SEEDSOURCE

'Developing best practice for seed sourcing of planted and natural regeneration in the neotropics'

SIXTH FRAMEWORK PROGRAMME
Call identifier: FP6-2002-INCO-DEV-1

PRIORITY A.2.1.
Managing humid and semi-humid ecosystems

SPECIFIC TARGETED RESEARCH PROJECT

Second Reporting Period
Periodic Activity Report

01/05/06 – 30/04/2007

Proposal/Contract no.:	003708
Project coordinator:	Stephen Cavers
Coordinating Institution:	CEH
Project start date:	01/05/2006
Duration:	4 years

Executive Summary - project description

The aim of SEEDSOURCE is to provide best practice policies for sourcing tree germplasm for use within a range of degraded landscapes to ensure the use of best adapted material, that maximises production, without eroding genetic and ecosystem diversity and long term adaptive potential.

Supply of appropriate germplasm is a critical factor for reforestation programmes. Use of inappropriately sourced material (due to lack of knowledge or availability) can lead to ecological and/or commercial failures, as trees die or fail to meet the particular objectives of a reforestation or restoration project. With recent interest in the conservation and restoration of native habitat, there is a growing trend towards planting trees with wider objectives than simply maximising production. Germplasm selection for production forestry is generally based on growth, form and quality criteria. In contrast, planting for ecological restoration requires an emphasis on different traits such as reproductive vigour, seed and seedling survival, and ability to compete with other species. Considerations of sustainability, ecological restoration and conservation of biodiversity also lead to promotion of 'local' seed sources for planting. However, the concept of 'local' is a relative one, depending on the scale over which adaptation occurs, and definition of 'local' seed collection zones is often arbitrary in the absence of adequate information about population delimitation.

The contrasting interests of production and ecological restoration mirror underlying scientific issues. The source of planting stock needs to be considered at both the population level and the individual level *i.e. which populations and which trees within that population?* The key scientific question is how gene flow and selection interact to influence population delimitation and reproductive fitness. The relationships between genetic diversity, habitat heterogeneity and the scale of adaptation in trees are complex, involving a variety of factors. Gene flow may counteract even fairly strong selection, preventing formation of locally adapted populations, although very strong environmental variation (hence selection pressure) may produce adaptive differences over short distances, despite continued high levels of gene flow. Since the genetic composition of seed is affected by patterns of pollen flow, the extent of localised adaptation and fitness may vary with pollen flow from differing environments. Human disturbance can thus have considerable and far-reaching genetic consequences through its effects on patterns of pollen flow. Furthermore, deforestation and other environmental changes may mean that previously well-adapted local populations become less so. In this context, the dangers of using inbred germplasm in tree species are clear. Trees generally carry heavy genetic loads (deleterious recessive alleles, *e.g.* Williams & Savolainen 1996), such that inbreeding, and in particular selfing, may lead to reduced fertility, slower growth in progeny and increased susceptibility to pests or diseases (*e.g.* Park & Fowler 1982, Sim 1984, Griffin 1991). Furthermore, deforestation and other environmental changes may reduce the adaptive potential of local populations, a dangerous scenario when coupled with altered patterns of pollen flow and reductions in genetic diversity.

A fuller understanding of population structure, delimitation and adaptation can provide a rational basis for selection of locally adapted and genetically diverse seed for planting, as well as for the management of natural regeneration. Different types of genetic studies provide input on the spatial dynamics of different genetic processes, and a full understanding requires the integration of a range of descriptive and experimental approaches. Neutral molecular markers are primarily influenced by gene flow and mating system. In contrast, adaptive differences are determined by differences in selection regime. There may be large genetic differences between

populations across the geographic range at the molecular level (*e.g.* because of genetic drift), but few significant adaptive differences (e.g. *Cedrela odorata*, Cavers *et al.* 2003b; Navarro *et al.* 2002). In other cases, field trials may reveal adaptive differences among populations where there is an absence of neutral marker differentiation (e.g. *Pinus sylvestris,* Garcia-Gil *et al.* 2003; *Araucaria araucana* Bekessy *et al.* 2003). At the regional scale, provenance trials can demonstrate the suitability of transplanted material but neutral molecular loci can also be effective markers of source areas for transplantation purposes and can provide important information about the evolutionary history of source populations.

Overall objective:

SEEDSOURCE will apply appropriate molecular and quantitative genetic tools to study both aspects of scale (populations and trees within them) in the sourcing of germplasm for varied use of widespread tree species of high socioeconomic importance in the neotropics.

Specific objectives.

1. The integration of climatic, topographic and substrate information with genetic differentiation and diversity estimates from non-coding and potentially coding genetic markers and adaptive performance from growth trials will produce appropriate translocation guidelines and seed source maps for each of the study species of the SEEDSOURCE project.

2. Appropriate application of hypervariable molecular markers will assess individual mating parameters and will be combined with quantitative assessment of the performance of seed sourced from a variety of forest landscapes (from continuous forest to remnant trees in farm land) and pollination conditions. Recommendations will be produced on the origin of germplasm to select for future tree establishment.

3. A metapopulation model will be developed to test the sensitivity of defined seed source areas/restrictions to translocations.

4. The ECOGENE model will be developed and used as tool to study genetic impacts within agroecosystems landscapes and relevant to the local environment of individual trees.

5. A combined field derived data and modelling approach will facilitate the development of informed management strategies for planting and natural regeneration for each study species.

6. Fifty of the most socio-economically important tree species within each of the Central American and South American tropics will be classified for their genetic and flowering/reproductive syndromes, and the most appropriate seed sourcing strategies identified for each under a variety of management scenarios. Dissemination of this information in a practical and relevant format will target relevant forestry and agroforestry stakeholders across tropical Latin America (e.g. policy makers, seed banks, forest management certifiers and educators).

Executive Summary - progress

Coordinating activities
The second coordination meeting was held 28/08-02/09/06 at CNR in Florence, Italy, hosted by partner 4 - Dr. GG Vendramin. All partners attended and a full progress report was made including detailed presentations by each partner to the assembled consortium. The opportunity was used to restructure reporting lines - requiring partners to report to Work Package leaders - to ensure that the project design was used to its full extent and facilitated rapid end-of-year reporting. This structure will be re-emphasised at subsequent meetings of the project group.

Activities in Work Packages
The project was structured in four core areas (CAs), each containing 3 work packages; in each CA the first work package comprises coordination duties ensuring focus across the core area. CA / WP leaders were assigned during project preparation and maintain responsibility for monitoring progress and reporting.

CA1 covers responsibilities for collections for the project as a whole, reciprocal transplant experiments and rangewide phylogeographic studies. A large effort has been made in the second period under **WP1** to gather as many samples to complete rangewide collection for all species. This effort has now obtained five substantial new field collections comprising additions to all target species, plus more than 1000 herbarium samples obtained by visits to eight herbaria. In parallel, agreements on material transfer and export permission have advanced, to allow sample exchange between partners. From a phylogeographic point of view substantial progress should now be possible in **WP3**, where collections can now be analysed using methods optimised for each species, for DNA extraction and cpSSRs. Efforts to identify optimal 'universal' loci for sequence data for comparative phylogeographic analysis have also progressed significantly. Under **WP2**, RTE sites have now been identified and established in Costa Rica for three species and, in Ecuador, efforts are well advanced for two species.

CA2 examines patterns of diversity at the landscape scale and the implications of landscape context for reproductive performance. In **WP5**, new markers for candidate genes have been developed: aquaporinsfor six target species and PepC for one, new microsatellites have been developed for eight target species (existing microsatellites are already available for all other target species). Full surveys of microsatellite variation have started in some species, and will commence with delivery of full collections in others. A wide range of acitivities are underway in **WP6**, involving almost all partners in some or all of phenological assessments, seed testing, seedling trials, outbreeding assessments and genetic analysis of inbreeding, addressing 13 of the target species plus 2 additional species.

CA3 will use new and developed simulation models to study the consequences of seed sourcing strategy and germplasm movement on gene flow and genetic diversity, and synthesise outputs from CA1 and 2. A consensus 'data compatibility template' was agreed under **WP7** to facilitate synthesis of results in downstream analysis, covering sample labelling and minium data requirements, to be implemented in the thrid reporting period following the coordination meeting. Under **WP8**, meta-analysis efforts are underway comprising 4 synthetic publications plus consolidation of data from previous projects. The EcoGene (**WP9**) model has undergone further refinement in preparation for simulation analysis in the second half of the project resulting in a new publication.

CA4 functions to consult the end-user community as to their requirements and expectations from the project, in terms of information and formats, and refine the target audience such that the effectiveness of message delivery is maximised. Efforts as part of **WP10** have refined the dissemination approach to target tertiary educators as the most sustainable and stable outlet for project outputs, with the highest probability of providing lasting benefits beyond the project lifetime. A review by the EC has identified SEEDSOURCE as an example of good practice in terms of stakeholder involvement in projects and it will be included in the inventory to be produced by the survey. During the second reporting period a survey ijn ecuador was completed (**WP11)**, elaboarting current practice in seed sourcing and reforestation in the country and a workshop has been planned to follow the coordination meeting. A series of workshops held in Central America (**WP12**) have provided a depth of end-user feedback with regard to dissemination materials. A number of clear initiatives have been identified as most effective and desirable from the point of view of the user community and outputs are now being planned and implemented to satisfy these needs.

Deviations

In general, progress has been hampered by the slower than expected gathering of collections. As a result, in the second period greater emphasis has had to be placed upon methodological development than was anticipated. However, good progress has now been made and and wide range of new and optimised markers (cpSSR, nSSR and SNP) are now available for all species. Full scale phylogeographical analysis and genetic diversity screening should proceed significantly during the third period, putting the project firmly on schedule.

Partner list

1. **CEH**: Stephen Cavers, Katherine Walker, Sam Davies
2. **OFI**: David Boshier, Paul Rymer, Jesus Cordero, Stephen Harris, Sarah Rendell
3. **INRA**: Henri Caron, Ivan Scotti, Caroline Scotti-Saintagne, Antoine Kremer, Remy Petit
4. **CNR**: G.G:Vendramin, A. Buonamici, F. Sebastiani, M.L. Racchi
5. **INPA**: Maristerra R. Lemes, Rogério Gribel, Christopher Dick, Alessandra P. Evangelista.
6. **CATIE**: Carlos Navarro, Bryan Finegan, Carolina Cascante, Gustavo Hernandez
7. **PUCE**: Renato Valencia, Galo Buitrón, Juan Iglesias , and Álvaro Pérez.
8. **UFRJ**: Rogerio Margis, Marcia Margis
9. **BFH**: Bernd Degen, Alexandre Sebbenn

Associated Institutions

UFRGS: Rogerio Margis, Marcia Margis
University of Adelaide: Andrew Lowe

Periodic activity report
Workpackage progress for period 2.

CORE AREA	**1**	**Adaptive variation & genetic differentiation at rangewide scale**
Work Package	1	***Collection and exchange of materials and methods***
Work Package	2	***Quantitative performance for replanting***
Work Package	3	***Evolutionary history and developing regional markers for species***
CORE AREA	**2**	**Diversity, reproductive performance & recruitment at the landscape scale**
Work Package	4	***Ensuring focus of quantitative and genetic studies***
Work Package	5	***Gene dynamics & quantitative seed performance in relation to landscape***
Work Package	6	***Estimate partitioning of non-coding and coding genetic diversity***
CORE AREA	**3**	**Analysis and prioritisation of regional and local sourcing strategies**
Work Package	7	***Data compatibility***
Work Package	8	***Meta-analysis of data***
Work Package	9	***Selection and definition of resource priorities***
CORE AREA	**4**	**Knowledge gathering, integration and dissemination of priorities**
Work Package	10	***Communication of biological and socio-economic information***
Work Package	11	***Knowledge gathering***
Work Package	12	***Preparation and dissemination of extension materials***

CORE AREA	**1**	**Adaptive variation & genetic differentiation at rangewide scale**
Work Package	**1**	***Collection and exchange of materials and methods***

1. Workpackage objectives and starting point of work at beginning of reporting period

- Manage collection and exchange of materials and methods
- Draw up IPR, germplasm exchange and data management agreements between partners
- Coordinate collection activities
- Ensure export and collection permits and procedures are followed
- Construct project website

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved.

Project administration
The University of Adelaide (UA) and Universidade Federal do Rio Grande do Sul (UFRGS) in amendments to the Technical Annex, as agreed with the Scientific Officer at the EC.

Collections summary
Sample collection has progressed significantly for all species during the second reporting period. As planned, existing collections have been rationalised and, where necessary, both fresh field collections and herbarium samples have been targeted: new field collections have been prepared in partner countries and elsewhere, whilst eight herbaria have been contacted and visited to sample accessions, yielding more than 1000 new samples across all target species.

Specific new field missions completed at the time of the report:

- PUCE: collections for fifteen of the target SEEDSOURCE species have been completed (>800 new samples) from both the Pacific coastal and Amazonian regions of the country as well as locations in Peru.
- INPA: samples from Panama, Bolivia, Ecuador and Brazil have been collected, representing contributions to collections for all fifteen of the target species and bringing INPA collections across all species to more than 1000 samples. In addition, new collections of *Swietenia humilis* have been made and will be incorporated in the phylogeographic studies (WP3).
- CATIE: Costa Rican populations (>80 samples) have been sampled for ten of the target species from sites at Sarapiqui and Turrialba.
- The Institute for Forest Studies, Cuba: in collaboration with CEH, linked funding has been secured using the SEEDSOURCE project to support the collection and analysis (by a visiting scientist) of a substantial new collection of *Cedrela odorata* from Cuba (>300 new samples), covering the natural range of the species on the island.
- The Insituto de Investigaciones de la Amazonia Peruana, in collaboration with CEH, have prepared collections of seven of the target SEEDSOURCE species (>100 samples) from their experimental station at Jenaro Herrera in the Peruvian Amazon region.

The official enquiries to herbaria worldwide returned positive reponses from eight, all of which will have been visited and sampled by the time of the annual coordination meeting in Quito. A summary of progress is given in Table WP1.1

Table WP1.1 Summary of herbarium samping progress

Herbarium	Collector	Sampled ?	Distrubuted
University of Michigan	INPA (Dick)	-	
OFI	OFI	Yes	No
NY Botanical Garden	INPA (Dick)	-	
Missouri Botanical Garden	INPA (Dick)	Yes	No
Royal Botanic Garden, Kew	OFI	-	
Guiana	INRA	Yes	Yes
INPA Manaus	INPA (Lemes)	Yes	Awaiting permit
CPATU Belem	INPA (Lemes)	Yes	Awaiting permit
National Museum Rio	UFRJ / UFRGS	-	-
University of Quito	PUCE	Yes	Awaiting permit
Utrecht Herbarium	CEH	Yes	Yes
Royal Botanic Garden, Edinburgh	CEH	Yes	Yes

IPR & material exchange agreement (all partners)

For the protection of Intellectual Property Rights and to ensure correct procedures are followed in the export and use of sampled tissue material, the text of a Material Transfer Agreement (MTA) was drawn up and agreed between partners, following legal screening by administrators in each of the partner Insitutions. The final accepted text is given in Appendix XX. The agreement will be signed by each Institution and final copies, including signatures of all Insitutions will be circulated to all project partners.

Export permissions
Export permissions for Brazilian and Ecuadorian samples are in the process of being secured. It is hoped that this process can be completed by the time of the annual coordination meeting in July 2007.

Website (OFI, CEH)
Jesús Cordero worked on maintaining the project website (www.seed-source.net) and translated it into Spanish. The latter coincided with follow up from the workshops run under WPs 11 and 12 in Central America (see report on these WPs for more detail). Occasional work on project MTA.

3. Deviations from the project workprogramme, and corrective actions taken/suggested

David Boshier pursued contacts with organisations in Colombia and Venezuela for obtaining further samples of priority species. There has been progress, but on a limited range of species which should yield new samples in 2007-08. These contacts will be continued in 2007-08 to see if this feasible for the full range of Seedsource species found in those two countries.

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p58)

CORE AREA	**1**	**Adaptive variation & genetic differentiation at rangewide scale**
Work Package	**2**	***Quantitative performance for replanting***

1. Workpackage objectives and starting point of work at beginning of reporting period

- Assess the scale of local adaptation under conditions of natural regeneration in four study species at seed germination, seedling establishment and the relation to genetic, environmental and geographic distance.
- Compare the patterns of adaptation under conditions of natural regeneration with those in plantation provenance trials.
- Relate evidence for the scale of adaptation to existing seed sourcing practices.

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

Activities in WP2 are primarily undertaken by partners CATIE and PUCE, reports on progress in RTE establishment and preliminary analysis are given below:

1. CATIE. We report on RTE establishment for three tropical forest tree species. The fruits for the first species, *Cedrela odorata*, were collected from January to April 2006, in six locations, and the experiments are now well established. The other species (*C. tonduzii, U. mexicana*) will be collected when fruits are mature. The locations for other species have been defined and the localization of mother trees will be determined in the coming months. Table WP2.1 indicates the location of collection zones for Rtes of *C. odorata*, *C. tonduziii* and *U. mexicana*. Table WP2.2. indiactes the corresponding planting sites for reciprocal transplant experiments.

Table WP2.1. Locations of collecting zones for Reciprocal Transplanting Experiments of WP2.

Specie/Population	**Longitude**	**Latitude**	**Altitude**	**Location**
Cedrela odorata				
Guanacast**e**	84.98000	10.25000	150	Las Juntas of Abangares
Pérez Zeledón	83.60000	09.380000	700	General Viejo
Puriscal	84.25000	09.90000	700	Puriscal, Ciudad Colón
San Carlos	84.48000	10.37000	200	Florencia
Sarapiquí	84.90000	10.23000	150	Horquetas, Puerto Viejo
Turrialba	83.70000	09.85000	700	Tucurrique, Turrialba
Cedrela tonduzzi				
Cartago	83.96700	9.70000	2000	El Empalme, Santa María of Dota.
Llano grande	83.91600	9.94400	2300	Llano Grande, Cartago
Zarcero	84.30014	10.189098	2000	Tapezco, Laguna,
Santa Cruz	83.73400	9.96800	1500	Santa Rosa, Santa Cruz
Ulmus mexicana				
Cartago	83.93200	9.80900	1400	Cervantes, Cartago
Turrialba	83.71400	9.87300	700	Chiz, Turrialba
Santa Cruz	83.44800	9.95800	1500	Santa Cruz

Table WP2.2. Locations of Reciprocal Transplanting Experiments of WP2.

Specie/Population	**Longitude**	**Latitude**	**Altitudemasl**	**Location/ Proprietary**
Cedrela odorata				
Guanacaste	84.98489	10.25452	108	Las Juntas, Abangares – Manuel Bonilla
Pérez Zeledón	83.66381	9.38984	790	General Viejo- Tulio Granados
Puriscal	84.25233	9.90340	700	Ciudad Colón- Ronald Madrigal
San Carlos	84.51179	10.37587	191	Santa Clara of Florencia- Ana Lía Quirós
Sarapiquí	84.03148	10.44266	150	Cristo Rey of Puerto Viejo- Abelardo Oconitrillo
Turrialba	83.65329	9.89756	597	CATIE
Cedrela tonduzzi				
Cartago	83.93250	09.80950	1550	El Guarco – Carlos and Uriel Navarro
Llano Grande	83.91346	9.44268	1700	José Sanabria
Zarcero	84.5000	10.2500	1600	Jaime Barrientos
Santa Cruz	83.75	9.9500	1500	La Pastora of Santa Cruz- Municipality of Turrialba
Ulmus mexicana				
Cartago	83.93250	09.80950	1550	El Guarco – Carlos and Uriel Navarro
Turrialba				CATIE
Santa Cruz	83.75	9.9500	1500	La Pastora of Santa Cruz- Municipality of Turrialba

Some preliminary results were obtained using SAS statistical systems: they show highly significant differences between provenances in adaptation to the different type of soils, there are clear differences between provenances of the dry area of Guanacaste and Puriscal compared with the rest of the populations, this preliminary analysis was done when the seedlings were just planted in all the six sites of origin of the seeds. The mortality at one month after planting in each of the different sites is shown in Table WP2.3:.

Table WP2.3. Mortality in C.odorata RTEs in Costa Rica.

Sitio	Guanacaste	Pérez Zeledón	Puriscal	San Carlos	Sarapiquí	Turrialba	Total
Guanacaste	49	47	59	70	54	71	350
Pérez Zeledón	8	13	31	10	9	17	88
Puriscal		4	3	8	3	4	22
San Carlos	24	33	54	24	35	42	212
Sarapiquí	3	13	21	21	16	22	96
Turrialba	7	6	29	10	7	7	66
Total	91	116	197	143	124	163	834

From preliminary statistical analysis of all the sites together, Turrialba is the best site, where provenances are growing almost 100 % more in height and diameter respect to the worst site Pérez Zeledón.

2. PUCE. We are working with *Cedrela odorata* (Spanish cedar) and *Ochroma pyramidale*, (balsa), two widely distributed species in different habitats and forest types in Ecuador. Herbarium records were initially used to identify potential sites for RTEs, followed up by site visits to either confirm sufficient populations for sampling or, where insufficient trees were located, to identify alternative sites.

Description of sites:

C. odorata is found in Ecuador in a variety of ecological gradients in the Coastal plains and the Amazon lowlands. In the Coast region: Las Mercedes, a pre-montane forest in the western foothills of the Andes; Chindul, a semi-deciduous forest; In the Amazonian region: Puerto Napo - Ahuano Road (NAR) and Yasuni Research Station (YSS), the former located at higher altitude and the latter a rainier site because it is closer to the Andean foothills where precipitation is usually higher (Fig. WP2.1; Table WP2.4).

O. pyramidale is an emblematic and common pioneer species in Ecuadorian disturbed areas in the Coast and Amazon regions. It prefers humid lowland areas being its highest altitudinal distribution at 2000 m in the Andean slopes. The species can be found frequently in road sides and secondary forests. Farmers leave balsa trees to grow in pasturelands in the coastal plains since the species is valuable and some timber companies buy the best pieces of balsa trees. Identifying suitable populations of this species was not a difficult task, however, trees in the coast are not fruiting synchronically and we are still waiting for fruits to extract seeds for RTEs.

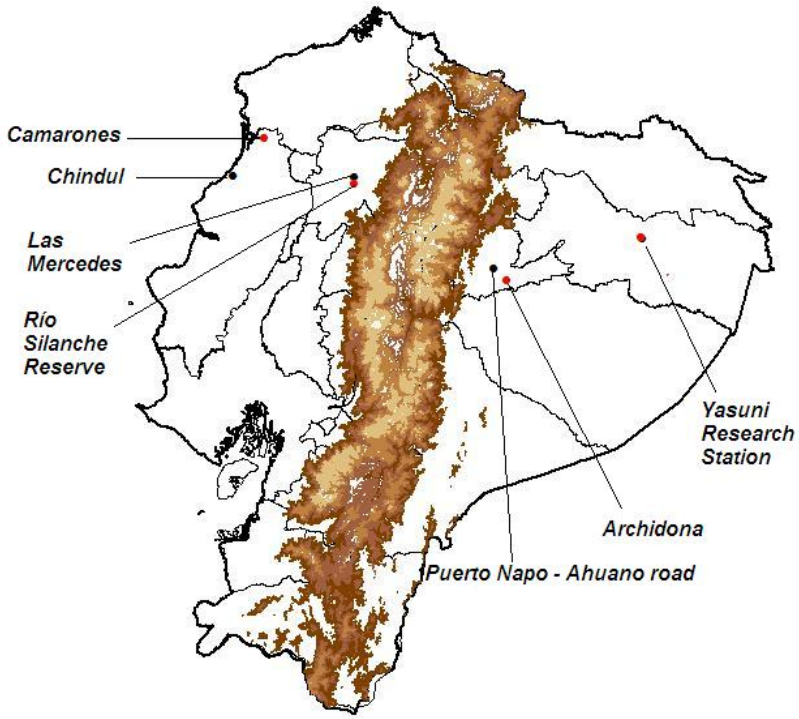


Fig. WP2.1. Map of sites selected to for RTEs: • *C. odorata* and • *O. pyramidale*. In brown, areas of Ecuador above 1000 m elevation.

Table WP2.4. Characteristics of sites selected to carry out RTEs in Ecuador. TPR = Tropical Pre-montane rainforest; TSF = Tropical Semi-deciduous forest; TER = Tropical Evergreen Rainforest. A map of these localities is presented in Fig. WP2.1.

Region	Locality	Elevation (m.a.s.l.)*	Climate	Rainfall (mm/yr)	Temp. °C	Vegetation type	Mother trees	Soil type	Species
Coast	Las Mercedes	711	Tropical Wet	2800 (1)	22	TPR	21	Volcanic	*C. odorata*
Coast	Chindul-La Quinta Road	164	Tropical Dry	890 (5)	24	TSF	21	No data	*C. odorata*
Amazonia	Pto. Napo-Ahuano road	428	Tropical Wet	3673 (0)	25	TER	22	Sedimentary	*C. odorata*
Amazonia	Yasuní Scientific Station	246	Tropical Wet	3000 (0)	25	TER	31	Clay	*C. odorata*
Amazonia	Yasuní Scientific Station	246	Tropical Wet	3000 (0)	25	TER	21	Clay	*O. pyramidale*
Coast	Silanche River Forest Reserve	627	Tropical Wet	5545 (0)	21	TER	30	Volcanic	*O. pyramidale*
Coast	Camarones*	370	Tropical Dry	890 (5)	24	TSF	31	No data	*O. pyramidale*
Amazonia	Archidona	820	Tropical Wet	6316 (0)	23	TPR	20	Sedimentary	*O. pyramidale*

Table WP2.5 Phenological records and mature fruits collected of *Cedrela odorata* in sites selected to carry out RTE's in Ecuador

	Pacific coastal sites				Amazon lowland sites					
Population	Chindul		Las Mercedes		YSS			NAR		
Phenology Status	Dec.	Mar.	Feb.	Mar.	Jan.	Mar.	Apr.	Jan.	Feb.	Mar.
Infertile	13	12	28	26	16	10	13	9	8	9
Flowers	3	1	1	3	12	1	0	3	1	0
Inmature fruits	4	7	0	0	2	12	13	9	10	10
Mature fruits	0	0	0	0	0	1	2	0	0	2
Falled fruits	1	1	0	0	1	0	0	0	0	0
Not found	0	0	0	0	0	8	5	1	3	3
Total	21	21	29	29	31	31	31	22	22	22

Amazon lowland sites:
Pto. Napo - Ahuano Road (NAR) (1° 3’ S, 77° 36’ W, 450 m.a.s.l). A suitable site for an RTE of Spanish Cedar was found in an ecological gradient of pastures, secondary and primary forest between Puerto Napo and Ahuano Road including Jatun Sacha Biological Reserve. This area is considered one of the keystone places for Spanish Cedar in Ecuador and it is located in the Napo river Watershed. It is a private reserve of 2500 ha of tropical evergreen rainforest property of Jatun Sacha Foundation, an environmental NGO. The soil is sedimentary with low hills and a bad drainage. The soils have low fertility, ph acid (5.1) and high contents of aluminium (Revelo & Palacios 2002). The vegetation is tropical evergreen forest and he canopy is 30 m high. Common tree species are *Virola duckeii*, *Brosimun* sp., *Otoba glycicarpa* and *Iriartea deltoidea*. Some forestry projects have been developed in the area by Jatun Sacha Foundation and a small Botanic Garden is maintained.

Yasuní Scientific Station (YSS) (0° 40' S, 76° 23' W, 246 m.a.s.l.). The second population of Spanish Cedar is located in the Yasuni Scientific Station area. YSS is placed in the south bank of Tiputini River inside of the Yasuni National Park, a protected area of 982,000 ha. Vegetation in the area is lowland evergreen forest dominated by terra firme primary forest. The flora of the area is high with a total of 1124 species recorded in a 25 ha plot (Yasuni Forest Dynamic Plot). Canopy is 25-30 m high with emergent trees of 40 m. Common species of trees are *Eschweilera coriaceae*, *Otoba glycicarpa* and *Iriartea deltoidea*. Detailed descriptions of the vegetation in the Yasuni are provided in Romero-Saltos et al. (2001) and Valencia *et al* (2004).

Archidona, is located in the western slopes of the Guacamayos Cordillera, on the eastern slopes of the Ecuadorian Andes. Balsa trees were collected along the road between Archidona and a Santo Domingo, a small Kichwa village settled in the Hollín River shores. Climate in the area is tropical humid but no data of rainfall and temperature are reproted. Soil is mainly volcanic and sedimentary. Vegetation is a premontane evergreen rainforest but original vegetation has been cleared with the exception of some small private reserves found in ecological lodges. Balsa trees were found in secondary forest remnants, in roadsides, and as isolated individuals in pastures. Common trees in the area are *Erythrina* sp., *Cordia alliodora*, *Socratea exorrhiza* and *Bactris gasipaes* (A. Perez & G. Buitrón pers. obs.).

Coastal plain sites:
Chindul (0° 13' N, 79° 52' W, 164 m.a.s.l.). It is a small town located in the slopes of the Mache-Chindul Coastal ridge in the Pacific lowlands. A remnant population of Spanish Cedar was located in this area approximate 20 km from Pedernales, the main town of the area. Cedars are located in the western border of the Mache-Chindul Ecological Reserve. Vegetation has not been surveyed although it seems closely to other coastal localities as Cerro Pata de Pájaro (RAP programm). The area has been intensively degraded and Spanish trees were found as isolated individuals in pastures or inside of small fragments of secondary forest.

Las Mercedes (0° 10 ' S, 79° 02 ' W, 711 m.a.s.l.). The last experimental population of Spanish Cedar was located in the Coast in the road between Santo-Domingo and Los Bancos, Pichincha province. The main numbers of individuals are located around the small town of Las Mercedes, 30 km approximate from Sto. Domingo. As Chindul, Spanish Cedars were found as isolated individuals in pastures, farms and secondary forest. However, some of the individuals found are 25-30 m high with abundant epiphytes. Vegetation in the area is highly secondary with ranch lands dominated the landscape. Common trees observed were *Iriartea deltoidea*, *Cordia alliodora*, *Pouteria* sp. and *Cedrela odorata*.

Silanche River Forest Reserve (SRFR) is located at 113 kilometres from Quito, in the western slopes of Andes. RSFR is a small reserve of 85-ha of primary forest surrounded by extensive timber plantations of several species (e.g., *Schizolobium parahyba*, *Parkia multijuga, Jacaranda copaia*). The small reserve consist in a primary forest maintained by logging company as a seed source of timber trees. Climate is tropical wet without dry months. The vegetation is tropical premontane rainforest with a canopy of 40 m high. Common trees in the reserve are *Carapa guianensis*, *Virola dixonii*, *Brosimum utile*, etc. A detailed description of Endesa Flora was published by Jorgensen & Ulloa 1989. Balsa trees selected were found inside of the reserve and in adjacent farmlands and secondary forest in roadsides.

Camarones. This locality is poorly explored and climate, soil or vegetation studies are not available. Original vegetation could be humid in the ridge tops and semi-deciduous in the foothills of the Chingon-Colonche mountain range, where this locality is located. *Chlorophora tinctoria*, *Tabebuia chrysantha* and *Cordia alliodora* are valuable timber species presents in the area. In vegetation surveys of nearby localities (Cerro Pata de Pájaro) botanists have recorded numerous endemic species of trees.

Cedrela odorata: Phenological studies

We established a monitoring programme to collect seeds and phenological records of Spanish cedar. To date, we have collected seeds of relatively few mother trees (summary in Table WP2.5). Monitoring information of the four Ecuadorian populations showed a relatively asynchronic pattern, with only few selected trees producing flowers or fruits. The populations in Chindul, NAR and YSS had few flowering or fruiting trees; the population in Las Mercedes showed one tree in flower alone (Table WP2.5).

Ochroma pyramidale: phenological studies

Early surveys in Archidona and YSS were done in May and October 2006, respectively. In Archidona and YSS, fructification was synchronic. Fruits were observed in trees during Dec. 06 and Jan. 07 in both localities. To date we have completed collections of seeds in the Amazon region. In the Coast, in Camarones, we have collected seeds of 15 mother trees (by Nov. 06) and seeds of 7 mother trees (by Apr. 07) in SRFR. In these two localities we have carried out monthly visits to record phenology and collect mature fruits since Jan 07. We have already extracted seeds of the majority of collected fruits. Preliminary results of size and weight variation of fruits and number of seed per fruit are summarized in Table WP2.6.

Table WP2.6. Morphometric measures of fruits and number of seeds of *Ochroma pyramidale*.

				Fruit characteristics (Average)			
Region	**Localities**	**Mother trees**	**No. fruits**	**length (cm)**	**wide (cm)**	**weight (gr)**	**Seeds per fruit**
Coast plains	Camarones	12	51	19,9 ± 5.0	2.5 ± 1.0	34.7 ±12	959 ± 294
I-A Valley	S. de Ibarra	5	16	17.7 ± 1.3	2.5 ± 0.3	29.7 ± 2.8	652 ± 124
W. slopes	Lita	4	19	15.9 ± 0.9	2.7 ± 0.4	31.2 ± 5.5	645 ± 142
Amazon	Archidona	4	14	23.6 ± 3.0	3.0 ± 0.5	32.8 ± 7.9	631 ± 266
lowlands	Yasuni S.S.	7	33	19.2 ± 6.8	2.4 ± 0.9	27.2 ± 12.5	554 ± 332

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

CATIE: Significant changes to the programme had to be made because of flowering and fruiting problems in both *Carapa guianensis* and *Vochysia ferruginea.* In these species, it was impossible to get seeds from the different populations at the same time; additionally, both species have recalcitrant seeds, so collection of different populations in different times of the year can not be done. As alternatives, *Ulmus mexicana* and *Cedrela tonduzii* were collected and they are in the nursery, with sites for planting are in preparation. Seedlings of *C. tonduzii* are around 10 cm tall, and will be planted in the RTEs in March- June 2007.

PUCE: Due to difficulties in finding *C. odorata* trees and asynchronic fruiting, RTEs establishment was delayed. However, seed collection is running and hopefully field trials will be established by February 2008. Seed collection of *O. pyramidale* is already completed in the two Amazonian localities but not in the coast because of asynchronic fructification. We expect to complete collections in the coastal sites soon and start the experiments by the end of 2007.

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p58)

CORE AREA	**1**	**Adaptive variation & genetic differentiation at rangewide scale**
Work Package	**3**	***Evolutionary history and developing regional markers for species***

1.Workpackage objectives and starting point of work at beginning of reporting period

- Evaluate chloroplast and nuclear nucleotide polymorphism and construct phylogeographic maps for the study species
- Evaluate DNA extraction methods from herbarium and woods samples for genotyping in comparison to DNA extraction of living tissues (for study species)
- Evaluate the possibility to identify species and range of species of interest based on genetic sequence data

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

As the focus of efforts in the project continued to be on securing the substantial collections necessary to conduct rangewide phylogeographic studies for 16 focal species, and these were ongoing throughout the second reporting period, activities in WP3 have again largely been in optimisation of methods using existing samples and on development of techniques for comparative analysis and for downstream applications such as timber monitoring and germplasm source control.

Herbarium & wood samples (INRA)

We have tested samples of dry wood (1 year to more than 5 years old) of 17 tree tropical tree species. We analysed herbarium samples (5 to 30 years old) of 5 species. In total, we tested 9 extraction protocols: 4 modified CTAB protocols, 4 modified Quiagen kit protocols and the Invisorb kit protocol. In all cases, the PCR products were purified using the GenElute PCR Cleanup Kit. We controlled the quality of the extracted DNA in several steps: first, the total DNA were run in an agarose gel; secondly, cpSSR (100-300 bp) and PCR-RFLP fragments of increasing length (500-1000bp) were amplified; and in the third step, they were sequenced. In each step, we used fresh material samples as control. We blasted sequences against Genebank databases.

<u>DNA extraction from herbarium samples</u>
As a rule, we obtained the better quality of DNA using Quiagen or Invisorb kits. But, results are uneven according the samples (quality of the drying, chemical treatments…).

<u>DNA extraction from wood samples.</u>
We succeeded in extraction for all species. We obtained good results in all cases, using Invisorb kit for the extraction and the GenElute PCR Cleanup Kit for the purification. CpSSR markers amplified in 86% of cases, and the rate of success decreased according to the fragment length for PCR-RFLP: 100% (~500pb), 83% (~800pb), and 73% (~1kb) (Table WP3.1). We succeeded to sequences fragments of 500 and 800bp length for several species.

Table WP3.1 : DNA extraction tests from wood samples of tropical tree species. ***Blank = no data***

		extraction	Amplification						
			ccmp2	ccmp3	ccmp4	trnH-psbA	trnS-trnG	psbB-psbF	trnD-trnT
Species	**samples**		< 200bp	< 200bp	< 200bp	~500pb	~800pb	~800pb	~1kb
Quercus petreae	*leaf*	OK	NO	OK	OK	OK	OK	OK	OK
Pinus pinaster	*needle*	OK	OK	NO	NO	OK	OK	OK	
Carapa guianensis	*Wood 1year*	OK	OK	OK	OK	OK	OK	OK	OK
Hymenea courbaril	*Wood 1year*	OK	OK	OK	OK	OK	OK	OK	OK
hymenea courbaril	*wood 5 years*	OK				*OK*			
Jacaranda copaia	*Wood 1year*	OK	OK	OK		OK	OK	OK	NO
Simarouba amara	*Wood 1year*	OK	OK	OK	OK	OK	NO	OK	OK
Virola sebifera	*wood >1year*	OK				OK	OK	OK	OK
Ceiba pentandra	*wood >1year*	OK					OK	OK	
Minquartia guianensis	*wood >1year*	OK				OK	OK	OK	NO
dipterix odorata	*wood 5 years*	OK				*OK*			
Tabebuia heterophylla	*Wood 1year*	OK			NO	OK	OK	NO	OK
Tabebuia sp	*wood 5 years*	OK				*OK*			
Dicorynia guianensis	*wood >1year*	OK							NO
Eperua falcata	*wood >1year*	OK				OK			NO
Aucoumea klaineana	*wood >5 years*	OK	NO	OK	NO	OK	NO	OK	OK
cedrelinga catenaeformis	*wood 5 years*	OK				*OK*			
Pinus radiata	*wood 5 years*	OK				*OK*			
guarea sp	*wood 5 years*	OK				*OK*			
khaya anthoteca	*wood 5 years*	OK				*OK*			

Phylogeography: development & testing of 'universal' phylogeography loci

a.) Screening of variation at cpSSR loci (CNR)
Primers from Weising and Gardner (1999) (ccmp1, ccmp2, ccmp3, ccmp4, ccmp5, ccmp6, ccmp7, ccmp10) were used to amplify genomic DNA. Amplified products were run using a MegaBace 96 capillary automatic sequencer, according to Heuertz *et al.* (2006). Seven species were studied. Results are given in table WP3.2 below

Table WP3.2: amplification, polymorphism, allelic richness and total variance partitioning in cpSSR test

Species	Samples size (no of pop)	No of amplified loci	No of polymorphic loci	No of haplotypes	%total variance among pop.
S.excelsa	78(7)	7	2	5	65
V.sebifera	42(1)	6	1	2	
J.copaia	10(1)	8	0	1	
C.alliodora	38(8)	8	4	9	87
S.humilis	30(6)	6	0	1	
B.quinata	36(7)	6	1	2	79

b.) Design of new cpSSR primers (INRA)
On the basis of the alignment of sequences of cp DNA fragments, new primers were designed in each side of the regions with mononucleotide repeats. Today, 2 new cp microsatellites in *Virola* species and one in *Carapa* species are polymorphic.

c.) Screening of variation at cpDNA loci (INRA / UoA / INPA)
Test for Direct Sequencing (INRA): Based on the literature (Shaw et al., 2005; Kress et al., 2005), ten chloroplast DNA regions were selected. In a first step we tested the quality of DNA amplification on 16 species (Table WP3.3). Excepting three primers(*trnS-trnG*, *trnS-trnfM*, *psbB-psbF*) for which an overlapping pattern was obtained, all others globally gave good results. In many cases one of the two primers (Forward or Reverse) gave a better pattern than the other. This could be explained by a more specific hybridization with DNA of one of the two primers during elongation. Finally, seven primers were selected to screen the inter-population polymorphism: *trnD-trnT*, *trnC-ycf6*, *trnH psbA*, *rpoB-trnC*, *rpl16*, *ycf6-psbM*, *rpl20-rps12*

Table WP3.3 : Proportion of species per cp primer pairs that give a single region amplification

Loci	***trnD-trnT***	***trnC-ycf6***	***trnS-trnfM***	***trnH-psbA***	***rpoB trnC***	***rpl16***	***ycf6 psbM***	***trnS-trnG***	***rpl20-rps12***	***psbB-psbF***
Amplification success	11/16	14/16	5/16	10/16	13/16	13/16	14/16	3/16	13/16	10/16

Success= number of species where a single band is observed on agarose gel

Screening of variation in populations (INRA): The screening for DNA variation at seven chloroplastic regions was performed on six individuals sampled from two different countries (3 individuals per country). For many species, individuals from a single population (one country) were available. Consequently for these species, only the intra-population polymorphism has been analysed (Table WP3.4). The quality of sequencing varied a lot between cp regions. In most of the cases the bad quality of sequencing was due to the presence of poly T. For several cp regions it was possible to sequence one of the two strands (*trnH-psbA*^{F}, Rpl16^{F}; *rpoB-trnC*^{R}) and for other regions the sequencing was bad in both senses (*PetA*^{F}/*PsbE*^{R}). Concerning

intra-population variation, among the 10 species analysed, the four regions which are polymorphic in the higher number of species are: *DT*, *rpl16, ycf6-psbM* and *trnC-ycf6*. At the interpopulation level, the no. of analysed species was not enough high to draw conclusions.

Table WP3.4: No. of haplotypes observed per cpDNA region for each species intra / inter population.

		trn**H** psb**A**	trn**C** ycf **6**	rpl**16**	rpo**B** trn**C**	trn **D** trn**T**	rpl**20-** rps**12**	pet**A** psb**E**	ycf**6** psb**M**
	Sequence length	300-600	900-1300	1000-1200	900-1200	800-1200	600-800	600-700	1000-1200
	Intra FG (3)	1	1	2**	1	2	1	1	1
	Intra PA (3)	1	1	1	Na	Na	Na	Na	Na
	Inter-pop (6)	1	1	2	Na	Na	Na	Na	Na
Laetia procera	Intra FG (3)	1	1	UR	1	UR	UR	UR	UR
	Intra FG(3)	UR**	2	2	2	2	1	UR	2
	Intra CR(3)	UR**	1	1	1	1	1	UR	1
	Inter-pop(6)	UR**	3	3	3	3	1	UR	2
	Intra EC(3)	1	1	1**	UR**	UR**	1	UR	1
	Intra PA(3)	1	1	2	UR**	1	1	UR	1
	Inter-pop(6)	2	1	2	UR**	UR**	1	UR	1
	Intra CR(3)	1	1	2	1	1**	1	1	1
	Intra CO(3)	UR**	1	1	1**	UR**	1	1	1
	Inter-pop(6)	UR**	2	3	2		1	2	2
Carapa guianensis	Intra FG(3)	1	1	1**	1	1**	1	1	1
Carapa procera	Intra FG(3)	2	2	1**	2	2**	2	2	2
	Intra M(3)	1	1	1	1	1	2	UR	1
	Intra CR(3)	1	1	1	1	1	1	UR	1**
	Inter-pop(6)	2!!	2!!	2!!	2!!	2	3	UR	2!!
Virola sebifera	Intra FG(3)	UR**	1	1**	1**	2	1	UR	2
Jacaranda copaia	Intra FG(3)	1	2	1	1	1	1	UR	1

Na= not analyzed, UR=Unreadable, ** = Part of the sequence is unreadable due an overlapping after a poly T , !! = More than 150 bp are not aligned between the sequences of the two populations. The species identification needs to be checked**.**

Screening the utility of universal cpDNA loci (UoA): Data from 131 different species studies, employing a range of locus and polymorphism screening methods (sequencing, RFLP and cpSSRs), reveal high variation in the level of individual locus polymorphism uncovered. A snap shot of the results are presented below (Fig WP3.1).

Fig. WP3.1: a. *Sequencing*

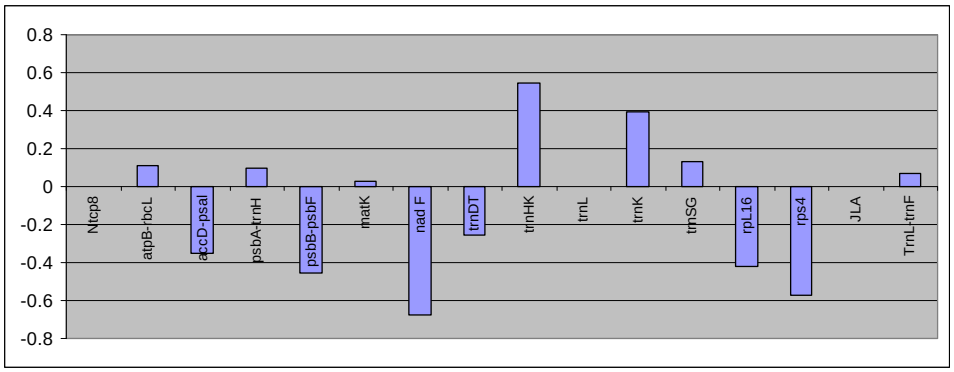


Fig. WP3.1: *b. RFLPs*

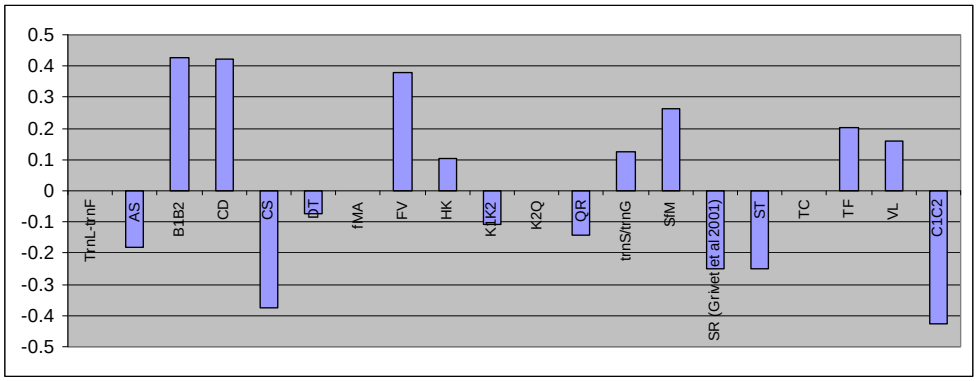
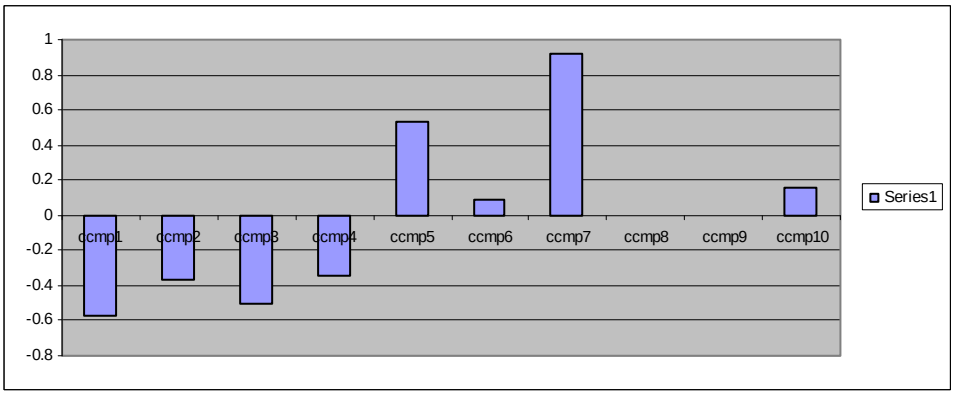


Fig. WP3.1: c. *cpSSRs*

Of the 10 most commonly available cpSSR loci (from Weising and Gardner), loci 5 and 7 were most polymorphic across species and 1-4 least.



Standardised study (UoA): Standardised DNA was distributed to CNR (cpSSR), INRA (sequencing) and UoA (RFLP) to more widely assess the relative polymorphism of a range of cpDNA screening methods. In addition UoA has been completing a literature review of all studies, which apply at least 2 cpDNA loci, to obtain within species diversity/structure measures for meta-analysis. This work will be written up as a single paper involving all project contributors.

Phylogeography: Optimisation and individual species studies
In general, CNR has been undertaking primer optimisation for most species, for chloroplast microsatellites. Primers from Weising and Gardner (1999) (ccmp1, ccmp2, ccmp3, ccmp4, ccmp5, ccmp6, ccmp7, ccmp10) were used to amplify genomic DNA. Amplified products were loaded on a 96 capillary sequencer MegaBACE 1000 (Amersham). Where full species collections have been available, some phylogeographic analysis has now begun.

1. *Simarouba amara* (CNR)
For this species primers ccmp 2, 3, 4, 6, 7, 10 worked. Only ccmp 4 showed a polymorphic pattern and allowed distinguishing two haplotypes: one was detected in samples from French Guiana and the other in Costa Rican populations.

2. *Minquartia guianensis* (CNR)
Analysis was performed on the samples received from UoA together with two samples from Ecuador received from Chris Dick and one sample from Costa Rica received from Carlos Navarro. Primers ccmp 1, 2, 3, 4, 6, 10 worked and showed two highly differentiated groups of haplotypes suggesting that the samples probably belong to two different species. The two samples of French Guyana and those of Ecuador and of Costa Rica share the same haplotypes. These five samples were polymorphic at ccmp 2, 3, 4, 6 showing 2, 2, 3, and 2 variants respectively, combining into four haplotypes (Table WP3.5). In the remaining samples, highly divergent haplotypes were detected (see green shaded cells of Table WP3.5).

Table WP3.5: Haplotypes identified in Minquartia guianensis

sample	Country	ccmp1	ccmp2	ccmp3	ccmp4	ccmp6	ccmp10
Fg1	French Guyana	128	140	142	230	138	117
Fg2	French Guyana	128	140	142	230	138	117
Fg3	French Guyana	128	140	142	230	138	117
Fg4	French Guyana	128	220	107	118	102	107
Fg5	French Guyana	128	220	107	118	102	107
CR1	Costa Rica	128	140	142	230	138	114
CR2	Costa Rica	128	140	142	230	138	114
CR3	Costa Rica	128	140	142	230	138	117
CR4	Costa Rica	128	140	142	230	138	114
CR5	Costa Rica	128	140	142	230	138	114
177	Ecuador	128	226	107	119	102	107
179	Ecuador	128	226	107	120	102	107
MinCR	Costa Rica	128	226	108	120	107	107

3. *Carapa guianensis* (CNR)
Ccmp 1 didn't amplify, ccmp 2, 3, 4, 5, 10 were monomorphic and ccmp 6 and 7 showed polymorphic patterns. Ccmp6 and 7 showed two and four variants respectively distinguishing five haplotypes. Haplotypes H1, H2, H3, H4 were present only in the population of French Guyana; H5 was shared by Costa Rica and French Guyana populations. Frequency of haplotypes per population is shown in Fig. WP3.2. Haplotype composition (in base pairs, ccmp 6 and ccmp 7) and haplotype frequencies are reported in Table WP3.6.

Fig. WP3.2

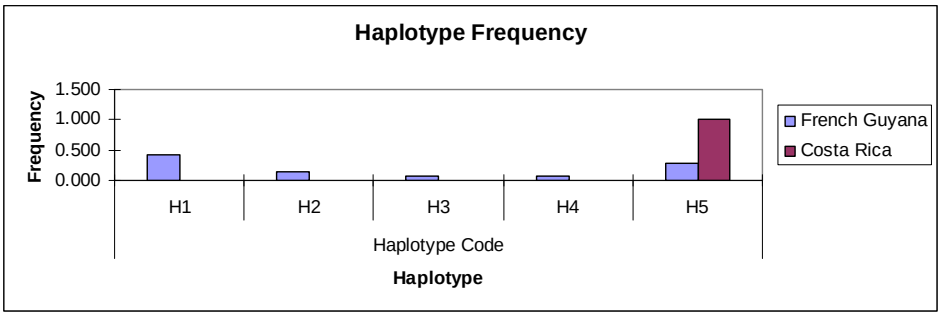
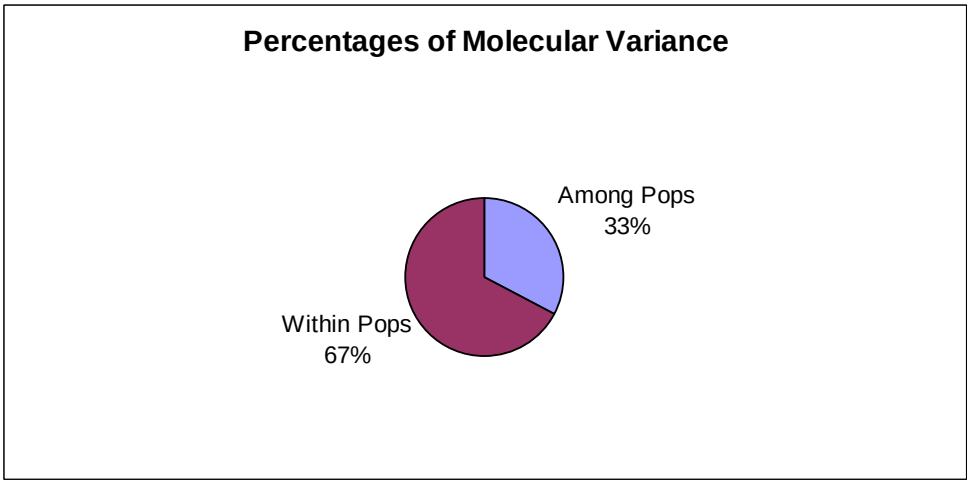


Table WP3.6

	French Guyana	Costa Rica
H1(123/128)	0.429	0.000
H2(123/129)	0.143	0.000
H3(123/135)	0.071	0.000
H4(125/127)	0.071	0.000
H5(125/128)	0.286	1.000

Analysis of molecular variance was performed using GenAlEx (Peakal and Smouse, 2005) and revealed a relatively low differentiation among populations, being only 33% of the total variance due to differences among populations (Fig. WP3.3).

Fig. WP3.3



4. *Socratea exorrhiza* (CNR)

Ccmp 1 didn't produce any amplification, ccmp 3, 5, 6, 7, 10 were monomorphic and ccmp 2 and 4 showed three variants each resulting in five haplotypes. The frequency of haplotypes per population is shown in Fig. WP3.4. Haplotype composition (in base pairs, ccmp 2 and ccmp 4) and haplotype frequencies are reported in Table WP3.7.

Fig.WP3.4

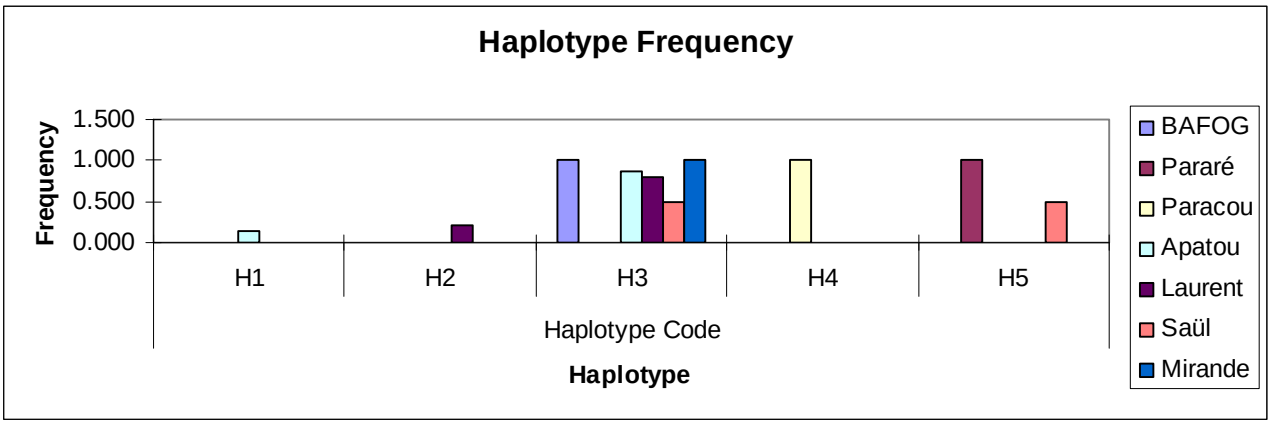
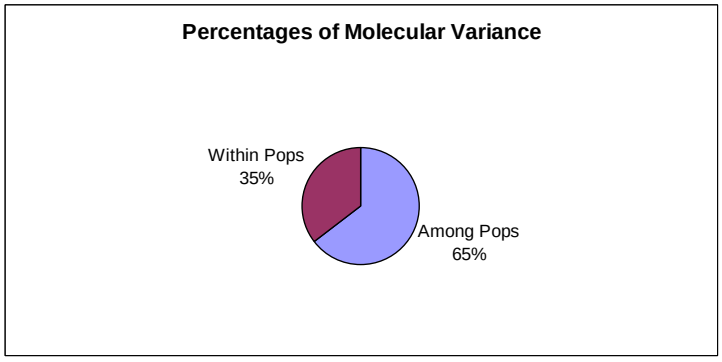


Table WP3.7

	BAFOG	Pararé	Paracou	Apatou	Laurent	Saül	Mirande
H1(205/187)	0.000	0.000	0.000	0.133	0.000	0.000	0.000
H2(205/188)	0.000	0.000	0.000	0.000	0.200	0.000	0.000
H3(206/187)	1.000	0.000	0.000	0.867	0.800	0.500	1.000
H4(206/188)	0.000	0.000	1.000	0.000	0.000	0.000	0.000
H5(207/194)	0.000	1.000	0.000	0.000	0.000	0.500	0.000

Analysis of molecular variance revealed a high differentiation among populations, being 65% of the total variance due to differences among populations (Fig. WP3.5).

Fig. WP3.5



5. *Virola sebifera* (CNR)
Ccmp 2, 3, 4, 6, 7, 10 amplified and ccmp3 was the only polymorphic one, displaying two haplotypes which allowed distinguishing the population of Iracoubo from the other populations of French Guyana.

6. *Jacaranda copaia*(CNR)
Primers ccmp1, 2, 3, 4, 5, 6, 7, 10 worked and none was polymorphic.

7. *Cordia alliodora* (CNR)
We analysed a total of 8 populations: 2 from Nicaragua, 2 from Honduras 1 from Costa Rica, 1 from Guatemala, one from Colombia and one from French Guyana. Ccmp 1, 3, 4, 6 were monomorphic, ccmp 2, 5, 7 and 10 showed 3, 3, 2, 2 variants respectively, combining into 9 different haplotypes. The frequency of haplotypes per population is shown in Fig. WP3.6 and in Table WP3.8.

Fig. WP3.6

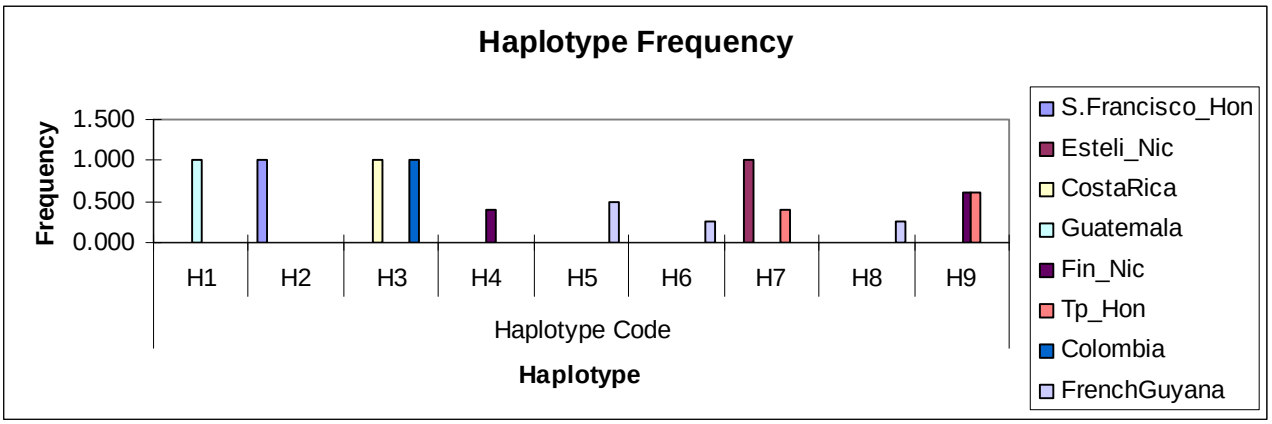
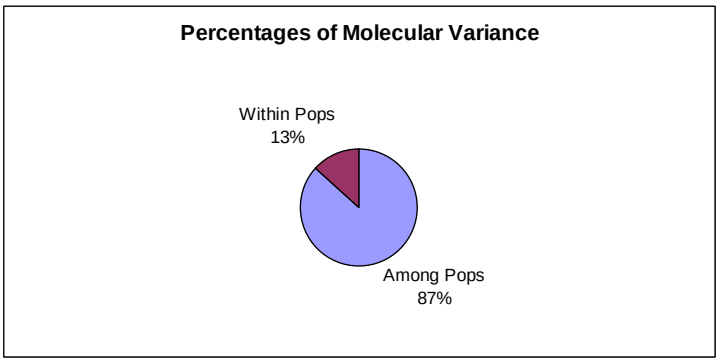


Table WP3.8

	S.Francisco Hon	Esteli_Nic	CostaRica	Guatemala	Fin_Nic	Tp_Hon	Colombia	French Guyana
H1	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000
H2	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H3	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000
H4	0.000	0.000	0.000	0.000	0.400	0.000	0.000	0.000
H5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500
H6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250
H7	0.000	1.000	0.000	0.000	0.000	0.400	0.000	0.000
H8	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250
H9	0.000	0.000	0.000	0.000	0.600	0.600	0.000	0.000

Analysis of molecular variance revealed that 87% of the total variance is due to differentiation among populations (Fig. WP3.7)

Fig.WP3.7



8. *Bombacopsis quinata* (CNR)

Two populations from Nicaragua, three from Costa Rica, one from Honduras and one from Colombia were analysed. Ccmp 1, 2, 3, 4, 6, 10 worked, but only ccmp6 was polymorphic producing two variants and two haplotypes (Fig. WP3.8). One haplotype was detected in Colombia, the other populations were fixed for the same haplotype except one population of Costa Rica where also the "Colombian haplotype" was detected. Haplotype composition (in base pairs, ccmp 6) and haplotype frequencies are reported in Table WP3.9.

Fig. WP3.8

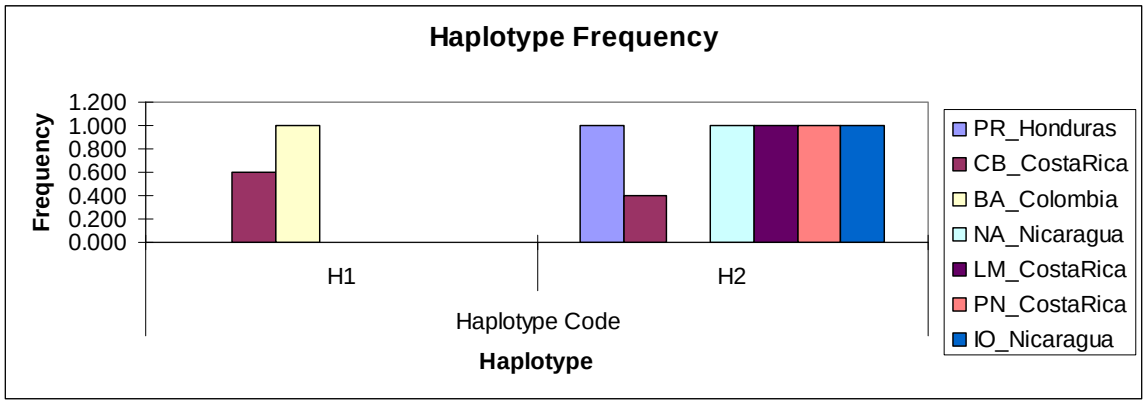
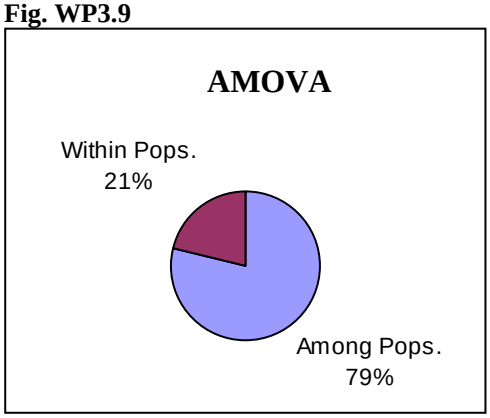


Table WP3.9

	PR_ Honduras	CB_ CostaRica	BA_ Colombia	NA_ Nicaragua	LM_ CostaRica	PN_ CostaRica	IO_ Nicaragua
H1(142)	0.000	0.600	1.000	0.000	0.000	0.000	0.000
H2(143)	1.000	0.400	0.000	1.000	1.000	1.000	1.000

Analysis of molecular variance revealed that 79% of the total variance is due to differences among populations (Fig. WP3.9).

Fig. WP3.9



9. *Swietenia macrophylla* (INPA).

PCR conditions were optimized for amplification of four non-coding regions of the chloroplast genome of *Swietenia macrophylla*. Table WP3.5 shows the cpDNA markers optimized and their characteristics.

TableWP3.5 – Characteristics of chloroplast non-coding markers analysed for *S. macrophylla.*

cpDNA Region	Sequence	T_A (ºC)	Fragment size (bp)	Reference
trnG - trnS	GAACGAATCACACTTTTACCAC GCCGCTTTAGTCCACTCAGC	56	700	Hamilton, 1999
psbA - trnH	CGAAGCTCCATCTACAAATGG ACTGCCTTGATCCACTTGGC	58	350	Hamilton, 1999
psbB - psbF	GTTTACTTTTGGGCATGCTTCG CGCAGTTCGTCTTGGACCAG	56	800	Hamilton, 1999
rps16_2xF-rps16_1x	GTGGTAGAAAGCAACGTGCGACTT TCGGGATCGAACATCAATTGCAAC	56	900	Oxelman *et al.*, 1997

10. *Bertholletia excelsa* (INPA).

From 19 non-coding markers of the chloroplast genome tested and optimized for phylogeography analysis in *B. excelsa*, reported previously, three were analysed here. We performed DNA sequencing of these regions for a total of 52 individuals from four populations of *B. excelsa*. Fig. WP3.10 shows the locations of the *B. excelsa* populations analysed so far for these chloroplast non-coding regions. A total of 52 individuals from four populations were sequenced for the three regions considering both directions (forward and reverse). No single polymorphism was found considering all individuals analysed for the three markers. Table WP3.6 shows the characteristics of the three markers analysed.

Table WP3.6 – Characteristics of chloroplast non-coding regions sequenced for individuals of *B. excelsa* from four populations in the Brazilian Amazon.

cpDNA region	T_A (ºC)	Fragment size (bp)
*rps*16	62	600bp
intron *trn L*	56	550bp
trn L - trn F	60	450bp

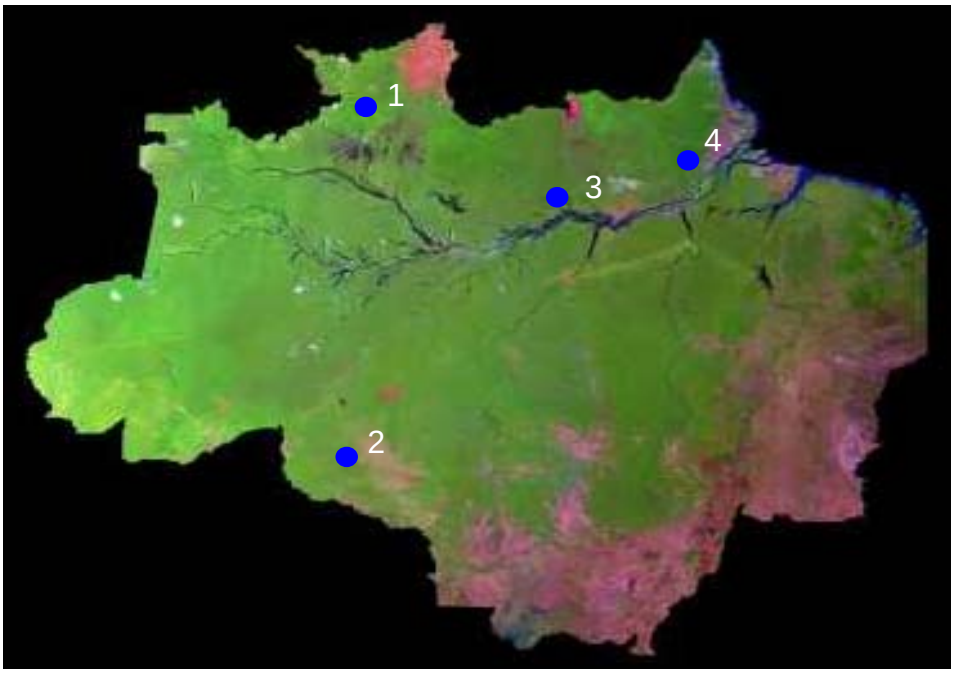


Fig. WP3.10 – Locations of four populations of *B. excelsa* - (1) PARES Serra do Aracá – AM, (2) Alto Jamarí – RO, (3) Igarapé Moura –FLONA Saracá Taquera – PA and (4) Laranjal do Jarí – AP

11. *Swietenia humilis* (CNR / INPA).

Ten cpDNA microsatellite markers (Weising and Gardner, 1999) were tested for *Swietenia humilis.* PCR conditions and annealing temperature were optimized for five out of 10 loci tested (Table WP3.7). Analysis of genetic diversity were carried out based on genotyping of 27 individuals of *S. humilis* collected in Honduras, Nicaragua, Costa Rica and El Salvador. All 27 individuals of *S. humilis* from different locations in Central America showed a single haplotype.

Table WP3.7 – Characteristics of chloroplast microsatellite loci optimized for *S. humilis*.

cpSSR locus	Sequence 5'-3'	Ta (ºC)	Fragment size (bp)
ccmp2	ATCGTACCGAGGGTTCGAAT FAMGATCCCGGACGTAATCCTG	56	211
ccmp3	GTTTCATTCGGCTCCTTTAT HEXCAGACCAAAAGCTGACATAG	56	104
ccmp4	CCAAAATATTBGGAGGACTCT TETAATGCTGAATCGAYGACCTA	56	123
ccmp5	AGGTTCCATCGGAACAATTAT FAMTGTTCCAATATCTTCTTGTCATTT	56	94
ccmp10	TTCGTCGDCGTAGTAAATAG HEXTTTTTTTTTAGTGAACGTGTCA	56	111

12. *Ceiba pentandra* (INPA).

Ten cpDNA microsatellite markers (Weising and Gardner, 1999) were tested for *Ceiba pentandra.* PCR conditions and annealing temperature were optimized for five out of 10 loci tested (Table WP3.8). Analysis of genetic diversity were carried out based on genotyping of 80 individuals from 10 populations of *Ceiba pentandra* (Tables 8, 9) at five cpSSR loci each.

Table WP3.8 – Characteristics of chloroplast microsatellite loci optimized for *Ceiba pentandra*

cpSSR Locus	Sequence 5'-3'	Ta (ºC)	Fragment size (bp)
ccmp 2	5'-GATCCCGGACGTAATCCTG-3' 5'-ATCGTACCGAGGGTTCGAAT-3'	56	139 - 141
ccmp 3	5'-CAGACCAAAAGCTGACATAG-3' 5'-GTTTCATTCGGCTCCTTTAT-3'	52	142 -146
ccmp 5	5'-TGTTCCAATATCTTCTTGTCATTT-3' 5'-AGGTTCCATCGGAACAATTAT-3'	54	104 -105
ccmp 6	5'-CGATGCATATGTAGAAAGCC-3' 5'-CATTACGTGCGACTATCTCC-3'	56	136 -138
ccmp 10	5'-TTTTTTTTTAGTGAACGTGTCA-3' 5'-TTCGTCGDCGTAGTAAATAG-3'	52	114 -117

For *Ceiba pentandra* 16 haplotypes were observed from 5 cpSSR loci over 80 individuals from 10 populations (Table WP3.9). The frequency of each haplotype is shown in Fig. WP3.11. AMOVA based on variation at five cpDNA microsatellite loci for 10 populations of *C. pentandra* showed equal partitioning of the genetic variatiion within (51%) and among (49%) populations. Median-joining analysis based on allele frequency revealed no clear pattern of phylogeographic structure for populations of *Ceiba pentandra* acroos its Neotropical range (Fig. WP3.12).

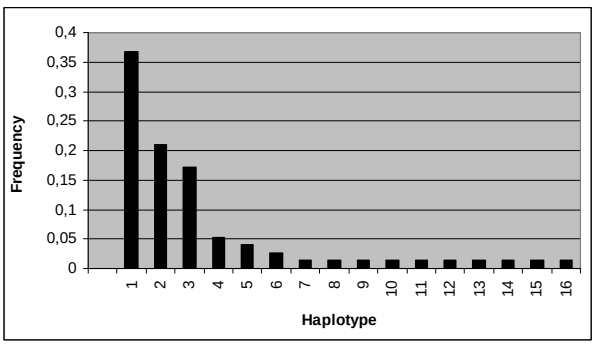


Fig. WP3.11 – Frequency of 16 haplotypes observed in 10 populations of *Ceiba pentandra* based on variation of five chloroplast microsatellite loci.

Table WP3.9– Chloroplast microsatellite haplotypes detected for 10 populations of *Ceiba pentandra*

Haplotype	Population	ccmp2	ccmp3	ccmp5	ccmp6	ccmp10	N
1	RP/A/RJ/RR/RM/AC/Manaus	140	143	104	137	116	28
2	A/RJ/R/RR/Manaus	140	143	105	137	117	16
3	RJ/AC/BC	140	143	104	137	115	13
4	Manaus	139	143	104	137	117	4
5	RP/RJ/BC	140	143	104	137	114	3
6	BC	139	143	104	137	114	2
7	RP	140	143	105	137	116	1
8	AC	140	143	105	137	115	1
9	RJ	141	143	104	137	117	1
10	PA	140	143	104	136	116	1
11	Manaus	140	142	104	137	116	1
12	Manaus	140	143	104	138	116	1
13	Manaus	139	142	104	137	117	1
14	Manaus	139	143	104	136	117	1
15	BC	139	143	104	137	115	1
16	RP	139	146	104	137	116	1

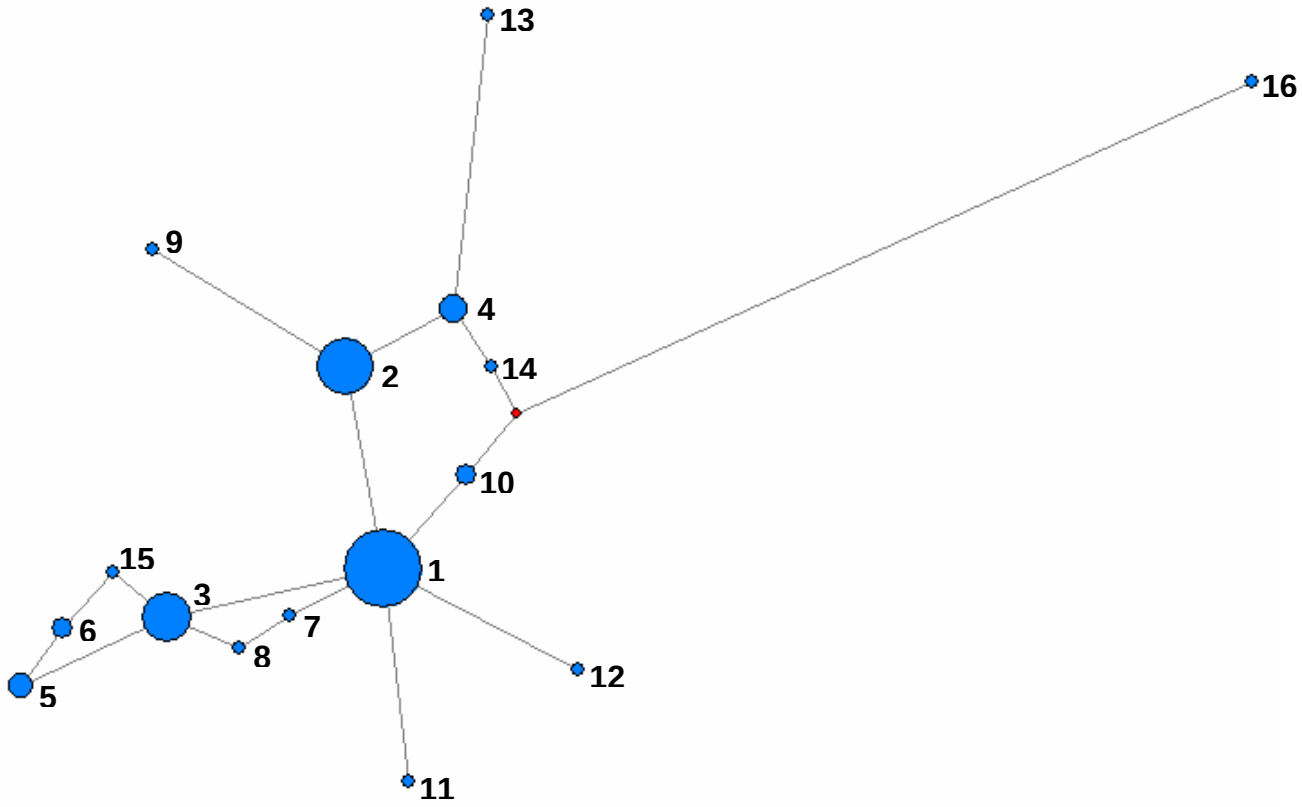


Fig. WP3.12 – Median-Joining network analysis based on 16 cpDNA microsatellite haplotypes observed for *Ceiba pentandra*. Numbers correspond to the haplotypes showed in Table WP3.9.

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

Progress of the work is dependent on the availability of samples (see WP1):

- The DNA extraction protocols from herbarium and wood samples have yet to be verified for all target species.
- The final choice among the seven cp fragments candidates for sequencing depends on the variability among populations
- The individual species studies will be able to start when all population from the whole distribution area will be available

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p58)

CORE AREA	**2**	**Diversity, reproductive performance & recruitment at the landscape scale**
Work Package	**4**	***Ensuring focus of quantitative and genetic studies***

1.Workpackage objectives and starting point of work at beginning of reporting period

- Ensure that criteria are agreed upon for design of sampling and experiments, and that response variables to be measured in order to characterize gene flow, genetic diversity, progeny performance and other response variables, are agreed upon by all partners in the project planning meeting.
- Prepare clear written guidelines for the research on the basis of agreements reached during the planning meeting, to be circulated to all partners and made available on the project website.
- Ensure that all WP leaders report regularly on progress and lessons learned to the leader of this CA, and that this information is shared among all those concerned.
- Try to make contact with other projects/institutes using similar genetic tools to quantify germplasm material.

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

WP4 functions as a coordinating activity and as such progress reported in WPs 5 & 6 represents output for WP4. See following WP summaries for progress.

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

None

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p58)

CORE AREA	**2**	**Diversity, reproductive performance & recruitment at the landscape scale**
Work Package	**5**	***Estimate partitioning of non-coding and coding genetic diversity***

1.Workpackage objectives and starting point of work at beginning of reporting period

- Development and optimisation of neutral and adaptive molecular DNA markers, with main emphasis on chloroplast and nuclear microsatellites and Single Nucleotide Polymorphism in candidate genes related to water stress responses and photoperiod induced phenology.
- Analysis of distribution of genetic diversity within species using neutral molecular markers
- Comparison of diversity in natural populations and in provenance trials for identifying hot spots of genetic diversity
- Analysis of diversity of candidate coding markers, and association between SNPs and Quantitative Traits Loci (QTL)

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved.

Development of SNP markers (CNR / INRA / INPA)
a.) Aquaporins (CNR/INRA)
A pair of universal primers for aquaporin, designed and provided by INRA (Ivan Scotti), was tested in six different species: *Cedrela odorata*, *Carapa guianensis*, *Bombacopsis quinata*, *Schizolobium parahyba*, *Virola sebifera*, *Ceiba pentandra*. The amplified products were cloned into plasmid vector and then sequenced from both ends using a *MegaBace* 1000 capillary sequencer.

The following sequences were performed:

- 44 sequences for *Cedrela odorata;*
- 46 sequences for *Carapa guianensis*;
- 46 sequences for *Bombacopsis quinata;*
- 34 sequences for *Ceiba pentandra*;
- 40 sequences for *Schizolobium parahyba*;
- 43 sequences for *Virola sebifera*.

The sequences of the six species were sent to Partner 3 (Ivan Scotti) to design gene-specific primers (Table WP5.1). Partner 3 designed an additional set of four primer pairs for *Eperua falcata*.

These primers were tested in all selected species, two samples each (except those of *Carapa guianensis*, tested by Partner 3). PCR was performed in a 12,5µL reaction volume containing 10 ng of DNA, 1×PCR buffer (Promega), 0,2 µM each primer, 0,2 mM each dNTP, 2,5 mM $MgCl_2$, 1 U Go *Taq* polymerase (Promega), 0.8% BSA, using the following thermal profile: initial denaturation at 95°C for 5', 30 cycles at 95°C 30'', 59°C 30'', 72°C 30''; final extension at 72°C for 7'.

Table WP5.1

Species	Name	Product Size (bp)	Primer length (bp)	Primer sequence 5' -> 3'		tm
Bombacopsis	BquPIP2.1+0017	657	20	F	GCCGGTATCTCTGGTGAGTG	60.68
	BquPIP2.1-0672		20	R	CCACGCCTTCTCTTTGTTGT	60.29
Bombacopsis	BquPIP2.2+0029	601	20	F	GGTTGTTTTTGGCCCGTAAG	61.59
	BquPIP2.2-0629		19	R	CGACCCAGAAGACCCAGTG	61.70
Carapa	CprPIP1.1+0018	763	19	F	CGGCATTTCAGGTCATCTC	59.18
	CprPIP1.1-0780		20	R	CCAACCCAGAAAATCCAGTG	60.34
Carapa	CprPIP2.1+0009	658	20	F	CTGCACAGCCGGTATCTCTG	61.95
	CprPIP2.1-0666		20	R	GATCATCCCAAGCCTTGTCC	61.78
Carapa	CprPIP1.2+0014	622	22	F	GCTTTGATGTGTTATTGCCTGA	58.53
	CprPIP1.2-0635		21	R	GAAAACGATGAAAAGGCTTGC	59.47
Cedrela	CodPIP2.1+0115	679	22	F	TGTGTAGGTGGGCACATTAACC	61.93
	CodPIP2.1-0792		20	R	CCCAGAAGATCCAGTGCAAC	60.66
Cedrela	CodPIP2.2+0028	670	21	F	GGTAAGTCCCAAACGGCAAAC	61.19
	CodPIP2.2-0697		20	R	GATCATCCCATGCCTTGTCC	61.01
Cedrela	CodPIP2.3+0120	697	18	F	GGCGCGTTTTGCTAACTG	60.54
	CodPIP2.3-0816		20	R	CCGACCCAGAAGATCCAGTG	62.98
Ceiba	CpePIP2.1+0106	608	22	F	GTTGTTTTGGTCACAGGTGGTC	61.63
	CpePIP2.1-0713		22	R	CAACGAGGGTTATACGAGGAAC	59.89
Ceiba	CpePIP2.2+0014	609	20	F	CAGCCGGTATCTCAGGTCAG	60.81
	CpePIP2.2-0804		20	R	CCCAGAAGATCCAGTGCAAG	60.79
Schizolobium	SpaPIP1.1+0006	696	19	F	CTACTGCACAGCCGGCATC	62.95
	SpaPIP1.1-0701		19	R	GATCGTCCCAGCCTTTGTC	60.62
Schizolobium	SpaPIP1.2+0016	757	17	F	GCCGGCATCTCAGGTTC	60.32
	SpaPIP1.2-0772		18	R	GCCACGCGTCCAGAAATC	62.79
Schizolobium	SpaPIP2.1+0009	658	19	F	GGTGACGTTTGGGTTGTTC	58.81
	SpaPIP2.1-0666		22	R	CACCACATCACATAAACACCTC	57.85
Virola	VsePIP2.1+0032	757	21	F	CGCGTATCTCTCTCTTCAACG	60.16
	VsePIP2.1-0788		19	R	CACACGCACACACACAATG	59.11

Results are shown in Fig.WP5.1.

- Primers for *Ceiba pentandra* CpePIP2.1 and CpePIP2.2 didn't amplify in this test: they were successively tested using more template and they generated weak amplification products (750bp and at 500bp respectively) at both 59°C and at 57°C (data not shown) (we think that the faintness of the bands is probably due to low quality DNA).
- Primers for *Cedrela odorata* CodPIP2.2 and CodPIP2.1 produced a strong single band each, while CodPIP2.3 displayed a weak band which appeared stronger decreasing PCR annealing temperature to 57°C.
- Primers for *Schizolobium parahyba* SpaPIP1.1, SpaPIP1.2 and SpaPIP2.1 produced a strong single band of the expected size.
- Primer pair for *Bombacopsis quinata* BquPIP2.1 generated a single strong band of the expected size, while BquPIP2.2 didn't amplify neither at 59°C nor at 57°C and at 55°C it produced a three band pattern.
- The primer pair for *Virola sebifera* VsePIP2.1 produced a single strong band of the expected size at 59°C (Fig. WP5.2).

Fig. WP5.1

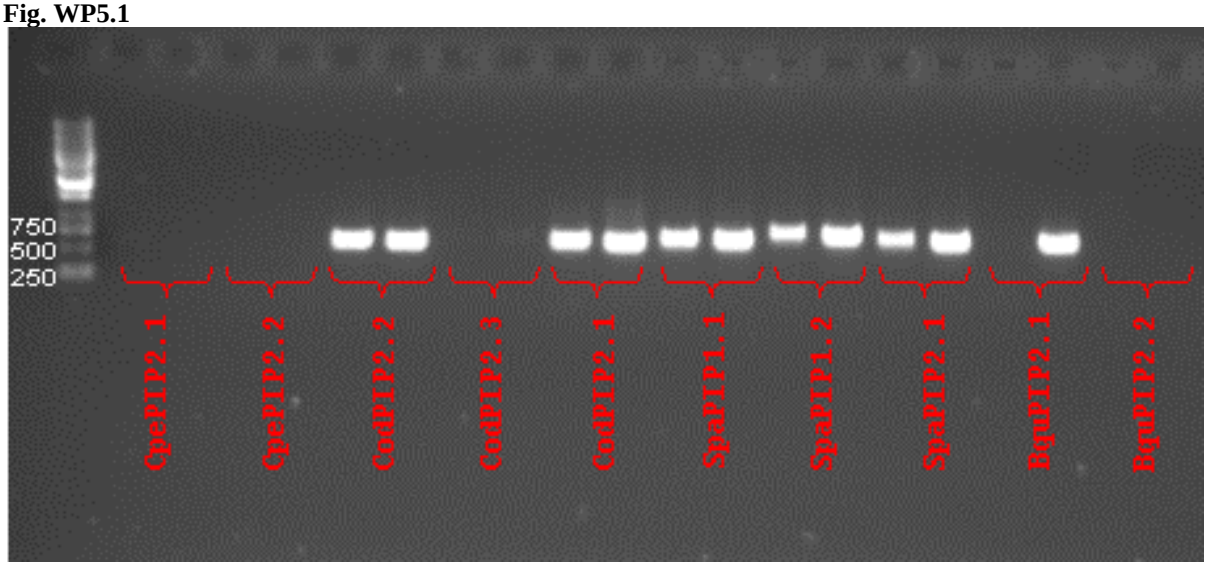
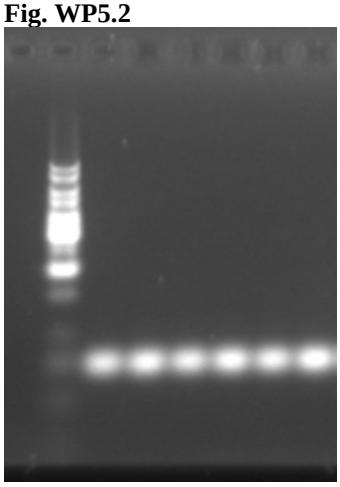


Fig. WP5.2



All 42 available samples of *Virola sebifera* were amplified with VsePIP2.1 using the following cycling conditions: initial denaturation at 95°C for 5', 30 cycles at 95°C 30'', 64°C 40'', 72°C 1'; and final extension at 72°C for 7' and sequenced from both ends. Sequences were aligned and screened for SNPs using the program CodonCodeAligner. Four SNPs were detected. Screening of nucleotide diversity within and among populations of *Bombacopsis quinata* and *Carapa guianensis* is in progress (OFI and INRA, respectively)

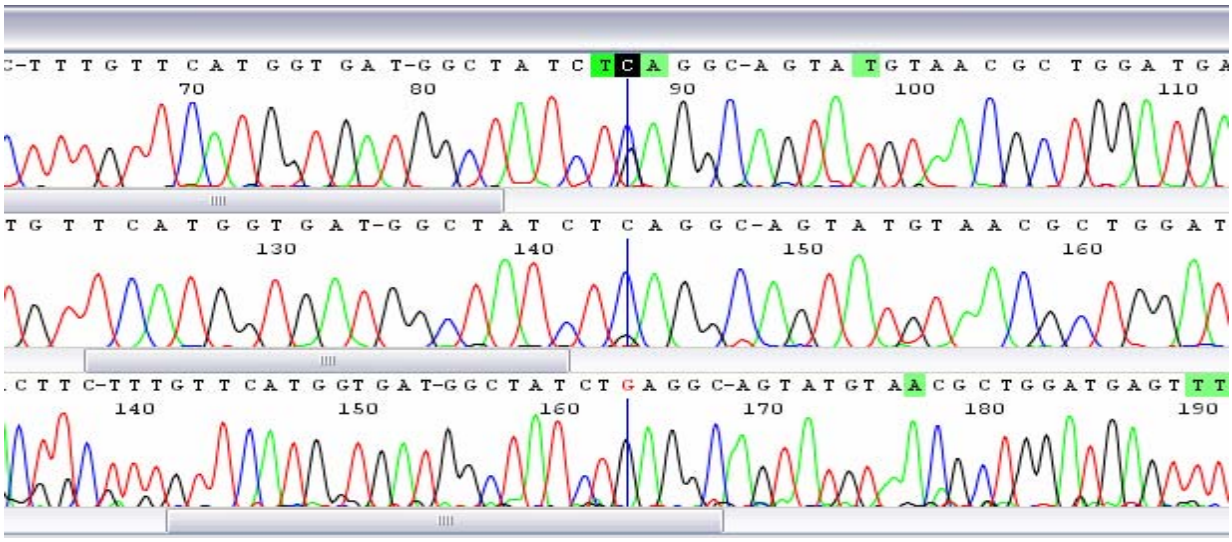


Fig.WP5.3 Example of a SNP detected in samples of *Virola sebifera*

b.) Preliminary analysis of polymorphisms at the nuclear gene *PepC* in *Swietenia macrophylla* (INPA).

Construction of genomic libraries - In order to verify the presence of single or multiple copies in the fourth intron of the *PepC* nuclear gene in *Swietenia macrophylla*; PCR products were cloned using the cloning system PROMEGA pGEM T-Easy (PROMEGA, 1999). For the construction of the libraries four ligations were performed using DNA of four individuals of *S. macrophylla* (two from different pops from the Brazilian Amazon and two from Central América*).* After transformation competent cells were grown in LB medium (peptone 1%, yeast extract 0.5%, NaCl 1% and agar 1.5%) with ampicillin and incubated at 37 C overnight. Colonies were collected and diluted in ultrapure water and denatured at 94 C for 3 min. Miniprep was performed for plasmid DNA extraction. Plasmid DNA was amplified using *M-13* (-20) forward and *M-13* reverse primers. **Sequencing** – PCR products were purified using ExoI (Exonuclease I) and SAP (Shrimp Alkaline phosphatase) enzymes and precipitated using ethanol 95% and $MgCl_2$ 2mM. The amplified products were sequenced using Big Dye Terminator III (ABI, Inc) following the manufcaturer´s instructions in a DNA ABI 377 Sequencer (ABI, Inc). **Analysis of SNP´s (Single Nucleotide Polymorphisms)** – The sequences were edited and aligned using Chromas and Bioedit softwares. The analysis of the sequences allowed the identification of three distinct *PepC* loci with 480, 466 and 407 bp showing high polymorphism (Table WP5.2). Fig. WP5.4 shows a SNP detected at position 190 of the *PepC* locus (407 bp). Upper panel shows a heterozygote individual (CG), lower shows a homozygote (CC).

Table WP5.2 – *PepC* loci, polymorphisms and number of heterozygotes obtained by analysis of clones from four individuals of *S. macrophylla* after construction of genomic libraries.

***Locus* PepC**	**No. of polymorphisms**	**No. heterozygotes**
407 pb	11	03
466 pb	24	04
480 pb	13	02

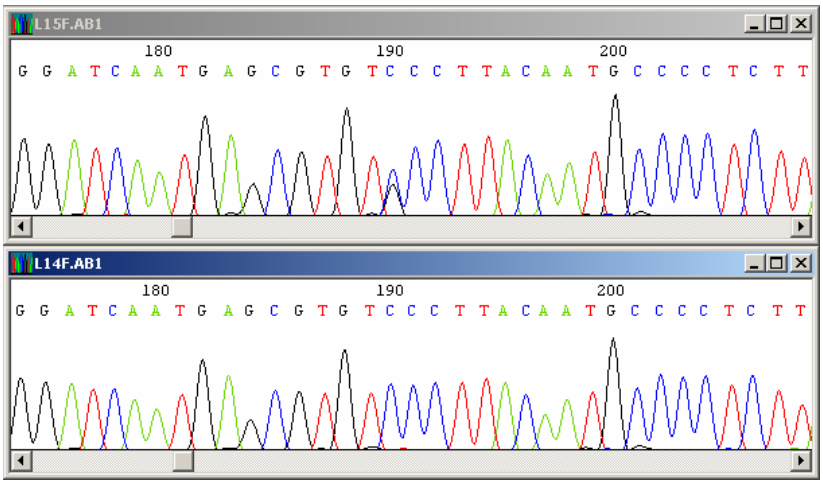


Fig. WP5.4 – Example of single nucleotide polymorphism (SNP) detected in the fourth intron of the nuclear gene *PepC* in *S.macrophylla*.

Development of nuclear microsatellite markers (CNR / CEH / UFRGS / INRA / INPA)

Cedrela odorata (CNR / CEH)
24 primers were designed using the software Primer 3 and sent to Participant 1 (Stephen Cavers) for testing them for quality and polymorphism. Nine gave clear, interpretable band patterns and were polymorphic. Polymorphism was tested in 487 individuals belonging to 12 populations distributed across Mesoamerica. The results are reported in a paper which is published online in Conservation Genetics (Hernandez G., Buonamici A., Walker K., Vendramin G.G., Navarro C. and Cavers S. (2007) Isolation and characterization of microsatellite markers for *Cedrela odorata* L. (Meliaceae), a high value neotropical tree).

Hymenaea courbaril (CNR)
From the library developed during the first year, ten primers were designed but none of them worked. For this reason a new library enriched only in GT and GA repeats was constructed: 6 primers were tested, 5 worked, but only 1 was polymorphic (but only six individuals are available for testing for variation). Another library was prepared using filter containing only tri-nucleotides and its screening is in progress.

Ochroma pyramidale (CNR)
From the library enriched with di, tri and tetra-nucleotides 10 primers were designed and three gave amplification products of the expected sizes: no variation was detected using these three primer pairs (but only ten individuals are available for testing for variation). A library enriched in GT and GA repeats was constructed also for this species: 12 primer pairs were tested, 4 worked, and 1 displayed variation. Also in this case a library enriched in trinucleotide repeats was prepared and its screening is in progress.

Minquartia guianensis (CNR)
A library enriched for di- (GA, GT, AT, GC), tri- (CAA, ATT, GCC) and tetra-nucleotide (CATA, GATA, ATAG) of *Minquartia guianensis* was constructed according to Edwards *et al*. (1996). Ten primers were designed and 5 were tested so far: 4 worked and the test for their quality and polymorphism is in progress.

Schizolobium parahyba and *Drimys* (CNR / UFRGS)
Two libraries enriched for di- (GA, GT, AT, GC), tri- (CAA, ATT, GCC) and tetra-nucleotide (CATA, GATA, ATAG) of *Schizolobium parahyba* and *Drimis* were constructed according to Edwards *et al*. (1996). For *Schizolobium parahyba,* 14 primer pairs were designed and sent to Rogerio Margis (Partner 8) who is testing them for their quality and variation. For *Drimys,* 200 clones are available to be sequenced and ready to be sent to Partner 8.

Simarouba amara / Virola sp. (INRA)
Enriched libraries were constructed for *Simarouba amara* and 3 *Virola* species. The primer pairs are currently testing in order to assess the level of polymorphism.

Bertholletia excelsa (INPA)
Ten nuclear microsatellite loci developed for *Bertholletia excelsa* (Collevatti et al. *in prep*) were tested for optimization of the PCR conditions and characterization of the loci. The annealing tempreatrue and other PCR conditions were optimized for eight out of 10 nuclear microsatellite loci tested.

Mapping the distribution of neutral diversity within and across natural populations

Preliminary studies of the distribution of the neutral diversity are currently being undertaken at for French Guianan populations for 3 species : *Carapa guianensis*, *Simarouba amara* and *Jacaranda ccopaia*. (INRA). Screening is in progress for Central American populations of *Cedrela odorata* using the newly developed microsatellites (CEH).

Screening and characterization of nuclear microsatellite loci developed for *Bertholletia excelsa* is in progress (INPA) – an example of the amplification of loci BEX-01 and BEX-02 is shown (Fig. WP5.5). Analysis of genetic diversity of one population of *B. excelsa* (Rio Purus, Brazilian Amazon) based on six nuclear microsatellite loci is shown in Table WP5.3.

Table WP5.3 – Genetic diversity in a natural population of *B. excelsa* from the Brazilian Amazon based on genotyping of 34 individuals at six nuclear microsatellite loci.

SSR Locus	n	A	He	Ho
BEX01	34	11	0.89	0.97
BEX02	34	3	0.57	0.65
BEX12	34	8	0.74	0.76
BEX22	34	3	0.47	0.35
BEX32	34	2	0.43	0.44
BEX37	34	8	0.78	0.65
Mean	**34**	**5.8**	**0.65**	**0.64**

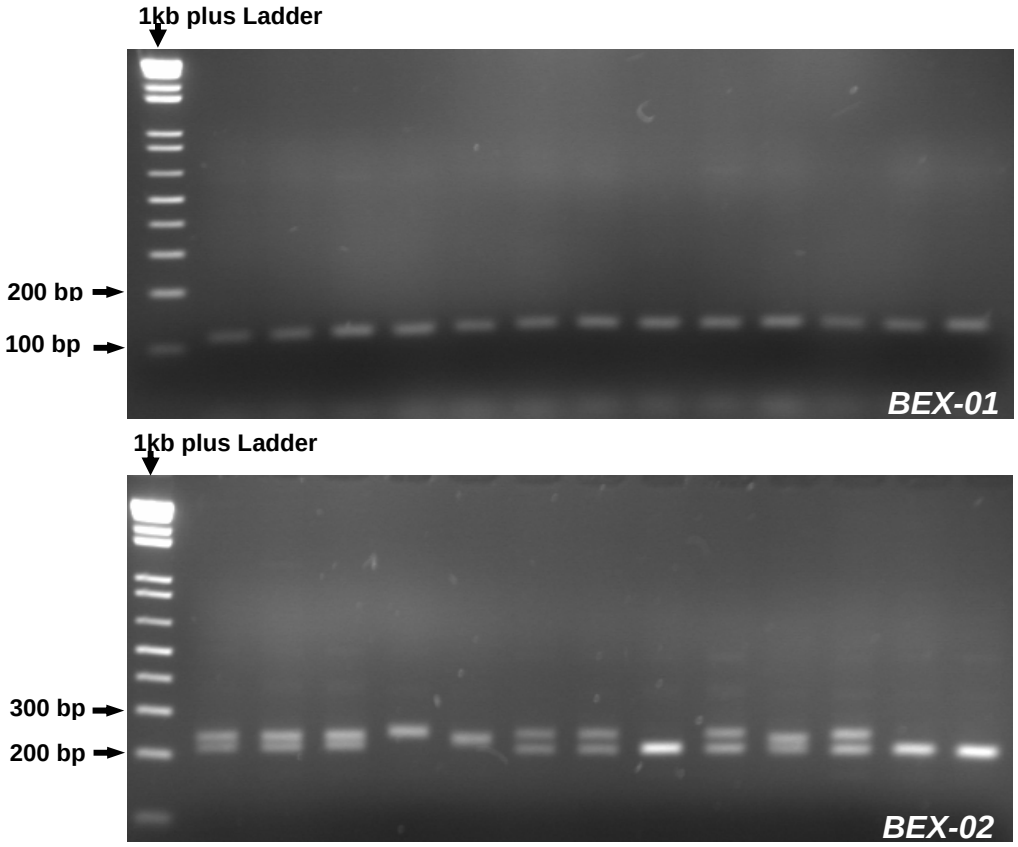


Fig. WP5.5 – Amplification of nuclear SSR loci *BEX-01* and *BEX-02* for *Bertholletia excelsa*.

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

Some delays in screening species for microsatellite and SNP variation have been experienced due to the lack of complete collections. For most species sufficiently large collections should be in place by the time of the annual meeting and full scale screening can begin.

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p58)

CORE AREA	**2**	**Diversity, reproductive performance & recruitment at the landscape scale**
Work Package	**6**	***Gene dynamics and quantitative seed performance in relation to landscape***

1.Workpackage objectives and starting point of work at beginning of reporting period

- Using polymorphic molecular markers compare the level of inbreeding for seeds, seedling recruits and adult trees for populations of three selected species occurring in primary forests and different man-made landscapes.
- Determine how landscape changes may affect the demography and the pollination system and their consequences for the mating system parameters.
- Determine how changes in local landscapes may affect the seed/fruit output of remnant adult trees and the population's recovery capacity.
- Relate how the mating system may contribute to shaping and maintaining any genetic structure of the populations
- Describe the implications of habitat disturbance and degradation on the long term conservation and management of selected tropical tree species
- Assess the impact of the mother trees local environment on seed set and progeny performance
- Assess the performance of selfed progeny relative to outcrossed progeny in species that show mixed mating
- Assess the impact of pollination distance on the quality of seed

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

Following the plot and sampling design outlined in the previous report, the following progress has been made during the last reporting period:

Jacaranda copaia, Minquartia guianensis, Carapa guianensis, Simarouba amara, Virola sebifera, *Dipteryx panamensis* (CATIE)

Flowering observations and field sampling (Bryan Finegan, Carlos Navarro and Carolina Cascante)

As predicted in last year´s report, the greater part of the work involving location of mother trees, phenological studies, seed collection and production of seedlings is being undertaken during the period July 2006 – December 2007. Due to the highly variable individual-tree phenology and erratic and usually low seed production of *Vochysia ferruginea* during this time period, it was decided to exclude this species from the study, and it was replaced with another light-demander, *Dipteryx panamensis*. The definitive list of study species and their characteristics is shown in the Table WP6.1 below.

Table WP6.1: Study species and ecological characteristics most relevant to the research.

Species and guild	Family	Sexual system	Seed dispersal	Seed viability	phenology	
					flowering	fruiting
Non-pioneer						
Carapa guianensis	Meliaceae	Monoecious	Terrestrial vertebrates	Recalcitrant	Jan-Apr	Mar-Sep
Minquartia guianensis	Olacaceae	Hermaphrodite	Flying and arboreal vertebrates	Recalcitrant	irregular	Feb-Apr
Virola sebifera	Myristicaceae	Dioecious	Flying and arboreal vertebrates	Recalcitrant	Jan-Mar	
Pioneer						
Dipteryx panamensis	Fabaceae	Monoecious	bats	recalcitrant	Being determined	Being determined
Jacaranda copaia	Bignoniaceae	Not yet determined	wind	orthodox	Aug-Sep	Jun-Aug
Simarouba amara	Simaroubaceae	Dioecious	Flying and arboreal vertebrates	recalcitrant	Oct-May	Nov-Apr

Sites for the planting experiment were identified during the year. These are all located on a single private property, La Ladrillera (see the 2006 report). Areas in which seedlings will be planted are on two soil types, Ultisols associated with hilly topography and Inceptisols formed on alluvial terraces. On each soil type, seedlings will be planted in both pasture and adjacent forest fragments. For each site, pH, acidity and cations were determined from soil samples.

Seedlings of four species – *Dipteryx panamensis, Minquartia guianensis, Simarouba amara* and *Virola sebifera*, are currently in the nursery set up close to the field site, and planting will begin during the coming weeks.

The following partners are ready to conduct genetic analysis for the following speices, once seed and leaf material are supplied by CATIE: Jacaranda copaia *(CNR)* Minquartia guianensis *(UoA)* Carapa guianensis *(INRA)* Simarouba amara *(INRA),* Virola sebifera *(UoA)* Dipteryx panamensis *(CNR/UoA). A student Melita de Vries has been appointed at the University of Adelaide to conduct this analysis, and CNR has developed additional SSR primers for* Minquartia guianensis *as part of WP5 which will be additionally be used in these studies.*

Carapa guianensis and *Jacaranda copaia* (PUCE)
In Ecuador, work was started on *Carapa guianensis*, a species restricted to the Pacific coast (Bilsa Bilogical Reserve) and *Jacaranda copaia*, a species restricted to the Amazon region (Yasuni Scientific Station).

Experimental populations

According to WP6 original design, experiments should include species with populations found in mature natural forest and one of the following disturbed environments: isolated pasture trees, fence lines, clumped pasture trees or populations that have different densities in natural forests. For *Carapa guianensis*, we selected 21 trees located in secondary forest and pastureland and 31 trees located in an adjacent primary forest (Table WP6.2). For *Jacaranda copaia* we selected 22 trees located in secondary forest (road side) and 24 trees located in an adjacent primary forest.

Table WP6.2: Size of selected trees of *Carapa guianensis* (N=52) at Bilsa Biological Station (BBS), located in the pacific cost of Ecuador and trees of *Jacaranda copaia* (N=46) at Yasuni Scientific Station (YSS), located in the Amazon lowlands of Ecuador.

	Height (m)			dbh (cm)		
Carapa guianensis (BBS)	mean	min.	max.	mean	min.	max.
Primary forest (N=31)	23.2	16	30	60.8	31	75
Secondary forest (N=21)	20.5	13	31	62.1	40	80
Jacaranda copaia (YSS)						
Primary forest (N=24)	23.5	20	30	50.5	32	90
Secondary forest (N=22)	20.4	15	32	41.7	28.5	82

Bilsa Biological Reserve is located in the eastern slopes of Mache-Chidul Ridge, in Esmeraldas province (00°21'N 79°44'W; between 400 and 600 m altitude). It is a 3000-ha private reserve managed by Jatun Sacha Foundation. Bilsa is adjacent to Mache-Chindul Ecological Reserve. The climate is tropical dry with an annual rainfall of 2000 mm and a dry season of six months, between July and December. Although the dry season is extended, the reserve is constantly covered in fog and mist during dry months. The average temperature is 25 °C (per. com. Felipe Arteaga; Clark, 1997). Access by a logging road is restricted to foot, horse, or mule during wet season. Vegetation is classified as tropical wet and semi-deciduous forest. The canopy is 35m high and dominated by species of *Otoba gordonifolia* and *Carapa guianensis* (Clark, 1997).

Yasuní Scientific Station is located in Yasuní National Park (YNP), in the Amazon lowlands of Ecuador (00º41'S 76º24'W; 200 m altitude). YNP and the adjacent Waorani Territory constitute the largest protected area in continental Ecuador, with 1.6 millions of ha (Valencia 2004). The climate is tropical wet with an annual rainfall of ~3000 mm and no dry season. Between April and August rains are less frequent and less intense, however, most of the time the weather is warm and humid, where neither rain or the temperature mark a defined season. Vegetation is classified as Tropical Rain Forest, the canopy is 45 m high. The floral composition is represented for a few common species numerously represented and many rare species, very rare to be found (Pitman, 1999 , Sánchez et al., 2002).

Notes on the taxonomy

Carapa guianensis. In March 2007, David Kempfak, who is revising the entire genus *Carapa* as part of his PhD thesis at the University of Saint Louis, Missouri, visited Ecuador and analysed the project samples and observed the species in the field. According to Kempfak, there are not reliable records of *C. guianensis* in Ecuador. Although exsiccate of *C. guianensis* determined by other specialists are deposited in different herbaria of Ecuador, it seems like there is a mixture of three different species among the specimens: two new species and *C.*

nicaraguensis. According to Kempfak we are working with one of those new, undescribed species.

Jacaranda copaia is a fast-growing tree that reaches 30 m in height and 90 cm dbh; its leaves grow in terminal bundles, erect on top of the crown. The tree grows without much demand for nutrients (Nieto y Rodríguez, 1994). Is a light-demanding pioneer tree that requires large tree fall gaps to regenerate. Seeds of *J. copaia* are non-dormant and do not forms a persistent seed bank in the soil. The pale purple and tubular flowers appear at the end of the tree; the inflorescence can be present for a month (Gentry 1974). The fruit is a dehiscent capsule that have 260 seed approximately; the seed are small (mass < 2 mg) (Dalling et al. 2002).

Phenological patterns

Starting in late March 2007, a thesis student, María Dolores Proaño, is following phenological patterns of *C. guianensis* in Bilsa. She will follow flower and fruit production once a month for one year. The first two surveys (in April and May) suggest that trees of *C. guianensis* produce flowers and fruits asynchronally. In two surveys, only one of the 52 trees had 2 mature fruits that were collected to start the experiments (Table WP6.3). Collection of fruits is incomplete.

Table WP6.3: Reproductive stage of 52 trees of *Carapa guianensis* monitored at Bilsa Biological Station. Trees might have immature and mature fruits at the same time.

Status	March-April	April-May
Infertile	40	43
Flowers	5	5
Inmature fruits	2	3
Mature fruits	0	1
Fallen fruits	4	0
Not found	1	1
Total	**52**	**52**

Jacaranda copaia is monitored by Daniela Cevallos Garzón who is doing a Licenciado thesis with this species. Phenological monitoring started in October 2006 in Yasuní. The first three surveys (October, 2006 February and April 2007) suggest that trees of *J. copaia* produce flower and fruits synchronically. According to information of herbarium samples, *J. copaia* flowers from July to January and fruits in February, March, and August (see Fig. WP6.1).

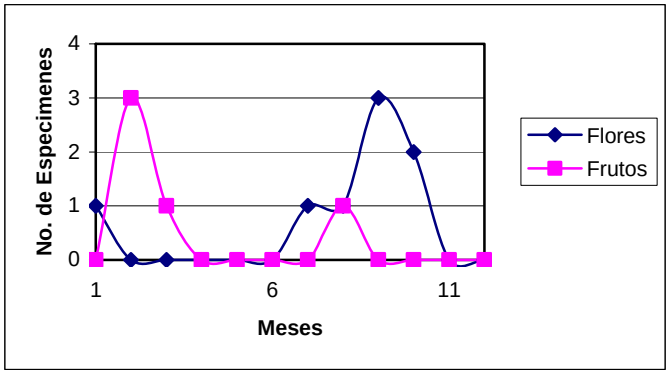


Fig. WP6.1: Flower and fruit production of *Jacaranda copaia* in YSS, Ecuadorian Amazonia (n = 31 herbarium samples QCA, MOBOT).

Size and weight of Jacaranda copaia fruits and collection of seeds

Seed collection of *J. copaia* was completed in April and the experiments will start soon. We plan to germinate seeds in a growing house then we will carry out garden experiments in the field. A summary of fruit sizes and weight is presented in the Table WP6.4 below.

Table WP6.4: Fruit size and weight of *Jacaranda copaia* collected in the surroundings of the Yasuni Scientific Station.

	Size									Weight (g)		
	Length (mm)			Wide (mm)			Thickness (mm)					
	mean	min.	max.	mean	min.	max.	mean	min.	max.	mean	min.	max.
Primary forest (N=24)	81.3	60.0	103.0	51.2	39.6	63.0	14.2	10.2	18.2	12.9	7.5	21.2
Secondary forest (N=22)	85.7	70.2	108.2	51.0	45.2	57.6	14.1	11.6	17.1	12.0	8.3	19.6

Symphonia globulifera (INRA)

Sampling mother tree :

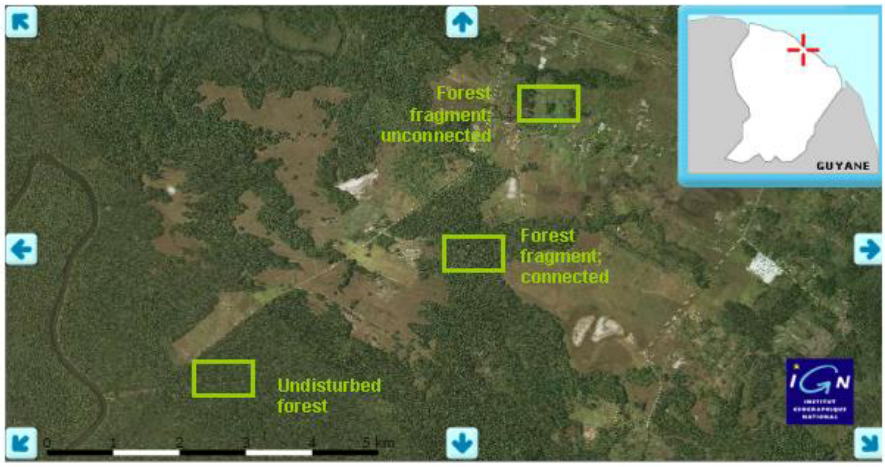
A zone, where the distribution of *Symphonia* shows a gradient of density, was delimited (see Fig. WP6.2). We then randomly selected mothers in the high and low density zone. Cambium is collected and the following informations are recorded: Exact GPS position, DBH, Distance to nearest 3 trees of same species, Foliage projective cover (canopy density) around tree, Basal area around tree, Fruit output

Sampling progeny arrays

Until now, between 20 and 40 seeds have been already collected on 5 mothers. Since the fructification is heterogeneous in time, the mother trees are observed each week. Collected seed material is planted in green house and will be raised for at least 6 months. The following set of characters will be noted for each progeny: Proportion of germination, Mortality, Regular (monthly?) growth measurement (height, diameter)

Fig. WP6.2

Choice of sampling sites for the study of landscape effects



Bombacopsis quinata (OFI)

David Boshier and Paul Rymer reviewed existing seed collections of *B. quinata*, from mapped trees in dry forest, pasture and fence lines and for use in studying mating system, gene flow and vigour. The collections were tested for viability using tetrazolium and germination tests. Despite tetrazolium tests indicating viability up to 70%, no germination was obtained and thus it will not be possible to look at vigour as part of the study. Carlos Navarro's group at CATIE attempted to carry out new seed collections from the same pasture and forest trees to allow a comparison between years and also allow the study of vigour in these species.

Paul Rymer carried out DNA extraction, PCR, sequencing and preliminary scoring for 24 seed from 20 trees in each of two populations in Costa Rica (dry forest - Lomas Barbudal Biological Reserve, pasture – Stewart Property). Results will be analysed in June/July 2007 for presentation at the ATBC meeting. Extraction of DNA from the seed coat was also tested as an aid to genotyping a greater number of parent for carrying out paternity analysis. Genomic DNA was visualised, but has yet to be genotyped to confirm alleles.

An existing trial of *B. quinata* in Honduras was thinned and controlled pollinations were carried out in Feb/March 2007. The trial is 10 years old and presents a unique opportunity to carry out F_2 generation crosses to examine the existence, scale and nature of outbreeding depression in this species. The scale of crosses was limited by poor flowering in the species, but some 150 crosses

Bertholletia excelsa (INPA)

The activities described below are part of a broader project entitled "Implantação e Caracterização Genética de um Banco de Germoplasma de Castanheira-do-Brasil (Bertholletia excelsa, Lecythidaceae) na Floresta Nacional de Saracá-Taquera, Municipio de Oriximiná, Pará". The project main goal is to establish a germplasm bank in the Saracá – Taquera National Forest in the Pará State (Central Amazon) that aims to preserve, characterize and quantify the genetic variability of the Brazil nut (*Bertholletia excelsa*). The project represents a partnership between Mineração Rio do Norte (MRN), the Brazilian Environmental and Renewable Resources Institute (IBAMA), and the National Amazon Research Institute (INPA). The Seedsource project has been partially supporting the genetic analysis of Bertholletia excelsa at INPA and the field expeditions for collection of genetic samples.

Fig. WP6.3

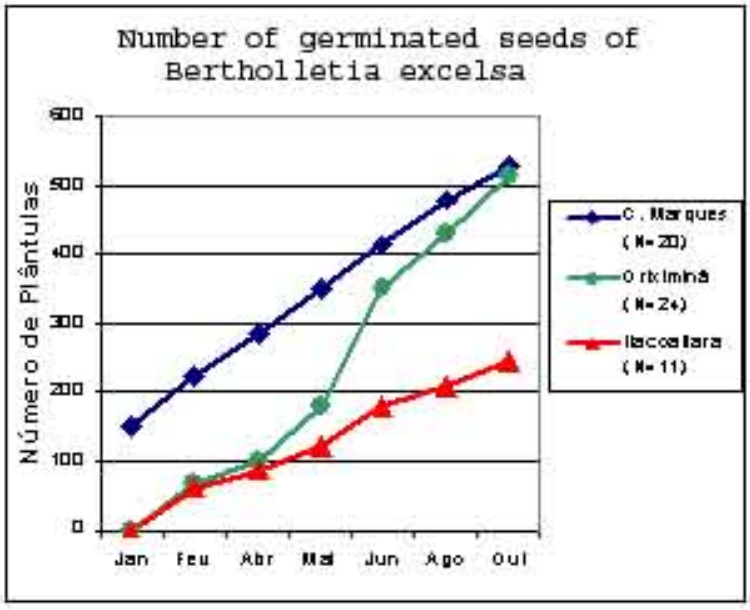
Pre-germination treatment of seeds

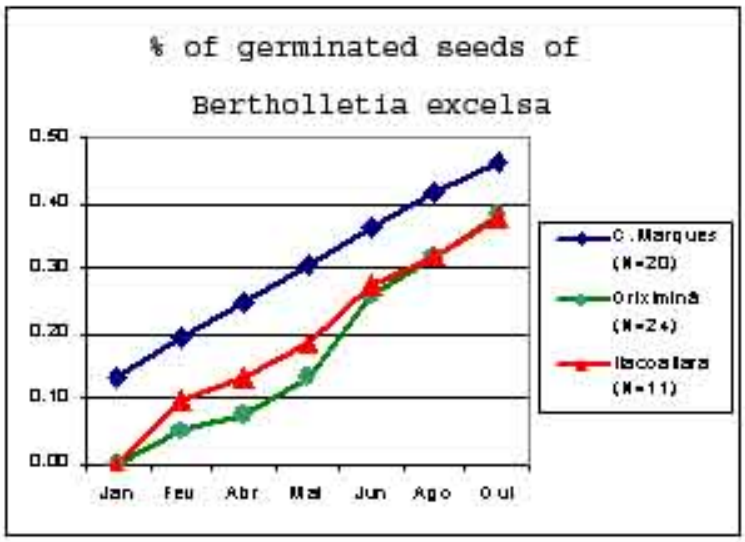
Germination in *B. excelsa* is delayed by the hard seed coat. Thus, seeds of *B. excelsa* were scarified to facilitate germination and also to ensure more synchronous germination. Scarification consisted in the removal of a shallow layer of the wood seed coat using a "guillotine" made with a machete attached to a wood platform (see Fig. WP6.3 above). The scarification should not go right through the wood coat reaching the endosperm, which usually cause fungi infection followed by the dead of the embryo.

Seed germination

The seeds of B. excelsa germinate slowly and unevenly. Scarified seeds start to germinate two months after sowing, but the last seeds germinate until 2-3 years later. This asynchrony results in a poor uniformity in terms of seedling age and size.

Fig. WP6.4





Eighteen months after sowing, 1287 seeds (41%) from the 55 mother trees had germinated. Seeds from the Costa Marques (RO) population germinated at a constant rate from the sixth month after sowing. Seeds from Oriximiná (PA) and from Itacoatiara (AM) started the germination 1-2 months later (i.e. in the 7th-8th month after sowing). After the 8th month the germination rate of the three populations was more or less constant, around 4-5% per month (Fig. WP6.4 above).

Pricking out and raising seedlings in the nursery

The *B. excelsa* young seedlings were pricked out into soft plastic pots (polypots) for their further growth in the nursery. The seedling pricking out occurred when the seedling exhibited two well developed leaves and reached around 10 cm height (Fig.WP6.5 below).

Fig WP6.5: Above: View of the seedlings at the proper stage to be pricked out – note the seedbed divided into compartments for the seeds from the same fruits. Right: polyembryonic seedling.

All B. excelsa seedlings raised in the nursery were identified by an aluminum tag where the mother-tree identification, date of sow, date of germination and date of pricking out were registered (Fig.WP6.6).

Fig. WP6.6: Seedlings of *B. excelsa*, showing the individual identification with aluminium tags.

Cordia alliodora (OFI), Schizolobium parahyba (INRA)***, Cedrelinga cataeniformis (PUCE)***
The status and inclusion of these species will be ascertained and agreed at the next coordination meeting, although CNR have developed SSRs for *Schizolobium parahyba* as part of WP5.

Cedrela odorata (CATIE, CEH), Swietenia macrophylla (CATIE, UoA, INPA, CEH), Vochysia ferruginea (CATIE, CEH)
CNR have developed SSRs for *Cedrela odorata* (WP5), which have now been screened and optimised by Gustavo Hernandez (CATIE), at CEH, as part of his Masters work. These have now been published in Conservation Genetics as Hernandez et al (2007). Using nine microsatellite primers, Gustavo completed screening of 70 open pollinated progeny arrays from 12 populations (including 2 phylogenetic lineages), including arrays from mother trees in forest and isolated landscapes. The results are currently being finalised and written up for submission as an M.Sc. dissertation in August 2007.

For *Swietenia macrophylla*, open-pollinated progeny arrays from a range of mother trees collected in different forest contexts (from intact forest to trees isolated in pasture landscapes) have been collected across Central America. Dr Carlos Navarro (CATIE) will visit the University of Adelaide during October/November 2007 to undertake molecular marker mating systems analysis on this material. In addition, data exist either from populations with different densities and logging regimes (CEH, *Swietenia macrophylla*), differential density post-logging site (INPA, *Swietenia macrophylla*), or differential densities and colonisation phase (CATIE and CEH, *Vochysia ferruginea*) and will be re-analysed for synthesis in this work package.

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p58)

CORE AREA	**3**	**Analysis and prioritisation of regional and local sourcing strategies**
Work Package	**7**	***Data compatibility***

1.Workpackage objectives and starting point of work at beginning of reporting period

- to coordinate information acquisition of molecular and phenotypic data (WP1-WP6), prior to data analysis (WP8).
- to organize data flow among partners through appropriate tools, for estimating relevant genetic, ecological parameters prior to modelling (WP10)

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

Three main subjects are of concern under the "data compatibility" header. During the second year of the SEEDSOURCE program, requirements for data collection and data output format were defined, and reports from partners actually collecting data and material, or expected to do so, were collected. The compilation of each partner's practices and practical constraints are the basis for a satisfactory compromise in data compatibility. The three areas requiring action for data compatibility are the following: (i) data format (ii) selection of data to be collected (iii) data conversion (for genotype data).

Data format

It was agreed that for all sampling, quantitative and molecular data, a universal data format should be used by all partners. This will allow easy transfer of data to feed the modelling action and will also set a base for common discussion across the partnership. A universal template will be prepared at the beginning of year 3 by Ivan Scotti and Bernd Degen. Default computer format for the template should be Excel, but there is no fixed choice and any spreadsheet format should in principle be accepted. For each measured quantity, a universally agreed unit of measurement will be chosen. Each partner will be responsible for converting data into the common units prior to data distribution / reporting / publishing.

A consensus was reached on the following code for samples: one letter for genus name, two letters for species name_two letters for site_three digits for sample number_three letters for collecting institute. So for example, the name of a Carapa procera sample collected here in Kourou would look like this: "Cpr_Ko_003_INR".

Data collection at sampling sites.

The following measures should be included in the mimimum data set at each collection site and for each tree:

- Exact GPS position
- DBH
- Distance to nearest 3 trees of same species
- Foliage projective cover (canopy density) around tree
- Basal area around tree
- Fruit output

In addition to this, and in order to ensure compatibility with EcoGene, the ratio of the basal area of the sampled species relative to the total basal area in a radius of 50 m² should be recorded. Preliminary data suggest the existence of adaptation to soil water availability and hypoxic stress, so paedological and hydrological parameters should be collected as well. The partners involved in Reciprocal Transplant Experiments have agreed on provide as much data as possible about soil conditions, within budget constraints. The same partners have also collected most of the required data, as listed above, for diversity and phylogeography samplings. The demanded data will be available, on the other hand, only for a subset of the collections needed for the two latter activities.

Coherence of genotyping data.

Microsatellite data will be collected as DNA fragment size throughout the partnership. Reference samples will be identified for each marker and each species to ensure coherence across laboratories, possibly beyond the duration of the present program. Methods to ensure the continuity and coherence between chloroplast PCR-RFLP data, collected in previous projects, and chloroplast sequence data are under evaluation. This problem does not have an easy solution and further reflexion is needed in this direction. Single nucleotide polymorphisms (SNPs) will be coded within and across species according to a uniform sequence reference. The exact form of this standardisation has yet to defined, but using a unique reference to published sequences (e.g. *Arabidopsis thaliana* homologs) appears as the best available option. This issue will be settled in Project year 3.

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

None

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p58)

CORE AREA	3	Analysis and prioritisation of regional and local sourcing strategies
Work Package	8	*Meta-analysis of data*

1.Workpackage objectives and starting point of work at beginning of reporting period

To analyse data produced in WP2 – 6 in a way that will be productive towards the goals of the project and useful for dissemination purposes, as identified in core area 4

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

Reviews of restoration, secondary forest and neotropical population genetic studies

Building on syntheses of the available literature two reviews have been prepared for journal submission, which will be disseminated to partners during the 3rd coordination meeting.

Linda M. Broadhurst, Andrew J Lowe, David Coates, Saul Cunningham, Maurice McDonald, Peter Vesk and Colin Yates. Maximising evolutionary potential in broadscale restoration. For *Conservation Biology*

Elizabeth Sinclair, Margaret Byrne, David Coates, Kingsley Dixon, Leslie Hammersley, Richard Hobbs, Stephen Hopper, John Koch, Siegfried Krauss, Andrew Lowe, David Venning, Stephen Vlahos, Colin Yates. Ecological Restoration Genetics – from Generalities to Practicalities. For *Conservation Biology*

Andrew Lowe (UoA) was invited to submit a review article on 'Dynamics of secondary forest population genetics' for *Genes, Genomes and Genomics*. Sam Davies (CEH) has agreed to drive this review and is being assisted by Andrew and Steve Cavers (CEH).

Chris Dick (UM) is preparing a synthesis of phylogeographic and population genetic studies for publication.

Generalities and syntheses from these reviews will be fed into the direction setting of the project.

GENEO-TROPECO meta-analysis

A final analysis of the GENEO-TROPECO AFLP data is being synthesised. In total AFLP data sets have been accumulated for over 40 species, together with information on their ecology, mating system and genome size. This synthesis will be presented for the first time at the third coordination meeting.

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

None

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p58)

CORE AREA	3	Analysis and prioritisation of regional and local sourcing strategies
Work Package	9	*Selection and definition of resource priorities*

1.Workpackage objectives and starting point of work at beginning of reporting period
Computer simulation studies will be done:

- to estimate the impact of different strategies of natural and artificial regeneration on the genetic structure of a tree population,
- to predict the impact of gene flow and local selection on the genetic structure of tree populations of adapted (autochthonous) and non adapted origin
- to design optimal seed harvesting strategies that maintain the genetic diversity of the original stands in the regeneration material.
- to estimate the impact of transfer of seed between different ecozones as defined by the quantitative assessment of provenance material and reciprocal transplant experiments (WP2) and genetic diversity and gene dynamic estimates (WP3 and 4).

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

The only contribution to WP9 came as planned from the BFH. The main objective of this WP is to perform a further development and application of the simulation model Eco-Gene and to develop a new model for simulations on a larger spatial scale.

Deliverable 23: During the second 12 months of the project additional changes have been made on the simulation model Eco-Gene. The model has been simplified and a new stand alone version including forest growth functions that can be installed and run independent of the Symfor model has been created. Eco-Gene has been applied to estimate the impact of selective logging on the genetic diversity and demographic structure of tropical tree species in the in Brazil.

Main output of these activities is a paper submitted to Forest Ecology and Management:

Alexandre M. Sebbenn ab, Bernd Degen b, Vânia C.R. Azevedo c, Marivana B. Silva d, André E.B. de Lacerda e, Ana Y. Ciampi c, Milton Kanashiro e , Francimary da S. Carneiro e , Ian Thompson e, Marilyn D. Loveless (submitted): Modelling the long-term impacts of selective logging on genetic diversity and demographic structure of four tropical tree species in the Amazon forest. Forest Ecology and Management

Deliverable 24: In the period from February 2006 to February 2007 Dr. Alexandre Sebbenn worked as a post doc from the Sao Paulo Forest Institute (Brazil) at the BFH. Part of his work addressed objectives of WP9 of the Seed Source project. A large progress was made to develop together with Bernd Degen a new multi-species, multi-scale version of Eco-Gene which is linked the Geographic Information System ArcView. A first running version has been programmed and first test simulations has been made. The main characteristics of this new model are:

- Grid cell approach
  - large scale simulations are possible
  - easy implementation in GIS

- Multi-species approach
  - each species a special layer
  - interaction between species (competition)
  - dynamics of inter and intraspecific genetic diversity

- Selection on quantitative traits
  - genetic + environmental variation
  - implementation of clinale selection possible

- Landscape dynamics
  - scenarios on deforestation

- As next and remaining steps has to be listed:
- Inclusion of functions on the competition among species/ species groups
- Implementation of real landscape structures
  - distribution of forest
  - distribution of selective environmental factors
- Module on seed harvesting strategies
- Module on landscape management
  - deforestation and reforestation
  - plantations with improved seed material
  - selective logging
  - clear cuts

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

None

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p58)

CORE AREA	**4**	**Knowledge gathering, integration and dissemination of priorities**
Work Package	**10**	***Communication of biological and socio-economic information***

1.Workpackage objectives and starting point of work at beginning of reporting period

- To act as a promotion pathway for the outputs of the research.
- To facilitate feedback from end-users to ensure the relevance of the research, and that the implications of the research can be incorporated as realistic management practices.
- To ensure the production of technically accurate extension materials

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

The Central American survey carried out in the first year identified critical issues with respect to the sustainability of past dissemination efforts related to forest genetic resources. David Boshier led discussion at the first year meeting (details given in the meeting report, August 2006) with respect to the implications for the target audience for SEEDSOURCE dissemination. It was agreed that the emphasis should be on tertiary education teachers where there is greater stability of personnel and a higher probability that any materials and training produced by SEEDSOURCE will be utilised beyond the life of the project. This has led to a clearer targeting of efforts within WPs 11 and 12 (see these WPs for more details).

Significant progress was made gathering information from Central American sources regarding most effective means for dissemination of project outputs (see workshop report and WP12). Clearly, best targets and formats differ between the Central American and South American regions and within different regions, but progress needs to be made to identify these pathways. Partners (PUCE, INPA, UFRGS) agreed to undertake surveys/workshops (or most appropriate means) to identify best routes to disseminate project outputs.

A response to a request for information was provided to a researcher working for an EC study regarding stakeholder involvement in projects. As a result of the information supplied SEEDSOURCE has been selected as an example of good practice, in the context of a study commissioned by DG Research on how Science and Society issues are being addressed in FP6, and will be included in the inventory of good practices to be produced by the study.

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

None

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p58)

CORE AREA	4	Knowledge gathering, integration and dissemination of priorities
Work Package	11	*Knowledge gathering*

1.Workpackage objectives and starting point of work at beginning of reporting period

- Produce list of potential species from which 12 will be chosen for case study in the project and 50 will be chosen for Central and South America for guideline dissemination according to analytical results of the project.
- To identify how germplasm collection and management practices vary with respect to species' regeneration characteristics, socio-economic importance, and the agroecosystems/forest types in which they occur.
- To provide feedback from end-users on research scope, methods and outputs to ensure that it is fine-tuned to the issues they face.

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

Description of work

The survey in Ecuador was completed, where responses from enquiries to 13 private companies, NGOs and public organizations showed germplasm collection and management practices vary between commercial and non-commercial activities. Reforestation programmes with commercial goals are carried out by private companies, with frequent planting of non-native species, with specific physical timber features and high economic performance, (e.g. *Pinus* spp., *Eucalyptus* spp. *Tectona grandis*). Native species like laurel (*Cordia alliodora*), caoba (*Swietenia macrophylla*), balsa (*Ochroma*), Spanish cedar (*Cedrela odorata*), cutanga (*Parkia multijuga*), chuncho (*Cedrelinga cataeniformis*), jacaranda (*Jacaranda copaia*) have good perspectives for commercial reforestation. In many cases the origin of seeds is from foreign enterprises.

Non-commercial reforestation (mainly for forest conservation and soil protection) is performed by NGOs, public institutions and community based organizations. These programs involve a higher number of native species (e.g., *Buddleja, Icana* and *Polylepis* species) than in commercial reforestation programmes. Nonetheless, several non-native species are frequently used over extensive areas. Germplasm (seeds, seedlings and reproductive tissues) of native species comes from collections done mainly in forest remnants or is sourced from commercial nurseries.

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

The workshop in Ecuador will now be held in July 2007 in conjunction with WP 12 and will follow the same format as those in Central America. Project personnel from CATIE (Carlos Navarro) and OFI (David Boshier) and will assist those from PUCE (Renato Valencia) in facilitating the workshop with the participation of tertiary educators from Ecuador.

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p58)

CORE AREA	4	Knowledge gathering, integration and dissemination of priorities
Work Package	12	*Preparation and dissemination of extension materials*

1.Workpackage objectives and starting point of work at beginning of reporting period

To produce and disseminate extension materials based on the projects (and other relevant research) results in a range of formats and levels, appropriate to a range of target end users.

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

Description of work

Jesus Cordero and David Boshier (OFI) gathered examples of existing dissemination materials related to forest genetic resources and worked on planning the content, designing material and exercises for workshops, along with Carlos Navarro and Carolina Cascante (CATIE). Three 1-1.5 day workshops were organized by the SEEDSOURCE project and held in November/ December 2006 in Nicaragua, Honduras and Costa Rica, with the following main objectives:

- to understand the information needs of tertiary education teachers and germplasm collectors on Central American native tree germplasm, and to explore different ways to present information from research, to thus obtain guidelines for future dissemination materials;
- to draw up a strategy to establish sustainable dissemination pathways, and to discuss the collaboration and task sharing necessary to implement the proposed strategy.

As such the workshops cover activities related to WPs 10, 11, and 12, but are reported here for convenience. Due to a higher employment stability and yearly influx of fresh students, teachers offer greater prospects for sustainability and outreach in disseminating messages on forestry genetics conservation and diversity, compared to fellow forestry professionals in both public and private organisations. Three SEEDSOURCE project members (David Boshier, Jesus Cordero, Carlos Navarro, plus Carolina Cascante in Costa Rica) facilitated the workshops. Between 13 to 25 teachers attended each workshop (a few students and seed collectors also participated), coming from the main relevant educational institutions in each country. Each workshop was divided into 4 sessions with participatory activities: presentations, individual surveys, revision of didactic materials, and plenary and group discussions.

The results showed ample scope for dissemination of existing and future SEEDSOURCE research results through a variety of current courses: Ecology, Natural Resources Conservation, Endangered Native Species Conservation, Silviculture, Forestry Seeds and Nurseries. Forest Genetics Conservation and Forest Genetics Improvement were considered as the most appropriate courses, but they are only offered in post graduate degree programmes.

Students have access to computing and the Internet, but resources are often insufficient, not free, out of date, or simply do not work. Teachers enjoy better access to these resources. There is in general free access for all to libraries and documentation, although the offer is somewhat limited and outdated. Teachers use a wide range of aids, according to individual institutional resources. In general, no differences were found between countries with respect to the courses and educational levels offered, computing and documentation access, or teaching aids. However, a huge resource gap was found between individual institutions and educational levels in each country.

The participants outlined the principal ideal characteristics of forestry related didactic materials for students and documentation for teachers. They grouped real examples of seed collection and management materials by their utility for students and/or teachers, setting some guidelines for future SEEDSOURCE research results dissemination materials. This identified strengths and weaknesses for transferring information as viewed by users.

A consensus was reached on teaching forestry genetics resources conservation and diversity in curricula across a range of courses, under the individual initiative of teachers or departments, introducing concepts in specific lectures or seminars within courses or modules. It was not deemed essential to create specific courses on this topic, or to include it within official degree programmes. Higher importance was given instead to a wider and far reaching dissemination of information, together with some sort of teacher training.

Participants were willing to start and participate in an informal network, so that they can meet fellow teachers and professionals working on this topic, exchange information, discuss topics, and as an access point to forestry genetics conservation and diversity resources. In practical terms, the following actions were proposed:

- establish a Seedsource users' forum via the project's website;
- make materials available on the website that were considered by teachers as the most relevant and appropriate as teaching aids– it will involve scanning of existing material;
- facilitate to libraries in Honduras and Nicaragua free access to peer reviewed journals through initiatives such as AGORA (www.aginternetwork.org) and OARE (www.oaresciences.org);
- development of case studies on a variety of topics related to Forest Genetic Resources. These will be suited for use in classes and will include teacher notes. SEEDSOURCE will also look at the possibility of running training workshops in Nicaragua and Honduras for teachers on the use of this material. In Costa Rica such workshops were not felt to be necessary.

In response to feedback at the three workshops in Central America, a bulletin board is being set up, using the YaBB opensource software. The *Foro de participantes en la Red Seedsource de diseminacion de resultados* will be open from June 2007 and will contain various sections:

- Pregunte al experto
- Noticias globales
- Documentacion disponible para descargar o donde se puede localizer
- Ayuda/General/Contactos (contactar a miembros de la red para solicitar ayuda o colaboraciones especificas, avisos de reuniones, conferencias, talleres, etc).

Carlos Navarro developed, tested and distributed a board game aimed at primary and secondary school pupils. The educational game includes the game in full colour plus a dictionary and cards called 'opportunity seeds'. So far, together with the Ministry of Education meetings with the teachers of secondary and primary education have been coordinated, in which they are instructed about the possibilities and use of the game. A powerpoint presentation is used to develop what is called in the education system 'technical donation' or "entrega técnica", which is not just giving them the education materials but also explaining the

use of them in extension and teaching. So far, we have worked with the indian communities and *telesecundarias* in Turrialba, Quepos and San Carlos in Costa Rica. Education games have also been sent to Nicaragua and Honduras.

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

As described above, on the basis of the survey in WP11 and the workshops held in Central America, the principal target audience for dissemination materials has been altered to university and technical school teachers. With that, the emphasis on the type of dissemination materials to be produced by the project has also altered, with consequent impacts on deliverable 28. Initial focus is now on making available on the project website existing materials that were assessed in the workshops as being of immediate use. Preparation of new materials will focus initially on the development of case studies on a variety of topics related to Forest Genetic Resources. Contacts have been made with staff at Bioversity International's (formerly IPGRI) Understanding and Managing Biodiversity Programme, who are also developing materials, with a view to exchange and mutual testing.

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p58)

Table: Deliverables List

List all deliverables, giving date of achievement and any proposed revision to plans.

No.	Deliverable name	WP no.	Date due	Actual/Forecast delivery date	Estimated indicative person-months *)	Used indicative person-months *)	Lead contractor
1	Initial project website established	1	3	7	2	2	OFI / CEH
2	Guideline of collection and exchange priorities	1	6	12	6	5	CEH
3	Reciprocal transplant experiments established	2	21	21	36	24	CATIE
4	Data from seedling reciprocal transplant experiments to WP7	2	36				CATIE
5	Paper on scale of adaptation: plant fitness in relation to genetic, environmental and geographic distance and in comparison to production variables	2	45	45	-	-	CATIE
6	Protocols for DNA extraction and analysis of herbariums and wood samples	3	36	24	4	4	INRA
7	Phylogeographic maps of study species	3	42	42	2+8	2+3	INRA
8	Database of DNA sequences to identify range and study species available	3	42	42	6	3	INRA
9	Written guidelines on standard project research procedures for CA2	4	9	18	1	1	CATIE
10	Enriched libraries in microsatellites for the selected species	5	12	12	9+61.5	9+30	CNR-IGV
11	List of new neutral (chloroplast and nuclear) and SNP markers for the selected species	5	18	18	1	1	CNR-IGV
12	Map of distribution of neutral diversity within and across natural populations	5	42	42	4.5	1.5	CNR-IGV
13	Method for tracing origin of plant material based on molecular markers assessed	5	42	42	-	-	CGR-IGV
14	Method for linking neutral and adaptive markers assessed	5	42	42	-	-	CNR-IGV
15	Database of molecular markers polymorphism	5	42	42	-	-	CNR-IGV

16	DNA collection from open pollinated progenies, seedling recruits, and mapped adult trees within range of landscapes	6	18	30	34.5+4	23	INPA
17	Common garden experiments established	6	21	21	36	12	INPA
18	Seed from outbreeding depression controlled pollinations	6	21	21	6	2	INPA
19	Gene dynamic parameters estimated for study species under different environmental conditions	6	42	42	24	6	INPA
20	Development of guidelines for data acquisition	7	18	18	1	1	INRA
21	Integrated species maps showing quantitative and molecular genetic discontinuities and variability	8	45	45	-	-	CEH
22	Correlations of gene dynamic and progeny performance parameters with biological and landscape features	8	45	45	-	-	CEH
23	Development of Eco-Gene model	9	45	45	-	2	BFH
24	Development of a GIS based Meta-population model	9	45	45	-	10	BFH
25	Reports on workshops covering survey results and draft extension materials	10	7	21/27	4.5	3+1	OFI
26	Reports on workshops covering project results	10	40	40	-	-	OFI
27	Report on germplasm collection & management practices	11	7	12/24	9.5	3.5	OFI
28	Trial extension materials based on existing research papers	12	4	36	6.5	2	CATIE
29	Extension materials produced	12	36	36	15	4+2	CATIE
30	Extension materials disseminated	12	48	48	15	4	CATIE

Table: Milestones List

List all milestones, giving date of submission and any proposed revision to plans.

No	Milestone name	WP no.	Date due	Actual/Forecast delivery date	Lead contractor
1.1	Updated website	1	3	3	CEH
1.2	Reference collection and exchange guidelines	1	6	6	CEH

2.1	Determination of the scale of local adaptation in three neotropical species during seed germination and seedling establishment, and how this relates to: (a) genetic, environmental and geographic distance; (b) existing seed sourcing practices for ecological restoration; (c) population variation in plantation production traits.	2	45	45	CATIE
3.1	Access to herbarium and wood samples	3	12	30	INRA
3.2	Access to population samples of widely distributed populations	3	18	30	INRA
3.3	Detection of variable regions in study species genomes	3	12	30	INRA
4.1	Definitive guidelines circulated among all partners and available on project website.	4	9	9	CATIE
4.2	Progress reports and modifications to guidelines agreed upon on the basis of experience during the execution of the project.	4	12	12	CATIE
4.3	End of planning meeting – draft guidelines prepared.	4	18	18	CATIE
4.4	Annual meetings – progress reports and updated guidelines circulated to all partners and available on project website.	4	12, 24, 36	12, 24, 36	CATIE
5.1	New microsatellite markers provided	5	12	12	CNR
5.2	List of candidate stress related genes as putative target for SNP detection provided, list of primers for their amplification prepared	5	12	12	CNR
5.3	Sequences of the identified candidate genes and of SNPs provided	5	18	18	CNR
5.4	Organisation of a technical meeting among WP4 participants	5	12	12	CNR
5.5	Populations diversity screened with microsatellites and SNPs	5	36	36	CNR
5.6	Assignment tests performed: organisation of a technical meeting among WP4 participants	5	45	45	CNR
5.7	Association tests with QTL performance and selected candidate genes performed	5	45	45	CNR
6.1	The impact of local environment of on seed set and progeny performance in all study species;	6	45	45	INPA
6.2	the impact of selfing on progeny performance in mixed mating species;	6	45	45	INPA
6.3	the extent of fitness loss through population mixing with respect to both geographical and ecological distance of the pollinating trees from mother trees.	6	45	45	INPA

6.4	How different local habitat condition affect the mating system and pattern of gene flow in selected tropical forest trees, the results of this WP will influence the decisions on management strategies in WP9 and the communication and dissemination activities of WP11 & 12.	6	45	45	INPA
7.1	Definition of genetic and ecological parameters to be estimated and analysed in the meta-analysis (WP8)	7	6	6	INRA
7.2	Ensure project-long data compatibility	7	45	45	INRA
8.1	Useable maps of distribution of variation within species for a range of quantitative, phylogenetic, diversity and coding loci.	8	45	45	CEH
8.2	General overview of how biology and landscape of species impacts on gene diversity and progeny performance	8	45	45	CEH
9.1	Thresholds for each studies species on the minimum number and spatial distribution of reproductive trees for forest management based on natural regeneration	9	45	45	INRA
9.2	A map of ecozonation across the range of each case study species which indicates areas within which seed transfer can occur but between which there are some performance consequences to transfer	9	45	45	INRA
9.3	Recommendations for the way of harvesting and the minimum number of individual trees that seed should be collected from to ensure maximum progeny performance	9	45	45	INRA
10.1	Documentation on current utilisation practices across the range of case study species	10	12	24	OFI
11.1	The survey is expected to reveal key factors in germplasm collection & management practices	11	12	24	OFI
11.2	Workshops held with stake holders and scientists in each of the regions	11	12	24	OFI
12.1	Workshops held with stake holders and scientists in each of the regions (months 5-6)	12	12	24	CATIE
12.2	Workshops held with stake holders and scientists in each of the regions (months 40)	12	5-6	18	CATIE

Plan for using and disseminating the knowledge

The preparation of dissemination materials began early in the project. Recent research on neotropical trees (from previous EU and other projects) are being revised for their implications for seed collection and other genetic resource management issues. Draft extension materials will be prepared based on key implications from this revision and trialled at initial workshops within the context of the survey (see WP 11). As analyses from Core Area 3 are produced and assimilated, results will be incorporated into extension materials. The presentation of extension materials (format, scope, style, coverage) and dissemination of resource management priorities will be informed initially by output from WP11 and through stakeholder workshops at various phases of the project. A variety of media (pamphlets, books, CDs, the web) will be used, depending on the target audience of each material and input from stakeholders. The academic community will be targeted by scientific papers and articles. Materials will be prepared in the languages appropriate to the intended geographical coverage of the material (e.g. Spanish, Portuguese, French, English for wide coverage materials, while only one of these for locally targeted material).

Extension materials will cover the following:

- Issues on genetic resource management with respect to land use and establishment systems (named species examples given within them).
- For species identified as of very high socioeconomic importance and where genetic resource management will vary with ecosystem and management scenario a detailed pamphlet containing life history and utilisation information will be prepared.
- A check-list will be produced for the 50 most socio-economically important species in each of Central and South America, detailing their current use, biology and recommendations for seed transfer and source collections (includes ecozonation across the range of each case study species)

Dissemination/extension materials will be circulated at a country and regional scale to a range of end users (government agencies, non-government organisations, foresters/agroforesters, teachers, extension workers) across the neotropics. Workshops will be organised with stakeholders at various stages of the project at locations within Central America and South America to disseminate results to both the resource management and scientific communities. The project will work through existing dissemination networks.

Dissemination of knowledge

A fundamental basis of the project is that research on seed source is directly applicable to issues of production, conservation and sustainability – both of ecosystems and human welfare. Thus an equal emphasis on dissemination and research along with involvement of a wide range of stakeholders will ensure a broad spread of the information, far beyond the immediate research community. Working through existing dissemination networks will ensure that the project outputs are placed within a much broader context and reach a wider public than would more normally be the case. Similarly the feedback process will ensure that the research is centred within that broader context. We anticipate that the approach and information developed and used in the project will be applicable to other developing country regions.

Planned/actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
15/04/05	*Conference*	*Higher education*	*Students from Honduras, Nicaragua, Bolivia, Perú, Costa Rica, Mexico,Colombia*	*15*	*6*
10/2006	*Workshops*	*Academic*	*international*	*100*	*6 and 2*
Ongoing	*Project web-site*	*Research/Higher education*	Neotropical		*OFI (J. Cordero)*
1-4/10/2006	*Poster*	*Research*	Worldwide	*~ 150*	*OFI (D Boshier, P Rymer)*
	Flyers	*General Public*	Ecuador		*PUSC (R. Valencia)*
	Didactic game	*School Children*	Neotropical		*CATIE (C. Navarro)*
30/11-1/12/2006	*Workshop*	*Higher education*	Nicaragua	*22*	*OFI (D. Boshier, J. Cordero) CATIE (C. Navarro)*
4/12/2006	*Workshop*	*Higher/Secondary education*	Honduras	*13*	*OFI (D. Boshier, J. Cordero) CATIE (C. Navarro)*
7/12/2006	*Workshop*	*Higher/Secondary education*	Costa Rica	*25*	*OFI (D. Boshier, J. Cordero) CATIE (C. Navarro, C. Cascante)*
30/05/07	*Conference*	*Higher education*	*Students from, Mexico,Colombia and Italy*	*5*	*6*
15-19/7/2007	*Conference*	*Research*	Neotropical	*~100*	*OFI (D Boshier)*
planned	*Workshops*	*End-users*	*Central / South America*	*>1000*	*OFI, INPA, CATIE, PUCE, UFRJ*
planned	*Extension materials*	*Universities, teachers*	*Central / South America*	*100-1000*	*All partners*

Publications

Dick CW, Bermingham E, Lemes MR, Gribel R (2007) Extreme long-distance dispersal of the lowland tropical rainforest tree *Ceiba pentandra* L. (Malvaceae) in Africa and the Neotropics. Molecular Ecology. 16 (14): 3039–3049.

Lemes MR, Grattapaglia D, Grogan J, Proctor J, Gribel R (2007) Felixible mating system in a logged population of *Swietenia macrophylla* King (Meliaceae): implications for the management of a threatened neotropical species. Plant Ecology. DOI 10.1007/s11258-007-9322-9

Hernandez G., Buonamici A., Walker K., Vendramin G.G., Navarro C. and Cavers S. (2007) Isolation and characterization of microsatellite markers for *Cedrela odorata* L. (Meliaceae), a high value neotropical tree. Conservation Genetics. DOI 10.1007/s10592-007-9334-y

Duminil J, Caron H, Scotti I, Cazal, S-O, Petit RJ (2006) Blind population genetics survey of tropical rainforest trees. Molecular Ecology. 15(12): 3505-3513.

Alexandre M. Sebbenn ab, Bernd Degen b, Vânia C.R. Azevedo c, Marivana B. Silva d, André E.B. de Lacerda e, Ana Y. Ciampi c, Milton Kanashiro e , Francimary da S. Carneiro e , Ian Thompson e, Marilyn D. Loveless (submitted): Modelling the long-term impacts of selective logging on genetic diversity and demographic structure of four tropical tree species in the Amazon forest. Forest Ecology and Management

Linda M. Broadhurst, Andrew J Lowe, David Coates, Saul Cunningham, Maurice McDonald, Peter Vesk and Colin Yates. Maximising evolutionary potential in broadscale restoration. For *Conservation Biology*

Elizabeth Sinclair, Margaret Byrne, David Coates, Kingsley Dixon, Leslie Hammersley, Richard Hobbs, Stephen Hopper, John Koch, Siegfried Krauss, Andrew Lowe, David Venning, Stephen Vlahos, Colin Yates. Ecological Restoration Genetics – from Generalities to Practicalities. For *Conservation Biology*

Davies S, Cavers S, Lowe AJ The genetic diversity consequences of reforestation and habitat restoration, *Genes, Genomes & Genomics.* In Preparation.

Communications /talks

Bernd Degen and Alexandre Sebbenn: Large-scale, multi-species model Eco-Gene, Workshop EMBRAPA, Belem, Brasil, 11-16/02/2006, addressed countries: Brazil, Size of the audience 20

Degen B. Impact of selective logging on genetic composition and demographic structure of seven tropical tree species in French Guiana and Brazil, 01.03.2007, Kourou, French Guiana, addressed countries: France / French Guiana

APPENDIX I:
Minutes of the second co-ordination meeting, 28 August - 02 September 2006

Participating Institutions

CEH	Centre for Ecology and Hydrology, Natural Environment Research Council
OFI	Oxford Forestry Institute, University of Oxford
INRA	Instit Nacional de la Recherche
CNR	Consiglio Nazionale delle Ricerche
INPA	Instituto Nacional de Pesquisas da Amazonia
CATIE	Centro Agronómico Tropical de Investigación y Ensenanza
PUCE	Pontificia Universidad Catolica del Ecuador
UFRJ	Universidade Federal do Rio de Janeiro
BFH	Institute of Forest Genetics

Members present

UA	Andrew Lowe (AL)
CEH	Stephen Cavers (SC), Katherine Walker (KW)
OFI	David Boshier (DB), Paul Rymer (PR)
INRA	Henri Caron (HC), Ivan Scotti (IS), Caroline Scotti-Saintange (CS)
CNR	Beppe Vendramin (BV), Anna Buonamici (AB)
INPA	Rogerio Gribel (RG), Maristerra Lemes (ML)
CATIE	Carlos Navarro (CN)
PUCE	Renato Valencia (RV), Álvaro Pérez (AP)
UFRJ	Rogerio Margis (RM), Marcia Margis-Pinheiro (MM)
BFH	Bernd Degen (BD)

Meeting Agenda

Monday	(28/08/2006)	Arrival of participants
Tuesday	(29/08/2006)	Lab Workshop 'Testing primers for amplification of genes'
Wednesday	(30/08/2006)	Lab Workshop 'Testing primers for amplification of genes'
Thursday	(31/08/2006)	SEEDSOURCE Coordination meeting
Friday	(01/09/2006)	SEEDSOURCE Coordination meeting
Saturday	(02/09/2006)	SEEDSOURCE Coordination meeting / Siena excursion

SEEDSOURCE Coordination Meeting
Thursday 31st
- General business - project deadlines, reporting etc - SC
- WP8: Scientific questions - 'Restoring evolutionary viability: myth-busting' – AL
- WP1: Export Permit situation – ML & RV
- WP1: Collections:
  - Review of collections made / planned for WP3: led by WP leader HC
  - Review of collections made / planned for WP5: led by WP leader BV
  - Review of collections made / planned for WP6: led by WP leader AL
- WP1: Review of marker development / initial screening (SSRs, SNP, cpDNA) – AB, BV
- WP1: Identification of cpDNA regions for sequencing – CS
- WP1: Development of extraction / PCR protocols for wood / herbarium samples – HC
- WP2: Sampling for Costa Rican RTEs - CN

Friday 1st
- WP10: Developing best practice in seed collection - DB
- WP1: Screening of species for cpDNA RFLP variation - AL
- WP1: Development of SNPs for water-stress related genes - MM
- WP3/5: Progress on Swietenia macrophylla / Bertholletia excelsa – ML
- WP3: Progress on Cordia alliodora / Bombacopsis quinata / Swietenia humilis – PR
- WP3: Progress on Cedrela odorata – KW
- WP3: Progress on Schizolobium – RM
- WP3: cpDNA and herbarium sampling: experiences with Cats Claw - AL
- WP5: Progress with Schizolobium SSRs – RM
- WP5: Progress on Cordia alliodora / Bombacopsis quinata / Swietenia humilis – PR
- WP6: Progress on Bertholletia excelsa – RG
- WP6: Progress with Carapa, Minquartia, Vochysia, Jacaranda, Simarouba, Virola - CN
- WP6: Progress on Cedrela odorata – SC
- WP6: Progress on Cordia alliodora / Bombacopsis quinata / Swietenia humilis – PR

Saturday 2nd
- General Business: Material Transfer Agreement, Species ID - SC
- WP7 & 9: Progress on ECOGENE – BD
- WP10-12: Survey of needs / opinions, and dissemination – DB, CN
- WP8: Scientific questions – AL
- Closing business - SC

ACTIONS AND UPDATES
Project Level: Consortium business, management structures and reporting

- It was agreed with partners that the project management structure should be more rigidly followed in future project interactions. Responsibilities assigned in the Core Area (CA) / Work Package (WP) hierarchy would be expected to deliver, and reporting for subsequent annual reports should follow this structure rather than a partner-by-partner approach. To clarify the lines of communication, CA / WP responsibilities were reviewed and agreed as below:

WP No	Workpackage title	Lead contractor No
CA1	**Adaptive variation and genetic differentiation at a range-wide scale**	**CEH**
1	Collection and exchange of materials and methods	CEH – SC
2	Quantitative performance for replanting	CATIE - CN
3	Evolutionary history and developing regional markers for species	INRA - HC
CA2	**Diversity, reproductive perf. and recruitment at the landscape scale**	**CATIE**
4	Ensuring focus of quantitative and genetic studies	CATIE - BF
5	Estimate partitioning of non-coding and coding genetic diversity	CNR-IGV – BV
6	Gene dynamics and quantitative seed performance in relation to landscape characteristics	UA – AL
CA3	**Analysis and prioritisation of regional and local sourcing strategies**	**INRA**
7	Data compatibility	INRA – IS
8	Meta-analysis of data	UM – CD / UA - AJL
9	Selection and definition of resource priorities	BFH – BD
CA4	**Knowledge gathering, integration and dissemination of priorities**	**OFI**
10	Communication of biological and economic information	OFI – DB
11	Knowledge gathering	OFI – DB
12	Preparation of extension materials and dissemination of resource management priorities	CATIE - CN

- to ensure all individuals involved are clear on responsibilties, SC will contact them in period following meeting to firmly establish lines of communication.

- University of Adelaide and University Federal do Rio Grande de Sul will be added to the Technical Annex as non-funded partners. University of Queensland and Pontífice Universidade Católica do Rio Grande do Sul will be removed. A letter communicating the changes will be sent to the Scientific Officer at the EC.

- A Material Transfer Agreement governing the exchange and use of samples between partners within the project will be drafted by SC and circulated to partners. This will specify limits for use of sample material, guidelines for authorship of downstream publications, and exchange of genetic data (including final location of samples).

- Reporting response times were very slow for this year and must be cut for future reports. Clarification of the reporting lines should help this, but in addition partners have been forewarned to expect a call for reports (financial and scientific in March 07, to ensure reports are submitted ahead of the contracual deadline.

Workpackage 1 (CEH): Collection and exchange of materials and methods

- SC will provide continual central coordination of collections during 2006/07 to ensure as many of the planned collections and exchanges are made. Samples being exported to Europe will all be directed to SC at CEH Edinburgh and will be subsequently dispersed from there. All partners will keep SC informed of collecting trips (timing and destinations)

- SC will also coordinate collection of herbarium samples (maintaining communications with individuals responsible for visits to herbaria, exchange of samples).

- Updated tables pinpointing collections to be made / already collected were updated at the meeting and are included under relevant WP headings below.

- It was agreed that, where it was not possible for project partners to collect, the possibility of contracted collection might be explored. This approach would be advanced by obtaining costed quotes from potential contractors then subsequent agreement with project partners. A Spanish collection protocol would also be necessary.

- Export permissions. ML reported that Brazilian law is due to change next month and export for academic purposes should become easier. She will communicate progress to partners, when information is available. Export permits from other collecting countries should be obtained by the collector. RV mentioned that in Ecuador export permits for DNA analyses may take 4 months and the paperwork is more difficult than export permits of herbarium specimens.

- Agreement was reached that an exchange would be made between partners for a number of species for which samples are immediately available, to facilitate screening for a 'universal phylogeography locus', coordinated by AL. The agreed exchange of material is detailed below:

Screening of variation at cpDNA loci to identify 'universal phylogeography locus'
Rationale
Need to screen across major phylogeographic divide: Panama Isthmus (PI) chosen. 8 species will be targeted for comparative analysis across region. Exchange DNA of 5 individuals from a population north of PI, preferably Costa Rica, and 5 individuals from a population south of PI, preferably French Guyane. Only 3 individuals from each population will be genotyped (allows for drop out). Total of 48 individuals for genotyping (6 individuals for 8 species). CNR will screen all available cpSSR loci on this material (10 spp, 10 indivs each), and have offered to sequence all different alleles, and one rep from each of CR and FG if same. INRA will screen sequence variation at main phylogenetic loci (AJB paper+ITS). UoA will screen SSCP variation at RFLP loci (Taberlet, Hamilton, Demesure, Petit+)

Full collections ready to go now for 8 target species

DNA exists for	***who has it***	***will be sent to***
Cedrela odorata (CR+FG)	CNR	INRA+UoA
Simarouba amara (CR+FG)	INRA	CNR+UoA
Minquartia guianensis (CR+FG)	UoA	CNR+INRA
Laetia procera (GENEO, CR+FG)	CEH	CNR+INRA+UoA
Tetragastris panamensis (GENEO, CR+FG)	CEH	CNR+INRA+UoA
Ochroma pyramidale (PA+EC)	CNR	INRA+UoA
Bombacopsis quinata (CR+CO)	CNR	INRA+UoA
Carapa guianensis (FG+CR)	INRA	CNR+UoA

Additional collections that could be supplemented if backup required

Material/species	***who has it***
DNA - Cordia alliodora (CR)	OFI
Leaf - Cordia alliodora (CR)	INRA
Leaf - Ochroma pyramidale (FG)	INRA?
Leaf - Ceiba pentrandra (FG)	INRA?
DNA - Schizolobium parahyba (BR)	CNR
Leaf - Schizolobium parahyba (CR)	CATIE?
DNA - Brosimum guianense (CR+FG)	INRA (not available, failed GENEO)
DNA - Ceiba pentrandra (GENEO, CR+BR)	UoA (export issues)
DNA - Swietenia macrophylla (GENEO, CR+BR)	UoA (export issues)

Workpackage 2 (CATIE): Quantitative performance for replanting

- WP2 is progressing well with RTEs currently being established in Costa Rica, Brazil and planned for Ecuador. All partners involved in establishment are happy with progress and plans and are maintaining close contact.

Workpackage 3 (INRA): Evolutionary history and developing regional markers for species

The Table detailing collections planned and made was updated during the meeting and is given below.

The lab responsible for each species will send DNA from five individuals in each of five widespread populations to CNR for preliminary screening with cpSSRs. DNA has already been screened for six species. Samples of the remaining 11 species will be sent to CNR pending export restrictions.

Herbarium samples.
As agreed at the startup meeting, primary collection would be supplemented by samples from herbarium collections. Progress has been made on contacting the original list of herbaria, and samples should now be taken. A protocol for moving forward with herbarium samples was agreed and responsibilities for each herbarium were established:

Herbarium Protocol
Send letter to request permission (Antoine/Henri)
Stage 1: visit/contact herbarium and get list of location sites for species in WP3
Stage 2: contact Stephen with list so that he can help contact partners
Stage 3: send list to collection/genotyping partner to make choice
Stage 4: make and distribute collection following
- sample leaf put in zip lock bag
- note down following info from specimen sheet:
    - species ID
    - person who last revised ID
    - sample location
    - lat and long
    - altitude
    - date of collection

- collectors name
- herbarium number/collectors number

Herbaria contacted and status of request :
U Michigan (contact: Chris Dick) – no letter sent, CD to sample ? (SC contact CD)?
OFI (contact: Stephen Harris) – yes, DB to sample?
NY Bot Garden (on web) – yes, visit, CD to sample ? (SC contact CD)?
Missouri Bot Garden (on web) - no reply (HC to prompt), CD to sample ? (SC contact CD)?
Kew (contact: check with Antoine) – full response waiting (HC to prompt), DB to organise
Guyana (contact: IS) – yes, CS to sample
INPA Manaus (contact: ML) – ML to ask and sample
CPATU Belem (contact: ML) – ML to ask and sample
National Museum Rio (contact: RM) – no response, RM to sample
U Quito (contact: RV) – HC to send official letter, RV to sample
Utrecht (contact: check with Stephen Harris), yes, SC will check what specimens are there
Edinburgh Bot Garden (contact: check with AL), yes, SC to sample

Other contacts:
Herbario de la Universidad de San Marcos, Lima, Peru – no reply, HC follow up
Herbarium Truxillense, Trujillo, Peru – no reply, HC follow up
Forestry Service in Martinique – no reply, HC follow up

We contact others too? HC to make contact in first instance
Colombia
Puerto Rico
Venezuela
Paris (African outgroups?)
Chicago field museum (DB to supply address, Chris to follow up? SC contact CD)
Museum of CR
EAP (Honduras, DB to follow up)
STRI (Chris to follow up? SC contact CD)

Table for Workpackage 3 Ideal distribution of collections to be made for phylogeographic study, including identification of previously collected and available material: update from second meeting.

Responsible partner*		CD/CN	CD/CN	CD/CN	CN	CN	CN	CN	STRI	DB	PUCE	PUCE	DB/INRA	INRA	CD	INPA/UFRJ/Andy/Chris	
Species		**ME**	**BE**	**EL**	**GU**	**HO**	**NI**	**CR**	**PA**	**CO**	**EC**	**PE**	**VE**	**GY**	**BO**	**BR**	Total
Bertholletia excelsa	INPA									1?		1?			4?	19	30
Carapa guianensis	INRA		2?			2	2	6	2	1?	1+3		1+1?	24		2+3?	45
Cedrela odorata	CEH	x	x	x	x	x	x	x	x	1?	3+3?	1+2?	?	1	1?	3+2	50
Hymenaea courbaril	CATIE	2?	2?	x	2?		2?	5	2	1?		1?	2?	3	2?	3	33
Jacaranda copaia	INRA		2?		2?	2?		5	2	1?	3+1	2?	2?	6	2?	2+3	33
Minquartia guianensis	INPA						1?	2+2	2	1?	1+3	1?	2?	3	2?	2+2	30
Simarouba amara	INRA	1?	1?	x	1?	2?	1?	4	2	1?	1+3	1?	2?	9	2?	1+2	33
Swietenia macrophylla	INPA	4	3	2	2	2	3	5	2	1?	1	1?	4?		2?	8+2	50
Symphonia globulifera	INPA	x	x	.	x	x	x	x	x	x	3	3	x	x	x	x	30
Virola sebifera	PUCE						1?	4	2	1?	3+1	3+1?	2?	2	2?	3?	30
Vochysia ferruginea	CATIE						5?	10	5	1?		1	5?			1	26
Ceiba pentandra	INPA	2?	2?	2?	2?	2?	2?	2	2	1?	4	2+2?	2?	3	2?	1+4	39
Ochroma pyramidale	PUCE	2?	2?		2?	2?	2?	4	4	1?	5	2+2?	2?		2?		36
Cordia alliodora	OFI	2?	1?	1?	2	3	2	5	2?	1	2	2	2?	1?	2?	2?	30
Socratea exorrhiza	PUCE	1?	2?	x	2?	2?	2?	4?	2	1?	3	3	2?	3	1?	3?	30
Bombacopsis quinata	OFI					1	3	4	1	1+1?			2?				30
Schizolobium parahyba	UFRJ	2?	1?	1?		1?	1?	4	2	1?	1+1	2?			1?	1+9	30

existing sample

? - indicates uncertainty as to whether or not collection can be made / need to access other sources than direct collection

People and partners responsible for getting contacts in particular target countries to facilitate collection indicated in first row, partner responsible for overall collection in column 2.

Workpackage 4 (CATIE): Ensuring focus of quantitative and genetic studies

This workpackage is responsible for identifying additional sources of funding for managing collaborations with other projects.

Potential funding initiatives

- EU-Supporting action - Sept 2005 – Proposal passed qualifying criteria but did not receive funding

Collaborators identified

- DENDROGENE - Milton Kanashiro to be invited to next coordination meeting

Workpackage 5 (CNR): Estimate partitioning of non-coding and coding genetic diversity

The Table detailing collections planned and made was updated during the meeting and is given below.

SNPs development. Significant progress was made during the year on identifying potential candidate genes for SNP development and BV / IS demonstrated the protocols and potential results during an initial 2-day workshop at CNR. The target species will be screened at CNR to develop primers specific to the aquaporin family of genes and will be applied to species by responsible partners in collaboration with partners responsible for marker development.

Table for Workpackage 5 Ideal distribution of collections to be made for genetic diversity study, including identification of previously collected and available material: update from second meeting.

Responsible partner*		CD/CN	CD/CN	CD/CN	CN	CN	CN	CN	STRI	DB	PUCE	PUCE	DB/INRA	INRA	CD	INPA/UFRJ/Andy/Chris	
Species		**ME**	**BE**	**EL**	**GU**	**HO**	**NI**	**CR**	**PA**	**CO**	**EC**	**PE**	**VE**	**GY**	**BO**	**BR**	Total
Bertholletia excelsa	INPA									1?		1?			?	5?	6(20)
Carapa guianensis	INRA		1?			1?	1?	4	1	1?	2	1?	1?	7		2	10(28)
Cedrela odorata	CEH	1?	1?	1?	1?	1?	1?	2?	1?	1?	2	1?	1?		?		14(40+)
Hymenaea courbaril	CATIE	1?	1?	X	1?		1?	2	2?	1?		1?	1?	1	?	1?	17(19)
Jacaranda copaia	INRA		1?		1?	1?		2	2?	1?	2	1?	1?	2	?	2	17(19)
Minquartia guianensis	INPA						1?	2	1	1?	1+1?	1?	1?	1	?	2	18
Simarouba amara	INRA	1?	1?	X	1?	1?	1?	2	2?	1?	1+1?	1?	1?	1+1?	?	2	20(22)
Swietenia macrophylla	INPA	4?	4?	1**	2?	2?	3?	5?	2?	1?	1?	1?	1?		?	8	8(41)
Symphonia globulifera	INPA	1?	1?	X	1?	1?	1?	2	1	1?	1+1?	1?	1?		?	1?	21
Virola sebifera	PUCE						1?	2	1	1?	1+1?	1?	1?	1	?		18
Vochysia ferruginea	CATIE						2?	4	2	1?			1?			1?	9(11)
Ceiba pentandra	INPA	1?	1?	1?	1?	1?	1?	2	2?	1?	1	1?	1?		?	1	21(23)
Ochroma pyramidale	PUCE	1?	1?		1?	1?	1?	2	1	1?	2	1?	1?		?		19
*Cordia alliodora**	OFI	1?	1?	1?	2	3	2	4	1?	1?	2?	1?	1?	X	?	2?	
Socratea exorrhiza	PUCE	1?	1?	X	1?	1?	1?	2?	1	1?	2	1?	1?	1+1?	?	1?	20
*Bombacopsis quinta**	OFI					1	3	4	1?	1+1?			2?				
Schizolobium parahyba	UFRJ	1?	1?	1?		1?	1?	2	1	1?	1	1?			?	6	20

existing sample

? - indicates uncertainty as to whether or not collection can be made / need to access other sources than direct collection

People and partners responsible for getting contacts in particular target countries to facilitate collection indicated in first row, partner responsible for overall collection in column 2..

- Material collected by DB
- ** *Swietenia humilis*

Workpackage 6 (UA): Gene dynamics and quantitative seed performance in relation to landscape

This workpackage aims to examine the mating system and fitness consequences of tree isolation across a landscape for a range of species. The analyses are based on an individual tree context such that detailed information on the landscape context for each mother tree will be collected and genetic, mating system, gene flow and fitness parameters recorded for progeny arrays, not the mature tree generation, will form the basis of analysis. The sampling design proposed is also designed to fit well with ECOGENE simulation modeling.

Species choice
Several species changes have been made, as per the table.

Population sample design
Two different population sample designs are proposed, the first design is the most preferable, but if there are limitations, the second can be considered:

1. A single population which exhibits a gradient of pressure involving all the two extremes of impact (e.g. dense forest to isolated pasture individuals, White et al, 2002, PNAS). In this case 40 mother trees should be sampled for progeny.
2. Where 2 populations exhibit different densities or pressures (e.g. intact forest, fragmented and isolated trees), then partners can consider sampling these separately (i.e. 20 mother trees from each). Although better statistical comparison will come from sampling 3 populations which demonstrate a gradation of the expected impact.

The spatial scale of the selected populations should be around 5-10km x 5-10km, corresponding to a landscape scale investigation.

Sampling mother trees
Population sampling should be 40 mother trees per site, if a single gradient site is chosen, and 20 mother trees from each of two sites, if sampling 2 separate sites (see next section above), where progeny are sampled according to the below mentioned criteria. Trees should be sampled at random across a site. For each tree the following information should be recorded by each partner:

- Exact GPS position
- DBH
- Distance to nearest 3 trees of same species
- Foliage projective cover (canopy density) around tree
- Basal area around tree
- Fruit output

Where partners have capacity, the following additional characters are also recommended:

- light reading around tree
- approx height
- canopy size
- tree form
- notes on regeneration
- demographic time series (if tree is part of permanent plot).

Where partners are interested, and have capacity, phenological data for each tree in the population can also be recorded. In addition supplementary pollen additions or crossing experiments can be undertaken if partners wish to test outcrossing depression issues or fitness of particular genotype combinations.

Sampling progeny arrays

For good progeny sampling, and to reduce variance, 20 seeds per progeny are suggested as the minimum sample number for genetic and fitness analyses. It is advisable also to sample leaf/cambium from mother trees to confirm the maternal genotype. Collected seed material will be germinated and raised by the collecting partner for at least 6 months, but preferably for up to 2 years to overcome maternal effects.

The following minimum set of characters should be noted for each progeny:

- Proportion of germination
- Mortality
- Regular (monthly?) growth measurement (height, diameter)

Additional characters can be recorded by partners, depending on capacity, and can include inbreeding characters like proportion albinism, polyembryonic sprouting.

Collection partners need to consider collecting at least 40 seed per tree to obtain 20 successfully germinated seedlings (assuming a 50% germination rate), and possibly more if additional analyses are planned. Leaf material will be sampled from seedlings in these fitness trials and sent to the genotyping partner.

An additional (optional) analysis was also suggested. It is possible that inbreeding depression will act to purge homozygotes from progeny arrays before the seedling establishment phase. Therefore if genotyping partners have the resources then an additional analysis can be performed. A comparison of genotypes can be made for 40 ungerminated and 40 germinated progeny, to examine heterozygostity levels before and after germination and establishment. This additional analysis can be limited to approximately half the mother trees for which seed progenies are collected. However to save wasted sampling, collection and genotyping partners should communicate about the likely number of samples to collect and types of analysis to be performed.

Collection and genotyping partners need to communicate about the likely volume of genotyping that will be possible to reduce wasted sampling effort. However in our experience more resources usually become available later on in a project for genotyping by which time it is too late to go back and make additional field collections.

Analysis

Where possible, results of previous outcrossing or fitness studies should be used, so as not to duplicate work. In some cases fitness assessments only need doing, since mating system and gene flow studies exist (e.g. *Swietenia, Symphonia, Vochysia, Ceiba*), or large scale fitness surveys only need some genotyping effort to generate complementary data (e.g. *Cedrela* and *Swietenia*). For future final analysis, additional complementary data sets should be identified (e.g. *Gliricidia, S. humilis*).

Analysis will assess mating system (outcrossing rate, correlation of paternity, using Ritland models and exclusion analysis, which give different estimates of outcrossing), gene flow (mostly using TWOGENER approaches), and fitness (germination, growth and mortality of seedlings up to one year of age) characteristics for progeny arrays collected from individual trees in a range of landscape contexts.

Table for Workpackage 6 (yellow highlight indicates completed within project, purple indicates completed as part of previous work)

Swietenia macrophylla	density/post l	1-gradient	1x25x16	INPA	min	no	no			INPA	yes	exclusion	Lemes
Swietenia macrophylla	density/loggir	1-gradient	1x30x20(x2 logg	INPA	min	no	INPA	yes	no	INPA	yes	yes	
Swietenia macrophylla	density/loggir	4-plots	4x400x20	CEH	min	no	no			CEH	yes	yes	Cavers
Swietenia macrophylla	tree isolation	20 frag pops	20x10x10	CATIE	min	no	CATIE	yes	no	UoA	yes	yes	Navarro
Carapa guianensis	fragmentation	1-gradient	1x40x20(40)	CATIE	max	yes	CATIE	yes	no	INRA	yes	two gener	yes
Jacaranda copaia	fragmentation	1-gradient	1x40x20(40)	CATIE	max	yes	CATIE	yes	no	CNR	yes	two gener	
Minquartia guianensis	fragmentation	1-gradient	1x40x20(40)	CATIE	max	yes	CATIE	yes	no	UoA	yes	two gener	
Simarouba amara	fragmentation	1-gradient	1x40x20(40)	CATIE	max	yes	CATIE	yes	no	INRA	yes	two gener	
Virola sebifera	fragmentation	1-gradient	1x40x20(40)	CATIE	max	yes	CATIE	yes	no	UoA	yes	two gener	
Vochysia ferruginea	fragmentation	1-gradient	1x40x20(40)	CATIE	max	yes	CATIE	yes	no	CEH	yes	two gener	Davies
Cedrela odorata	tree isolation	10 frag pops	10x10x10	CATIE	min	no	CATIE	yes	no	CEH	yes	two gener	
Schizolobium parahyba	fragmentation	1-gradient	1x40x20(40)	UFRJ	min	?	UFRJ	yes	no	UFRJ	yes	two gener	
Symphonia globulifera	density/loggir	1-gradient	1x40x20(40)	INRA	max	no	no			INRA	yes	exclusion	Degen
Symphonia globulifera	density/loggir	1-gradient	1x40x20(40)	INRA	max	?	INRA	yes	no				
Cordia alliodora	density	1-gradient	1x44x20	OFI	min	yes	no (but still have seed)			OFI	yes	exclusion	Boshier
Bombacopsis quinata	fragmentation	1-gradient	1x40x20(40)	OFI	min	yes	OFI	yes	no	OFI	yes	two gener	
Carapa guianensis				PUCE?			PUCE?			UoA/CNR			
Cedrelinga cataeniformis				PUCE?			PUCE?			UoA/CNR			
Cedrela odorata				PUCE?			PUCE?			UoA/CNR			
Ochroma pyramidale				PUCE?			PUCE?			UoA/CNR			

Dipterix panamensis	logging	dendrogene
Carapa guianensis	logging	dendrogene
Hymenea courbaril	logging	dendrogene
Bagassa guianensis	logging	dendrogene
Symphonia globulifera	logging	dendrogene
Dipterix odorata	logging	dendrogene
Jacaranda copaia	logging	dendrogene
Manilkara huberi	logging	dendrogene

Workpackage 7 (INRA): Data compatibility

- Significant advances have been made with the ECOGENE model and several new features have been added. BD has agreed to refine and send to SC a list of necessary data requirements for modelling. IS has agreed to coordinate data compatibility questions as a whole across the project.

Workpackage 8 (UA – AJL / UM - CD): Meta-analysis of data

Practical questions
Tackle the myths of restoration and conservation

Myth 1. Overharvesting might diminish recruitment in the collecting area, or might erode genetics of the site
SEEDSOURCE – not specifically tackled, but ISP data (WP6) and modeling (WP9) could usefully inform this debate

Myth 2. Sampling seed from 1 or 2 individuals captures a representative proportion of intra-specific genetic variation
SEEDSOURCE – Information from ISPs (WP6) and range-wide surveys of SSRs (WP5) provide ideal information to tackle this issue

Myth 3. Local is best
SEEDSOURCE – This is a core focus of the project. Combining information on local adaptation and outbreeding depression (WP2, WP6), evolutionary history of species (WP3), and the contemporary connectivity and distribution of key adaptive genes (WP5) provide key points to inform debate

Myth 4. Genetic pollution and hybridization are bad
SEEDSOURCE – not specifically tackled, but ISP data (WP6) on gene flow distance provides some useful information

Myth 5. We should aim to restore historic communities
SEEDSOURCE – Not tackled specifically but could generate guidelines from RTE (WP2) and range wide SSR/SNP analyses (WP5) in terms of informing restoration/plantation in a world influenced by climate change

Myth 6. The context of a seed sourcing tree (isolated vs forest) is not important
SEEDSOURCE – Information from the ISPs (WP6) specifically tackles this issue by linking mating system and progeny fitness changes associated with tree context

Myth 7. We only need to leave a few reproductive adults in a post-logging landscape
SEEDSOURCE – Information from the ISPs (WP6) and ECOENE modeling (WP9) specifically tackles this issue by linking mating system and progeny fitness changes associated with tree density and postlogging impact.

Myth 9. It is too difficult to track illegally harvested timber
SEEDSOURCE – Development of species phylogenetic markers and DNA extraction protocols from wood (WP3) can held produce DNA origin identification tools

Scientific questions and potential meta-analysis papers subjects

Phylogeography (WP3 & WP5)
- Is there generally higher genetic diversity of Amazon populations compared with populations in Mesoamerica (WP5)? This may result from (i) larger habitat area/population sizes in Amazonia, (ii) less habitat restriction during the Pleistocene

- Do the genetic divergences of populations separated by the Andes suggest a vicariant or dispersal origin?

- Are there major phylogeographic breaks common to cross-Amazon populations? Such breaks have been difficult to identify.

- Is there a single locus(loci) that is phylogeographically informative across species?

Adaptation (WP2, WP5 and WP6)
- What is the spatial scale of local adaptation? Are plants sufficiently adapted to local conditions to fair poorly in RTEs? Is there a range of plasticity in environmental response?

- Does outbreeding depression exist for trees

- Do species phylogroups relegated to Pacific and Atlantic forests express differential drought tolerance, as found in *Cedrela odorata*?

- Can we generate universal SNP markers for adaptive gene screening for non-model species

Gene flow (WP5 and WP6)
- Does the spatial scale of gene dispersal (neighbourhood area, slope of isolation by distance) correspond to expected dispersal abilities of study species?

- How do gene dispersal dynamics at local (WP6) and range wide scales (WP5) compare across landscapes and species

- Is there a mating system/progeny fitness threshold for density/fragmentation impact in natural populations

Workpackages 10 (OFI): Communication of biological and economic information

Significant progress was made gathering information from Central American sources regarding most effective means for dissemination of project outputs. Clearly, best targets and formats will differ between the Central American and South American regions and within different regions, but progress needs to be made to identify these pathways: partners (PUCE, INPA, UFRGS) agreed to undertake surveys / workshops (or most appropriate means) to identify best routes to disseminate project outputs.

APPENDIX II.

MATERIAL TRANSFER AGREEMENT

FOR RESEARCH-ONLY PURPOSES

[LEGAL NAME OF ORGANISATION], a signatory partner of the Specific Targeted research Project SEEDSOURCE agrees to the exchange of certain tangible materials and / or information , hereinafter named as "the Material(s)", with other members of the consortium, for the sole purpose of conducting the research specified in Annex 1 of SEEDSOURCE Contract (Technical Annex) ('the Research' or 'Project'), subject to the conditions specified below. The members of the consortium have signed with the Commission a contract, hereinafter the "Contract" (Contract number 003708) [LEGAL NAME OF ORGANISATION] that acknowledges that all Materials, whether directly or indirectly enclosed therein as well as extracts, replications, summaries, or derivatives thereof, may not be used for any form of commercial exploitation howsoever. The agreement will be taken to cover exchange of any plant tissue materials or any extracts, replications, summaries, or derivatives thereof, including genetic data. Hereinafter, "data" or "information" refer to outputs resulting from SEEDSOURCE project activities only.

The provisions of the Contract shall apply to the present agreement and shall prevail over any contradictory provisions. This Material Transfer Agreement does not imply any direct or indirect license or warranty whatsoever with regards to the Material and use thereof nor does it guarantee not to infringe any rights or claims from third parties with regards to the Materials or the Materials' suitability, novelty or safety for any purpose whatsoever. In consideration for partners in the consortium providing [LEGAL NAME OF ORGANISATION] access to the Materials and the right to utilise them for the Research, [LEGAL NAME OF ORGANISATION] agrees to the following conditions:

1. Any plant tissue material collected as part of the work programme of the project will be used only by partners within the consortium and no part of the Materials or any extracts, replications, summaries, or derivatives thereof will be transferred or distributed to any third party howsoever.

2. Any plant tissue material or any extracts, replications, summaries, or derivatives thereof collected as part of the work programme of the project will be used only for the purposes indicated in the original project plan or, where project activities differ from the original plan, only for studies explicitly agreed between consortium partners.
[LEGAL NAME OF ORGANISATION] undertakes to use the Material according to the national and international laws and regulations and will make his business of obtaining all authorisations needed to the conduct of its research and experiment.

3. Any use of the collected material or any extracts, replications, summaries, or derivatives thereof additional to the project remit, or beyond the project lifetime, must be subject of explicit written agreement between the original collectors and those wishing to use the samples.

4. DNA and genotype data generated within the project should be used only for the purposes indicated in the original plan and within the project, any other use of the data should be subject of explicit written permission from the researchers generating the data.

5. [LEGAL NAME OF ORGANISATION] undertakes not to disclose any information whatsoever with regards to the Material and use thereof, without the prior written approval of partners involved in its generation.

6. Documents to be published concerning outputs of the project should include both collectors and researchers generating genotypic data, where both have had intellectual input, and reflect the relative contributions. An agreement between the authors should be obtained.

7. In a spirit of mutual cooperation, where collection and analysis of a species is carried out by more than one partner, complete sets of DNA extracts and genotypic datasets will be exchanged between partners involved for that species, for samples for which both partners have had involvement.

8. To hold harmless [LEGAL NAME OF ORGANISATION] and its governors, officers, employees and agents from any and all liabilities or claims brought by third parties resulting from the transfer to and use of the Materials by the Recipient.

9. This Agreement is personal to [LEGAL NAME OF ORGANISATION] and not capable of assignment.

10. This Agreement is subject to Law of the Contract.

On behalf of and for [LEGAL NAME OF ORGANISATION]
Date:
Signature:
Name (print):..... .....
Title:.....

The agreement will cover the following species in the first instance:
Bertholletia excelsa
Carapa guianensis
Cedrela odorata
Hymenaea courbaril
Jacaranda copaia
Minquartia guianensis
Simarouba amara
Swietenia macrophylla
Symphonia globulifera
Virola sebifera
Vochysia ferruginea
Ceiba pentandra
Ochroma pyramidale
Cordia alliodora
Socratea exorrhiza
Bombacopsis quinata
Schizolobium parahyba