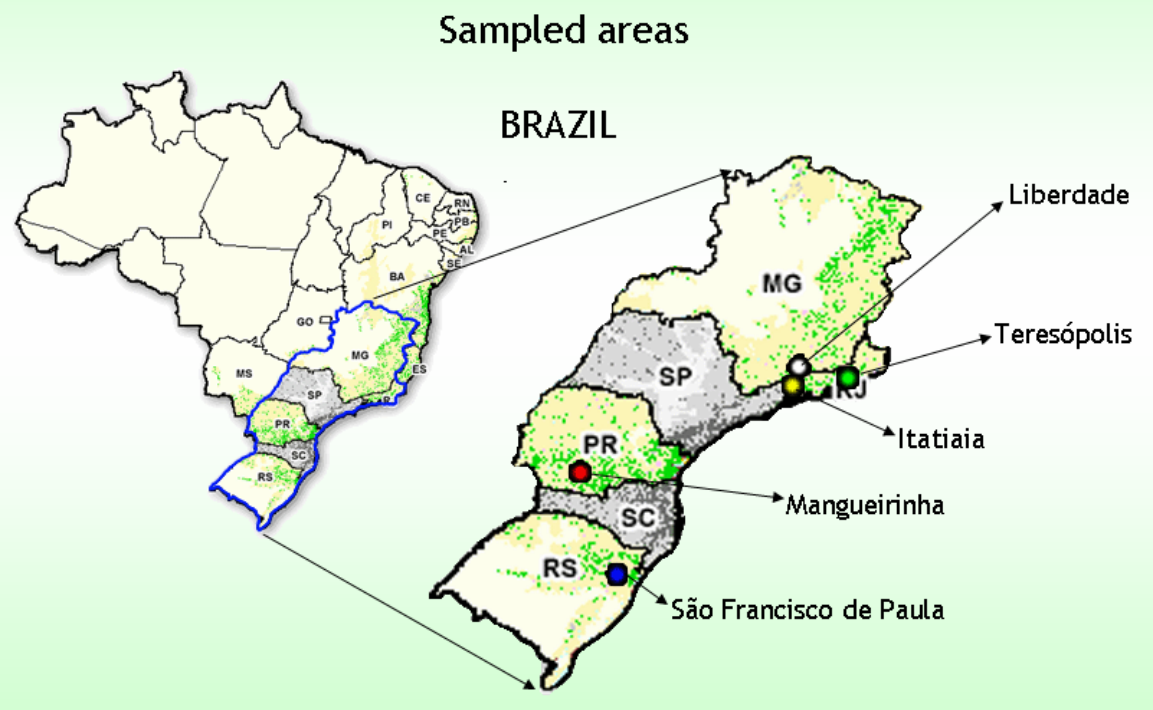


Table. *A. angustifolia*: analyzed populations, acronyms, coordinates and sample number.

Populations	Acronym	Lat	Long	Number of trees	Sample tissue
1) Itatiaia-RJ	ITA	22°24'	44°50'	44	leaves
2) Teresópolis - RJ	TERE	22°24'	42°57'	35	cambium
3) São Francisco de Paula - RS	SFPA	29°30'	50°10'	84	cambium
4) Mangueirinha - PR	MANG	25°56'	52°10'	51	cambium
5) Liberdade - MG	LIB	21°50'	46°51'	29	leaves

AFLP polymorphism levels in *Araucaria* populations

Primer pairs Eco+2/Mse+4	Number of loci	% polymorphic loci				
		ITA (n=44)	TERE (n=35)	SFPA (n=84)	MANG (n=51)	LIB (n=29)
CT/GACC	97	44,3	31,9	46,3	59,7	53,6
CG/GACC	106	60,3	44,3	72,6	80,1	34,9
CC/GACC	106	48,1	42,4	51,8	65,0	52,8
CA/GACC	132	47,7	34,0	53,7	46,2	24,2
CG/GCAC	87	42,2	52,8	81,6	91,9	66,6
CA/GCAC	155	54,2	42,5	87,7	56,1	26,4
TOTAL	683	50,0	44,9	66,6	65,4	40,4

Table. Intra and interpopulation genetic diversity in *A. angustifolia*.

Primer pairs	*He*ITA	*He*TERE	*He*SFPA	*He*MANG	*He*LIB	*Ht*	*Fst*
CT/GACC	0,14	0,11	0,14	0,19	0,17	0,17	0,17 (±0,022)*
CG/GACC	0,18	0,15	0,21	0,24	0,11	0,21	0,14 (±0,015)*
CC/GACC	0,15	0,14	0,16	0,21	0,19	0,18	0,12 (±0,014)*
CA/GACC	0,17	0,12	0,18	0,16	0,08	0,17	0,15 (±0,014)*
CG/GCAC	0,14	0,18	0,29	0,29	0,22	0,26	0,15 (±0,015)*
CA/GCAC	0,19	0,15	0,29	0,18	0,10	0,23	0,15 (±0,010)*
TOTAL	0,17	0,14	0,22	0,21	0,14	0,20	0,15 (±0,006)*

He = expected heterozigosity, *Ht* = total heterozigosity and *standard error after 1.000 replications.

Genetic distance among Araucaria populations using UPGMA

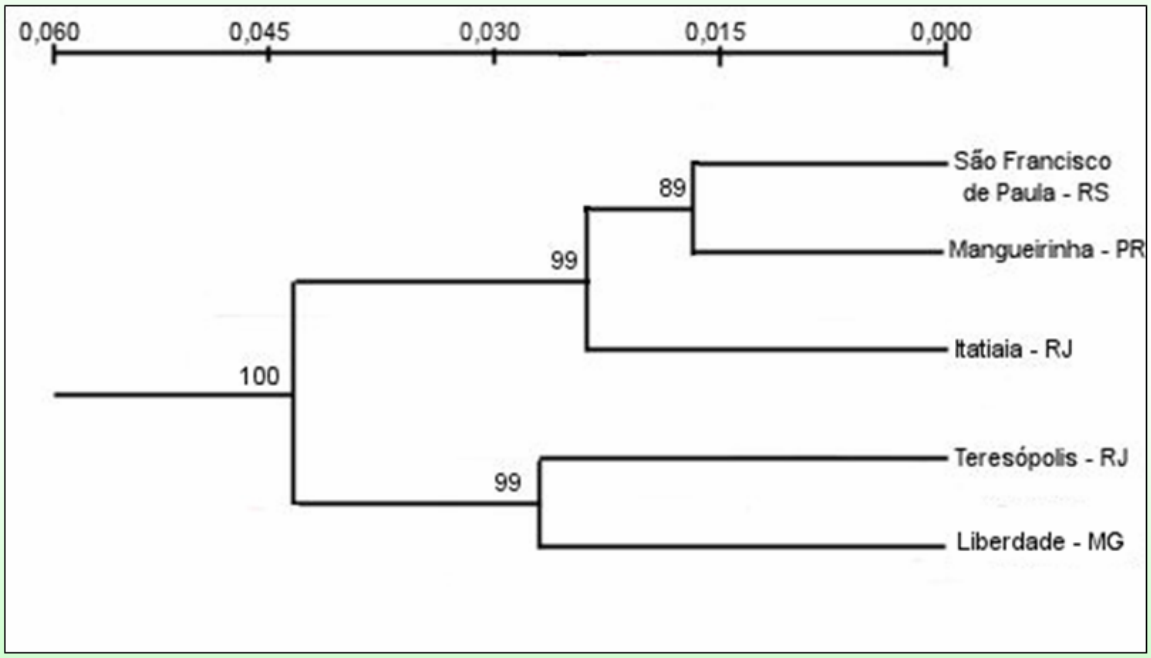
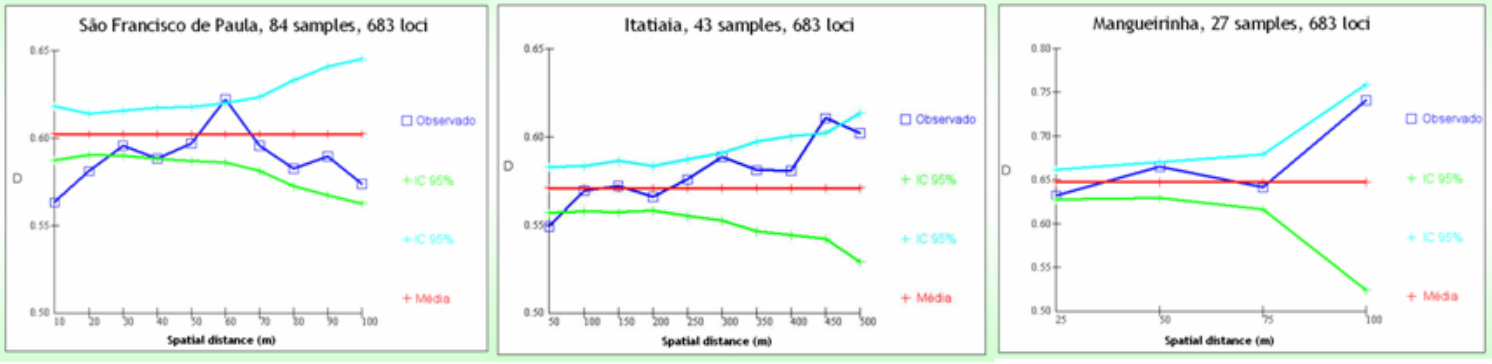


Table. Correlation between genetic distance(Nei, 1978) and spatial distance (in Km) among the five studied populations.

Populações	ITA	TERE	SFPA	MANG	LIB
ITA	--	171	964	866	54
TERE	0,047	--	1092	1021	150
SFPA	0,030	0,055	--	371	1012
MANG	0,027	0,030	0,025	--	911
LIB	0,075	0,033	0,068	0,038	--

Upper right: geographic distance (Km); lower left: genetic distance

Analysis of intrapopulation structure: spatial correlation by SGS



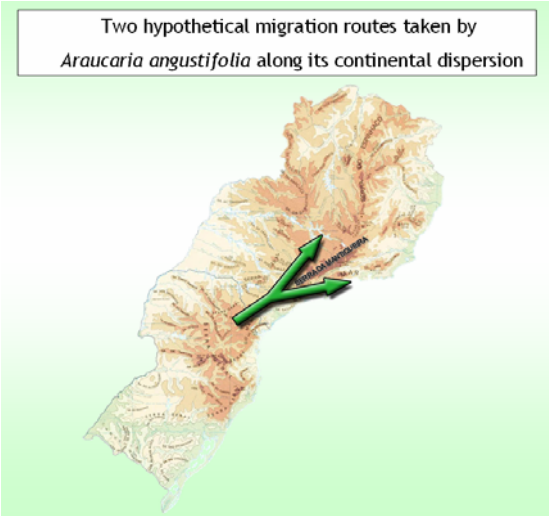


Table. Analysis of Araucaria populations genetic diversity

Source of variation	g.l.	Adition of Squares	Components of variance	% of variation
Among population	4	3827,324	18,86931 Va	19,96
Intrapopulation	238	18012,034	75,68081 Vb	80,04
Total	242	21839,358	94,55013	
Φst = 0,19*				

* Degree of significance(p< 0,001).

Conclusions:

- The genetic diversity observed using the AFLP dominant marker was higher inside each population than among the populations of *A. angustifolia*;
- The species *A. angustifolia*, even if considered in risk of extinction, still presents an intermediate level of genetic diversity;
- Differentiation and genetic structure were found among populations from South and Southeastern of Brazil, except to Itatiaia population, probably due to events occurred along the Quaternary;
- Genetic structure was 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.

Results concerning *Calophyllum brasiliense* populations using AFLP markers

Table: Characteristics of each population studied

Population	Acronym	Number of samples	Material	Latitude	Longitude
Macaé 1	MC1	63	leaves	22º23'S	41º45'
Macaé 2	MC2	15	leaves	22º23'S	41º45'
Oriximiná	PA	50	leaves	1° 40' S	56°
Tiradentes	TR	39	leaves	21º S	44º
Guápiles	CR	40	cambium	9 ° N	83º30'

Table: Genetic diversity in populations of *C. brasiliense*

Primers	*Ht*	*He*					*Fst*
		PA	CR	MC1	MC2	TR	
CC/GCAC	0,23	0,16	0,22	0,18	0,18	0,20	0,32 (±0,02)*
CC/GACC	0,22	0,12	0,26	0,18	0,18	0,22	0,25 (± 0,02)
CT/GCAC	0,26	0,11	0,29	0,23	0,25	0,26	0,28 (± 0,02)
CT/GACC	0,33	0,31	0,44	0,19	0,19	0,27	0,24 (± 0,01)
Para as 4 combinações	0,27	0,19	0,32	0,19	0,20	0,25	0,26 (± 0,01)

* Standard Error

Table: List of primer pairs, number of detected loci and percentage of polymorphicsin each population of *C. brasiliense*

Primers Eco+2/Mse+ 4	Number of *loci*	% de *loci* polymorphic				
		PA (n = 50)	CR (n = 40)	MC1 (n = 63)	MC2 (n = 15)	TR (n = 39)
CC/GCAC	93	48,4	67,7	48,4	47,3	60,2
CC/GACC	117	50,4	97,4	50,4	50,4	71,8
CT/GCAC	135	39,3	90,4	66,7	70,4	77,8
CT/GACC	174	96,6	100	55,1	50,0	93,1
TOTAL	519	62,6	91,1	55,9	54,9	78,4

Table: Estimates of *Fst* using TFPGA

Populations	**PA**	**CR**	**MC 1**	**MC 2**
PA	-----	-----	-----	-----
CR	0,2361 (±0,01)	-----	-----	-----
MC 1	0,2992 (±0,02)	0,2841(±0,01)	-----	-----
MC2	0,3896 (±0,02)	0,2715 (±0,01)	0,0699 (±0,01)	-----
TR	0,1737 (±0,01)	0,2620 (±0,01)	0,6710 (±0,02)	0,6691 (±0,02)

Dendrogram corresponding to the genetic distance (Nei,1978) among the five populations of *C. brasiliense* (UPGMA).

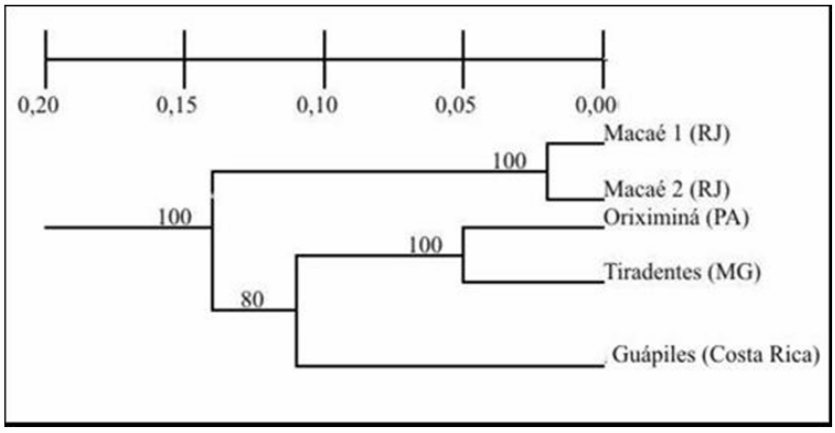


Table:Genetic distance (Nei, 1978) and spatial distances (Km) among *C. brasiliense* populations

Population	PA	CR	MC 1	MC 2	TR
PA	-----	4000	2700	-----	2400
CR	0,1041	-----	5400	-----	5100
MC 1	0,0939	0,1303	-----	3	300
MC 2	0,1641	0,1550	0,0281	-----	-----
TR	0,0583	0,1494	0,1125	0,1755	-----

AMOVA – Genetic diversity of *C. brasiliense* populations

Source of variance	G.L *	Soma dos quadrados	Component of variance	Percentage of variance	P
Among populations	4	5970.927	35.72837	34.7	<0,001
Inside populations	202	13596.232	67.30808	65.3	<0,001
Total	206	19567.159	103.03645		

Conclusions:

The genetic diversity observed using the AFLP dominant marker was higher inside each

- No spatial structure was found for the genetic diversity in the analyzed populations.
- The polymorphism was elevated in all populations
- The intracontinental populations (Oriximina and Tiradentes), despite the large spatial distance presented a higher genetic similarity than to their regional counterparts placed in the oceanic areas.

Partner 6: Institute for Plant Biotechnology for Developing Countries (IPBO).

Scientific team involved: G. Gheysen, N. Colpaert, Tina Kyndt and D. Broucke

Milestones and deliverables:
Deliverable/milestone 8: AFLP analysis of samples: almost finished, delayed due to late delivery of plant material, interpretation of results ongoing – to be finalised in final report.

Research activities during the fourth reporting period:

Workpackage 2: Identifying biological determinants of genetic diversity

At the last annual meeting in Costa Rica it was decided that IPBO should concentrate on the flow cytometry for genome size analysis (as one of the factors that could influence genetic diversity) and should finalize AFLP analysis on the following species: *Goethalsia meiantha, Goupia glabra, Lecythis ampla, Maranthes panamensis, Pseudobombax munguba* and *Tapirira guianensis.*

Table 1 gives a list of the plant species for which material has been grown to obtain suitable material for flow cytometry. Several experiments have been performed already to compare some of these plants with standard plants with a known genome size. To optimize the protocol, several buffers and staining dyes and several types of flow cytometers have been tested. The final measurements will be performed once all the plant material is ready (end of february 2006). Table 2 summarizes the current state (end of january 2006) of the performed AFLP analysis. Unfinished experiments on *Pseudobombax munguba* and *Tapirira guianensis* are still ongoing.

For *Goethalsia meiantha, Goupia glabra, Lecythis ampla, Maranthes panamensis,* the AFLP gels have been scored and analysis has been performed. These results are summarized below. We can conclude that for these species intraspecific diversity is more present within than among populations (low levels of genetic differentiation between populations) although *Goupia glabra* and *Goethalsia meiantha* have a larger differentiation between populations than the other 2 species. *Maranthes panamensis* has low levels of gene diversity.

Table 1: List of species for flow cytometry.

Species	**sent by**	**comments**
Eugenia uniflora	our greenhouse	
Symphonia globulifera	INRA	
Eperua grandiflora	INRA	
Heavia guianensis	our greenhouse	
Pinus oocarpa	CATIE	
Vouacapoua americana	INRA	
Goupia glabra	INRA	
Dicorynia guianensis	INRA	
Jacaranda copaya	INRA	
Simarouba amara	INRA	
Cecropia sciadophylla	INRA	
Chrysophyllum sanguinolentum	INRA	
Carapa guianensis	CATIE	
Callophyllum brasiliense	CATIE	
Virola Michellii	INRA	
Cedrela Odorata	CATIE	
Monorobea	INRA	
Ceiba pentandra	CATIE	no results for analysis due to viscous extracts. Could not be measured
Eperua falcatta	INRA	
Tabebuia heterophylla	INRA	
Pseudobombax ellipticum	our greenhouse	no results for analysis due to viscous extracts. Could not be measured
Swietenia macrophylla	CATIE	
Maranthes	CATIE	no germination
Vochysia	CATIE	no germination
Lecythis ampla	CATIE	no germination
Tetragastris	CATIE	no germination
Goethalsia meiantha	CATIE	no germination
Hyeronima	CATIE	no germination
Schefflera	INRA	no germination
Eschweilera	CATIE	no germination

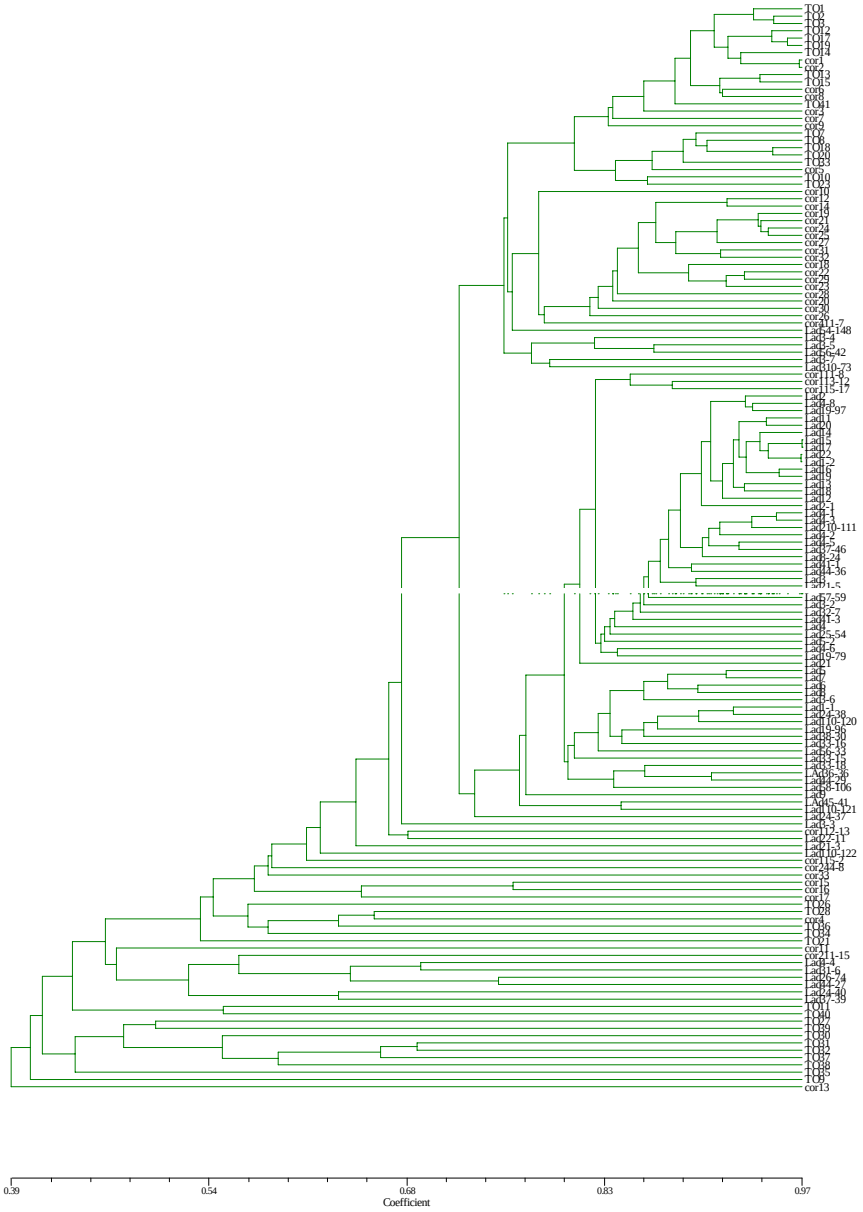
Table 2: complete collections, material missing, activities such as grinding, DNA-extractions, screening for primer combinations, AFLP's and scoring for all species.

	Material received	Material missing	No. of individuals	Grinding	DNA-extraction	Screening for primer combinations	AFLP's	Scoring
Goupia glabra	1x80 INRA 1x80 TAPAJOS 1x40 INPA	Collection complete	205	Done	Done	Done	Done	Done
Maranthes panamensis	CATIE	Collection complete	178	Done	Done	Done	Done	Done
Goethalsia meiantha	CATIE	Collection complete	163	Done	Done	Done	Done	Done
Laetia procera	2x40 CATIE 1x 45 INPA	Collection complete		Samples transferred to partner 1				
Eschweilera costaricensis	CATIE	Collection complete	215	Done	Samples transferred to partner 1			
Lecythis ampla	CATIE	Collection complete	191	Done	Done	Done	Done	Done
Tapirira	2x40 CATIE 1x40 TAPAJOS 1x40 INPA	Collection complete	227	Done	Done	Done	In progress	To do
Schefflera morototoni	1x40 INRA	1x80 INRA lost in mail		Samples transferred to partner 1				
Caryocar glabrum	1x40 INRA 1x80 INRA 30 Paracou	Collection complete		Samples transferred to partner 1				
Ceiba pentandra	2x40 CATIE 1x80 INPA			Samples transferred to partner 1				
Pseudobombax munguba	2x40 INPA 1x45 INPA	Collection complete	129	Done	Done	Done	In progress	In progress

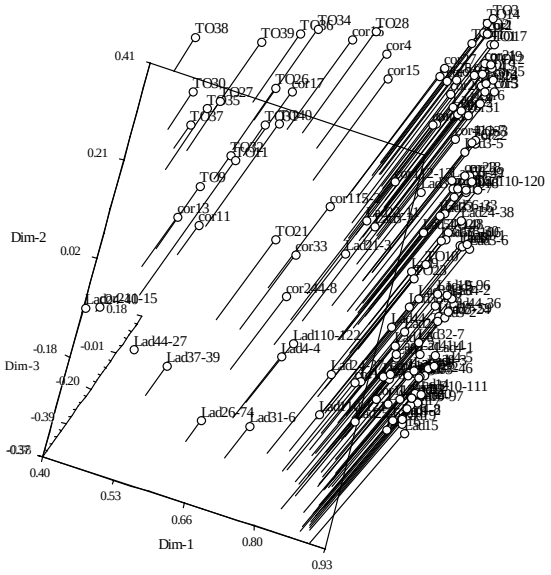
Results AFLP data *Goethalsia meiantha*

164 samples were sent: 26 were deleted because of bad AFLP results or probable misidentification. 148 samples used for further analysis. AFLP analysis was carried out with 5 primer combinations (E+GT/ M+GCGT, E+GT/ M+GCGG, E+GT/ M+GCGA, E+ GA/M+CAGC, E+ GA/ M+CAGT) and obtained 217 scored markers, of which Polymorphic markers: 213 (98.2%). 3 populations were analysed : Tosi: 41 samples sent → 33 used (code: To), Corinto: 41 samples sent → 41 used (code: Cor), Ladrillera3: 82 samples sent → 74 used (cod: Lad)

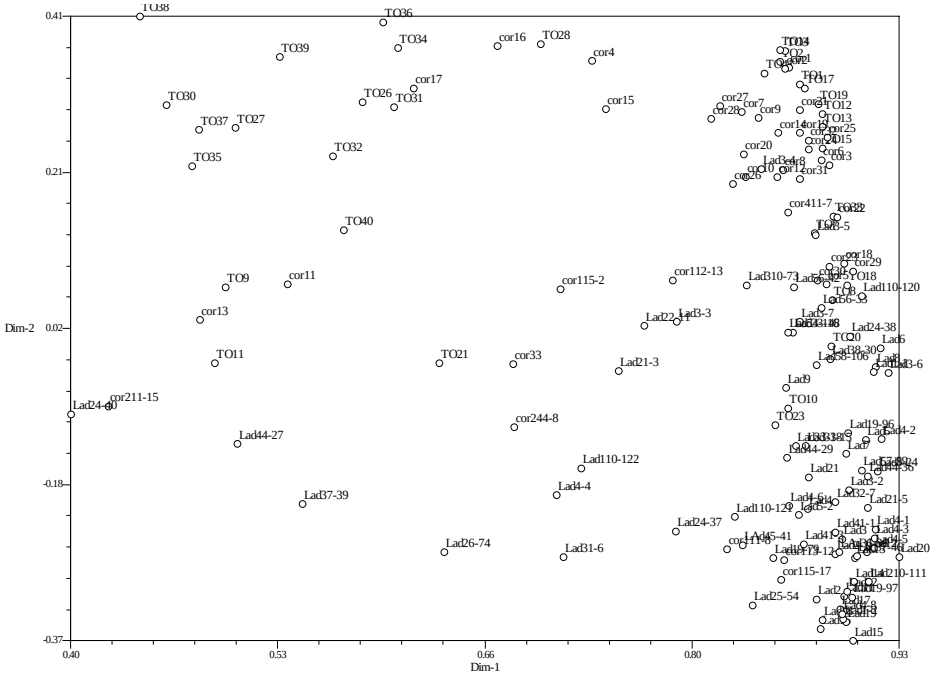
1/ UPGMA based on similarity coefficient of Jaccard
software: NTSYS-pc



2/ Principal coordinate analysis based on similarity coefficient of Jaccard
software: NTSYS-pc
1st eigenvector: 66.8 % of variance explained
2nd eigenvector: 5.31%
3rd eigenvector: 2.97%
PCO plot in three dimensions



PCO plot in first two dimensions



3/ Analysis of molecular variance (AMOVA)
Software: Arlequin

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	2	590.063	5.92579 Va	21.52
Within populations	145	3132.660	21.60455 Vb	78.48
Total	147	3722.723	27.53034	

Significance tests (1023 permutations). Va and FST : P(rand. value > obs. value) = 0.00000, P(rand. value = obs. value) = 0.00000, P(rand. value >= obs. value) = 0.00000+-0.00000

4/ Calculation of gene diversity and population genetic structure
Software: AFLPsurv. Bayesian method with non-uniform prior distribution with a predefined value of Fis (0.0 if H&W assumed) = 0.000. The number of permutations for test on Fst is: 1000. Total number of fragments recorded: 217. Mean number of fragments per individual: 137.7. Total number of segregating fragments : 213 (98.2%)

Table 1: Population data [Lynch & Milligan method]

Population	n	#loc.	#loc_P	PLP	Hj	S.E.(Hj)	Var(Hj)	VarI(Hj)	VarI%	VarL(Hj)	VarL%
1	28	217	216	99.5	0.44631	0.00644	0.000041	0.000009	22.7	0.000032	77.3
2	39	217	215	99.1	0.39185	0.00799	0.000064	0.000015	24	0.000048	76
3	68	217	168	77.4	0.27663	0.01055	0.000111	0.000013	11.8	0.000098	88.2

Table 2: Gene diversity within populations [Lynch & Milligan method]

n	Hw	S.E.(Hw)	Var(Hw)	VarI(Hw)	VarL(Hw)	VarP(Hw)
3	0.3716	0.05002	0.002502	0.000004	0.000020	0.002478

Table 3: Population genetic structure [Lynch & Milligan method]

n	Ht	Hw	Hb	Fst
3	0.4176	0.3716	0.046	0.1082
S.E.		0.050018	0	0.145195
Var		0.002502	0	0.021082

Table 4: Permutation test for genetic differentiation among populations
Resampling statistics based on 1000 random permutations of individuals among populations
Fst = 0.1082 (lower 95% limit -0.0059, upper 95% limit 0.0139)

Pairwise Fst between populations

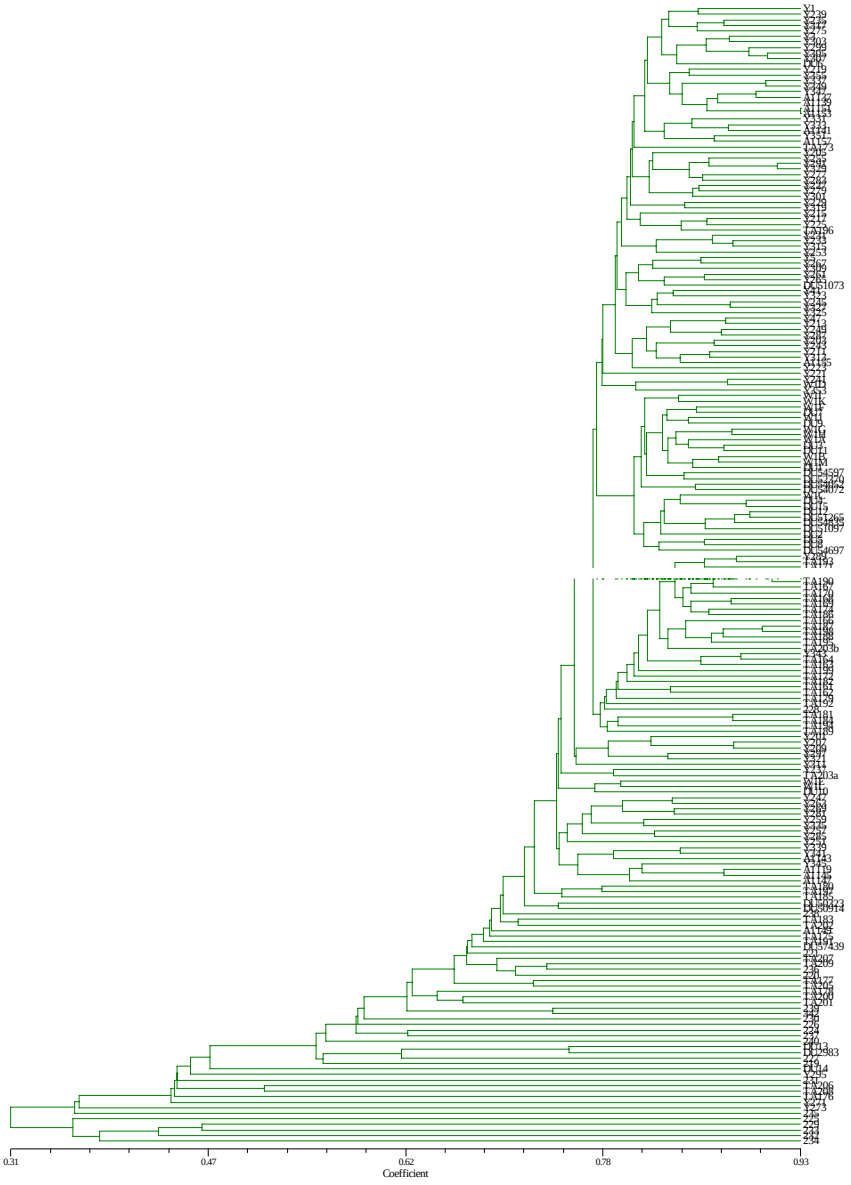
1	0	0.0646	0.1668
2	0.0646	0	0.0989
3	0.1668	0.0986	0

Mantel test: Geographic distance (Euclidean) – 1-r distance (as calculated by AFLPsurv)
Matrix correlation: r = 0.37453 (= normalized Mantel statistic Z)
Approximate Mantel t-test: t = 7.8931, Prob. random Z < obs. Z: p = 1.0000
Out of 100 random permutations: 100 were < Z, 0 were = Z, and 0 > Z
(The observed comparison is not included in these counts.)
The one-tail probability is: p[random Z >= observed Z] = 0.0099

Results AFLP data *Goupia glabra*

207 samples were sent, 1 was deleted because of bad AFLP results or probable misidentification, 206 samples used for further analysis. AFLP analysis was carried out with 6 primer combinations (E+CA/ M+ACTA, E+CA/ M+ACCA, E+TC/ M+CAGC, E+TC/ M+CAGT, E+TC/ M+ACTA, E+CA/ M+ACAC), yielding 201 scored markers, of which polymorphic markers: 195 (97.0%). Three populations were analysed:INRA: 95 samples sent → 94 used (code: Y, and A), Manaus Reserva Ducke: 41 samples sent → 41 used (code: DU & W), Tapajos: 70 samples sent → 70 used (cod: TA or no code)

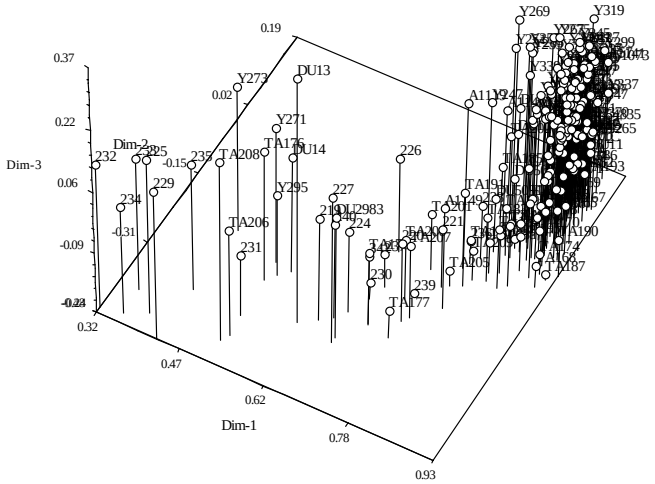
1/ UPGMA based on similarity coefficient of Jaccard
software: NTSYS-pc



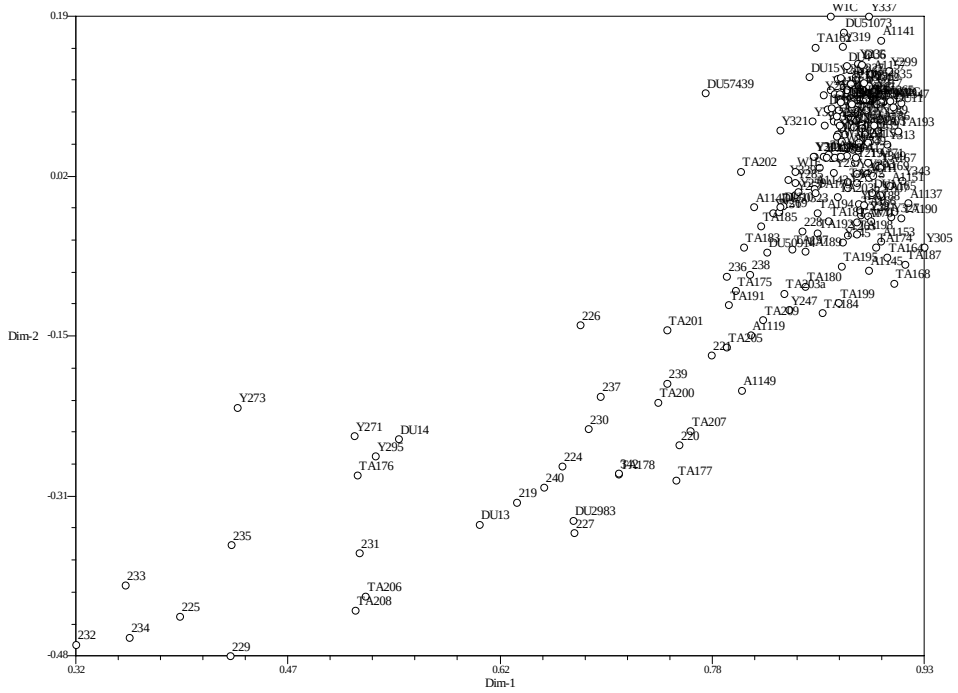
2/ Principal coordinate analysis based on similarity coefficient of Jaccard
software: NTSYS-pc

1st eigenvector: 69.8 % of variance explained
2nd eigenvector: 2.17%
3rd eigenvector: 1.39%

PCO plot in three dimensions



PCO plot in first two dimensions



3/ Analysis of molecular variance (AMOVA)
Software: Arlequin

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	2	413.076	2.89106 Va	13.26
Within populations	202	3820.870	18.91520 Vb	86.74
Total	204	4233.946	21.80626	

Significance tests (1023 permutations). Va and FST : P(rand. value > obs. value) = 0.00000
P(rand. value = obs. value) = 0.00000. P(rand. value >= obs. value) = 0.00000+-0.00000

4/ Calculation of gene diversity and population genetic structure
Software: AFLPsurv
Bayesian method with non-uniform prior distribution with a predefined value of Fis (0.0 if H&W assumed)= 0.000. The number of permutations for test on Fst is: 1000. Total number of fragments recorded: 201. Mean number of fragments per individual: 124.6. Total number of segregating fragments : 195 (97.0%)

Table 1: Population data [Lynch & Milligan method]

Population	n	#loc.	#loc_P	PLP	Hj	S.E.(Hj)	Var(Hj)	VarI(Hj)	VarI%	VarL(Hj)	VarL%
1	87	201	169	84.1	0.28988	0.00996	0.000099	0.000014	13.7	0.000086	86.3
2	37	201	185	92	0.29622	0.01095	0.00012	0.000026	21.6	0.000094	78.4
3	62	200	183	91.5	0.36426	0.01059	0.000112	0.000008	7.1	0.000104	92.9

Table 2: Gene diversity within populations [Lynch & Milligan method]

n	Hw	S.E.(Hw)	Var(Hw)	VarI(Hw)	VarL(Hw)	VarP(Hw)
3	0.3168	0.02381	0.000567	0.000005	0.000032	0.00053

Table 3: Population genetic structure [Lynch & Milligan method]

n	Ht	Hw	Hb	Fst
3	0.3514	0.3168	0.0346	0.0985
S.E.		0.023807	0	0
Var		0.000567	0	-0.00485

Table 4: Permutation test for genetic differentiation among populations
Resampling statistics based on 1000 random permutations of individuals among populations

statistic	Fst
observed	0.0985
lower 95% limit	-0.0043
upper 95% limit	0.0131
lower 99% limit	-0.0051
upper 99% limit	0.0193
P value (low)	1.0000
P value (high)	0.0000

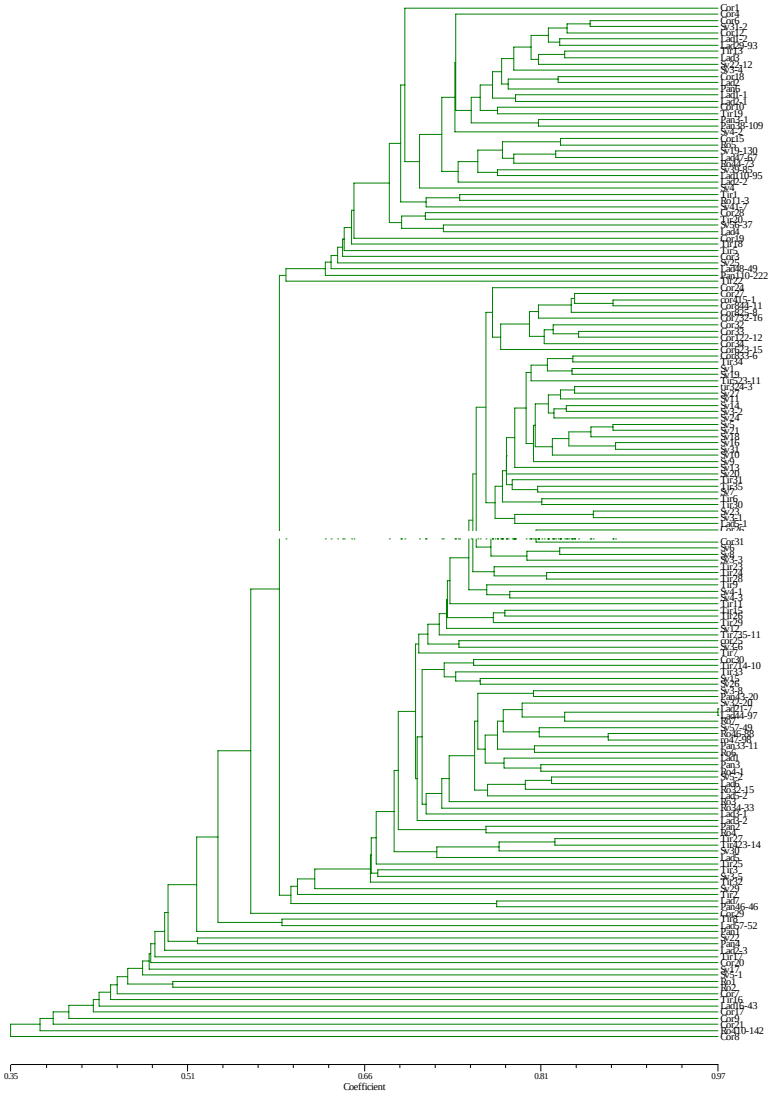
Pairwise Fst between populations

1	0	0.0596	0.1134
2	0.0596	0	0.1161
3	0.1134	0.1161	0

Results AFLP data *Lecythis ampla*

191 samples were sent, 25 were deleted because of bad AFLP results or probable misidentification, 166 samples used for further analysis. AFLP analysis was carried out with 6 primer combinations (E+AG/M+ACTA, E+GA/ M+ACGC, E+TC/ M+ACCA, E+CA/ M+ACCC, E+AT/ M+ACTA, E+GT/ M+ACCA), yielding 242 scored markers, of which Polymorphic markers: 240 (99.2%). Six populations were analysed : Corinto: 41 samples sent → 33 used (code: Cor), Tirimbina: 42 samples sent → 35 used (code: Tir), Sela Verde: 55 samples sent → 48 used (code: Sv), Ladrillera 1: 24 samples sent → 24 used (code: Lad), Paniagua: 12 samples sent → 11 used (code: Pan), Rojomacha: 17 samples sent → 15 used (code: Ro)

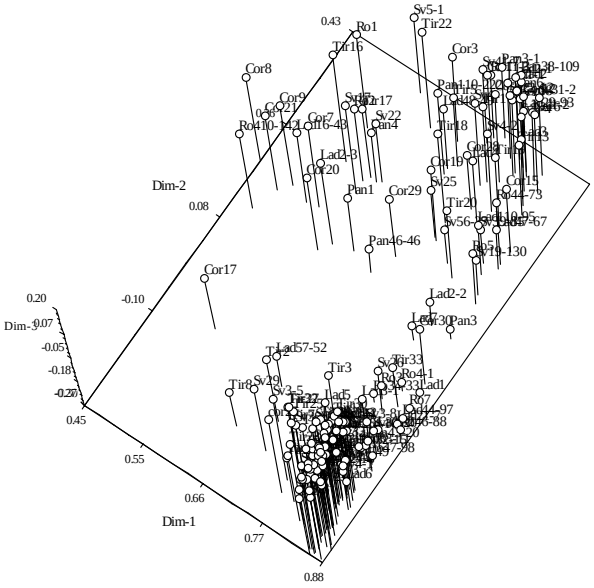
1/ UPGMA based on similarity coefficient of Jaccard
software: NTSYS-pc



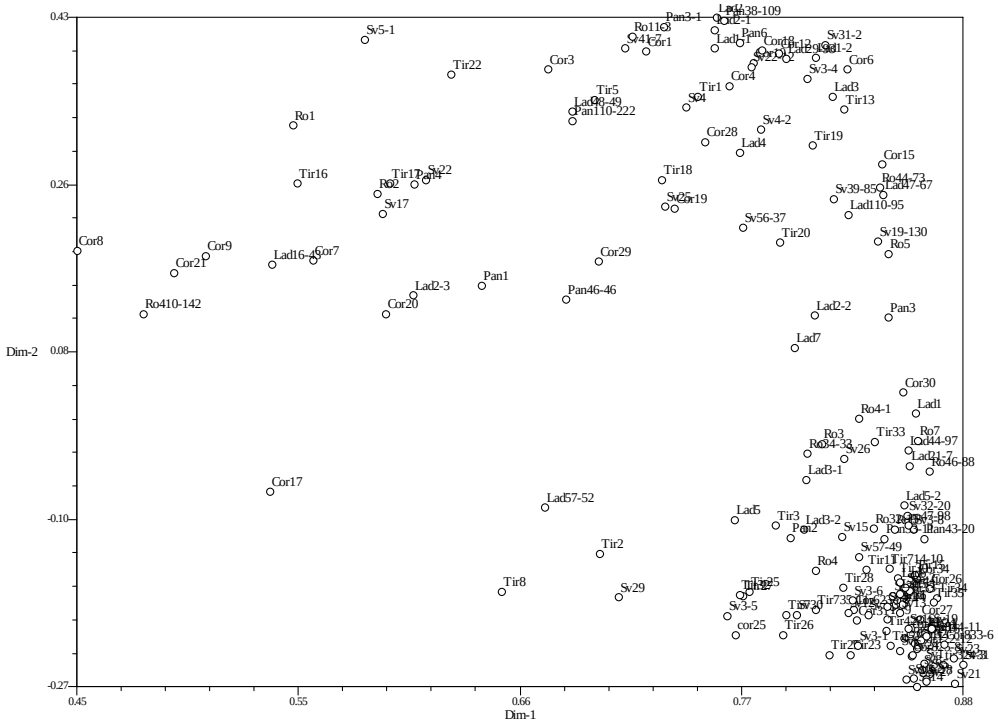
2/ Principal coordinate analysis based on similarity coefficient of Jaccard
software: NTSYS-pc

1st eigenvector: 62.0 % of variance explained
2nd eigenvector: 5.59%
3rd eigenvector: 1.39%

PCO plot in three dimensions



PCO plot in first two dimensions



3/ Analysis of molecular variance (AMOVA)
Software: Arlequin

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	5	408.367	1.85222 Va	5.39
Within populations	160	5206.820	32.54262 Vb	94.61
Total	165	5615.187	34.39485	

4/ Calculation of gene diversity and population genetic structure
Software: AFLPsurv. Bayesian method with non-uniform prior distribution with a predefined value of Fis (0.0 if H&W assumed)= 0.000. The number of permutations for test on Fst is: 1000. Total number of fragments recorded: 242. Mean number of fragments per individual: 145.6. Total number of segregating fragments : 240 (99.2%).

Table 1: Population data [Lynch & Milligan method]

Population	n	#loc.	#loc_P	PLP	Hj	S.E.(Hj)	Var(Hj)	Varl(Hj)	Varl%	VarL(Hj)	VarL%
1	31	242	238	98.3	0.39679	0.0069	0.000048	0.000012	25.7	0.000035	74.3
2	33	242	237	97.9	0.3536	0.00808	0.000065	0.000016	23.8	0.00005	76.2
3	46	242	234	96.7	0.32888	0.00813	0.000066	0.000015	22.1	0.000051	77.9
4	23	242	231	95.5	0.38217	0.00787	0.000062	0.000021	33.4	0.000041	66.6
5	10	242	238	98.3	0.41993	0.00706	0.00005	0.00003	60.6	0.00002	39.4
6	14	242	233	96.3	0.38746	0.00835	0.00007	0.000027	38.9	0.000043	61.1

Table 2: Gene diversity within populations [Lynch & Milligan method]

n	Hw	S.E.(Hw)	Var(Hw)	Varl(Hw)	VarL(Hw)	VarP(Hw)
6	0.3781	0.0132	0.000174	0.000003	0.000007	0.000164

Table 3: Population genetic structure [Lynch & Milligan method]

n	Ht	Hw	Hb	Fst
6	0.3882	0.3781	0.01	0.0258
S.E.		0.013198	0	0.070311
Var		0.000174	0	0.004944

Table 4: Permutation test for genetic differentiation among populations
Resampling statistics based on 1000 random permutations of individuals among populations
Fst = 0.0258, lower 95% limit -0.0064, upper 95% limit 0.0118, lower 99% limit -0.0079, upper 99% limit 0.0176. P value (low) 1.0000, P value (high) 0.0000.

Pairwise Fst between populations

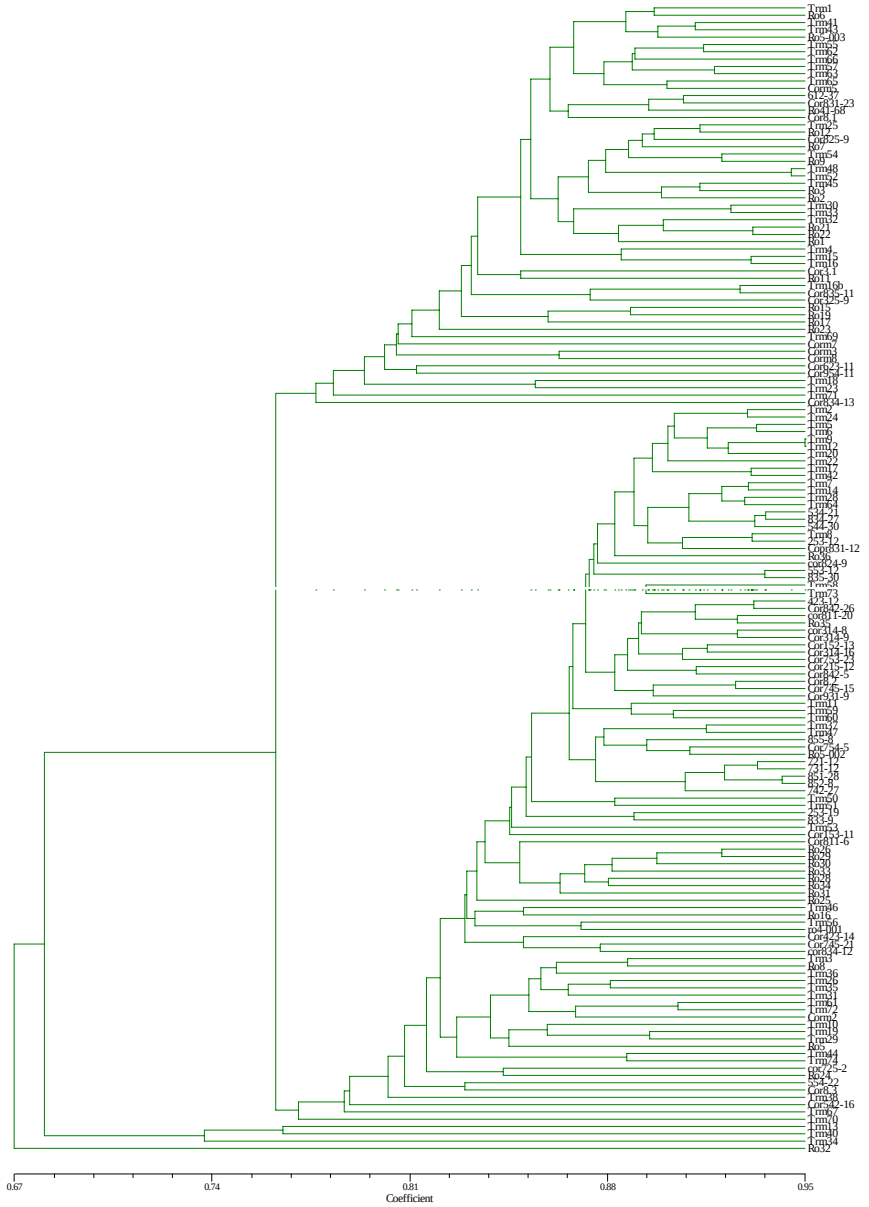
1	0	0.0165	0.0294	0.0305	0.019	0.0215
2	0.0165	0	0.0142	0.0414	0.041	0.036
3	0.0294	0.0142	0	0.037	0.0572	0.0461
4	0.0305	0.0414	0.037	0	0	0.0042
5	0.019	0.041	0.0572	0	0	0
6	0.0215	0.036	0.0461	0.0042	0	0

Results AFLP data *Maranthes panamensis*

178 samples were sent, 21 deleted because of bad AFLP results or probable misidentification

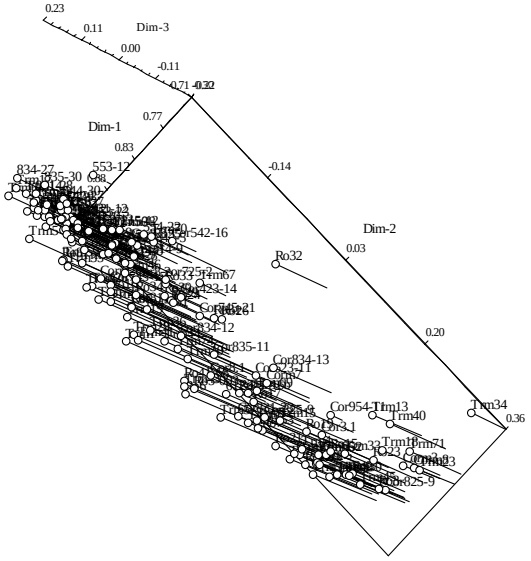
157 samples used for further analysis. AFLP analysis was carried out with with 6 primer combinations (E+GT/M+TACA, E+CA/M+CAGT, E+CA/M+CAGA, E+GT/M+TACG, E+CA/M+CACG, E+CA/M+CAGC), yielding 226 scored markers, of which Polymorphic markers: 166 (73.5%). Three populations were analysed : Tirimbina: 93 samples sent → 87 used (code: Trm or no code), Corinto: 44 samples sent → 37 used (code: Cor), Rojomacha: 43 samples sent → 33 used (code: Ro).

1/ UPGMA based on similarity coefficient of Jaccard
software: NTSYS-pc

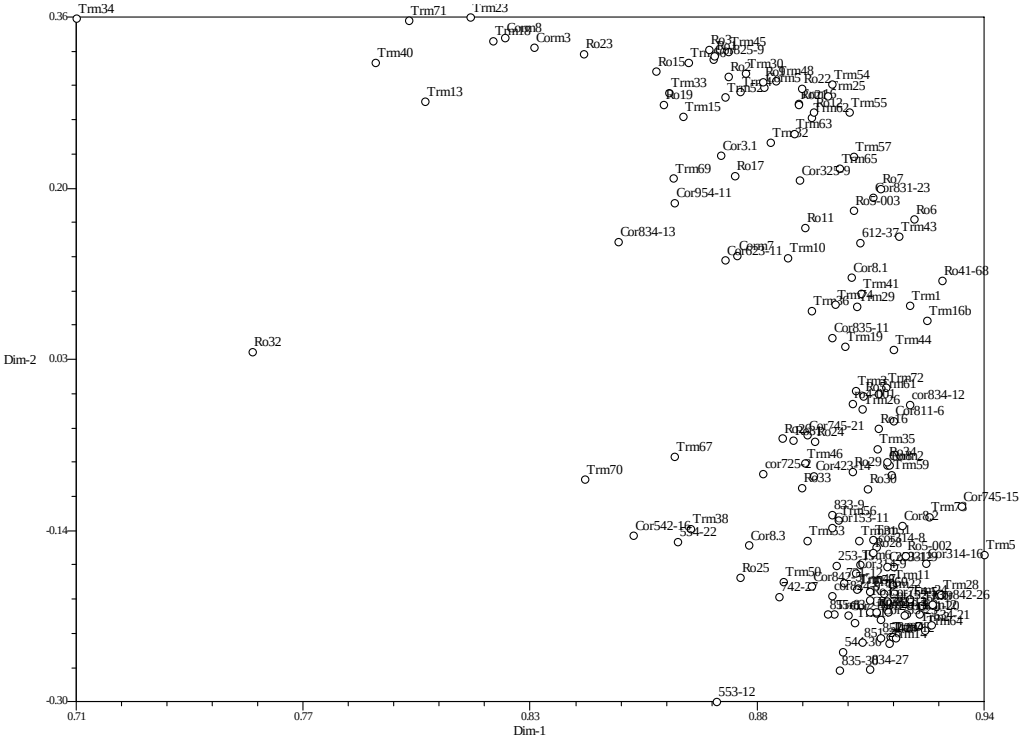


2/ Principal coordinate analysis based on similarity coefficient of Jaccard
software: NTSYS-pc
1st eigenvector: 79.9 % of variance explained
2nd eigenvector: 4.17%
3rd eigenvector: 0.95%

PCO plot in three dimensions



PCO plot in first two dimensions



3/ Analysis of molecular variance (AMOVA)
Software: Arlequin

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	2	128.292	0.98907 Va	5.18
Within populations	154	2785.549	18.08798 Vb	94.82
Total	156	2913.841	19.07705	

4/ Calculation of gene diversity and population genetic structure
Software: AFLPsurv. Bayesian method with non-uniform prior distribution with a predefined value of Fis (0.0 if H&W assumed)= 0.000. The number of permutations for test on Fst is: 1000. Total number of fragments recorded: 226. Mean number of fragments per individual: 167.2. Total number of segregating fragments : 166 (73.5%).

Table 1: Population data [Lynch & Milligan method]

Population	n	#loc.	#loc_P	PLP	Hj	S.E.(Hj)	Var(Hj)	VarI(Hj)	VarI%	VarL(Hj)	VarL%
1	83	226	138	61.1	0.2257	0.01042	0.000108	0.000015	13.4	0.000094	86.6
2	36	226	118	52.2	0.22834	0.01028	0.000106	0.00003	28	0.000076	72
3	32	226	116	51.3	0.2428	0.01102	0.000121	0.000032	26.2	0.00009	73.8

Table 2: Gene diversity within populations [Lynch & Milligan method]

n	Hw	S.E.(Hw)	Var(Hw)	VarI(Hw)	VarL(Hw)	VarP(Hw)
3	0.2323	0.00532	0.000028	0.000008	0.000029	-9E-06

Table 3: Population genetic structure [Lynch & Milligan method]

n	Ht	Hw	Hb	Fst
3	0.2393	0.2323	0.007	0.0293
S.E.		0.005316	0	0.036132
Var		0.000028	0	0.001306

Table 4: Permutation test for genetic differentiation among populations
Resampling statistics based on 1000 random permutations of individuals among populations
Fst = 0.0293. lower 95% limit -0.0085. upper 95% limit 0.0043. lower 99% limit -0.0100, upper 99% limit 0.0082, P value (low) 1.0000, P value (high) 0.0000

Pairwise Fst between populations

1	0	0.0331	0.0293
2	0.0331	0	0.0259
3	0.0293	0.0259	0

Mantel test: Geographic distance (Euclidean) – 1-r distance (as calculated by AFLPsurv)
Matrix correlation: r = 0.02831 (= normalized Mantel statistic Z)
Approximate Mantel t-test: t = 0.6469 (Prob. random Z < obs. Z:p = 0.7412, Out of 100 random permutations: 78 were < Z, 0 were = Z, and 22 > Z, p[random Z >= observed Z] = 0.2277)

3. Project Management and Coordination

Final Coordination Meeting & Dissemination Workshop

The final coordination meeting was held in Turrialba, Costa Rica hosted by CATIE (Partner 2). Partners reported progress against the objectives agreed and targets set at the first coordination meeting and identified areas of difficulty. The following minutes report the meeting program, and objectives and action points as agreed by all partners. The minutes were also made available to all partners through the project website.

FINAL COORDINATION MEETING: MINUTES

GENEO-TROPECO
Monday 3rd – Friday 7TH October 2005
CATIE, Turrialba, Costa Rica.

Present:

CEH:	Andrew Lowe (AL), Stephen Cavers (SC), Katy Walker (KW), Sam Davies (SD)
CATIE:	Carlos Navarro (CN), Bryan Finegan (BF), Gustavo Hernandez (GH), Pablo Madriz (PM)
INRA:	Henri Caron (HC)
INPA:	Rogerio Gribel (RG), Maristerra Lemes (ML)
UFRJ:	apologies (flight problems)
IPBO:	Godelieve Gheysen (GG), Tina Kyndt (TK)

Programme for the 4th GENEOTROPECO meeting.

Monday 3rd:

Summary by partners of the work completed since the last coordination meeting

CEH WP1 Inter-lab AFLP comparison (SC)
WP2 AFLP screening (AL)
WP3 Microsatellite analysis of four *Swietenia macrophylla* populations (KW)
WP3 Sampling and microsatellite analysis of *Vochysia ferruginea* from Costa Rica (SD)

CATIE WP3 Pinus oocarpa (PM, CN)

INRA WP1 Development of SSR loci and softwares (HC)
WP2 AFLP screening (HC)
WP3 Sampling strategy and microsatellite analysis of *Symphonia globulifera* from French Guiana (HC)

INPA WP2 Collections (ML)
WP3 "Gene flow and genetic diversity in a managed population of mahogany (*Swietenia macrophylla*, King) in Eastern Amazonia" (ML)
WP3 "Pollinator management in the self-incompatible fruit tree *Theobroma grandiflorum* (Sterculiaceae)" (RG)

IPBO WP1 Genome size analysis (GG)
WP2 AFLP analyses (TK)

Discussion session on Friday Workshop: obtain main messages and operationalize the results of Geneotropeco and other scientific publications. presentation.

Tuesday 4th

Summary of Work Packages, outputs & plans for finalising

Summary of work completed versus deliverables promised, and actual and potential outputs including details of publications. Plans for completing work programme. WP5 will be dealt with first to allow time for indentification of data requirements and their collection during the meeting.

WP5 Designing management strategies to maximize diversity CATIE

Deliverables:

Leaflets produced in local languages to explain practical outputs of the project, management strategies and the importance of managing forest genetic resources, Month 42

Papers drafted for publication in high quality, refereed, international scientific journals, Month 48

Hold workshop for Forestry workers in Central America. If accompanying measure application is successful organise international conference to cover the whole of Latin America, Month 48

Outputs:

Genetic diversity game for schools and high schools.
Species management plans – form ? (journal papers, booklet ?)
Leaflets
Workshop

WP1 Development of molecular techniques IPBO / CEH

Deliverables:

New SSR primers will be made available to partners through website
Developed molecular methods and software will be available to all partners through website

Outputs:

Cambium sampling paper
Inter-lab comparison

WP2 Identifying biological determinants of genetic diversity CEH

Deliverables:

Full list of species and locations of forests to be analysed to be placed on website
Meta-analysis: Analysis of results will be made available in a useful form for comparative regression analysis and processing in WP 4 and 5

Outputs:
AFLP Meta-analysis
Single-species papers ?

WP3 Effect of human-mediated processes on genetic diversity **INRA**

3.1 Impact of fragmentation: Mahogany

3.2 Logging and sustainability of management: *Araucaria, Symphonia*

3.3 Secondary regeneration: *Vochysia*

3.4 Domestication: *Theobroma*

Deliverables:

Location and description of species and forests analysed to be placed on website,

Range of growth and genetic diversity and dynamic parameters will be measured for undisturbed and managed systems for each case study. Data will be in format ready for WP4 and WP5

Outputs:
Papers on: *Swietenia* (Belize) *Swietenia* (Brazil) *Araucaria, Symphonia, Vochysia, Theobroma,* General paper ?

Wednesday 5th. Field trip to Sarapiquí.

Thursday 6th:
WP4 Simulation modelling of population genetic dynamics **INRA**

Deliverables:

ECO-GENE model adapted for use with case studies of WP3

Simulation models for exploited ecosystems and single species produced. For each case management strategies that maintain the genetic resource base by maximising gene flow will be identified

Outputs:
Individual species papers incorporating modelling ?
Or synthesise with WP 3 papers ?

Friday 7th: Workshop INBIO

Status of WPs against deliverables and agreed actions for final project period.

The Project progress was reviewed against the deliverables set out in the Technical Annex and plans for finalising the delivery of outputs were made. In all WPs, the project is on course to achieve delivery within the lifetime of the project.

Work Package 1

Deliverables

New SSR primers will be made available to partners through website

Developed molecular methods and software will be available to all partners through website

Existing Outputs

Colpaert, N., S. Cavers, E. Bandou, H. Caron, G. Gheysen and A. Lowe, 2005: Sampling tissue for DNA analysis of trees: trunk cambium as an alternative to canopy leaves. Silvae Genetica accepted, In Press.

The Inter-lab comparison of AFLP analysis has been completed incorporating contributions from CEH, IPBO and INRA. The principal conclusion was that diversity level comparisons are valid given that certain restrictions are placed on scoring and analysis procedures (score all bands, acore 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. Although in each case (IPBO, CEH, INRA) differentiation within the sample set was detected, the precision of AFLP fingerprinting was not sufficient to resolve common sets of samples under principal cooordinates analysis: however, this is most likely a statistical effect due to the sensitivity of PCO and the datasets will be re-examined using pairwise 'relatedness'.

Agreed Actions

- Finalise comparative analysis, using pairwise relatedness.
- Prepare comparison as a publication by December 2005.

Work Package 2

Deliverables

Full list of species and locations of forests to be analysed to be placed on website

Meta-analysis: Analysis of results will be made available in a useful form for comparative regression analysis and processing in WP 4 and 5

Existing Outputs

AFLP analyses have now been fully or partially completed for approximately 30 species with a further 8 expected to be completed by the end of the project (for which samples

have been collected but analysis has not yet been undertaken). To ensure the maximum number of datasets are obtained, the remaining eight collected species were re-assigned to partners in the best position to conduct AFLP analysis in time for the project close. SC will organise re-distribution of samples as soon as possible.

List of re-assignments:

Cecropia sciadophylla	INRA
Carapa guianensis	CEH
Schefflera morototonii	UFRJ (pending RM agreement)
Caryocar glabrum	UFRJ (pending RM agreement)
Ceiba pentandra	UQ
Monorobea coccinea	INRA
Eschweilera costaricensis	UQ
Laetia procera	CEH

Agreed Actions

In anticipation of the completion of AFLP analysis, a strategy for publication was devised, to ensure maximum exposure for project results but also allow each partner to retain ownership of part of the analysis of the dataset. Rather than proceeding with multiple publications featuring datasets from individual species, it was decided to target a single, high-profile publication with a complete overview, plus a series of additional papers focussing in detail on different aspects of the data. Each of the subsidiary papers would be lead by one partner in collaboration with one other (each partner therefore has a lead paper plus a collaborative paper), with authorship apportioned appropriately. The series would be prepared together and targeted to appear as a focus issue in a high-profile journal, preferebaly Molecular Ecology or Heredity. The agreed themes and responsibilities (pending agreement of absentees) were:

- Meta-analysis overview: A. Lowe to lead
- Fine scale genetic structure: CEH, INPA
- Differentiation at local scale (gene flow): INPA, CATIE
- Differentiation across wider range (biogeography): UFRJ, INRA (confirm Rogerio Margis)
- Diversity within populations - Measure of genetic and nucleotide diversity: INRA, CEH
- Diversity within species - Genome analysis: IPBO, UFRJ (confirm Rogerio Margis)
- Phylogenetic patterns of diversity: CATIE, IPBO

Work Package 3

Deliverables

Location and description of species and forests analysed to be placed on website,

Range of growth and genetic diversity and dynamic parameters will be measured for undisturbed and managed systems for each case study. Data will be in format ready for WP4 and WP5

Existing Outputs
High-quality datasets have been completed for all of the case study species and analysis is underway. Data from each species (apart from *Theobroma grandiflorum*) will be prepared for integration with ECOGENE.

Agreed Actions
It was agreed that each case study would prepare individual publications on population genetic analysis. Paper list:

- *Vochysia ferruginea*
- *Araucaria araucana*
- *Swietenia macrophylla* (Belize)
- *Swietenia macrophylla* (Brazil)
- *Theobroma grandiflorum*
- *Symphonia globulifera*

Work Package 4
Deliverables

ECO-GENE model adapted for use with case studies of WP3

Simulation models for exploited ecosystems and single species produced. For each case management strategies that maintain the genetic resource base by maximising gene flow will be identified

Existing Outputs
ECOGENE is ready to be used for simulation using datasets from WP3. Each partner has a copy of the software and has received initial training in its use. Datasets from WP3 are now being prepared as input for the model.

Agreed Actions
ECOGENE simulation studies will either be incorporated with the case study paper (WP3) or published as an individual study. In the case of *Swietenia macrophylla*, CEH and INPA agreed to publish individual papers on population genetic analysis but cooperate to produce a joint ECOGENE-based study. Bernd Degen will be approached to assess the viability of producing a single synthesis covering the various ECOGENE analyses. Paper list:

- Synthesis paper covering ECOGENE simulations (confirm with BD)
- *Vochysia ferruginea*
- *Araucaria araucana*

- *Swietenia macrophylla* (joint CEH / INPA covering both Belize and Brazil)
- *Symphonia globulifera*

Work Package 5
Deliverables

Leaflets produced in local languages to explain practical outputs of the project, management strategies and the importance of managing forest genetic resources, Month 42

Papers drafted for publication in high quality, refereed, international scientific journals, Month 48

Hold workshop for Forestry workers in Central America. If accompanying measure application is successful organise international conference to cover the whole of Latin America, Month 48

Existing Outputs
Following the deliverable targets:

Leaflets:It was agreed that results derived from WP3 case studies would be made available to CATIE as they are obtained for development of leaflets identifying important information for management of each species. The template for the leaflet is in place and CATIE are just awaiting results but these must be finalised.

Papers: a complete publication strategy was identified for the closing stages of the project with the individual papers listed and target journals identified. Some papers are already in press and others will be ready for submission towards the end of 2005.

Workshop: The final day of the 2005 meeting was held as a workshop presenting results and applications derived from the GENEO-TROPECO project. The workshop was held at INBio in San Jose, Costa Rica and included representatives from the Costa Rican government's Biodiversity Commission. The Workshop schedule and delegate list are attached below. A feedback form was circulated during the meeting requesting opinions and formats and key data for dissemination pruposes. The results were collated during the meeting and a final session was held at the end of the day to discuss the key points. It was agreed that presentations from the meeting would be collated on the project website.

Additional: a Genetic Diversity board game for schools and high schools, that received input from project partners during the meeting week, is being devised by CATIE (see attached prototype). Finally, a Specific Support Action bid has been submitted to the EC to take the dissemination activities further.

Agreed Actions
- Data from case studies / ECOGENE simulations to be finalised and circulated to CATIE

4. EXPLOITATION AND DISSEMINATION 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 questionnnaire 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.

Accompanying measures application to FP6 INCO SSA call

As an agreed action of the project, to promote dissemination and communication of project results, CEH undertook to prepare and submit 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, an outcome is awaited.

Workshop Programme:

Presentación de estudios sobre diversidad genética de especies amenazadas y nativas, de interés económico.

Viernes 7 de octubre. INBio

Actividades

8:00 am	Apertura y reseña del proyecto. Presentado por Andrew Lowe
8:20 am.	Rescate y conservación de especies forestales nativas, Costa Rica. Rescue and conservation of native tree species in Costa Rica ". Presentado por *M.Sc. Ileana Moreira G. y M.Sc.Elizabeth Arnáez S. Escuela de Biología. ITCR*.
8:45 am	Flujo génico y diversidad genética en una población manejada de caoba (*Swietenia macrophylla*, King) en el este amazónico. Gene flow and genetic diversity in a managed population of mahogany (*Swietenia macrophylla*, King) in Eastern Amazonia" por *Maristerra R. Lemes - INPA, Brazil*.
9:10 am	Manejo de polinizadores en el árbol frutal autoincompatible *Theobroma grandiflorum* (Sterculiaceae)" "Pollinator management in the self-incompatible fruit tree *Theobroma grandiflorum* (Sterculiaceae)" por *Rogério Gribel - INPA, Brazil*
9:35 am	Receso
9:50 am	El uso de marcadores moleculares y cuantitativos en estudios de diversidad genética: Experiencia con *Cedrela odorata*, *Swietenia macrophylla* and *Lonchocarpus costaricensis* "The use of molecular and quantitative markers in genetic diversity studies. Experiences with *Cedrela odorata*, *Swietenia macrophylla* and *Lonchocarpus costaricensis" por Carlos Navarro*
10:15 am	Presentación general de Geneotropeco "Geneotropeco general presentation" por Andrew Lowe.
10:40 am	Flujo génico en *Symphonia globulifera* "Gene flow in *Symphonia globulifera"* por *Henri Caron. INRA, France.*
11:05 am	Análisis genético de poblaciones de *Goupia glabra, Maranthes panamensis, Goethalsia meiantha* and *Lecythis ampla* utilizando AFLP. "AFLP-based population genetic analyses of *Goupia glabra, Maranthes panamensis, Goethalsia meiantha* and *Lecythis ampla"* por Tina Kyndt
11:30 am	Almuerzo
	Macroanálisis de AFLP: estudio de casos. "AFLP meta analysis: *Case studies"*. *por* Andrew Lowe, Godelieve Gheysen y Rogerio Margis.
12:30 pm	- *Swietenia macrophylla*. por Stephen Cavers.
12:50 pm	- *Vochysia ferruginea*. por Samantha Davies.
2:05 pm	Taller
3:30 pm	Clausura.

DELEGATE LISTING: INBio Workshop, San Jose.

GENEO-TROPECO: Maristerra Lemes, Rogerio Gribel, Andrew Lowe, Tina Kyndt, Stephen Cavers, Henri Caron, Godelieve Gheysen, Samantha Davies, Katy Walker, Bryan Finegan, Carlos Navarro

Elizabet Arnaes. Biology Dept. Instituto Tecnológico de Costa Rica

Iliana Moreira. Biology Dept. Instituto Tecnológico de Costa Rica

Carolina Cascante - CATIE

1. Msc. **Quirico Jiménez Madrigal**. Parliament. (Diputado) Apartado 39-1013. Asamblea Legislativa, San José.

2. **José Joaquín Campos**. Deputy Director CATIE.

3 . **Marta Liliana Jiménez**. Director CONAGEBIO. Comisión Nacional para la Gestión de la Biodiversidad (CONAGEBIO).

María Isabel Chavaría. Ministry of the Environment (MINAE)

Gilbert Canet – Sistema Nacional de Áreas de Conservación SINAC

Carmen Roldan Chacón – Sistema Nacional de Áreas de Conservación (SINAC)

Rafael Bolaños – CCT

Francisco Ramírez – MINAE

Eugenio Corea – UNICEFOR

Dagoberto Arias - Instituto Tecnológico de Costa Rica

Francisco Mesén. Consultant CATIE

Fabiana Rojas. ITCR.

Carlos Sandí – EARTH

Ana Cristina Tamayo D.Universidad EARTH - Costa Rica

Glenn Rivara – ACG (Conservation Area Guanacaste)

Milena Gutiérrez Leitón – ACG

Publications in refereed journals

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

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

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:

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

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

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

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

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

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

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.

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.

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

Navarro C, Stephen Cavers, Ari Pappinen, Peter Tigerstedt, Juha Merilä, Andrew Lowe (2005) Ecotypic differentiation and variability at both quantitative traits and neutral markers in Mesoamerican *Cedrela odorata*. *Silvae Genetica* 54(6): 281-292.

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.

Thesis

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.

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.

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.

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.

Poster presentations

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.

Submitted

Lemes, M. R., Reis, V. M., Martiniano, T. M., Faria, C. P. & Gribel, R. (2006) Cross-amplification and characterization of microsatellite loci for three species of *Theobroma* from the Brazilian Amazon. *Molecular Ecology Notes.*

Lowe AJ, Cavers S, Davies S, Copestake- Goodall W, Navarro C (Submitted) Fine-scale genetic structure within Costa Rican populations of *Vochysia ferruginea* Mart., at different colonisation stages. *Molecular Ecology*.

Margis R , Felix D, Caldas JF, Salgueiro F, Margis-Pinheiro M (Submitted) The *Eugenia uniflora* population diversity increases along the Brazilian coastal Atlantic rain forest. *Molecular Ecology*.

In prep.

Lowe AJ, Cavers S, Margis R, Caron H, Kremer A, Navarro C, Petit R (In prep) Chloroplast genome diversity and differentiation within neotropical trees.

M.C. Bottino, D.B.Felix, F. Salgueiro, F. Scarano, M. Alves-Ferreira and R. Margis (2006). Assessment of genetic diversity in populations of *Calophyllum brasiliense* Camb using AFLP markers. *Genetics and Molecular Biology* (in preparation).

M.I.F. de Souza, F. Salgueiro, D.B.Felix, J.V.M. Bitencourt, F. Scarano, M. Alves-Ferreira and R. Margis (2006). Genetic diversity of *Araucaria angustifolia* [Bert.] O. Kuntze using AFLP markers *Biodiversity and Conservation* (in preparation).

Summary of exploitation and dissemination activities (4th period)

1.Dissemination activities		Totals
Number of communications in conferences (published)		0
Number of communications in other media (internet, video etc)		website
Number of publications in refereed journals (published)		14
Number of articles/books (published)		0
Number of other publications		0
2.Training		
Number of PhDs		1
Number of MScs		7
Number of visiting scientists		8
Number of exchanges of scientists(>3 months)		0
3.Achieved results		
Number of patent applications		0
Number of patents granted		0
Number of new prototypes/products developed		0
Number of new tests/methods developed		0
Number of new software/codes developed		0
Number of production processes		0
4.Industrial aspects		
Industrial contacts		no
Financial contribution by industry		none
Industrial partners	large	none
	small	none

5. ETHICAL ASPECTS AND SAFETY PROVISIONS

There are no special ethical considerations associated with this project and all activities adhere to national health and safety guidelines.