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Genetic diversity and population structure of trees in fragmented dry zone forests of Central America.

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FINAL REPORT

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1. Executive Summary

Project Purpose

Sources of biodiversity depletion identified & strategies to minimise impact developed & promoted.

Mesoamerican dry forests are amongst the world's most threatened habitats: a mosaic of habitats, which under human influence have become highly fragmented and localised. The same forests are the source of many tree genera that are used, or show potential for use, worldwide. Conservation here must be pro-active, but may take different forms; *e.g.* ecological restoration, or active *in situ/circa situm* conservation of specific, economically important, tree species through local use. The project aimed to provide data for genetic management by; a) assessing effects of fragmentation on intra-specific genetic diversity, mating system & gene flow, b) relating habitat, population size & proximity of alternative populations/habitats to intra-specific diversity, c) devising strategies for conservation of mesoamerican dry forest tree species.

Research Activities

The project studied intra-specific genetic diversity, mating system, gene flow, phenology, localised adaptation and inter-provenance crossing in "undisturbed" and fragmented forest for four species (*Bombacopsis quinata*, *Cedrela salvadorensis*, *Leucaena salvadorensis*, *Swietenia humilis*).

Outputs

For the species and degree of separation studied (≤4.5km), there was no genetic barrier between remnants, but altered and proportionately greater inter-fragment gene flow over longer distances. The results contrast with traditional views, where increases in spatial isolation and population size reduction were considered to reduce inter-fragment gene flow. Although there was evidence for localised adaptation at a 50km scale, there was no evidence for outbreeding depression from the altered levels of gene flow. There was also increased seed production under increased disturbance for *S. humilis*, although not for *B. quinata*, whose conservation appeared to be limited by poor regeneration.

The genetic impacts of fragmentation are complex, but in some tree species pollination occurs over far greater distances and more frequently than previously thought, such that connectivity may be high across landscapes with little forest cover. With adequate gene flow and seed production, some remnant forest patches and trees may be important contributors to connectivity and conservation more generally. Their description as 'isolated' or 'living dead', with little or no conservation value, is for some species misleading and more human perception than a true reflection of any biological reality. It is more realistic to view remnant forest patches not as islands, but as existing within a mosaic of land uses which differ in their capacity to provide habitat or permit movement. Conditions in many large lowland estates are less propitious than on steep land under subsistence production. There will be a distance beyond which genetic isolation occurs, but determination is experimentally problematic, thresholds will vary between species, depending on pollinator characteristics and availability, and specificity of the tree-pollinator relationship. Self-compatible species that normally show some level of outcrossing are likely to show much lower thresholds than self-incompatible species. Declines in pollinator populations may also affect gene flow, reproduction and hence viability of remnant tree populations.

Contribution to DFID's development goals

The project identified, with others (*e.g.* R6913), some of the potential for, and limitations to, biodiversity conservation at the forest/agriculture interface. Outputs were disseminated through courses, workshops and publications to a range of users. There is a need to alter perceptions of conservation planners more used to traditional *in situ* conservation methods, to consider the possibility that trees outside protected areas have a role in biodiversity conservation. This requires the direct involvement of development organisations and an effective two-way communication between them and 'traditional' conservation organisations to ensure both conservation and development benefits. There is a need to raise awareness amongst development professionals of the value of natural regeneration both as socio-economic and conservation resources. Promotion of the planting and use of multi-purpose and/or exotic tree species which does not take into account the range of forest products available to farmers from natural regeneration may have deleterious effects on both farmer welfare and conservation.

List of acronyms

CATIE	Centro Agronomico Tropical de Investigación y Enseñanza, Costa Rica
CIFOR	Centre for International Forestry Research
CITES	Convention on International Trade in Endangered Species
COHDEFOR	Corporación Hondureña de Desarrollo Forestal
CONSEFORH	Proyecto de Conservación y Silvicultura de Especies Forestales de Honduras
ESNACIFOR	Escuela Nacional de Ciencias Forestales, Honduras
FAO	Food and Agriculture Organization
FRP	Forest Research Programme of DFID
LEUCNET	Leucaena Research Network
MINAE	Ministerio del Ambiente y Energia (formly MIRENEM), Costa Rica
MIRENEM	Ministerio de Recursos Naturales, Energia y Minas, Costa Rica
NGO	Non-governmental organization
SCRI	Scottish Crops Research Institute
UNAH	Universidad Nacional Autonoma de Honduras

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2 Background

RESEARCH PROGRAMME PURPOSE: Biodiversity of the forest/agriculture interface conserved
RESEARCH PRODUCTION SYSTEM: semi-arid
COMMODITY BASE: Timber, non-timber forest products, wildlife
BENEFICIARIES: Central American, Mexican and Global community, smallholder farmers, ecotourists
TARGET INSTITUTIONS: International: CIFOR, IPGRI, IUCN, WCMC; Regional: CATIE;
National: COHDEFOR, INIFAP, SARH, SPN, NGO's
GEOGRAPHIC FOCUS: Mesoamerica

2.1 The Importance of Mesoamerican Dry Forests

The dry forests of Central America and Mexico are amongst the world's most threatened habitats (< 2% remains, with 0.1% in protected reserves). They consist of, contrary to the views of H.M.S. Sulphur's botanist, a mosaic of floristically rich habitats, which owing to human influence have become highly fragmented and localised. Indeed the same forests are the source of many tree genera which are used, or show potential for use, both locally and worldwide for timber and multipurpose trees (*e.g. Gliricidia*, *Leucaena*, *Prosopis*), while several important timber species (*e.g. Astronium graveolens* and *Bombacopsis quinata*) are almost commercially extinct and some (*e.g. Swietenia humilis*) are on the CITES endangered species list.

2.2 Genetic Diversity in Tropical Trees and the Problem of Fragmentation

The realisation of conservation objectives through strategies emphasising the role of remnant forest patches and trees in farming systems (*circa situm*), is favoured by a growing awareness of their possible importance in sustaining not only gene pools, but also environmental services and rural livelihoods. Given the general inadequacy of resources available to support conservation *per se*, incorporation of genetic conservation criteria into forest and farm management practices will offer, in many cases, the best prospects for achieving conservation goals. It is only recently that genetic conservation strategies have sought to capitalise on such practices (*e.g.* del Amo 1992, Hellin & Hughes 1993). Yet knowledge of the effects of exploitation (harvesting and fragmentation) on tree gene pools is poor and insufficient to prescribe genetic management in such situations, limiting the effectiveness of on-going projects. Consequently the linkages between development and conservation in many "integrated" programmes are often too diffuse to ensure mutual success. Establishing the potential and limitations of *circa situm* strategies, to "last desperate effort" conservation scenarios, is of vital importance and relevance, under the increasing rate of deforestation worldwide. This will increase the possibilities for active conservation by local communities, through utilisation and maintenance of the resources and in turn permit the development of strategies to optimise the potential for conservation, within the constraints imposed by management for production in the forest/agriculture interface.

Much recent work has sought to apply our improving understanding of forest population structure and dynamics to the conservation of forest genetic resources (*e.g.* Adams *et al.* 1992, Bawa & Krugman 1991, Kemp 1993, Ledig 1992, Ledig *et al.* 1990). However, lack of information characterising both species distribution and frequency, and meta- and sub-population structure is a major caveat to generalisations (Bawa & Ashton 1991, Moran & Hopper 1987). Given the seminal importance of mating systems and patterns of gene flow in determining levels, patterns and dynamics of genetic diversity (NRC 1991), recent research using isozyme and DNA markers has focused on these topics. The size of the task in relation to the limited resources available dictates that researchers concentrate on; a reduced group of taxa taken to be representative of others sharing similar attributes, and studies that will inform more generally about issues of highest priority (Moran & Hopper 1987, NRC 1991). Better definition of metapopulation structure and dynamics, and clarification of the effects of fragmentation and its longer-term consequences, are two priorities about which consensus is emerging (*e.g.* Murawski 1995, Young 1995). Information however remains both limited and biased towards coniferous taxa of the northern temperate forests (Hamrick 1992, Muona 1989, NRC 1991). Although recent tropical studies (reviewed by Murawski

1995) have begun to redress the balance they have also shown that sampling strategies, adapted from recommendations for temperate trees are inappropriate to the tropics.

Understanding of gene flow, genetic spatial structure and mating systems of tropical forest trees has progressed over the last 30-40 years (Corner, 1954; Federov, 1966; Bawa, 1974). Tropical forests, characterised by high species richness and low to medium species densities (Hubbell, 1979; Gentry, 1992; Phillips *et al.*, 1994; Richards, 1996), contain species which were thought likely to be self-pollinated, since large interplant distances and asynchronous flowering would reduce the chance of successful cross-pollination (Corner, 1954; Federov, 1966). Consequently, the predominant view was that populations would be highly genetically structured due to genetic drift. Similarly, Ashton (1969) argued that tropical forests would be genetically structured, although structuring was most probably due to limited seed dispersal, a high degree of selfing and near neighbour pollinations along with habitat selection. More recent studies have shown that a high proportion of tropical tree species are naturally outcrossing (*e.g.* Bawa 1974, Bawa *et al*., 1985), with the associated risks from inbreeding of reduced fertility, growth, environmental tolerance and susceptibility to pests or diseases (*e.g.* Sim 1984, Griffin 1990). Maintenance of genetic diversity is therefore vital for the long term viability and adaptability of populations of many tree species.

Gene flow through pollen movement is dependent upon numerous factors, including the pollinator, sexual system and mating system (Loveless & Hamrick, 1984). The type of pollen vector (*e.g.* wind, bees, hummingbirds and bats) may disperse pollen over different distances (Loveless & Hamrick, 1984). Furthermore, pollinator behaviour, such as traplining (in which the animal moves preferentially along corridors or between precociously flowering trees) as seen in some bee, bat and butterfly species (Frankie & Baker, 1974; Gilbert, 1975; Ackerman *et al.*, 1982), has a significant effect on pollen dispersal patterns. Tropical trees have a diverse array of sexual systems including hermaphroditic, monoecious, dioecious, dichogamous and heterostylous species (Bawa & Beach, 1981; Loveless and Hamrick, 1984; Bawa *et al.*, 1985; Loveless, 1992) which have implications for pollen flow (*e.g.* dioecious species are unable to self-pollinate). Furthermore, the mating system may be predominantly outcrossing as both pre-zygotic and post-zygotic (late-acting) incompatibility systems in hermaphroditic and monoecious species may ensure outcrossing (Richards, 1986). In general, isozyme studies have found the majority of species to be highly outcrossed (Stacy *et al.*, 1996; Nason *et al.*, 1997; Nason & Hamrick, 1997), although some species with mixed mating systems have been identified (*e.g.* Murawski *et al.*, 1990, 1994; Murawski & Hamrick, 1992a, b). Finally, the distribution and density of mature trees (Murawski *et al.*, 1990; Murawski & Hamrick, 1991, 1992a) along with the flowering synchrony (Boshier *et al.*, 1995b) have been shown to influence pollen dispersal and hence outcrossing rates.

Gene flow, through pollen movement, has been shown to occur over larger distances than that of seed (Boshier *et al.*, 1995a; Nason *et al.*, 1997). In the species studied so far, high proportions of pollinations have been found to be affected by near neighbours, although significant amounts of pollen have been detected from distances of 1km (Stacy *et al.* 1996; Nason *et al.*, 1997). These results have important implications for the level and distribution of genetic variation over larger spatial scales. In general, the amount of differentiation among populations of tropical trees has been shown to be distance dependent. Gene flow among populations separated by 1-8km has been found to be relatively high (Schierenbeck *et al.*, 1997; Loveless and Hamrick 1987; Hamrick and Loveless, 1989). However, studies that extend to 100km indicate that the genetic distances between populations generally reflect their geographic separation (Hall *et al.*, 1994a,b; Rocha and Lobo, 1996). Thus genetic structuring may exist from the near neighbour level within populations to between populations that are separated by hundreds or thousands of kilometres.

The potential effects of fragmentation on remnant stands and trees, and their consequent conservation value, are complex and therefore the subject of debate (*e.g.* Heywood & Stuart 1992, House & Moritz 1991, Janzen 1986, Saunders *et al.* 1991, Young 1995, Young *et al.* 1996). Fragmentation may represent an erosion of diversity almost as serious as that due to species loss, with the possible destruction of allelic complexes of locally adapted populations through altered patterns and levels of gene flow (Ellstrand 1992, Ledig 1992). Whilst fragmentation may reduce populations below critical size and gene flow to a level below that necessary to prevent genetic drift, it may also increase or change patterns of gene flow between remnant populations, and hence maintain or increase within-species diversity (Bawa 1994, Hamrick 1992, Young 1995). At the pessimistic extreme are views, such as that enunciated by Janzen (1986), of the genetic conservation value of remnant trees in agroecosystems, which he characterised as "the living dead". More optimistically, the extensive and effective gene flow maintained between isolated trees of many taxa, even those in severely fragmented landscapes, suggests that remnant forest patches and trees can be effective and important in conserving genetic diversity (Hamrick 1992). Preliminary studies (Prober & Brown 1994, Young *et al.* 1993), and Young's (1995) review, of the effects of fragmentation on forest tree populations, though restricted to temperate regions, suggested that the major impact of fragmentation may be the reduction of genetic diversity in remnant populations through the generation of genetic bottlenecks.

The constraints identified above (fragmentation, loss of dry forest, sampling strategies, role of forest remnants/trees for *in* and *circa situ* conservation) and the need for research to resolve them, were identified at a variety of levels from international (*e.g.* CIFOR) to local government in Central America (*e.g.* CONSEFORH, Honduras; MINAE, Costa Rica; Hughes, 1989) and NGO's (*e.g.* Estudios Rurales y Asesoria, Mexico), and logically led to the research undertaken through this project. In addition the species chosen for investigation in this project have been shown to be of high priority to local people, on the basis of end use preferences (*e.g.* Colindris *et al*., 1995).

3 **Project Purpose**

The overall aim of the project was to study intra-specific genetic variation in four tree species, *Bombacopsis quinata, Cedrela salvadorensis, Leucaena salvadorensis*, *Swietenia humilis* under the prevailing conditions of habitat fragmentation within the Central American dry forest ecosystem. This report covers the project's research activities, collaborations, outputs (publications, courses, workshops, trials) and the implications of the results for future research and forest management.

Specifically the project aimed to:
a) assess the effects of fragmentation on intra-specific genetic diversity, mating system and gene flow (within and between fragments);
b) relate habitat and population size to intra-specific diversity;
c) relate diversity to proximity of other populations/habitats;
d) devise appropriate sampling strategies for the four species under the prevailing conditions.

The four "marker" species, represent different types of breeding system, pollination mechanism, seed dispersal mechanism and successional status. The species are also of socioeconomic importance, to local people and, in some cases, as exotics in other parts of the world.

B. quinata, from Central America, Colombia and Venezuela, is well known for the high value of its timber; which is mainly used in carpentry, interior panelling, window and door frames. Due to general deforestation in its natural distribution and felling for its timber, it has been classified, by the FAO Group of Experts on Forest Genetic Resources, in the highest priority for genetic conservation, exploration and evaluation (FAO, 1993). It has also been singled out as a species severely threatened at the population level (FAO, 1986).

C. salvadorensis, from Central America and Mexico, is restricted to calcareous outcrops in dry forest ecosystems. A remnant of dry forest, discovered in the Comayagua valley (Cerro de Manzanillos), has the only known population of *C. salvadorensis* in Honduras. COHDEFOR/ CONSEFORH are seeking to help conserve this forest, as the best remaining area of dry forest.

L. salvadorensis has a narrow, and now highly fragmented, natural distribution from the north-west of Nicaragua, southern Honduras, to the east of El Salvador. The species' continued existence is probably due to its use on farms, as the majority of forest in which it was originally found has long since disappeared (Hellin & Hughes, 1993). Evaluation of the level of intra-specific genetic variation was started under R.4524 (Intensive study of *Leucaena* genetic resources in Central America & Mexico) and the activities of CONSEFORH, but was limited by lack of material from the western part of its distribution due to the political situation prevailing at the time.

S. humilis occurs on the Pacific watershed of Central America and Mexico, and has been placed on appendix 2 of the CITES endangered species list. This in itself indicates both the social and economic importance of the species. There is no information on the level of intra-specific variation in *S. humilis*, although there is some limited evidence of interspecific hybridization with *S. macrophylla* at the southern extreme of its distribution in Costa Rica.

4 Research Activities

The project examined various scenarios for the possible effects of fragmentation on within species diversity, the possible destruction of allelic complexes in locally adapted populations through altered patterns and levels of gene flow, and the genetic conservation value of remnant forest patches and trees in agroecosystems. For the species/habitats under study, genetic diversity is not constant, but in a state of flux as populations adapt, expand, contract and even disappear. Even if time were available, it would not have been possible to study the change of diversity with time by artificially fragmenting the forest (*cf.* study in Brazilian amazon, Lovejoy *et al.*, 1986) as such pristine habitat no longer exists for this ecosystem. To counter this, populations were studied where there was evidence of past status. The study of mating system, gene flow *etc.* allowed discrimination between populations with similar levels of diversity, but which are, in genetic terms, moving in different directions.

The initial project plan of work was followed, namely;

1) Identification of suitable areas and fragments of forest containing populations of selected species under differing conditions. Populations were mapped and individual trees marked.

2) Phenological observations were carried out in stands over four years to look at year to year variation in flowering. Where little was known about a species' flowering phenology, detailed observations were carried out on trees.

3) Controlled pollinations to study/confirm the absence/presence/type of self incompatibility.

4) Seed was collected from trees to carry out starch gel electrophoresis. 60 seed per population assayed, with genetic diversity, structure and gene flow assessed among populations.

5) Mating system and gene flow studied using seed arrays.

6) Results synthesised, along with those from on-going ecological research, to give an integrated picture of genetic viability of dry zone trees under conditions of fragmentation.

The project worked in close cooperation with R6080 (Development & application of molecular assays for tree populations), R6168 (A study of the reproductive biology & population differentiation of *B. quinata*), R6913 (CUBOS) with interchange of both information and material, and joint work at some sites. During the project major delays were caused by; i) a fire at Lomas Barbudal, such that seed of *S. humilis* could not be collected from sufficient trees until 1997; ii) initial failure to find *L. salvadorensis* in El Salvador delayed collections; iii) failure of the collaboration from the National University of Honduras.

Table 1. Details of populations mapped and seed collected at sites (MS = mating system, GF = gene flow, PD = population differentiation, * = possibly *S. macrophylla* or *S. macrophylla* x *S. humilis*)

Species	Site	Site Type	Country	No of trees collected	Purpose
B. quinata	Lomas Barbudal Biological Reserve	Protected Area	Costa Rica	53	MS/GF
	La Mula, Paloverde National Park	Protected Area	Costa Rica	32	MS/GF
	Playa Nancite, Santa Rosa National Park	Protected Area	Costa Rica	52	MS/GF
	Islas Murcielagos, Santa Rosa National Park	Protected Area	Costa Rica	5	PD
	Reserva Absoluta Natural Cabo Blanco	Protected Area	Costa Rica	27	PD
	Hacienda Ciruelas, Guanacaste	Farm	Costa Rica	20	MS/GF
	Punta Ratón, Choluteca	Private Forest/Farm	Honduras	110	MS/GF
C. salvadorensis	Cerro Los Manzanillos, Comayagua	Private Forest	Honduras	14	MS/GF
L. salvadorensis	San Antonio del Norte, La Paz	Farm	Honduras	10	PD
	Calaire, Choluteca	Farm	Honduras	81	MS/GF
	Nueva Esparta, La Union	Farm	El Salvador	9	PD
S. humilis	Lomas Barbudal Biological Reserve*	Protected Area	Costa Rica	12	MS/GF
	Playa Nancite, Santa Rosa National Park	Protected Area	Costa Rica	63	MS/GF
	Comayagua Valley	Farm	Honduras	20	PD
	Punta Ratón, Choluteca	Private Forest/Farm	Honduras	39	MS/GF
	Cerro Las Tablas, Choluteca	Private Forest/Farm	Honduras	53	MS/GF

4.1 Study site populations

Various areas and fragments of forest, containing populations of the selected species, were selected in Guanacaste (Costa Rica) and in Choluteca (Honduras) on the basis of characteristics such as, number of trees of the marker species and separation from one fragment to another. The selected sites are summarised in Table 1. The conditions ranged from continuous "undisturbed forest" (Lomas Barbudal); fragments in mosaic situations (Bosque La Mula, Cerro Las Tablas, Punta Ratón); highly isolated patches (Cerro Manzanillos, Playa Nancite, Islas Murcielagos); trees on farms (Cerro Calaire, Hacienda Ciruelas, Punta Ratón, Nueva Esparta). At each site trees of *B. quinata*, *C. salvadorensis*, *L. salvadorensis* and *S. humilis/S. macrophylla* were numbered, position surveyed and classified roughly for age/maturity. Maps were produced using TREMA (R5648). Topographic maps and aerial photos of differing ages helped in verification of site history over the last 40 years. At several sites fires and tree felling during field work (table 3 in Boshier *et al.*, in press) confirm the threat to this ecosystem, even when the forest is within a "protected" area. During two short trips to eastern El Salvador, an extensive area of the ex "war zone" was covered, with only one group of *L. salvadorensis* (16 trees) found. Herbarium specimens of both *L. salvadorensis* and *L. shannonni* were collected and represent the first collections of *L. salvadorensis* in El Salvador since the type specimen in 1924. The farmer on whose land the trees stand commented that the species is very useful, but his were the only examples that he knew of in the area.

4.2 Phenology and controlled pollination studies

Flowering and fruiting phenology (stage & quantity) were studied for the three flowering seasons within the populations of *B. quinata*, C. *salvadorensis*, *L. salvadorensis* and *S. humilis*. In the case of *S. humilis* the relatively short flowering period of individual trees (approx. 30 days) and the considerable degree of within population flowering asynchrony inevitably results in reduced mating possibilities and within population structuring. In contrast the extended flowering of both *B. quinata* (approx. 70 days) and *L. salvadorensis* (>100 days) leads to higher flowering synchrony and mating possibilities. Incorporation of this information with the isozyme data increased understanding of population substructure and effective size. Detailed phenological observations were made on individual trees of *L. salvadorensis* and *S. humilis*.

L. salvadorensis

Phenological and controlled pollination studies of *L. salvadorensis* were carried out in a seed orchard (CONSEFORH trial E16/88). To study the nature of the incompatibility mechanism, controlled crosses, between and within families, were made in the same seed orchard. Problems were encountered with use of the controlled pollination techniques developed in Hawaii (Wheeler, 1991), and modifications were necessary to achieve an acceptable success rate under the more tropical conditions in Honduras. On the basis of the information from both the phenology and incompatibility studies, recommendations were made to the CONSEFORH project to improve the management and production of the seed orchard.

S. humilis

Thirty trees of *S. humilis* close to the CONSEFORH La Soledad trial site were used for reproductive biology studies. Superficially the flowers appear hermaphrodite, but more careful examination reveals that they are functionally either male or female, such that the trees are monoecious (see White & Boshier, 2000). Given the small size of the flowers and higher proportion of male flowers, it is difficult to locate female flowers within the trees. Careful observation (every 2-3 days) of these trees over several weeks (1995, 1996), revealed a temporal pattern in the production of male and female flowers in disagreement with that previously reported (Lee, 1967; Styles, 1972). The difference is probably due to an overemphasis in previous studies on herbarium material. Controlled pollinations were made (self and cross) to examine incompatibility in the species. In the former case the seed takes eleven months to mature, and the seed was collected to permit confirmation of allelic segregation in the molecular markers. As gene flow occurs through both pollen and seed, measurements were made of seed dispersal.

4.3 Optimal Outcrossing Distance

Fragmentation may alter pollinator movements and hence pollen flow. While molecular studies provide data on pollen flow, also of importance is the determination of what effect different patterns have on species fitness. Mixing of gene pools (*e.g.* provenances) may lead to outbreeding depression, and so experiments were carried out to determine the effect of pollination distance in *B. quinata* and *S. humilis*.

For *B. quinata* controlled pollinations were carried out in one natural population (Hacienda Ciruelas, Guanacaste) and one trial site (La Soledad, Comayagua). At each site flowers were pollinated using pollen from a single source of known distance. In Guanacaste 529 pollinations were performed on 11 trees with pollen from 0m (self) to 500km away, giving 21.2% capsule formation. At La Soledad 581 pollinations were performed on 28 trees (southern Honduras origin), with pollen from 0m (self) to 1,000km away, giving 34.1% capsule formation. Mature capsules were collected with capsule size, number of viable and non-viable seed recorded as initial measures of fitness. Seed from each capsule was planted in soil from the maternal environment, with germination, height and number of leaves recorded. The seedlings were planted in a replicated trial in the maternal environment for future evaluation of fitness. In addition the same material was planted in a; a) green house trial and b) common garden trial with measurement of the same fitness parameters. For *S. humilis* controlled pollinations were carried out in one natural population in the Comayagua Valley, with flowers pollinated with pollen from 0m (self) to 120km away. Pollinations were carried out on a total of 478 thyrses on 15 maternal trees, with pollen ranging from 0m (self) to 120km away. Pollinations were monitored at approximately one, two and 12 months to determine capsule set. Mature capsules were collected with capsule size, number of viable and non-viable seed recorded as initial measures of fitness. Seed from each capsule was raised in a greenhouse trial with germination, height and diameter recorded as measures of fitness.

4.4 Localised adaptation

Reciprocal transplant experiments were established over two spatial scales (1-55km & 400km) to examine the scales at which localised adaptation may occur in both *B. quinata* and *S. humilis* populations in relation to patterns of genetic diversity, gene flow and habitat heterogeneity. Edaphic differences among the sites were examined by taking soil samples from each seed collection site. In addition, where the reciprocal transplant trials survived a soil sample was taken from within the trial. Survival, height and diameter were recorded for each plant, at nine and 21 months after sowing.

4.5 Collaborative Agreements

Agreement COHDEFOR/CONSEFORH/ESNACIFOR/OFI

Within Honduras the project's activities were carried out under an access agreement drawn up with CONSEFORH, COHDEFOR and ESNACIFOR. The agreement was used as a template for agreements with other FRP projects active in Honduras and marks a step forward in terms of COHDEFOR's desire and ability to control and influence the research carried out by foreigners in Honduras. As part of the agreement, D. Boshier gave a different lecture each year to ESNACIFOR students. The project document was also translated into Spanish for use by collaborators. At the request of the CONSEFORH Directorate, D. Boshier participated in various discussions with staff on the seed collection, experimental design, trial management *etc*. Talks were also given to CONSEFORH staff on OFI activities in Central America, and more specifically on the implications of results from R4724, R5729 and R6516 for CONSEFORH.

Universidad Nacional Autonoma de Honduras (UNAH)

In 1995 phenology and controlled pollination studies were carried out in collaboration with a researcher of the National University of Honduras, a recent graduate of the MSc taxonomy course at Reading University. Support was provided for field costs and a small amount of laboratory consumables and equipment. This was initially an ideal way to provide both continuity to the fieldwork, and to encourage development of local research capacity. After a successful initial period of fieldwork, the UNAH staff failed to continue the agreed work programme, without informing OFI, which consequently led to the loss of seed from controlled pollination and the repetition of some work. Whilst such collaboration is desirable, for a number of reasons it is often surprisingly difficult to achieve despite there being many such staff without funding to carry out research.

5 Outputs

Research results and products are described in relation to the main research topics. A synthesis of the main findings is given here, while readers interested in more detail should refer to the publications related to each topic (listed at the end of each section) and available in appendix 3.

5.1 Impacts of fragmentation on mating patterns and gene flow

The levels of genetic diversity, genetic structure and gene flow, were studied across the native range of both *B. quinata* (Colombia to Honduras) and *S. humilis* (Costa Rica to El Salvador). Remnant stands of secondary dry forest, and remnant trees in pastures in the Punta Ratón region of the Honduran Pacific alluvial coastal plains were used, along with “control” plots in more continuous areas of forest in both Honduras and Guanacaste, Costa Rica to assess the impact of fragmentation. The fragments varied in size (7-150 trees) and degree of spatial isolation (1-4.5 km).

The levels of diversity and among population differentiation for *B. quinata* and *S. humilis* were similar to those reported for other neotropical tree species (*e.g.* Chase *et al*., 1995; Hall *et al.* 1994a, b, 1996; Rocha & Lobo, 1996). Both species showed decreasing genetic similarities between populations with distance, although over 500km among population genetic differentiation was less, and gene flow more in *B. quinata* (F_{ST} = 0.044, N_m = 5.4) than *S. humilis*, (F_{ST} = 0.173, N_m = 1.2), possibly reflecting differences in their dispersal modes. The greater gene flow in *B. quinata* may be due to the kapok-enclosed seed dispersing further on occasions than the winged *S. humilis* seed, and/or reflect the ability of bats to move greater distances than the small insect pollinators of *S. humilis*. Both species exhibited a hierarchical population genetic structure with genetic similarity related to geographic distribution, indicating that isolation by distance was responsible for the observed structure, as in other neotropical trees (*e.g.* Hall *et al.* 1994a,b; Rocha & Lobo, 1996). Indeed, for both species the clustering of populations identified "northern" (El Salvador and Honduras) and "southern" (Costa Rica) groups with the geographic intermediate Nicaragua populations lying in either of these groups. Grouping of two populations of *S. macrophylla* (Lancetilla, Honduras; Upala, Costa Rica). The higher level of among population gene flow in *B. quinata* was not associated with increased heterozygosity levels as the average observed heterozygosity was 0.171, compared to 0.283 for *S. humilis*.

For both species, genetic markers (isozymes or microsatellites) showed a lack of genetic differentiation between fragments (<5%), consistent with the relatively low heterogeneity observed in allele frequencies among populations of tropical trees separated by one to several kilometres (*e.g.* Loveless 1992, Schierenbeck *et al*. 1997). However, as a large proportion of the trees were present prior to fragmentation, the distribution of genetic variation was largely a reflection of the previous more continuous forest, current levels of genetic variation providing little indication of a population’s ability to retain genetic variation in the long term. Rather, the extent of current gene flow within and among the remnant patches will influence the long term effects of fragmentation, and whether any immediate rare allele loss through reduced population size, will be followed by more extensive theorised genetic depletion (Sork *et al*. 1998). Smaller fragments did show a higher loss of rare alleles, a sampling effect, that was consistent with results from other studies (Young & Clarke, 2000). *L. salvadorensis*, similarly, maintained high levels of genetic diversity even among the highly fragmented populations. The results for *L. salvadorensis* are presented as a case study (see appendix 3), which is used as a teaching exercise in all the conservation genetics courses.

Using allozyme markers, both *B. quinata* and *S. humilis* were found to be highly outcrossing (t_m> 0.95), with genetic structuring over similar scales (50-60m and 40-80m, respectively) and over 50% of pollinations effected within a 200m radius. There was no evidence for an increase in inbreeding, in particular selfing, as a result of fragmentation, with all fragments shown to be outcrossed (t_m> 0.90), reflecting the strong self-incompatibility mechanisms in each species. Direct measures of current mating patterns showed altered patterns of pollen flow both within and between fragments. Common to both controls and fragments for both species was a high frequency of pollen flow from within the first 300 m of the maternal tree, indicating the predominance of near neighbour mating. A large proportion of pollen donors were, however, from outside of each fragment and located across the sampled area, indicating an extensive network of gene exchange over this spatial scale (16 km^2). So in two fragments (22 and 44 *S. humilis* trees), 62% and 53% respectively of pollen donors were from within the fragment, while 24% and 34% were from distances greater than 1.5 km and 3.6 km respectively. There was no evidence for increased inbreeding within fragments, with both species continuing to show high levels of outcrossing even in the smallest fragments. Indeed, a *S. humilis* tree, 'isolated' by 1.2 km from the nearest flowering trees, showed 100% external pollen sources, in agreement with the species' self incompatibility, with over 70% from a range of trees from the main area of forest, over 4.5 km away. The increase in spatial isolation promoted long distance gene flow between it and all the fragments, in contrast with predictions that spatially isolated trees are more likely to deviate from random mating and receive pollen from fewer donors (Murawski & Hamrick, 1991).

For both species, at the degree of separation studied, fragmentation did not impose a genetic barrier between remnants. Rather it appeared to promote altered and proportionately higher levels of inter-fragment gene flow over longer distances, facilitated by the ability of pollinators to move between spatially isolated stands of trees (*e.g.* Raw, 1989). Such enhanced levels of long distance gene flow into the smaller fragments, also found in *Spondias mombin* (Nason & Hamrick, 1997), promote increased genetic mixing at this spatial scale and will potentially restore, maintain, or even increase, levels of genetic variation for populations of these species within this modified environment. This contrasts with traditional views of the genetic effects of fragmenting populations, where increases in spatial isolation and population size reduction have been considered to reduce gene flow between fragments (*e.g.* Templeton *et al*., 1990; Saunders *et al.*, 1991). The results are in agreement with Hamrick's conclusion (1992) that in neotropical trees two contrasting trends produce the general mating patterns observed: a) individual trees appear to receive pollen from relatively few pollen donors, but the genetic composition of the pollen received varies greatly from tree to tree; b) although a high proportion of fertilisation is effected by nearest neighbours, a significant proportion of pollen movement occurs over relatively long distances. The genetic impacts of fragmentation are complex, but for some neotropical tree species under fragmentation, pollination may occur over distances that are much greater than previously considered. The distance found for *S. humilis* is >10 times greater than that which was considered as distant by Chase *et al*. (1996) and is more in accord with the distance extremes previously identified by zoologists (*e.g.* Janzen 1971; Frankie *et al*., 1976). There will, however, be some distance beyond which genetic isolation will occur, followed by losses in genetic diversity and associated problems for population viability and adaptation (see discussion in Young *et al*., 1996). Although determination is experimentally problematic, thresholds will vary between species, depending on pollinator characteristics, availability, the specificity of the tree-pollinator relationship, and the presence and strength of any self-incompatibility mechanism (Boshier, 2000). Self-compatible species that normally show some level of outcrossing (*e.g.* Murawski & Hamrick 1992a), are likely to show increased levels of inbreeding at much shorter distances of separation (lower thresholds) than self incompatible species.

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WHITE, G.M., BOSHIER, D.H. and POWELL, W. (1999) Genetic variation within a fragmented population of *Swietenia humilis* Zucc. *Molecular Ecology,* **8**: 1899-1910. (Peer reviewed paper)

WHITE, G.M., BOSHIER, D.H. and POWELL, W. (in press) Pollen flow counteracts fragmentation in a tropical forest. *Proceedings of the National Academy of Sciences* (Peer reviewed paper)

5.2 Impacts of fragmentation on adaptation, inbreeding/outbreeding depression and population viability

The occurrence of localised adaptation has been promoted as a major factor in determining suitable seed sources for re-afforestation (*e.g.* Campbell, 1986; Frankel *et al.*, 1995), while the use and definition of ‘local’ seed sources is currently under debate (*e.g.* Linhart, 199x; Ennos *et al.*, 1998). The spatial scale of genetic variability is dependent upon the relative strengths and scales over which gene flow and natural selection occur. High levels of gene flow tend to have a homogenising effect, whilst restricted gene flow increases the likelihood of genetic structure. Similarly, differential selection between locations may result in genetic structure. This study was designed to examine the interaction between gene flow, localised adaptation and pollination distance on fitness (evidence for inbreeding and/or outbreeding depression) in tree species with contrasting dispersal strategies (*B. quinata*, bat pollinated with "kapok”, wind dispersed, seed; *S. humilis*, insect pollinated with winged, wind dispersed, seed). Thus the study directly addressed issues related to seed sources for ecological restoration, provenance delimitation and mixing from altered pollen flow dynamics following fragmentation.

Although the reciprocal transplant experiments of *B. quinata* failed to survive, those of *S. humilis* identified localised adaptation (home seedlings with greater size after 21 months growth) over a range of spatial scales, with the level of adaptation reflecting both the degree of geographic and genetic separation. Over 55km the mean home seedling advantage for volume was 45%, whilst over 400 km it was 84%. Although when selection pressures are high, environmental heterogeneity may determine the scale of localised adaptation (*e.g.* May *et al.*, 1975), other studies have identified adaptation over similar distances to those of gene flow (Stratton, 1995; Ronsheim, 1997). For many plant species gene flow appears to occur over scales greater than or equal to that of environmental heterogeneity, such that the level of gene flow is primary in determining the scale of localised adaptation. The scale of population differentiation and localised adaptation identified in both *B. quinata* and *S. humilis* is dependent on the geographic distance between populations, such that conservation of their genetic diversity will require maintenance of a number of populations stratified by geographic distance.

Recent studies have shown that fragmentation may lead to increased gene flow (*e.g.* this study; Young *et al.*, 1993) which could result in outbreeding depression from the mixing of genetically distinct populations. Although restricted seed dispersal and/or selection lead to genetic structure, pollination distance had little or no effect on the fitness traits measured (capsule formation, size and progeny growth). Both species were shown to be self-incompatible. *B. quinata* formed very few, small, capsules when selfed, with the progeny showing reduced growth, while all *S. humilis* selfs failed to form mature capsules. Related matings showed no reduction in fitness for either *B. quinata* or *S. humilis,* and only in the 1800 km *B. quinata* pollinations was any significant distance effect observed (smaller capsules with fewer seed than all pollination distances except selfs). However, 1800 km pollinations formed as many capsules as other distances, with no other evidence of outbreeding depression (germination or growth in trials). After 1800 km the most distant pollinations in *B. quinata* (400 km) were insufficient to result in smaller capsules, while in *S. humilis* the most distant pollinations (130km) failed to produce outbreeding depression. These studies suggest that either reduced or increased gene flow due to fragmentation would have no direct effect on genetic viability of fragments, as pollination distance had no effect on capsule formation or progeny fitness.

Other studies have found pollinations between related individuals show reduced success and/or offspring fitness (*e.g.* Ritland & Ganders 1987, Waser & Price 1991, 1994, Trame *et al.*1995, Hardner & Potts, 1995), with reduced fitness for the longest pollination distance evaluated, except in the only other tree species studied to date (Hardner *et al.*, 1998). The finding of localised adaptation in *S. humilis* without outbreeding depression may be due to a number of factors, not least those associated with estimating fitness. Estimating the fitness of trees is problematic as differences identified in seedlings (*e.g. S. humilis*) may not necessarily reflect adaptation to adult phase. Localised adaptation identified in *Pinus ponderosa* was not apparent until 20 years of age, coinciding with the onset of intense competition for water (Namkoong & Conkle, 1976). Early growth measurements in both *S. humilis* and *B. quinata* may, however, be reasonable indicators of future growth. Trials showed significant correlations in both *B. quinata* (height, 1 & 6 years; height and circumference, 8 & 26 years, Melchior *et al*. 1996; height and diameter with flower and capsule formation, Sandiford 1998) and *S. humilis* (height, 2 & 7 years). For both *B. quinata* and *S. humilis* there were also significant correlations between above ground volume and fresh and dry shoot mass, and fresh and dry root mass.

The extent of localised adaptation and lack of any pollination distance effect on offspring fitness may also be due to the ancient polyploid nature of the two species (Bawa, 1973). Increased chromosome numbers may buffer the dilution of alleles such that polyploids exhibit relatively low levels of localised adaptation and outbreeding depression, although the effect of ploidy on the latter has rarely been addressed (Grant, 1981). Polyploid species generally have wider geographical ranges and higher tolerances to ecological variables (*e.g.* pests, pathogens, drought) than diploid relatives, and as allelic richness may increase following polyploidisation, polyploids may have more adaptive ability and show low levels of localised adaptation (Grant, 1981). If the dominance model is assumed (as most data suggest) then inbreeding depression in tetraploids should be half that of the diploid progenitors. The failure to identify outbreeding depression in either *B. quinata* or *S. humilis* suggests that the break-up of polyploid co-adapted gene complexes contributed little or nothing to the fitness of individuals, although this may become apparent in the F_2 and subsequent generations.

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5.3 Prospects for *circa situm* tree conservation in Mesoamerican dry forest agro-ecosystems and more general perspectives for *circa situm* conservation

In situ conservation of the remaining areas of mature Mesoamerican dry forest is likely to be the most effective and efficient means of safeguarding a substantial part of the threatened specific and subspecific tree diversity of this forest type. However, many species and populations of concern are not well or not at all represented in these areas and require a different approach. Habitat restoration may also have a role, but the socio-economic conditions that make this a possibility are not common and costs may be prohibitive. *Circa situm* conservation has much to offer in these situations, but also significant limitations; in order to be effective its application must be guided by information obtained from multi-disciplinary research of the type outlined in section 6.5. The integration of this project with the "Conservation of tree species diversity in Mexico & Honduras" (CUBOS, R6514) project, includes socioeconomic aspects of communities' and farmers' practices with respect to trees and tree germplasm. This represents a unique attempt to understand both the causes and patterns of biodiversity depletion/maintenance to optimise the potential for conservation of inter and intra specific diversity within the constraints imposed by management for production. The contrasting examples of *B. quinata* and *S. humilis* illustrate that we cannot permit ourselves the luxury of assuming that all species are likely to persist in an agricultural mosaic until proven otherwise. However, neither should we underestimate the capability of many species to persist in large numbers in these agro-ecosystems under current practices, as this could lead to the misdirection of limited conservation resources towards species not under threat. *Circa situm* conservation of tree diversity is already happening, albeit as a side effect of the practices of resource-poor farmers. Given apparently similar conditions across large parts of the dry zone foothills of El Salvador, Nicaragua, and Guatemala *circa situm* populations may represent a considerable conservation resource. If conservation planners were to allow themselves to take this resource into consideration how many species currently assumed to be threatened by habitat loss might prove to be thriving (Vandermeer & Perfecto 1997)?

An enhanced future role for *circa situm* conservation in the dry tropics of Mesoamerica will depend principally on there being species of conservation importance in the agro-ecosystems and forests in question. Species of widespread occurrence whose status does not appear about to change and species well represented in adequately protected areas should not attract more of the scarce resources available for conservation. Are then agro-ecosystems likely to contain the types of species we wish to conserve? Agro-ecosystems are highly disturbed in comparison with the original vegetation, and the species that dominate are likely to be either ‘pre-adapted’ to disturbance (*i.e.* weedy, pioneer and/or coppicing), and/or positively selected for by farmers. Indeed many species owe part of their ‘value’ (both locally and globally) to their ability to survive in habitats that have been drastically altered from their original state and hence are often not threatened. The long distance dispersal of the propagules of these typically pioneer type species, coupled with ever increasing disturbance, ensure that they will generally not be conservation priorities. One priority must therefore be the relatively few species with much narrower distributions, endemic to areas now completely converted to agro-ecosystems (*e.g Leucaena salvadorensis*, Hellin & Hughes 1993). For these species *in situ* is *de facto* not a conservation option. A second priority would be those species and species assemblages whose continued *in situ* conservation would be enhanced by their conservation, *circa situm*, in agro-ecosystems between reserves.

An understanding of which tree species are where says nothing about the species’ viability within an agro-ecosystem. The effects of fragmentation and the regeneration of patches of secondary forest are likely to be dynamic and we must carefully consider the reproductive and regenerative capacities of priority species and the perpetuation of management practices that allow natural or artificial regeneration, to ensure we are dealing with populations that have a long-term future. The level of intraspecific variation actively conserved may be related to the range of farming practices into which any one species is incorporated, which can be quite diverse (*e.g.* Raintree & Taylor 1992). Yet knowledge of the effects of human interventions (*e.g.* harvesting and fragmentation) on tree gene pools is relatively poor. We still do not know at what stage forest fragments become genetically isolated from each other, nor do we fully understand the consequences of gene flow between managed and remnant natural populations. The selection pressures exerted by farmers, either intentionally or unintentionally, at the species, population and within-population levels are also little understood and their compatibility with conservation objectives are unclear.

In the case of the species studied here in southern Honduras it appears that to describe the remnant trees and forest patches in which they occur as ‘isolated’ or ‘living dead’ (Janzen 1986a), with little or no conservation value, would be misleading and more a conditioning of human perception than a true reflection of actual gene flow and any biological reality. It is more realistic to view remnant forest patches not as islands, but rather as existing within a mosaic of land uses which differ in their capacity to provide habitat or permit movement for any organism. In this sense it is important to recognise the complementary habitat role that maintenance of trees on farms is already playing to *in situ* conservation of the dry forest. To date, this and other studies suggest that for many genes, species, individuals and populations of tropical forest species, connectivity may in fact be high across a landscape with little forest cover. With adequate geneflow and seed production, some remnant forest patches and trees may be important contributors to connectivity and conservation, both *in situ* and *circa situm*, more generally. The capacity of some insect pollinators to move long distances has been shown (Webb & Bawa 1983), although the potential to move between patches will depend on their behavioural response to such a mosaic. Some bat species have been shown to move preferentially down forest tracks and pathways (*e.g.* Estrada *et al.* 1993). Whereas some bee species are generally restricted in their movements (Powell & Powell 1987), most show some long distance movements and some will move across agricultural landscapes over 4 km between forest fragments (*e.g.* Raw 1989). With a range of non-specialist pollinators the dynamics of pollen flow in *S. humilis* are probably far less susceptible to habitat disturbance than tree species with more specialist pollinator relationships; although *Ficus spp*, with species-specific wasp pollinators, may apparently form extensive metapopulations in fragmented landscapes (Nason *et al*. 1998). Changes in pollinator assemblages in fragmented landscapes may strongly affect patterns of gene flow and reproduction in remnant tree populations, and concerns that declines in pollinator populations in such altered agroecosystems may eventually limit tree reproduction require monitoring of numbers, as well as evidence for pollinator limitation (Allen-Wardell *et al*. 1998).

Active protection of trees by farmers is strongly dependent on their perceptions of the trees’ potential value to them and on recognition of farmers’ long-term ownership rights to the trees by other community members. However, the case of *B. quinata* clearly demonstrates that even if a species is valued its conservation is not guaranteed. In fact, *use value* may lead to a species’ decline until a threshold is reached where farmers are obliged to modify their practices if they wish to ensure a continued supply. It appears that at this point the rate of decline of many species tends to level out as mature individuals are managed on a more rational basis and removals replaced by natural regeneration which receives active protection by farmers. However, if regeneration is poor in the agroecosystem environment, as appears to be the case for *B. quinata*, it may continue to decline and disappear. Alternatively farmers may switch much of their demand for a particular product to another species.

Hughes (1998) suggests that *circa situm* conservation is probably limited to species that tolerate disturbance and are actively used and preferred by farmers. However, while it does indeed favour disturbance-tolerant species, the examples from southern Honduras and those provided by Kass *et al.* (1993) suggest that it is applicable to a broader range of species than just the actively preferred ones. However, the survival of non-preferred species may to a large extent depend on the technology level of land management practices; *e.g.* a greater number of stumps of non-valued coppicing species is allowed to persist in the low-intensity swidden agriculture of southern Honduran hillsides, than in the ploughed fields of the central valleys of Oaxaca, Mexico. Considerations of social justice argue against proposing ecosystem-level approaches that may deny farmers the opportunity to adopt more profitable practices, although in reality the opportunity cost of conservation may be minimal over much of the Mesoamerican dry zone, given the significant biophysical constraints (*e.g.* topography, resource availability) faced by farmers seeking to intensify production. At least in southern Honduras there appears little immediate risk of agricultural intensification occurring and leading to massive extinctions as Janzen (1986b) warns. *Circa situm* conservation strategies are more likely to be effective where planned on a species by species basis, within the farming systems context of a particular area, with resources focused on target species in order to minimise socio-economic impacts. The ‘area’ or ‘management unit’ over which such strategies would operate would be measured in numbers of participating households or numbers of land units in which target species conservation is successfully incorporated into existing, or acceptable, farm management practices.

The prospects *circa situm* for *S. humilis* appear good, but less so for *B. quinata*, and it is ironic that of these two species the former is the one afforded more protection by international convention, being listed under appendix II of CITES. However, for it or similar species, some form of monitoring may be advisable to ensure that its ‘niche’ in the farming system is maintained in the face of changing socio-economic trends. Current trends, at least in some areas, involve an iterative process of property subdivision and an elimination of forest and fallow areas with a corresponding increase in numbers of trees in grain fields and pastures. It is possible that farm size will eventually reach a threshold beyond which farmers are unwilling to further divide properties amongst their heirs, and increased dependence on off-farm sources of income will divert pressure from land and tree resources, leading eventually to a stable situation. However, it is unclear at what point this threshold will be reached, to what degree this decision will be influenced by the availability of off-farm income, and whether the reduction in farm size leading to this point will lead farmers to increase tree densities on farm to satisfy tree product needs, or reduce them to increase per unit yields of basic grains. The challenge and opportunity offered by *circa situm* conservation is to adapt to these changes over a relatively wide geographic area, rather than seek to halt these processes within a confined area such as a reserve. To this end there is a need to raise awareness amongst development professionals of the value of natural regeneration both as socio-economic and conservation resources. Efforts to promote the planting and use of multi-purpose and/or exotic tree species which do not take into account the range of forest products available to farmers from natural regeneration may have deleterious effects on both farmer welfare and conservation.

BOSHIER, D.H., GORDON, J.E. and BARRANCE, A.J. (in press) Prospects for *circa situm* tree conservation in Mesoamerican dry forest agro-ecosystems. In: *Biodiversity Conservation in Costa Rica, Learning the Lessons in the Seasonal Dry Forest.* Frankie, G.W., Mata, A. and Vinson, S.B. (Eds.) University of California Press, Berkeley, California, USA. (Book Chapter)

BOSHIER, D.H. and YOUNG, A. (2000) Limitations and future directions. pp. 289-297. In: *Forest conservation genetics: principles and practice.* Young, A., Boshier, D.H. and Boyle, T.J. (Eds.) CSIRO, Melbourne, Australia/CABI Publishing, Wallingford, UK. (Book Chapter)

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KANOWSKI, P.J. and BOSHIER, D.H. (1997) Conservation of tree genetic resources *in situ*. pp. 207-219. In: *Plant conservation: the in situ approach.* Maxted, N., Ford-Lloyd, B.V., and Hawkes, J.G. (Eds.) Chapman and Hall, UK. (Book Chapter)

YOUNG, A., BOSHIER, D.H. and BOYLE, T.J. (Eds.) (2000) *Forest conservation genetics: principles and practice.* CSIRO, Melbourne, Australia/CABI Publishing, Wallingford, UK. 350 pp. (Book)

5.4 Germplasm transfer and trial establishment

Seed transfer. Under existing agreements, collected seed was made available to collaborators in Central America, and as requested and where able, assistance, technical and/or financial, given to establish trials with the material. In this way a total of 0.5 kg of *B. quinata* and 5 kg of *S. humilis* seed was passed to collaborators. Negotiations are currently underway, as part of the rationalisation of OFI seed collections (R6551), for remaining seed lots of *B. quinata* and *S. humilis* to be held at CAMCORE and CATIE, so that they are still available for the establishment of provenance and progeny trials.

Herbarium specimens. A total of 61 voucher herbarium specimens, principally *S. humilis*, were collected to aid confirmation of tree identity. Duplicates were deposited in the National Museum (Costa Rica), UNAH, ESNACIFOR and Kew.

Progeny trial of *S. humilis*. Under an agreement with the Forest Restoration and Silviculture Programme (Guanacaste Conservation Area-ACG) of MINAE (Costa Rica) and CATIE's Tree Improvement Project (partially supported by R5729), a *S. humilis* progeny trial was established at Horizontes Experimental Station in 1995, using seed from the Playa Nancite collections. The trial serves both as an *ex situ* conservation stand for this rare population and as a possible future seed source for restoration and reforestation work in the ACG. Trial measurements by ACG staff have been passed to D. Boshier with a request for assistance in analysis.

***Ex situ* conservation stands of *Cedrela salvadorensis*.** CONSEFORH established *ex situ* conservation stands of *Cedrela salvadorensis* at its two research stations (La Soledad, Comayagua; Santa Rosa, Choluteca) using seed collected by the project from Cerro Manzanillos. It was not possible to complete molecular analysis of the population within the time frame of the project.

6 Contribution of Outputs

6.1 Dissemination Outputs

A total of: 1 book, 6 book chapters, 3 peer-reviewed papers, have been published (or in press) to date, as project outputs. A full list of the project's research outputs is given below. Four courses were taught in Honduras and Australia (total of 45 people from 16 countries). Participants held senior posts in government or NGOs, covering national park planning/management, forestry research, natural resources management, *etc*. Under the project the purchase and distribution of 200 copies of the textbook *Forest Conservation Genetics: principles and practice* was funded to key libraries and teachers in universities, forestry schools and research organisations. A summary of the distribution by country is given in appendix 1. Development and use of teaching material during short courses, included case study material for the development of a genetic conservation strategy for *L. salvadorensis* and a check list to guide compilation of information for the development of species specific genetic conservation strategies. These materials have proved useful in teaching forest conservation genetics and would warrant further development, probably in conjunction with a second edition of the *Forest Conservation Genetics* text book. In addition to presentations in congresses and training courses, four oral presentations were given to CONSEFORH to discuss application of results, and ESNACIFOR students on a range of topics (see list below). A list of other meetings attended, where a poster covering the R6516's work was presented, is given in appendix 2.

List of Publications, reports, workshops, training courses and oral presentations

Publications

BILLINGHAM, M.R. (1999) Genetic structure, localised adaptation and optimal outcrossing distance in two neotropical tree species. DPhil thesis. University of Oxford. 177pp. (PhD)

BOSHIER, D.H. (2000) Mating systems. pp. 63-79. In: *Forest conservation genetics: principles and practice.* Young, A., Boshier, D.H. and Boyle, T.J. (Eds.) CSIRO, Melbourne, Australia/CABI Publishing, Wallingford, UK. (Book Chapter)

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WHITE, G.M. and BOSHIER, D.H. (2000). Fragmentation in Central American dry forests - genetic impacts on *Swietenia humilis.* pp. 293-311 In: *Genetics, demography and the viability of fragmented populations.* Young, A. G. and Clarke, G. (Eds.) Cambridge University Press, Cambridge, UK. (Book Chapter)

WHITE, G.M., BOSHIER, D.H. and POWELL, W. (1999) Genetic variation within a fragmented population of *Swietenia humilis* Zucc. *Molecular Ecology,* **8**: 1899-1910. (Peer reviewed paper)

WHITE, G.M., BOSHIER, D.H. and POWELL, W. (in press) Pollen flow counteracts fragmentation in a tropical forest. *Proceedings of the National Academy of Sciences* (Peer reviewed paper)

YOUNG, A., BOSHIER, D.H. and BOYLE, T.J. (Eds.) (2000) *Forest conservation genetics: principles and practice.* CSIRO, Melbourne, Australia/CABI Publishing, Wallingford, UK. 350 pp. (Book)

Internal Reports

In addition to quarterly/annual reports to FRP, the following report was also written for collaborators;

BOSHIER, D.H. and BILLINGHAM, M.R. (1996) Proyecto "Diversidad genética y estructura de poblaciones de árboles en fragmentos del bosque seco de América Central" R.5729, R.6516. Informe de actividades de campo realizados en Costa Rica y Honduras, 17/1/96-10/5/96. Report R6516. Oxford Forestry Institute, Oxford. 12pp. Spanish. (BTOR)

Workshops, courses, oral presentations and training materials

BOSHIER, D.H., YOUNG, A., and KANOWSKI, P.J. (1996-1999) Forest conservation genetics: principles and practice. [3, one-week Training Courses for 30 people from 11 countries] (Training course)

BOSHIER, D.H. (1998) El bosque seco mesoamericano – conservación de sus recursos genéticos a través del uso. [one-week Training Course for 15 people from 5 countries] Spanish (Training course)

BOSHIER, D.H. (1999) Trees; their genes and habitats. British Ecological Society Annual Symposium, Ecological Consequences of Habitat Heterogeneity. 23-25 March. (Scientific Presentation)

BOSHIER, D.H. (1999) The dynamics of mating in a fragmented population of *Swietenia humilis* (Zucc.) using SSRs as a marker system. Forest Biotechnology 99. Joint meeting of the International Wood Biotechnology Symposium and IUFRO Working Party 2.04.06 Molecular Genetics of Trees. 11-16 July. (Scientific Presentation)

BOSHIER, D.H. (2000) Mantenimiento de poblaciones arbóreas en el bosque seco fragmentado Taller de Maduración del Proyecto CUBOS. Escuela Agrícola Panamericana, Honduras. 3-5 July. [Maturity workshop involving 15 representatives of NGOs and Government agencies from 4 Mesoamerican countries] Spanish. (Oral Presentation)

BOSHIER, D.H. (1996) Porque es importante la biologia reproductiva para forestales? Escuela Nacional de Ciencias Forestales, Honduras. [lecture to ≈60 third year students] Spanish. (Oral Presentation)

BOSHIER, D.H. (1997) El bosque seco mesoamericano – su importancia y conservacion. Escuela Nacional de Ciencias Forestales, Honduras. [lecture to ≈60 third year students] Spanish. (Oral Presentation)

BOSHIER, D.H. (1998) Ventajas y desventajas de sistemas agroforestales: arboles de sombra y cultivos perenes. Escuela Nacional de Ciencias Forestales, Honduras. [lecture to ≈60 third year students] Spanish. (Oral Presentation)

BOSHIER, D.H. (1998) CONSEFORH staff on the implications of results from R4724, R5729, R6516 for CONSEFORH. Honduras. [lecture to project staff] Spanish. (Oral Presentation)

BOSHIER, D.H. (2000) *Leucaena salvadorensis* – case study. Oxford Forestry Institute, Oxford. 9pp. English and Spanish. (Training Course)

BOSHIER, D.H. (2000) Case study check list – Forest Conservation Genetics. Oxford Forestry Institute, Oxford. 2pp. English and Spanish. (Training Course)

6.2 Conservation of La Mula corridor - genetic factors cited in Presidential decree.

This forest corridor was programmed for felling and conversion to rice fields. D. Boshier wrote a note "Pochote en el corredor de la Mula" as part of a technical document, prepared to help the local communities in their efforts to protect the forest, through the legal process. The planned felling was a clear example of the problems the project is aimed at, and would have been expected to have a large deleterious effect on the long term future of *B. quinata* in the area. The corridor has now been protected by its incorporation into the Palo Verde National Park, with its importance as a genetic resource of *B. quinata* cited in the Presidential decree (La Gaceta no.22, 31/1/95).

6.3 Follow up action and research

The following specific and more general types of action/studies are desirable or on-going.

A study of the taxonomy of the *Swietenia* genus

Given the suggestion from this study, and elsewhere (Chalmers *et al.*, 1994; Helgason *et al.*, 1996) of the lack of a clear distinction between *S. humilis* and *S. macrophylla*, there is a great need for a comprehensive, range wide, study of the systematics of the genus *Swietenia*, involving assessment of both molecular and morphological traits. This would potentially have policy implications, given the different status of protection afforded the three species under CITES. The necessary seed and herbarium collections for such a study already exist, as a result of a number of projects (funded from a variety of sources) that have made collections in recent years (*i.e.*R6516, CATIE). Although recent restrictions on germplasm movement imposed by IBAMA (Brazil) would make the setting up of a study more complex. The study would make a suitable PhD, or two year post doctoral project.

Regeneration of *B. quinata*

There is a specific need for more detailed work on *B. quinata* to determine the particular limitations to natural regeneration, compared to its relative success and ease of establishment as a plantation species.

Data analysis of trials

It would be desirable to make further measurements and data analysis of the trials to confirm the preliminary results, as well as to aid collaborators in statistical analysis (specifically ACG). This is dependent on continued funding to D. Boshier, which is currently not available.

Dissemination and training (see also section below on *circa situm* conservation)

D. Boshier's role on the Dendrogene technical advisory panel and an IPGRI workshop provide fora for further dissemination of the project's general findings into more general research and policy agendas. Initial responses to publication of the Forest Conservation Genetics textbook indicates that there is interest and scope for training courses of the type run in Australia and Honduras, that emphasise the broader relevance of genetic issues to conservation and forest management.

Modeling of human disturbance - studies of self incompatibility, sexual systems *etc.*

1) Detailed genetic and demographic studies of a small number of "model species" that are ecologically and evolutionarily representative of the range of life histories. Data regarding the response of these species to forest changes can be extrapolated to other species with which they share characteristics.
2) Continued accumulation of accurate baseline biological data on a broad range of tree species.

To fully assess the relative merits of different management/conservation strategies, there is a need to integrate genetic, ecological, silvicultural and sociological information to reflect the complex interactions of natural systems. The lack of information, the limitation of resources and the need for more immediate action in many situations, necessitate pragmatic "best guess" approaches. Such limitations also dictate the need for studies that permit extrapolation of appropriate strategies for the majority of taxa from results on a few. Identification, by a validated model, of the factors that leave species genetically susceptible to human disturbance requires extensive reproductive/regeneration ecology and genetic data for selected model species (see EMBRAPA/DFID Dendrogene project). The ability to extrapolate from model results to make more general recommendations for species management guilds (*e.g.* combination of ecological group, spatial distribution, aspects of reproductive biology) depends on the existence of basic biological information (*e.g.* incompatibility & pollination mechanisms, dispersal, seedling regeneration) that enables species to be so classified. The potential of models to develop and incorporate genetic indicators for assessing sustainable management into management guidelines also depends on a full integration with management operations, so that genetic indicators are developed, with testing and feedback, that are usable in day to day operations.

The integration of landscape models, that use spatially explicit information habitat type mosaics, with metapopulation models, that describe a set of connected populations within a landscape, offers a means to examine the influence of landscapes and habitats on genetic processes and structure of populations. Connecting genetic and demographic models at landscape scales, requires the adoption of more relevant scales of study than those over which migration is currently measured, and that are sensitive to recent changes in gene flow. Direct parentage analysis methods estimate current rates of gene flow, but have generally been applied over modest spatial scales, while recent studies (*e.g.* this study) suggest that under fragmentation pollen flow distances may increase by factors greater than ten. Current analytical models have not been used to their maximum capabilities to resolve such long distance pollen dispersal events. In addition, while pollen and seed movement may influence genetic structure differentially, from the perspective of demographic processes (*i.e.* colonisation) in metapopulation/landscape models, seed dispersal data may be as important as pollen dispersal, requiring a range of markers for direct comparisons between relative gene flow levels owing to pollen and seed dispersal.

***Circa situm* conservation potential – research and training**

For *circa situm* conservation to have anything but a minor role in the conservation of tropical forest resources priorities amongst species and forest types will need to be set and short-cuts found. Below are recommendations for research and dissemination priorities for the further development of *circa situm* conservation. The list is neither prescriptive nor exhaustive and reflects primarily the situation in southern Honduras. It is presented to illustrate the types of information demands that *circa situm* prescriptions will make. However, overriding such specificity is the need for conservation planners, more accustomed to *in situ* methods of conservation, to consider the possibility that populations of trees found outside of protected areas have a role in the conservation of biodiversity. This will in turn require the direct involvement of development organisations in biodiversity conservation and an effective two-way communication between them and 'traditional' conservation organisations to ensure both conservation and development benefits.

- Species distribution patterns need to be established not only at a coarse-grained level, so as to identify species that are rare and therefore a conservation priority, but also at a finer resolution to identify which species are not conserved *in situ* and which land-uses within an agro-ecosystem are most appropriate for each species.
- Targeted tree populations need to be described in terms of size class distributions with respect to reproductive maturity. We cannot assume that a *circa situm* population contains the same proportion of reproductively active individuals as an *in situ* population.
- Fragmentation thresholds for gene flow need to be determined and the possible selection pressures exerted by farmers, further elucidated.
- Species of conservation concern within agro-ecosystems need to be understood and classified in terms of farmers' perceptions of their value. Species guilds need to be defined, combining biological and socio-economic variables to reduce, by extrapolation, the information demands of *circa situm* conservation.
- Education and training curricula need to be broadened so that more conservation biologists recognise the potential role of on-farm conservation and more rural development practitioners recognise their role in biodiversity conservation.
- The habitat benefits for conservation of different agroecosystems need to be evaluated, recognised and promoted.
- The techniques of rapid rural appraisal have to be adapted and tested for their ability to provide adequate and cost effective information for *circa situm* management planning.
- Strategies need to be explored on a species by species basis to ensure effective use of resources and minimum disruption to farming practices.
- The costs and benefits to farmers of actively promoting diversity conservation through a *circa situm* approach need to be carefully balanced and, if necessary, forms of compensation considered.
- Effective systems for long term monitoring of the biological (*e.g.* pollinator levels, regeneration levels) and socio-economic dynamics (*e.g.* management practice changes) of conservation need to be established.

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Appendix 1 Distribution of Forest Conservation Genetics text book (addresses available)

Country	Collaborators/ Training course participants	Current/ex DFID projects	Research incl CGs	Education/training	total
Argentina			1		1
Australia	2			1	3
Bangladesh			2		2
Bolivia				1	1
Botswana			1	1	2
Brazil		2	4	6	12
Brunei	1				1
Cameroon		1	1		2
China			2		2
Colombia			3	1	4
Congo			1		1
Costa Rica	2	4		2	8
Cote D’Ivoire			1		1
Cuba			1		1
El Salvador	1		1		2
Ethiopia			2		2
Fiji	1		1		2
Gabon			1		1
Ghana			3	1	4
Guatemala	1				1
Guinee			1		1
Honduras		1		4	5
India		4	10	6	20
Indonesia			4	3	7
Italy			2		2
Jamaica		1		1	2
Kenya			3		3
Liberia			1		1
Madagascar			1		1
Malawi			2		2
Malaysia			2	2	4
Mexico			6	4	10
Mozambique			1	1	2
Myanmar			1		1
Namibia			1		1
Nepal		1			1
Nicaragua			1		1
Nigeria			2	2	4
Pakistan			2		2
Papua NG			1	1	2
Philippines			1	2	3
Reunion			1		1
Rwanda			1		1
Samoa	2				2
Senegal			1		1
Sierra Leone			1	1	2
Solomon Is	1	1			2
South Africa			6	3	9
Sri Lanka		1	2	1	4
St Helena			1		1
Sudan			1		1
Switzerland			1		1
Tanzania			1	1	2
Thailand			2	3	5
Tonga	1				1
Trinidad/Tob			1		1
Uganda			2		2
UK	14	3		2	19
USA	1				1
Vanuatu	2		1		3
Venezuela				1	1
Vietnam			2		2
Zaire			1		1
Zambia			2	1	3
Zimbabwe			5	1	6
Totals	29	19	99	53	200

Appendix 2 Meetings attended

In addition to the meetings at which D. Boshier was a speaker (listed in section 6), the following meetings were attended. Posters covering the work of R6516 were presented at all meetings.

- "Measuring and monitoring biodiversity in tropical and temperate forests" IUFRO Symposium, Chiang Mai, Thailand 25/8 - 5/9/94. Included two CIFOR/IPGRI meetings to discuss collaborative work on habitat fragmentation.

- "The future for the genus *Swietenia* in its native forests" Linnean Society Meeting, London; 8/9/94.

- "The role of genetics in conserving small populations" British Ecological Society, Ecological Genetics Group, York; 18-19/9/95.

- "Reproductive biology 96" Royal Botanic Gardens Kew; 1-5/9/96.