

GENETIC DIVERSITY AND POPULATION STRUCTURE OF
PEACH PALM (*Bactris gasipaes* Kunth) IN AGROFORESTRY
SYSTEMS OF THE PERUVIAN AMAZON

By

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Abstract of Thesis Presented to the Graduate School
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Peach palm (*Bactris gasipaes* Kunth) is an important component in Peruvian agroforestry systems, and is cultivated for its fruit and ‘heart of palm.’ Based on observations of farming practices in the Peruvian Amazon, the genetic diversity of peach palm appears to be vulnerable to farmer selection, inbreeding and founder effects in traditional swidden-fallow agroforestry systems.

This study used microsatellite molecular markers to assess the genetic diversity and population structure of peach palm in the agroforestry systems of eight riverine communities in northeastern Peru, comprising two study areas 160 km apart (four colonist and four indigenous communities per study area). In addition, an analysis of a peach palm germplasm collection of diverse geographical origin provided a basis of comparison for estimates of genetic variability and population structuring.

Farmers were surveyed on their seed selection practices for peach palm in swidden-fallow agroforestry systems. An average of only four maternal parent palms were reported to have been selected to provide seed for the establishment of the swiddens and fallows sampled in both study areas. However, seeds of peach palm obtained from

different regions have recently been migrating into populations in the two study areas at contrasting rates. A metapopulation approach was used to describe migration within and among regions, implying a hierarchical structure of gene flow which could maintain relative levels of genetic diversity in peach palm over time— offsetting founder effects and strong phenotypic selection observed in traditional agroforestry systems.

Seed migration was occurring over larger distances and at a higher frequency in the indigenous metapopulation, and a proportionally greater number of alleles were found (with respect to the colonist metapopulation). The indigenous farmers were also preserving remnant peach palms through successive swidden generations, which may contribute to the maintenance of alleles within the metapopulation by reducing founder effects. Population differentiation was reduced in the indigenous study area with respect to both the colonist metapopulation and the comparison sampling from the peach palm germplasm collection. In general, all groups of populations sampled had relatively limited genetic structure, which is believed to result from the inter- and intra-regional exchange of seeds over long periods of time. Pollen flow, over distances greater than expected, has probably played a role in reducing population differentiation as well. The facilitation of long distant seed migration among populations in the recent past is believed to have attributed to levels of neutral microsatellite diversity observed at present; thus, the preservation of evolutionary processes which actively create and maintain general levels of genetic diversity on-farm seems warranted.

CHAPTER 1 INTRODUCTION

Bactris gasipaes Kunth (syn. *Guilielma gasipaes* Kunth), also known as peach palm, is well adapted to the nutrient-poor acid soils of the Neotropics. This fully domesticated palm reaches heights of over 20 m at maturity, and is cultivated as an upper-story component in agroforestry systems for its starchy, nutritious fruit (Clement 1989). The fruit is consumed after cooking, or processed into a variety of products depending on variations in texture, flavor, oil and starch content of the fruit mesocarp. Processed products include fermented beverages, flour for infant formula and baked goods, cooking oil and animal feed (Clement et al. 2004b). The palm is also managed in high-density plantations for its meristem, or ‘heart of palm,’ which was a profitable international export for both Costa Rica and Ecuador in the recent past (Mora-Urpí and Echeverría 1999), but which is now suffering from over-production (C.R. Clement, March 2003, personal communication).

Profits from the sale of peach palm fruit in subsistence-based markets make it one of the most valuable crops currently grown in the Peruvian Amazon (Weber et al. 1997, Labarta and Weber 1998). *Bactris gasipaes* was listed as the number-one priority tree species for agroforestry research and development in the Peruvian Amazon during the late 1990s based on farmer-preference surveys conducted by the International Centre for Research in Agroforestry (Sotelo-Montes and Weber 1997).

Vulnerability of Genetic Diversity in Peach Palm Agroforestry Systems

Biodiversity is defined as the sum total of all biotic variation, including all flora and fauna species from the scale of an ecosystem down to that of individual genes and alleles (Purvis and Hector 2000). Genetic diversity within a species is thus at the lowest hierarchy of biodiversity— which enhances, not diminishes, its importance. Without genetic diversity, a plant population loses its ability to adapt to its environment and evolve with changing climates or cultural systems. Such concerns are vitally important for a multipurpose tree like peach palm.

It has been estimated that *Bactris gasipaes* has been in the process of domestication for the past 10,000 years (Clement 1988, 1992). Based on observations of farmer practices in the Peruvian Amazon, the genetic diversity of peach palm appears to be vulnerable to farmer selection and founder effects in traditional swidden-fallow agroforestry systems. Swidden-fallow agroforestry, or shifting agriculture, has been the main mode of peach palm cultivation in post-contact Amazonia (Patiño 1963). These agroforestry systems contain spatial, genetic and cultural factors that have the potential to diminish the palm's diversity and alter its genetic architecture (Clement 1988). Over time, the combined forces of genetic drift, selection and inbreeding in the context of domestication decrease genetic diversity in those traits being selected and increase divergence among populations if selection is differential.

The reproductive biology of *Bactris gasipaes* is an important factor when considering its population genetic distribution, as pollen gene flow is believed to be limited and local. Anthesis of male and female flowers is not normally synchronous within a given palm, thus selfing occurs very rarely (Listabarth 1996). The principal pollinators of *Bactris gasipaes* in the Peruvian Amazon, beetles of the genus *Phyllotrox*

(Curculionidae) and *Epurea* (Nitidulidae), have a flight range believed to be only 150 to 200 m between palms (Mora-Urpí and Solis 1980, Mora-Urpí 1982, Listabarth 1996), and the pollen has a relatively short viability period of 1 to 2 days (Miranda and Clement 1990). A molecular marker investigation using progeny arrays within a completely genotyped isolated population is currently underway, which will help to determine a more accurate pollen-dispersal distance for this species (C.R. Clement, June 2004, personal communication). Patterns of seed dispersal produced by forest wildlife (*e.g.* avian predators in the Psittacidae family) have not been investigated, but the dispersal distance is thought to be limited.

If the scattered isolation of peach palm populations in the forest inhibits pollen-mediated gene flow, the relatively small size of the swidden-fallow clearings (1 to 3 hectares on average) would also limit the breeding population sizes of this allogamous palm (Clement 1988). When preparing to plant a new swidden, farmers tend to choose seed for the next generation of palms from a small selection of preferred individuals from a base population that is limited in number to begin with. Few farmers realized that genetic quality might decline through repeated selection of only the best trees (Weber et al. 1997). Thus, a loss of within-population heterozygosity is expected to occur with each founding event (Nei et al. 1975, Maruyama and Fuerst 1985), with particular impact on the elimination of rare recessive alleles and those not favored by selection. Since there is a high likelihood that half or full sibs will be planted together in groups (Clement 1988), in subsequent generations inbreeding or sib-mating occurs; increasing the frequency of homozygous genotypes at the expense of the heterozygous genotypes which can lead to inbreeding depression (Hartl 2000).

Genetic Forces Preserving Diversity within Peach Palm Populations

Farmers sometimes collect seed from palms on neighboring farms, and select desirable palm fruits in the local markets to obtain seed for planting (Weber et al. 1997). This results in migration of genetic material among neighbouring or even distant populations, and may counteract genetic drift, selection and inbreeding, which work to reduce genetic variation within populations and increase divergence among them. Thus, migration could produce sufficient gene flow to restore relative levels of genetic diversity, through a process that resembles interaction within a metapopulation— loosely defined as a population of interacting subpopulations (Hanski 1999). This phenomenon was discussed by Louette (2000) in terms of the management of Mexican maize (*Zea mays* spp. *mays*) landraces. Recent genetic analysis of peach palm growing in the Yurimaguas region of Peru found moderate to high levels of genetic diversity with low population differentiation, lending support to the theory that seed exchange is countering the genetic divergence and fixation of populations (Adin et al. 2004).

Main Objectives of Study

We do not know to what extent farmer seed selection, seed sourcing and management of agroforestry systems in general lead to a reduction or maintenance of genetic diversity in peach palm; yet, this knowledge is fundamental in any effort to preserve crop genetic resources *in situ*. This study employs microsatellite molecular markers to examine the genetic diversity and population structuring of peach palm cultivated in swidden-fallow agroforestry systems of the northeastern Peruvian Amazon. An exploration of the interplay between farmer seed selection and seed migration (interaction within and among metapopulations) was the underlying thread of this investigation, which compared the agroforestry management practices of two study areas.

CHAPTER 2 REVIEW OF THE LITERATURE

Domestication Process in Peach Palm

It has been estimated that *Bactris gasipaes* has been in the process of domestication for the past 10,000 years (Clement 1988, 1992). The species has historically been cultivated from central Bolivia to northeastern Honduras and from the mouth of the Amazon River to the Pacific coast of Ecuador and Columbia (Mora-Urpí and Clement 1988). The analysis of Rodrigues et al. (2004a) suggested two migration routes out of a possible source area in southwestern Amazonia where the purported wild progenitor *Bactris gasipaes* var. *chichagu* is found (Henderson 2000). The first route is to the northeast, in the direction of eastern Amazonia; the second is to the northwest, in the direction of western Amazonia, eventually crossing the Andes to reach Central America. The lack of consistent preferences driving traditional *in situ* breeding for fruit and stem characteristics, coupled with extensive germplasm exchanges (seed migration), has served to maintain a wide range of variation in *Bactris gasipaes* (Clement 1988).

Mora-Urpí and Clement (1988) have morphologically characterized and mapped a complex landrace pattern divided into oriental and occidental subcomplexes, based principally on vegetative differences as well as a 3-tier hierarchy to discriminate landraces by the weight of their fruit:

- Microcarpa: 10 to 30 g per fruit
- Mesocarpa: 30 to 70 g per fruit
- Macrocarpa: 70 to 250 g per fruit

Rodrigues et al. (2004a) further clarified these landrace classifications with molecular genetic analyses (2-1).

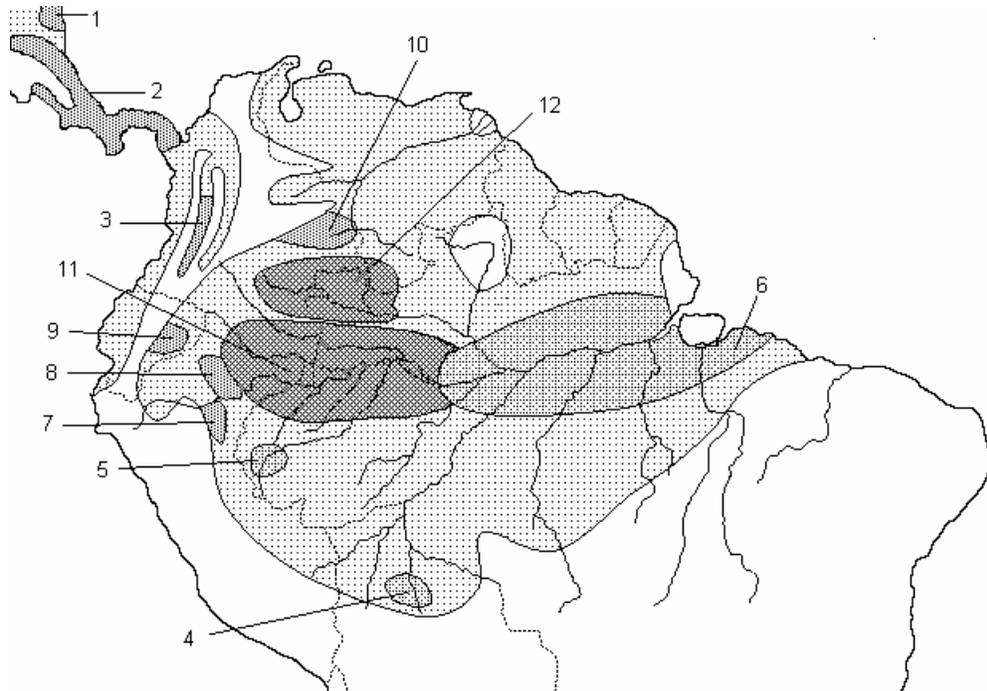


Figure 2-1. Approximate distribution of *Bactris gasipaes* landraces in the lowland Neotropics (light shading indicates general distribution of species). A) Microcarpa landraces - 4. Temb , 5. Juru , 6. Par . B) Mesocarpa landraces - 1. Rama, 2. Utilis, 3. Cauca, 7. Pampa Hermosa, 8. Tigre, 9. Pastaza, 10. Inirida. C) Macrocarpa landraces - 11. Putumayo, 12. Vaup s.
Source: Rodrigues D.P. 2001. An lise das morfo-ra as primitivas de pupunha (*Bactris gasipaes* Kunth) mantidas no banco ativo de germoplasma de pupunha com marcadores moleculares RAPDs. Master's Thesis, Universidade de Bras lia, Bras lia.

The size and starchiness of the fruit reflect the degree of modification that occurred during the course of domestication of *Bactris gasipaes* from its wild progenitor, *B. gasipaes* var. *chichagu* (Clement 1988, Rodrigues et al. 2004a). Macrocarpa fruits are extremely starchy and dry, which helps to account for their large increase in size relative to the smaller, oilier fruits of microcarpa *Bactris gasipaes* and *B. gasipaes* var. *chichagu* (Clement 1992). Other expected attributes of domestication found in macrocarpa include an increased pulp-to-fruit ratio (97% in macrocarpa vs. 85% in microcarpa), increased

bunch weight (8 kg in macrocarpa vs. 3 kg in microcarpa), an increased fruit to bunch ratio (95% in macrocarpa vs. 90% in microcarpa) and an increased frequency of spineless leaves and trunks (Clement and Mora-Urpí 1988a).

These modifications in *Bactris gasipaes* illustrate that a repetitive, continual process of artificial selection (domestication) has the capacity to extend the phenotypic extremes occurring in a plant well beyond the range of variation found in its natural progenitors (Hartl 2000). At the same time a general loss of heterozygosity, and the associated genetic variation often occurs during the domestication of any species as a direct result of selecting for these phenotypic extremes (Doebley 1989). A reduction in the frequencies of those alleles not favored by selection should only occur at loci affecting selected traits (Hartl 2000).

Molecular Analyses of *Bactris gasipaes* Landraces

The first molecular analysis of *Bactris gasipaes* on any significant scale used isozymes (Clement 1995). Isozyme analyses observe allelic variation at isozyme loci using electrophoretic techniques (Weeden and Wendel 1989). The bands in the gels represent the protein products of specific alleles, usually at loci involved in intermediary metabolism (Newberry and Ford-Lloyd 1997). Clement (1995) analyzed 270 plants from nine progenies of the Putumayo ‘macrocarpa’ landrace variety, sampled from the germplasm collection of Instituto Nacional de Pesquisas da Amazônia (INPA) in Manaus, Brazil (established as a result of the 1983-84 USAID expeditions, which collected seed of *Bactris gasipaes* from farms throughout the geographical range of the palm). He found an extremely low mean heterozygosity and moderate to high inbreeding coefficients, evidently due to recent selection for spinelessness in the palm. He then went on to infer

that the experimental collection sampled was established using a limited genetic base to begin with (Clement 1995).

In 1997, Clement et al. examined isozyme variation in three spineless populations of *Bactris gasipaes* (361 plants) maintained in INPA's germplasm collection, and identified the apical meristem of a lateral shoot for optimal extraction of enzymes (and subsequently, DNA). The low mean heterozygosity in the results obtained was attributed to the management history (apparent inbreeding) of the germplasm, prior to its collection in the experimental population sampled (Clement et al. 1997).

The first molecular analysis of DNA from *Bactris gasipaes* was carried out in 2001 by Sousa et al. using RAPD (Random Amplified Polymorphic DNA) markers, followed by an AFLP (Amplified Fragment Length Polymorphism) marker analysis by Clement et al. in 2002. RAPD and AFLP techniques use arbitrary primers with PCR (polymerase chain reaction), resulting in DNA fragments that are then separated on agarose gels. Genetic diversity in RAPD and AFLP data is visualized on the gels as the presence or absence of band fragments resulting from sequence differences in primer binding sites (Karp et al. 1997, Hartl 2000). Genetic difference between any two plants is indicated by the dissimilarity of their banding patterns (Karp et al. 1997). In these two studies, which both used germplasm obtained from INPA's collection, the marker analysis revealed that populations which were morphologically determined to represent three separate landrace types by Mora-Urpí and Clement (1988) corresponded to only two genetically differentiated landraces (Sousa et al. 2001, Clement et al. 2002, Rodrigues et al. 2004a).

Rodrigues et al. (2004a) confirmed this same revision of landrace differentiation using a RAPD analysis of seven landraces from INPA's collection, and also went on to

estimate the partitioning of genetic diversity among the different landraces sampled as 15% among landraces and 85% within them, suggesting that the different landraces are closely related. These values are similar to those estimated for other allogamous plant species in the tropics (Bawa 1992). However, it remained to be determined how the 85% gets distributed throughout the various populations that constitute a given landrace of *Bactris gasipaes*.

Mora-Urpí et al. (1997) hypothesized that most of the genetic variation within a landrace may occur among numerous small sub-populations due to genetic drift and differential selection, and that within these sub-populations there may be relatively low diversity due to founder effects and selection. Recently, genetic analysis of Pampa Hermosa landrace populations using AFLP markers has suggested otherwise, finding low genetic differentiation among populations with relatively high levels of genetic diversity within them (Adin et al. 2004). When population genetic structure is established using molecular methods, the extent to which population subdivision and inbreeding influence patterns of variability within and between populations can be inferred (Loveless and Hamrick 1984).

Farmer Selection of Tree Seed

In Peru, some farmers discriminate peach palm varieties by the color of the fruit mesocarp, for the qualitative traits of the fruit associated with each color. For instance, fruits with a red waxy coat are said to be higher in oil than are fruits with a yellow (or red) non-waxy coat (Weber et al. 2001). If farmers who prefer the palm as a source of starch are only planting out seed from yellow non-waxy fruit, their selection might be dramatically eroding diversity at loci influencing this trait (especially as yellow fruits occur less frequently than red).

Brodie et al. (1997) surveyed farmers in two separate regions of the Peruvian Amazon in an investigation of the origins of fruit and timber tree germplasm on-farm. They found that 87% of the fruit trees were planted from seed the farmer had personally selected. Sixty percent of these seeds were selected from on-farm germplasm and the rest were obtained from off-farm sources in the near vicinity. Lengkeek (2003) conducted similar surveys amongst farmers in Kenya and found that an influx of germplasm from a distant source occurred only rarely for both indigenous and non-indigenous tree species.

Brodie et al. (1997) reported that the farmers' selection criteria focused on the size, sweetness, texture, taste and seed characteristics rather than on overall yield of the tree. Since the sources of germplasm for the first generation of trees were primarily local, with presumably low levels of diversity, this study concluded that the farms were at risk of experiencing reduced yields (via inbreeding depression) and pest or pathogen outbreaks as a result of the narrow genetic base of their trees. They also concluded that farmers' perceptions are short-term and that they generally do not appreciate the value of variation in the tree species they cultivate. Few farmers realized that genetic quality might decline through repeated selection of only the best trees (Weber et al. 1997, Lengkeek 2003).

Bora Swidden-Fallow Agroforestry

A swidden is an agricultural field that is cleared from out of the forest, usually by hand with axes, saws and fire, and then planted for one or a few years with nutrient demanding crops before being left fallow for a period of time (Conklin 1957). The indigenous Bora farmers living near Pebas Peru have been reported to manage their swiddens and fallows by protecting useful vegetation, both spontaneously occurring and intentionally planted, from the encroachment of the surrounding forest. A substantial

proportion of the useful vegetation occurring in a given fallow was not necessarily planted by the farmer in the previous swidden (Denevan and Treacy 1987).

Bactris gasipaes was listed by Denevan and Treacy (1987) as a “planted or protected perennial species” in the Bora swiddens and fallows, and recent field research in 2002 and 2003 corroborates this claim. A large proportion of the palms sampled for the current study were claimed to be over 50 years old, originally planted in previous generations of swiddens. These palms re-sprout and maintain production over the years as a result of the farmers’ systematic clearing of the same land again and again.

Ten to twenty years was reported to be the minimum length of fallowing used by the farmers (Denevan and Treacy 1987). If left to compete with the rapid regeneration of secondary forest vegetation, *Bactris gasipaes* perishes from a lack of photosynthetic activity after about 20 years (Clement 1990). The palm was present in Denevan and Treacy’s (1987) sampling of a 19-year old fallow, albeit at a lower density compared to the younger fallows sampled.

Metapopulation Dynamics

Many populations of plant species are subdivided into local breeding units, a situation which has the potential to lead to the genetic differentiation among these units through differential selection and drift. Theories of population structuring often contrast the trend toward differentiation with the rate of gene flow among sub-populations (Slatkin 1985, Hartl 2000). The exchange of genes in plants takes place through direct migration via seed dispersal or flow of pollen. This movement of genetic material plays an influential role in determining the spatial scale of observed genetic differentiation in populations (Slatkin 1985).

Gene flow in the context of metapopulation dynamics is a useful way to model the effects of seed dispersal, or migration, in the genetic structuring of *Bactris gasipaes* populations. The term metapopulation was originally coined to describe a population of populations— a higher hierarchical level of the population concept (Levins 1970). While populations are defined as assemblages of interacting individuals each with its own finite lifetime, metapopulations are assemblages of interacting populations with distinct finite lifetimes, or expected time to population extinction (Hanski and Glipin 1991). In this manner an assemblage of populations can persist for a much longer period of time than the lifetime of any one individual population. The ‘classical’ metapopulation concept described by Levins is strictly associated with the dynamics of population turnover, involving both the extinction and recolonization or establishment of new sub-populations. Subsequently, the use of the metapopulation concept has broadened to include any assemblage of populations in which genes are exchanged among discrete sub-populations through migration or dispersal (Hanski 1998).

A metapopulation landscape is conceptualized as a network of idealized habitat patches or fragments, surrounded by uniformly unsuitable habitat, in which species are distributed in discrete local populations linked through dispersal among them (Hanski 1998). In this thesis, the concept of a metapopulation landscape will be extended to describe the population structure of the domesticated tree crop peach palm, which is unable to indefinitely survive outside of managed agricultural environments (Clement 1990) and whose sub-populations turnover at irregular intervals. In this case, the subpopulation turnover is caused by the swidden-fallow cycle of rainforest farming, in which areas of fallowed land are intermittently cleared and replanted over and over.

Migration occurs when a new tree crop is planted after clearing the forest fallow and new seeds are brought in from distinct sub-populations. This will inherently involve a degree of artificial selection taking place during population 'founder events'.

Artificial Selection within the Metapopulation

The role of artificial selection in the process of diverging sub-populations is obvious, as any calculation of changes in gene frequency in a population due to selection will show (*e.g.* Falconer 1996, pp.30). When population size is $> 15-30$ individuals, the role of random genetic drift in diverging sub-populations can largely be ignored relative to selection (Hartl 2000). Strong selection regimes are important for the maintenance of genetic variation in a metapopulation when gene flow among sub-populations is irregular and unequal in reciprocal directions (Jain and Bradshaw 1966).

Reduced Gene Flow within the Metapopulation

The theoretical models of Sewall Wright (1943, 1946) show that strong gene flow between sub-populations relative to selection pressures would homogenize the set of sub-populations, while the complete absence of gene flow would allow the sub-populations to exhibit the deleterious effects of inbreeding. A reduced level of gene flow between partially isolated populations is necessary for the long-term persistence of genetic variability (Wright 1946, Nagylaki 1976, Zhivotovsky and Feldman 1992). Slatkin (1981) simulated the efficiency of selection in a subdivided population with migration between populations and found that the time to fixation through genetic drift always increased with decreasing migration between populations to a minimum.

Pollen-Based Gene Flow within the Metapopulation

It is generally assumed that for out-crossing species, pollen-based gene flow will have a greater impact on population differentiation or homogenization than will gene

flow by seed dispersal; yet, it is often difficult to distinguish between the effects of each (Levin and Kerster 1974, Ennos 1994). Individual sub-populations within a metapopulation often receive pollen from several neighboring sub-populations, and even if the levels of gene flow are limited from each source, they are additive, so their sum has the potential to have considerable impact on genetic structure (Levin 1981).

Pollen dispersal is likely to be taking place to varying degrees even among populations that appear to be completely isolated by distance from one another; this would be particularly true for tree species with taller statures and longer life spans (Hamrick and Godt 1996). According to Hamrick and Nason (2000), pollen flow can often be quite extensive at distances as great as one kilometer for most temperate and tropical species. Gaiotto et al. (2003) found pollination distances of up to 22 km for another Amazonian heart of palm species (*Euterpe edulis*), when the main pollinator was previously thought to be a bee with a relatively limited flight distance. Since the actual pollination dispersal distance is unknown for *Bactris gasipaes*, we cannot underestimate the role pollen flow might play in the dynamic nature of peach palm metapopulations.

Migration within the Metapopulation

Varvio et al. (1986) modeled the effects of migration among sub-populations and found that the values of total population heterozygosity and measures of subpopulation differentiation depend not only on migration rates but also on the subdivision pattern, and on the interaction between the two factors. Li (1976) showed that the effect of an individual's migration between two sub-populations is smaller when the number of sub-populations increases. The larger n is within a subpopulation, the greater the genetic composition of the immigrants diverges from that of nonimmigrant individuals (Varvio et al. 1986). Therefore, smaller sized sub-populations with higher rates of migration lose

differentiation among sub-populations more rapidly. Levin (1988) concluded that a variable migration rate homogenizes neutral allele frequencies less effectively than does a uniform rate of migration with the same mean.

Another factor to consider is that if individuals from a given population immigrate into a nearby subpopulation, their gene frequencies would differ less from the target subpopulation than would immigrants originating from farther away. This violates a commonly used model of population structure, the stepping stone model of Kimura (1953) in which only adjacent populations exchange migrants. Kin-structured migration, in which individuals disperse amidst their relatives, will similarly disrupt the assumptions of this model (Levin 1988).

Population Turnover within the Metapopulation

The recurrent turnover of populations is a chronic disturbance capable of keeping a metapopulation from obtaining the theoretical equilibrium between drift and gene flow (Wright 1931). Slatkin (1977) modeled this effect assuming that for an assemblage of interacting populations, a proportion e of populations go extinct at random each generation and are immediately replaced by an equal number of new populations, each founded by k individuals. The populations then immediately grow to a constant size N , denoted in Wright's Nm . Wade and McCauley (1988) found that the relative number of founders (k) to the number of migrants exchanged among populations (Nm) plays a substantial role in determining the effects of extinction-recolonization cycles. These models also assume that fitness of the colonizing and pre-existent individuals in a population are more or less equal. Of course, a more likely metapopulation structure is one in which the extinction rate depends upon the size of individual sub-populations,

which most certainly are not of a constant, equivalent size, yet this classical model is still a useful starting point from which to assess the associated genetic implications.

Pannell and Charlesworth (1999, 2000) state that population turnover in a metapopulation will decrease genetic diversity both within populations and in the total metapopulation measured as a whole. Gilpin (1991) purports that in a metapopulation with both gene flow into existing populations and extinction-recolonization from adjacent populations, genetic variation will be depleted at a rate close to the population half-life of the large local populations in the system. Yet Gilpin's calculations do not account for the effects of recolonization from discrete and non-adjacent sub-populations, which is sometimes the case with peach palm. In addition, with peach palm the founders (k) sometimes originate from well outside the metapopulation (another region altogether perhaps).

Slatkin (1977) contrasts two modes of colony formation, an important consideration when evaluating the consequences of these repeated colonization bottlenecks. The 'migrant pool' mode is when a colonizing group represents a mix of genotypes drawn from the metapopulation at large. Variation among new populations is derived from the binomial sampling of the genetic variation contained within the entire metapopulation, and is proportional to $1/2k$. At the other extreme, the 'propagule pool' mode of colony formation, each set of genotypes involved in founding a new population is drawn from just one possible source population (Slatkin 1977). Whitlock and McCauley (1990) illustrated an intermediate mode between 'migrant' and 'propagule pool.' They introduced the term ϕ as the probability that two gene copies present in the founding group are drawn from the same source population, such that ϕ of zero equals the

‘migrant pool’, \emptyset of one the ‘propagule pool’, and $1 > \emptyset > 0$ cases that are intermediate between the two extremes (Whitlock and McCauley 1990). With ‘propagule pool’ colonization, extinction-recolonization increases genetic differentiation and erodes genetic variation (under most conditions) much more intensely than occurs with ‘migrant pool’ colonization (McCauley 1991, 1993).

However, these models will not adequately describe cases in which founding events occur successively in the absence of extinctions (Le Corre and Kremer 1998). In such cases, the cumulative effects of founding events depends as much on the counterbalancing action of gene flow, i.e. the number of migrants (Nm) exchanged among existing populations, as it does on the strength of each founding event, i.e. the number of colonists founding new populations. It is also affected by the relative contributions of populations of different ages constituting both the migrant and colonist pools of individuals, with the cumulative effect having the most impact when colonists arrive only from recently founded populations and migrants originate strictly from populations of similar ages (Le Corre and Kremer 1998).

Overlapping Generations and Remnant Trees within the Metapopulation

Overlapping generations slow the rate of loss through genetic drift (Hamrick and Nason 1996). The effect of generational overlap is amplified by the longevity of tree species and the maintenance of remnant individuals from previous generations, which reduces the effects of bottlenecking. In addition, the occurrence and the associated implications of inbreeding in a population (*i.e.* increase in homozygosity) are postponed (Johnson 1977, Choy and Weir 1978). The relevance of this ‘storage effect’ (Chesson 1985, Seger and Brockman 1987) for the maintenance of genetic variation is that the recessive alleles of heterozygotes are preserved in long-lived individuals, where hidden

variation may reside and flow into more recently founded populations over multiple generations of fluctuating selection (Hairston et al. 1996).

Long-lived woody perennial species have a higher proportion of polymorphic loci, more alleles per locus and more genetic diversity than other vegetative life forms. Hamrick et al. (1992) reviewed the studies published over a 20 years period reporting allozyme variation for woody plant species with different life history characteristics. They found that the mean genetic diversity for long-lived woody perennial species is 55% higher than that of short-lived woody species, 42% greater than of herbaceous perennials and 15% higher than of annual species (Hamrick et al. 1992). In addition, long-lived woody perennials have more genetic diversity within their populations as well— 38% higher than annuals and 51% to 80% higher than short-lived herbaceous and woody species (Hamrick et al. 1992). These higher rates of genetic diversity within individuals and populations may partially be due to the fact that long-lived out-crossing tree species generally have a higher potential to be receptors for long-range gene flow (Nybom and Bartish 2000), which they then incorporate into the local gene pool of multiple, overlapping generations.

Austerlitz et al. (2000) also conclude that the effects of founder events can be substantially reduced when the specific life cycle of tree species is taken into account, even under conditions of limited gene flow. However, they emphasize that this is not due to the overlapping generations of trees as much as to their delayed reproduction, which allows for an increase in the number of founders of the breeding population before reproduction actually begins (Austerlitz et al. 2000). The effective size of a population bottleneck is often estimated using only the genetic diversity contained in the initial

colonists of a given area. These estimates will be biased low because they do not include the cumulative effects of successive founding events in the colonization of new habitat (see Eastel 1985, Baker and Moeed 1987).

Microsatellite Molecular Markers

Microsatellites are currently the predominant marker system for population genetic analysis (Newbury and Ford-Lloyd 1997, Goldstein and Schötterer 1999). Data acquired from microsatellite markers is much more informative than data obtained from either a RAPD or AFLP analysis. Microsatellite primers are designed to amplify specific targets in the genome through PCR (polymerase chain reaction) by flanking the targeted sequence (Karp et al. 1997). These targets are core repeating sequences of two to nine base pairs which vary in the number of repeats occurring between plants— this variation serves as the measurement for genetic diversity by coding for discrete alleles at a given locus (Karp et al. 1997, Karp 2002). Microsatellites are co dominant markers, which is a major advantage over RAPD markers because heterozygous and homozygous genotypes can be distinguished. This allows direct calculation of all genotype and allele frequencies without the assumption of Hardy-Weinberg equilibrium

The Mechanics of PCR and Microsatellite Markers

Amplification of targeted sequences is carried out through polymerase chain reaction (PCR) technology in a thermocycler. This involves a series of automated temperature changes that unravel the DNA, and then the microsatellite primers are combined with the enzyme *Taq* polymerase to isolate and amplify the specific sequences we want to analyze for their variability (Sobral and Honeycutt 1994). The PCR product obtained from each DNA sample-primer pair combination is then run through an automated gene sequencer for analysis.

Genetic polymorphisms are visualized in the sequence data as differences in the length of PCR products, representing the subtraction or addition of simple sequence repeat units within each sample. Since microsatellites are multi-allelic and co dominant, each allele yields a distinct allele peak in the data output; genotypes that are heterozygous yield two allele peaks (identified within an expected allele size range), those genotypes that are homozygous yield only one allele peak (Karp et al. 1997, Karp 2002).

CHAPTER 3 MATERIALS AND METHODS

Populations and Sampling

Leaf material for DNA extraction was collected from *Bactris gasipaes* grown in four sub-populations (agricultural communities) from each of two river drainages in the Peruvian Amazon. Both drainages fall within the geographical territory of the ‘Putumayo’ peach palm landrace (Rodrigues 2001). The Tamshiyacu-Tahuayo Rivers both join the Amazon River near Tamshiyacu, Peru (40 km southeast of Iquitos) (Figure 3-1A, Table A-1). The Yahuasyacu-Ampiyacu Rivers both join the Amazon River at Pebas, Peru (120 km northeast of the city of Iquitos) (Figure 3-1B, Table A-2). Ten farms (farmers) were sampled in each of the two river systems (metapopulations), two or three farms per community or population. Between 24 and 81 palms were sampled from each community— 221 palms from the Tamshiyacu-Tahuayo communities and 165 palms from the Yahuasyacu-Ampiyacu communities, for a total of 386 plant samples. An average of 22.1 (between 14 and 30) DNA samples were collected from Tamshiyacu-Tahuayo farms and an average of 16.5 (between 5 and 34) DNA samples from Yahuasyacu-Ampiyacu farms.

Communities within each of the two study areas were separated between five and twenty-five km apart. Given that the flight range of the pollinators is believed to be a maximum of only 150 to 200 m (Mora-Urpí and Solis 1980, Mora-Urpí 1982), the probability of long distance pollen flow occurring between any two communities is very low.

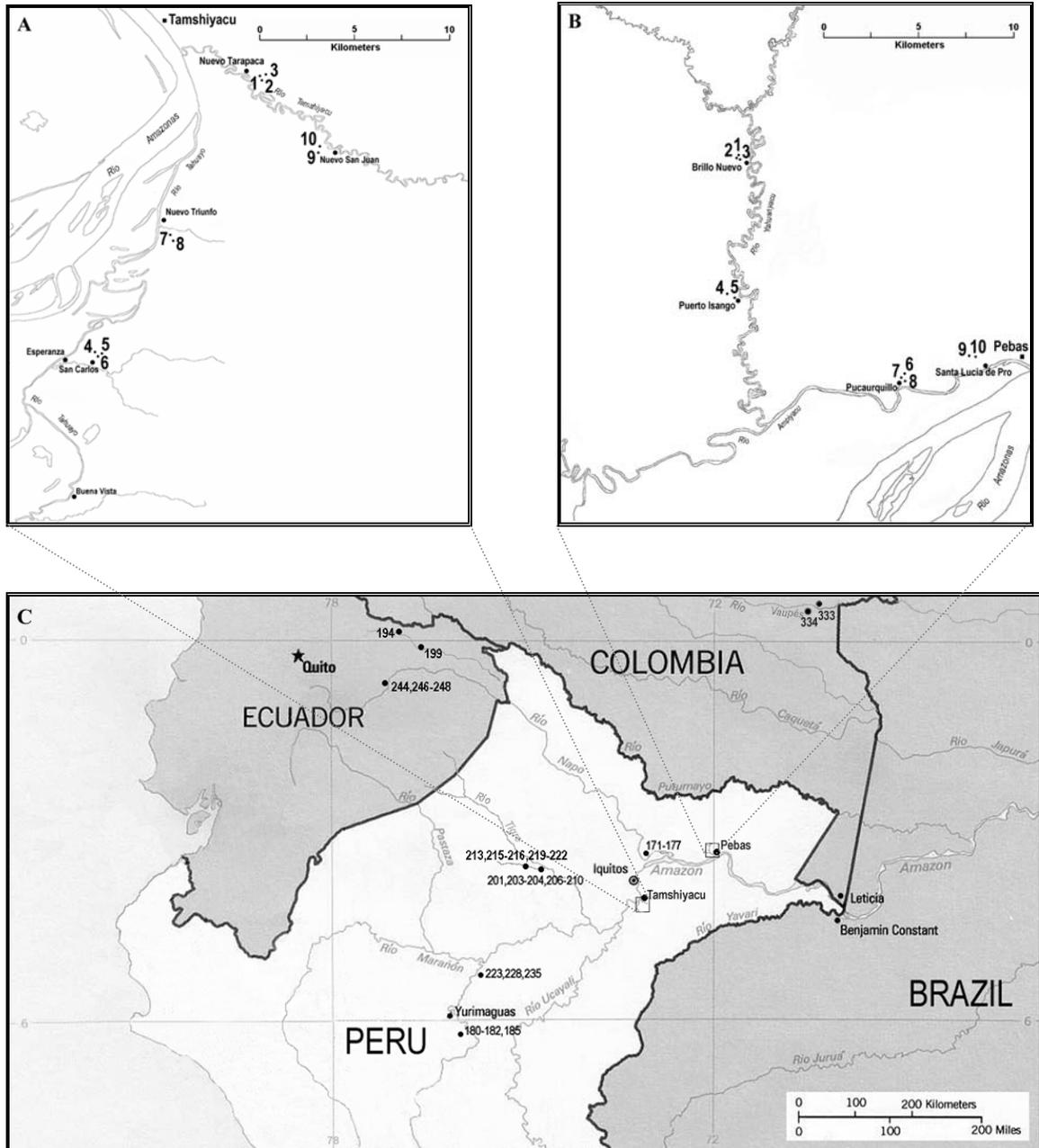


Figure 3-1. Relative locations of two study areas, metapopulations (communities) and farms sampled. A) Colonist and B) indigenous metapopulations. C) Eight populations of 5 peach palm landraces sampled from El Dorado collection. Numbers 1-10 on maps A and B indicate location of farms sampled and numbers 171-334 on map C indicate source location for germplasm sampled from El Dorado collection.

The Tamshiyacu-Tahuayo study area is inhabited by people whose ancestors are a mix of both recent immigrants as well as those indigenous to the region, who for the most part do not affiliate themselves with any one particular ethnicity. The Yahuasyacu-Ampiyacu study area lies within the boundaries of an indigenous federation of Huitoto, Bora, Yahua and Ocaina peoples, Federación de Comunidades Nativas del Ampiyacu (FECONA). Therefore, the Tamshiyacu-Tahuayo communities, farmers and palms will hereafter be referred to as ‘colonist’ and the Yahuasyacu-Ampiyacu communities, farmers and palms ‘indigenous.’

These labels, ‘colonist’ and ‘indigenous,’ are for the purpose of identification only. This study does not intend to imply that there is anything intrinsically *better* about the indigenous nature of a particular style of agroforestry management. In fact, both the colonist and indigenous styles of management observed were remarkably similar.

In addition to the samples collected from the colonist and indigenous communities, a control comparison of 37 samples was collected from the *Bactris gasipaes* germplasm reservoir maintained by Instituto Nacional de Investigación Agraria (INIA) at their El Dorado station located near Iquitos. These individuals were collected during the 1983-84 USAID expeditions from eight populations of five peach palm landraces in Peru, Ecuador and Colombia (Figure 3-1C, Table A-3), and will provide a baseline of a broader genetic sampling against which to compare the results obtained from the two study areas.

Informal Farmer Surveys

Before collecting DNA tissue of peach palm, each farmer was informally surveyed to determine their seed selection criteria (preferred palm and fruit types) and the average number of maternal parent palms used to establish swidden-fallow agroforestry systems. When collecting DNA tissue with these farmers, the recollected origin of the seed (which

grew into the palms sampled) and the approximate age of all palms (whose tissue was sampled) were recorded. Sampled palms perceived by a farmer to have been planted during a pre-existing cycle were identified, to infer the degree of generational overlap taking place on a given farm.

Collection and Extraction of DNA

The DNA was collected as 8 cm leaf cuttings from lateral shoots, for ease and efficacy of extraction according to previously documented experience (Clement et al. 1997, Rodrigues 2001). When lateral shoots were not present, juvenile leaf tissue was used. Each sample was placed in a separate paper envelope and zip lock bag with silica gel desiccant in an airtight container, and the samples were then periodically transferred into cold storage (4 to 8°C) at the facilities at INIA, San Roque (Iquitos), during the duration of fieldwork. Upon returning to the Soltis Molecular Systematics Lab on the University of Florida campus, genomic DNA extraction followed a standard CTAB (Cetyltrimethyl ammonium bromide) procedure (Doyle and Doyle 1987) with minor revisions (Appendix B). DNA quantification was carried out by comparison with known concentrations of a DNA standard (λ -Hind III) in ethidium bromide-stained 2% agarose gels.

Microsatellite Marker Genetic Analysis

Microsatellite Loci for *Bactris gasipaes*

Ten microsatellite markers have recently been isolated, optimized and characterized for *Bactris gasipaes* at Universidade Federal do Amazonas in Manaus, Brazil (Rodrigues et al. 2004b) in addition to a different set of 18 markers previously developed at the Biotechnology Research Unit of Centro Internacional de Agricultura Tropical (CIAT) in Bogota, Colombia (Martínez et al. 2002). At first, six of these microsatellite marker loci

were chosen to genotype all individuals sampled. Three of these markers were labeled with blue florescent dye, and three with green, at the five-prime end of the forward primer. This allowed multiplexing of multiple marker loci during sequencing by distinguishing loci and alleles in the data output whose size ranges overlapped one another. In the end, only three of these marker pairs could be used due to inconsistent PCR (polymerase-chain reaction) products for the three additional loci originally chosen (Table 3-1).

Table 3-1. Microsatellite primer pairs used for population genetic analysis of peach palm

Locus	Primer sequence (5'-3')	Repeat motif	Allele size range	Expected product
Bg02-19	F: GCGTTCAGACTTGCATACACA R: CCCACATGCAGGAGTCGTAT	(CT) ₂₃ (CA) ₆	149-205 bp	182 bp
Bg02-24	F: AAACCTGATCCGATTGGCTA R: CACCACCACCACCTCCAC	(GA) ₁₇	119-155 bp	135 bp
Bg55	F: TTCTGGGTGCGGTGGTAG R: ATGATGGACTGAAGAGATGGAATAG	(GT) ₂ GC(GT) ₃ GC(GT) ₅	281-306 bp	278 bp

Isolation and characterization of Bg02-19 and Bg02-24 described in: Rodrigues D.P., Vinson C., Ciampi A.Y., Farias I.P., Lemes M.R., Astolfi-Filho S. and Clement C.R. 2004. Ten new microsatellite markers for *Bactris gasipaes* Kunth (Palmae). Mol. Ecol. Notes, in press; Bg55 described in: Martínez A.K., Gaitán-Solis E., Duque M.C., Bernal R. and Tohme J. 2002. Microsatellite loci in *Bactris gasipaes* (Arecaceae): their isolation and characterization. Mol. Ecol. Notes 2, 408-410.

PCR Amplification

Separate PCR amplifications were performed for each of the three primer pairs, for each of the 423 DNA samples (a total of 1,269 reactions), in a 25.0 µL volume containing (prepared in order listed): 1 µM each of forward and reverse primers, 1X PCR Buffer (Promega), 2.5 mM MgCl₂, 250 mM dNTPs, 1 unit *Taq* polymerase (Promega), 2.5 µL 1:50 DNA dilution was then added.

PCR amplifications of Bg02-19 and Bg02-24 loci were performed separately for each DNA sample-primer pair combination using a Biometra T3 thermocycler with the following conditions: initial denaturation at 94°C for 2 min; 25 cycles of 94°C for 10 s, the primer specific annealing temperature (58°C for Bg02-19 and 64°C for Bg02-24) for 10 s, 72°C for 30 s; 10 cycles of 94°C for 10 s, 50°C for 10 s, 72°C for 30 s; ending with 72°C for 30 min. PCR amplification of Bg55 locus was performed separately for each DNA sample-primer pair combination on the Biometra T3 with the following conditions: initial denaturation at 94°C for 3 min; 35 cycles of 94°C for 15 s, the primer specific annealing temperature (50°C for Bg55) for 15 s, 72°C for 15 s; ending with 72°C for 5 min. These conditions were optimized by Rodrigues et al. (2004b) and Martinez et al. (2002) to minimize stutter banding (PCR artifacts that could be misidentified as actual alleles) in the sequence results.

DNA Sequencing

For each of the three DNA sample-primer pair combinations, 2.0 μ L of PCR product was added to 25.0 μ L SLS sequence buffer + 0.5 μ L 400 base pair size standard. This 3-primer pair multiplex was then visualized on a Beckman-Coulter CEQ 8000 automated capillary sequencer, one lane for each DNA sample. Allele sizes were estimated using the CEQ 8000 version 7.0 software, and then visually inspected taking into consideration the expected allele size in base pairs for each of the three loci and the original DNA clones from which the microsatellite loci were developed. Stutter bands were identified distinct from the actual alleles, to correct any errors made when the software called and sized the alleles.

Statistical Analyses

To estimate genetic diversity, the following measures were calculated for all populations using Microsatellite Analyser MSA v. 3.15 (Dieringer and Schlötter 2003): expected (H_e) and observed (H_o) heterozygosity per locus and the number of alleles and allele frequency distribution per locus. These populations were tested for departure from Hardy-Weinberg equilibrium, performed with GENEPOP v. 3.4 (updated from Raymond and Rousset 1995) using the U -test for a hypothesis of heterozygote deficiency; exact P values were determined by a Markov chain method (Guo and Thompson 1992).

The extent and significance of the genetic differentiation among populations was also investigated with MSA, which provided unbiased estimates of Wright F -statistics (Weir and Cockerham 1984) at each locus, and averaged over multiple loci. Estimates were obtained for the following parameters: F , the overall inbreeding coefficient or the correlation of allele frequencies within individuals in different populations; f , the within population inbreeding coefficient or the correlation of allele frequencies among individuals within populations; and θ , an estimator of Wright's fixation index F_{st} , measuring the correlation of allele frequencies between individuals within populations, calculated over all populations and for each pairwise population comparison (Cockerham 1969, Weir and Cockerham 1984). Statistical significance of F , f and θ was tested by bootstrapping over loci with a 95% confidence interval. To test the significance of pairwise θ -values, MSA permuted genotypes among groups with Bonferroni corrections (Dieringer and Schlötter 2003).

Isolation by Distance version 1.52 (Bohonok 2002) was used to test for the presence of an isolation-by-distance relationship using the pairwise θ -values, as was demonstrated by Rousset (1997). Significance in the isolation-by-distance relationship

was tested using a Mantel test. This test assesses whether the pairwise genetic distance matrix is correlated with the pairwise geographic distance matrix.

Reduced Major Axis (RMA) regression techniques were then used to estimate the slope and intercept of the isolation-by-distance relationship. Reduced Major Axis (RMA) regression is more appropriate than Ordinary Least Squares (OLS) regression when the independent variable x is measured with error (Sokal and Rohlf 1981). Error in the independent variable leads to biased estimates of slope. Hellberg (1994) specifically suggested that for analysis of isolation-by-distance, RMA is a more appropriate estimator of slope than OLS.

SAS (SAS Institute) was used to characterize multilocus genotypic associations within each population overall and among 2 and 3 loci within each population (Yang 2000, 2002). These genotypic (zygotic) associations were defined on the basis of gametic and allelic frequencies (Yang 2002).

Most contemporary studies using microsatellite loci report $R_{st}(\rho)$, an estimator of genetic differentiation accounting for variance in allele size and defined for genetic markers (like microsatellites) undergoing a stepwise mutation model (Slatkin, 1995). Since there is no way to be certain that mutation in any species follows a strict stepwise mutation model, R_{st} has limitations to its use (Slatkin 1995, Balloux et al. 2000). In addition, Balloux and Goudet (2002) report that when populations are very weakly structured with high rates of gene flow, Weir and Cockerham's F_{st} (1984), provides a more accurate estimator than Slatkin's R_{st} (1995). Therefore, this study relied solely on F-statistics for an analysis of population structure.

CHAPTER 4 RESULTS AND DISCUSSION

Farmers' Seed Selection, Sourcing and Management of Palms

Within the two metapopulation study areas there was a strong tendency among both groups of farmers to limit the number of founding individuals per population. The average number of maternal parent palms reported to have been selected to provide seed for the establishment of the swiddens and fallows sampled was 4.3 (range 1 to 10) palms among the 10 colonist farmers, and 1.5 (range 1 to 2) palms among the 10 indigenous farmers. In addition, 19 % of all palms sampled from both study areas (N= 386 palms) grew in swiddens and fallows established by a given farmer using only one maternal parent palm as a seed source. As a point of comparison, Brown and Marshall (1995) recommend using a minimum of 50 maternal parents to minimize founder effects when establishing a new population. Therefore, the practices of these farmers could potentially lead to founder effects, genetic bottlenecks and drift.

The dynamic movement of peach palm seed and the distances over which it had taken place in the recent past appeared to be an important variable between the two metapopulation study areas (Figure 4-1). Within the colonist metapopulation, fifty-one percent of peach palms sampled (113 out of 221 palms) grew from seed selected from a farmers' own swidden or fallow, and an additional 20% (44 out of 221 palms) grew from seed selected from an immediate neighbor (within the same community). Fifteen percent of the sampled palms (33 out of 221 palms) grew from seed that had originated from one

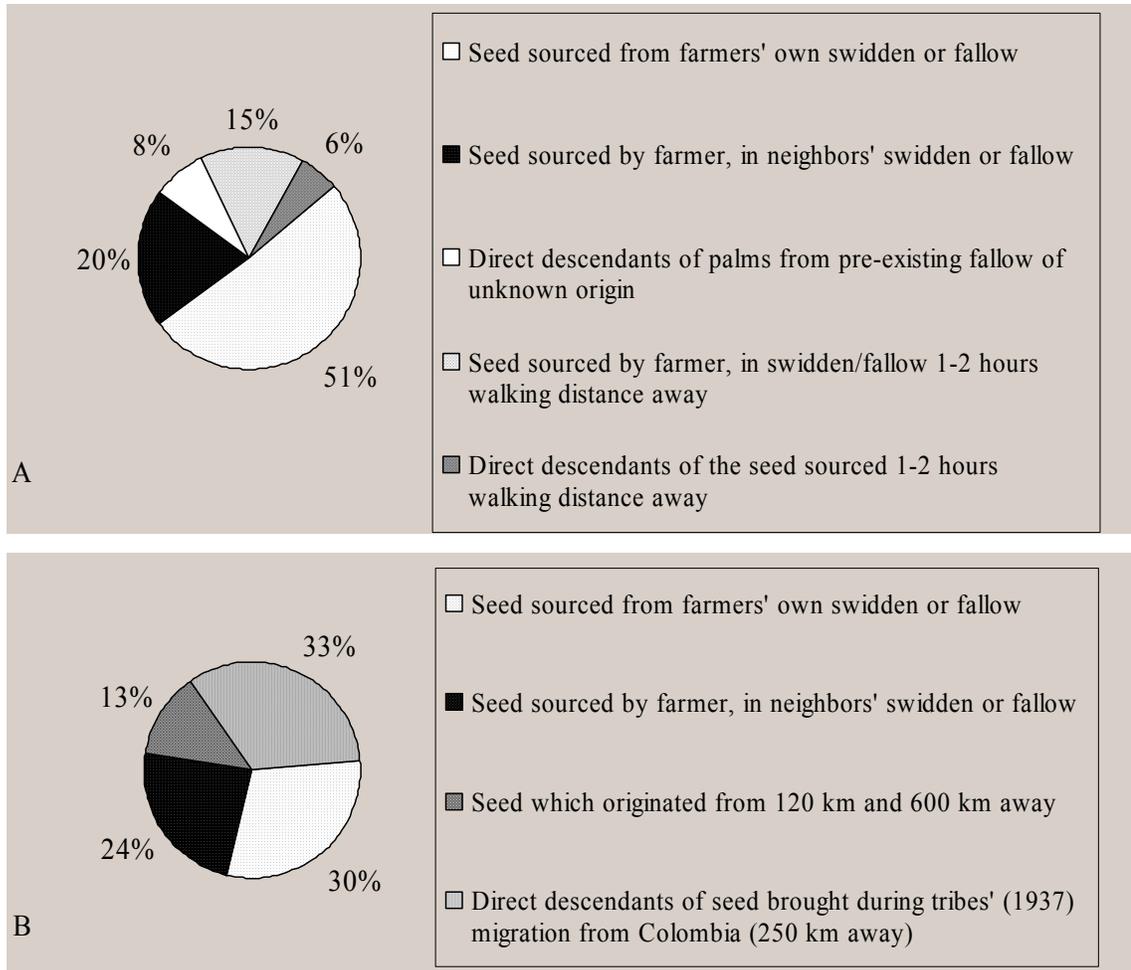


Figure 4-1. Seed sourcing for peach palms sampled from the colonist and indigenous metapopulations. A) 221 colonist palms. B) 165 indigenous palms. All palm seed for a given planting event was reportedly obtained from only one single source location at a time.

to two hours walking distance away, and an additional 6% (13 out of 221 palms) were direct descendants (progeny) of that 15% (Figure 4-1A).

In sharp contrast, within the indigenous metapopulation the scale of the movement of genetic material was much larger; thirteen percent of the palms sampled (21 out of 165 palms) grew from seed that had originated from 120 to 600 km away (Figure 4-1B). Of these 21 palms, two were spineless whose seed had originated in Iquitos, 120 km upriver. An additional 19 palms were sampled which were found growing in the context of a

Peruvian governmental program (Fondo Nacional de Compensación y Desarrollo Social) to promote spineless heart of palm cultivation in the area using peach palm seed originating from the Yurimaguas region, almost 600 km upriver from the communities.

In addition, within the indigenous metapopulation 33% of the palms sampled (55 out of 165 palms) were said to be descendants of seed originating from the homeland of the grandparents and parents of the farmers, 250 km to the north in the Putumayo region of Colombia (Figure 4-1B). These people fled in 1937 with their families (along with seeds and cuttings of their traditional crops) to their present location in Peru to escape the lingering slave trade that had decimated the region's tribes at that time. The average age of these 55 palms sampled was 33.3 (range 3 to 50+) years.

A comparison of the average age of all palms sampled from the two metapopulation study areas exhibits a divergence in their age distribution (Table 4-1). Twenty-eight percent of all palms sampled (47 out of 165 palms) from indigenous farms were attributed to planting events 30 to 50 years earlier, compared to only 1% of all palms sampled (3 out of 221 palms) from previous (unknown) planting events observed and sampled on colonist farms. Most of the colonist farmers claimed that the production of peach palm fruit deteriorates in fallow over time (as discussed in Clement 1990), so they made little effort to maintain (protect) older remnant palms from previous

Table 4-1. Average age of peach palms sampled from colonist and indigenous metapopulations

	Ten colonist farms	Ten indigenous farms
Number of palms sampled	221 palms	165 palms
Average age of all palms sampled	6.0 years (range <1-17 years)	19.2 years (range <1-50+ years)

generations once the palms had been overcome by forest re-growth. These farmers preferred to simply plant a new generation of palms, if possible. Thus, palms managed by the colonist farmers rarely survived through the fallow period until the next swidden clearing cycle. While this may have been a factor somewhat enthusiastically over-exaggerated by the indigenous farmers surveyed, a strong divisional trend in management practices is apparent over all twenty farms sampled.

Molecular Marker Results

Genetic Diversity and Hardy-Weinberg Equilibrium

The three microsatellite marker loci carried 49 alleles in the indigenous metapopulation ($n = 165$), while only 43 alleles were found in the colonist metapopulation ($n = 221$). The frequency of observed heterozygosity averaged over loci was somewhat higher in the indigenous metapopulation as well (Table 4-2). Expected heterozygosities throughout both metapopulations were generally higher than observed heterozygosities at individual loci, with one minor exception where a landrace population consisted of only two individuals (Table 4-2). Of the 19 tests of conformity to Hardy-Weinberg proportions, 12 groups of populations showed a significant deficiency of heterozygotes at the 0.01% level, and 3 groups were deficient at the 1% and 5% levels (Table 4-2). This is also apparent in the values obtained for the inbreeding coefficient f , an estimator measuring the effects of nonrandom mating within populations on a scale of -1, indicating an excess of heterozygotes, to 1, indicating an excess of homozygotes, relative to proportions expected in a Hardy-Weinberg equilibrium population.

Within the two metapopulation study areas, observed heterozygosities in the colonist communities ranged from 0.639 to 0.698, while those of the indigenous

Table 4-2. Microsatellite diversity and heterozygote deficiency in colonist and indigenous peach palm metapopulations; landraces sampled from El Dorado collection included for comparison.

	<i>N</i>	# of pops.	# of alleles per locus			Heterozygosity		<i>f</i>
			Bg02-19	Bg02-24	Bg55	Expected	Observed	
Colonist Metapopulation:								
Communities	221	4	21	14	8	0.833	0.678	0.194***
Community:								
Nuevo Tarapaca	42	3	17	7	7	0.823	0.698	0.156***
San Carlos	81	3	15	13	8	0.806	0.639	0.210***
Nuevo Triunfo	60	2	15	11	8	0.824	0.684	0.171***
Nuevo San Juan	38	2	13	11	7	0.847	0.697	0.191***
Indigenous Metapopulation:								
Communities	165	4	22	16	11	0.806	0.693	0.152***
Community:								
Brillo Nuevo	55	3	15	13	5	0.758	0.664	0.121**
Puerto Isango	24	2	13	9	6	0.826	0.741	0.107*
Pucaurquillo	58	3	21	14	10	0.802	0.625	0.207***
Sa. Lucia de Pro	28	2	14	12	7	0.792	0.708	0.105**
El Dorado Collection:								
Landraces	37	5	17	14	7	0.766	0.713	0.155***
Landrace:								
Putumayo	7	1	9	7	6	0.869	0.746	n.a.
Pampa Hermosa	7	2	7	7	5	0.848	0.833	0.024
Pastaza	6	2	7	6	3	0.793	0.667	0.09
Tigre	15	2	11	8	6	0.809	0.619	0.248***
Vaupés	2	1	2	2	3	0.5	0.667	n.a.

Each metapopulation analyzed among communities and farms (populations) pooled within communities; sampling from El Dorado germplasm collection analyzed among geographical landraces and populations of origin pooled within landraces. Expected (H_e) and observed (H_o) heterozygosities averaged over all three loci. The unbiased measure of inbreeding f (Weir and Cockerham 1984) considers the effects of nonrandom mating within populations (averaged over loci). Significant heterozygote deficiency over all loci, relative to Hardy-Weinberg expectations at: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

communities ranged from 0.625 to 0.741 (Table 4-2). As a point of comparison, the 37 individuals of 5 different landraces sampled from the El Dorado germplasm collection revealed similar numbers of alleles per locus, and a larger observed heterozygosity (0.713) than was estimated for either of the two metapopulation study areas (Table 4-2).

Private alleles in the context of this study are alleles found only in one of the two study metapopulations; all of the other alleles totaled in Table 4-2 were shared between them. There were more than twice as many private alleles, occurring at a higher average weighted frequency in the indigenous metapopulation than in the colonist. However, in general these private alleles were rare in both metapopulations, all occurring at frequencies of 0.028 or less (Table 4-3).

Testing for a genotypic association among all three loci within the two metapopulations and the El Dorado populations found no significant association using chi-square (P values 0.12 and higher). However, within the colonist metapopulation there was a significant association (at the 0.01% level) between loci Bg02-19 and Bg02-24.

Genetic Differentiation

In an analysis of the genotypes observed at marker loci, genetic differentiation estimated among colonist farms was low ($F_{st} = 0.03$) yet significantly different from zero ($P < 0.0001$) (Table 4-4). The genetic differentiation estimated among indigenous farms was even lower ($F_{st} = 0.017$), yet still significant ($P < 0.001$). Genetic differentiation among the eight El Dorado populations of five landraces (Vaupés, Pampa Hermosa, Pastaza, Putumayo and Tigre) appeared greater ($F_{st} = 0.05$), revealing more moderate levels of significant ($P < 0.01$) population differentiation consistent with the relatively larger geographical scale of this particular sampling (Table 4-4). Analyzing the farms in

Table 4-3. Weighted allele frequencies at individual loci for colonist and indigenous metapopulation study areas. ‘Private alleles’, alleles unique to one or the other metapopulation study areas, are shown in bold.

Colonist Metapopulation: N = 221						Indigenous Metapopulation: N = 165					
Bg02-19	Freq.	Bg02-24	Freq.	Bg55	Freq.	Bg02-19	Freq.	Bg02-24	Freq.	Bg55	Freq.
152	0.002	128	0.002	282	0.061	152	0.028	114	0.003	282	0.006
154	0.236	130	0.009	286	0.207	154	0.15	130	0.012	284	0.019
156	0.037	132	0.064	288	0.293	156	0.034	132	0.037	286	0.18
158	0.002	134	0.025	290	0.039	160	0.003	134	0.019	288	0.559
162	0.062	136	0.169	296	0.011	162	0.097	136	0.118	290	0.009
164	0.122	138	0.139	300	0.098	164	0.038	138	0.199	294	0.003
166	0.002	140	0.112	302	0.075	166	0.019	140	0.115	296	0.006
168	0.005	142	0.059	304	0.216	170	0.006	142	0.115	298	0.003
170	0.03	144	0.071			172	0.103	144	0.118	300	0.087
172	0.073	146	0.014			174	0.006	146	0.019	302	0.028
174	0.011	148	0.002			176	0.019	148	0.009	304	0.099
176	0.034	150	0.041			178	0.053	150	0.025		
178	0.14	152	0.014			180	0.028	152	0.006		
182	0.007	158	0.279			182	0.034	154	0.009		
184	0.014					184	0.094	156	0.012		
186	0.039					186	0.088	158	0.183		
188	0.057					188	0.022				
190	0.05					190	0.122				
192	0.048					192	0.047				
194	0.025					198	0.003				
198	0.002					200	0.003				
						204	0.003				

Two private alleles, allele 200 at Bg02-19 (indigenous) and allele 128 at Bg02-24 (colonist), were also found in the El Dorado populations at low weighted frequencies (0.014 and 0.029 respectively).

the two metapopulation study areas as two discrete panmictic populations, the genetic differentiation between them was estimated as $F_{st} = 0.027$, which is low but still significant ($P < 0.0001$) for these two groups of farms located approximately 160 km apart.

Isolation-by-Distance

Pairwise estimates of F_{st} (Weir and Cockerham 1984) were plotted against the geographical distance between populations, performed separately for each of the two metapopulation study areas. Genetic material was assumed to travel linearly along the trails and rivers to obtain estimates of geographical distance. The absence of a statistically significant isolation-by-distance relationship up to 27 km distance in the indigenous metapopulation further stresses low population genetic differentiation (Figure 4-2A). Accordingly, the values of pairwise estimators were low for the indigenous populations, and only 4% of these were significant ($P < 0.05$) (Table C-2).

In contrast, the colonist farms exhibited a significant ($P < 0.01$) isolation-by-distance relationship up to 35 km distance (Figure 4-2A). The pairwise values obtained for the colonist populations were generally higher than those obtained for the indigenous populations, and a higher percentage of these values (29%) were significant at the 1% and 5% levels (Table C-1).

The values of pairwise population genetic differentiation among the 8 El Dorado populations of 5 landraces were higher than those of either of the two metapopulations. These populations illustrated a significant ($P < 0.01$) isolation-by-distance relationship up to 1250 km, calculated as the shortest linear distances along rivers, which in a few cases included trails connecting watersheds (Figure 4-2B). However, none of these pairwise F_{st} values were significant ($P < 0.01$) when testing for population differentiation, probably due to the small population sizes analyzed (Table C-3).

Table 4-4. Unbiased estimates of Wright F -statistics for colonist/indigenous metapopulations; landrace populations sampled from El Dorado collection included for comparison.

	N	# of pops.	F -statistics at individual loci									F -statistics over all loci		
			Bg02-19			Bg02-24			Bg55			F	f	θ
			F	f	θ	F	f	θ	F	f	θ			
Colonist farms	221	10	0.202	0.181	0.026	0.127	0.088	0.042	0.308	0.292	0.023	0.211	0.186	0.03***
Colonist communities	221	4	0.204	0.189	0.019	0.129	0.108	0.023	0.312	0.291	0.03	0.213	0.194	0.024***
Indigenous farms	165	10	0.151	0.129	0.024	0.19	0.181	0.01	0.136	0.121	0.016	0.161	0.146	0.017**
Indigenous communities	165	4	0.152	0.14	0.014	0.191	0.184	0.009	0.138	0.125	0.014	0.162	0.152	0.012**
El Dorado populations	37	8	0.283	0.266	0.024	0.05	-0.02	0.066	0.243	0.195	0.059	0.192	0.15	0.05*
El Dorado landraces	37	5	0.284	0.272	0.017	0.061	-0.02	0.08	0.247	0.206	0.052	0.197	0.155	0.05*
Colonist vs. Indigenous	386	2	0.195	0.178	0.02	0.159	0.151	0.009	0.286	0.244	0.056	0.21	0.188	0.027***
Colonist vs. Indigenous vs. El Dorado	423	3	0.203	0.187	0.019	0.149	0.146	0.009	0.279	0.243	0.049	0.208	0.188	0.025***
Colonist and Indigenous vs. El Dorado	423	2	0.204	0.195	0.02	0.149	0.146	0.003	0.275	0.262	0.017	0.207	0.198	0.011*

Weir and Cockerham's (1984) unbiased estimates of Wright F -statistics defined as follows: F = total heterozygote deficiency (overall inbreeding coefficient), f = deficiency within individuals within populations (inbreeding coefficient) and θ = deficiency among populations (an estimate of fixation index or Wright's F_{st}). Each metapopulation analyzed among farms and among farms pooled within communities; El Dorado germplasm analyzed among populations of origin and among populations pooled within geographical landraces. Estimates of F -statistics also calculated between groups of populations (colonist, indigenous, El Dorado). Significant population differentiation at: * $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$.

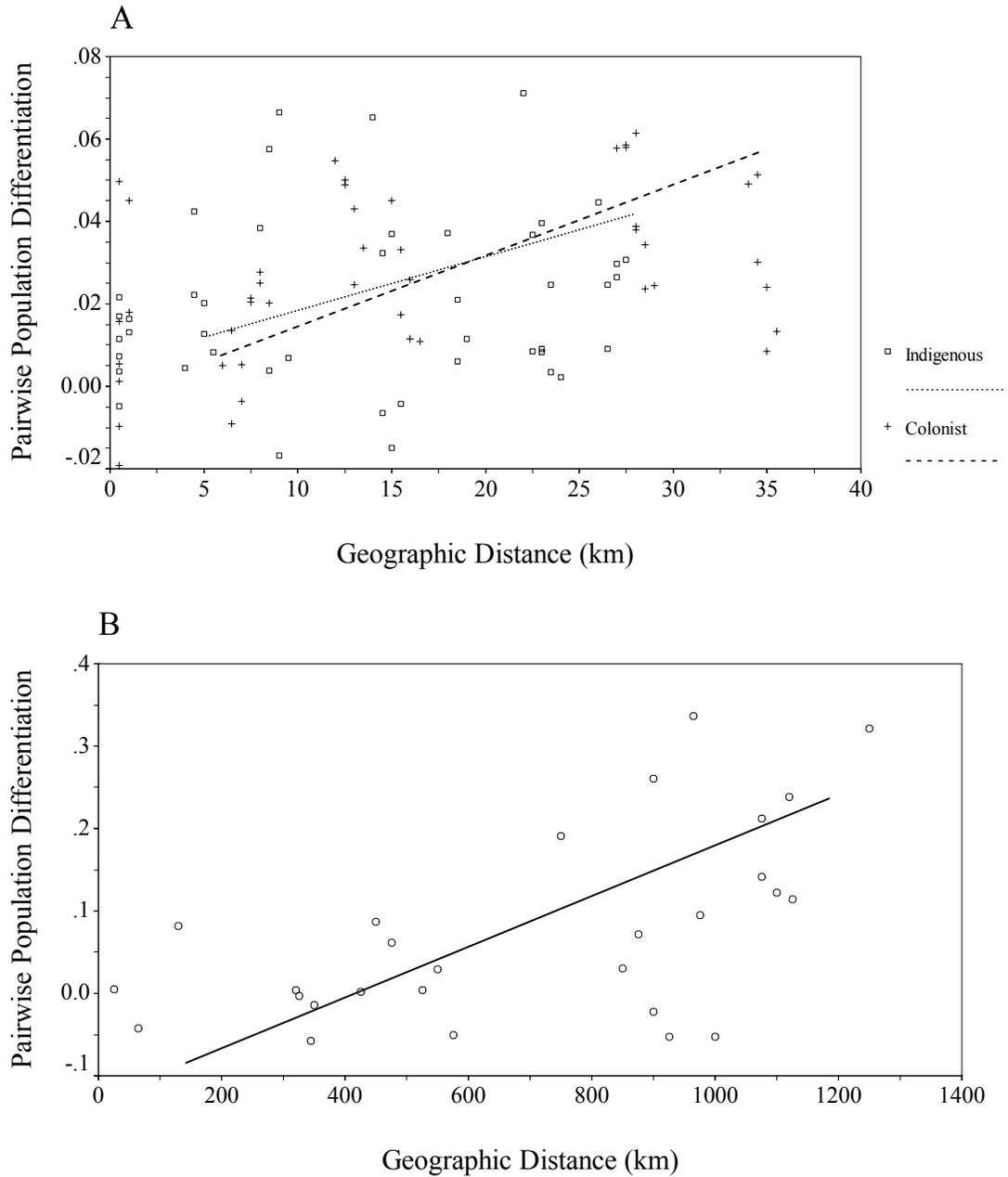


Figure 4-2. Testing for an isolation-by-distance relationship among farms within each of the two metapopulations and among populations analyzed from the El Dorado collection. A) Correlation between unbiased estimates of pairwise population genetic differentiation [$F_{st}(\theta)$ over all loci] and geographic distance, $R^2 = 0.1948$ and Mantel test of correlation, $P < 0.0029$ for colonist populations; $R^2 = 0.0301$ and $P < 0.0896$ for indigenous populations. B) Correlation, $R^2 = 0.3346$ and Mantel test, $P < 0.009$ for El Dorado populations.

Discussion

The high inbreeding coefficients (f) calculated for both metapopulations (Table 4-2) are the result of recurrent sib-mating occurring over many generations, as inbreeding coefficients measure the probability that two alleles are identical by descent (Hartl 2000). Clement et al. (1997) and Clement (1995) both reported similar levels of inbreeding in populations of *Bactris gasipaes*. This kind of selection is the very essence of crop domestication, necessary to amplify the specific traits one prefers in a given species (Doebley 1989), yet ideally there should be a balancing factor in the agroecosystem to allow for a continuation of the crop's evolution. This balance appears to be facilitated in part by the migration of genetic material with and among metapopulations.

Seed Migration within a Metapopulation

The migration of seed has the potential to introduce 'new' alleles into a population, thereby increasing its genetic diversity and slowing the rate of divergence within the metapopulation. Additional migration across time via the descendants of introduced seed is taking place among 6% (13 palms) and 33% (55 palms) of the total palms sampled from the colonist and indigenous study areas respectively (Figure 4-1). These palms might still carry alleles introduced into the population by their immigrant ancestors, and could contribute to their continued maintenance. Yet the impact that the immigration of genetic material has on a metapopulation will primarily depend upon the scale of distance from which it originated, as a comparison of isolation-by-distance relationships in Figures 4-2A and 4-2B reveals.

Migration within a peach palm metapopulation occurs among a group of populations in both the immediate (neighbor's swidden) and close vicinity (1 to 2 hours away), as well when seed is brought in from another distant metapopulation (120 to 600

km away) (Figure 4-1). In other words, the boundaries of seemingly localized metapopulations are in fact permeable, open to immigration from outside. At a greater scale, the swiddens and fallows comprising entire regions of Peru, Ecuador and Colombia would theoretically form one massive peach palm metapopulation, connected by the historic flow of seed over large distances. Rodrigues et al. (2004a) use the genetic relationships among landraces to discuss the possible migration of peach palm seeds throughout South America and up through Honduras out of a single source area of domestication, previously proposed for southwestern Amazonia (Huber 1904, Ferreira 1999). As illustrated in this study and elsewhere, a certain degree of seed migration continues even today at a similarly extensive scale, particularly concerning genetic material of the spineless Pampa Hermosa landrace from the Yurimaguas region of Peru (Figure 4-1B) (Adin et al. 2004).

Although the seed for planting a new swidden generation was always obtained from just one source population (Figure 4-1), and sometimes comprised groups of half-sib individuals originating from only one maternal parent, each farm sampled within a given community is assumed to be an interacting unit of a metapopulation (*i.e.* colonist community) within a larger group of metapopulations (*i.e.* colonist metapopulation study area). This is due to the relative proximity of neighboring farms (between 250 and 500 m apart; see Figure 3-1), which are potentially interchanging genetic material via long-distant pollen-mediated flow within the community metapopulation. And as previously discussed, farmers are also directly exchanging genetic material among neighbors, and presumably between communities. Therefore a given source of seed is a mix of

genotypes drawn from the metapopulation at large, and the migration of seed resembles the ‘migrant pool’ mode of gene immigration (Slatkin 1977).

Population Structuring and Genetic Differentiation

One direct consequence of migration within a metapopulation, particularly via the ‘migrant pool’ mode, is that population genetic differentiation is substantially reduced. A comparison of the overall F_{st} estimates for colonist and indigenous farms (Table 4-4) has little meaning, however these estimates are both consistent with high rates of gene flow among populations, *i.e.* exchange of genetic material among farmers along the course of the two river systems over a long period of time. Indeed, in Figure 4-1 there appears to be comparably high rates of exchange among neighboring farmers in both of the two study areas. Adin et al. (2004) came to similar conclusions regarding G_{st} estimates obtained for two riverine groups of peach palm populations from the Yurimaguas region of Peru, which were similarly low (G_{st} is an equivalent estimator of the parameter F_{st} which considers the ratio of inter-population genetic variation to the total variation).

Pairwise population differentiation was higher in the colonist metapopulation than in the indigenous (Table C-1 and C-2), and there exists a statistically significant isolation-by-distance relationship among them (Figure 4-2A) — thus there is some indication that the structure of the colonist metapopulation is more defined than the indigenous. The high incidence of seed migration into the indigenous metapopulation (Figure 4-1B) might be causing this discrepancy, as genetic divergence among sub-populations would be reduced if immigrant seeds were a pool of genotypes from the larger (regional) metapopulation structure.

The overall F_{st} estimate obtained among the eight El Dorado populations of five landraces ($F_{st} = 0.05$, $P < 0.01$; Table 4-4) was somewhat lower than the among population

differentiation estimated for regional-scale samplings of other allogamous plant species in the tropics (typically between 0.10 and 0.15; Bawa 1992, Hamrick et al. 1992). However, it was still substantially higher than the population differentiation estimated for the two metapopulation study areas. Therefore, as distances increased between populations the gene flow among them was less. For instance, Rodrigues et al. (2004a) analyzed 220 peach palms from a much larger geographical selection of six landraces, representing close to the entire range of the species (from Central America through Peru to the mouth of the Amazon in Brazil), and estimated genetic differentiation among populations as $G_{st} = 0.16$.

It is interesting to note that the lowest F_{st} estimate overall was obtained in a grouping analysis between the two metapopulation study areas (Group 1) and the El Dorado populations (Group 2) ($F_{st} = 0.011$, $P < 0.01$) (Table 4-4). The low genetic differentiation between the study areas when considered as two discrete panmictic populations is relevant here as well ($F_{st} = 0.027$, $P < 0.001$). Mora-Urpí and Clement (1988) proposed that populations near Iquitos and Pebas might represent heterogeneous mixtures of Putumayo, Pampa Hermosa, Pastaza and Tigre landraces, among others. This is because the region is at the crossroads of several major river systems, which would have facilitated the exchange of different landraces over long periods of time; the results presented here would seem to support their hypothesis.

Maintenance of Remnant Trees on the Indigenous Farms

As competition for light and nutrients increases, older palms in fallow do not produce the fruit yields of younger palms in swidden clearings (Clement 1990). Yet in northwest Amazonian indigenous cultures, remnant peach palms provide a sense of generational continuity and play an important role in revisiting memories of the people

(often direct ancestors) who planted them. This symbolic association with peach palm has been reported by anthropologists (*e.g.* Chaumeil 2001, Erickson 2001 and Rival 2002) as being broadly shared by at least five specific tribes of the northwest Amazon. One of these ethnographies (Chaumeil 2001) took place in the same indigenous communities sampled for this population genetic analysis of peach palm.

It is more a matter of custom with the indigenous than with the colonist farmers surveyed, regardless of the utilitarian value of the remnant palms. Yet this is not to generalize that only indigenous Amazonians would find the preservation of remnant palms worthwhile; if other groups of indigenous and non-indigenous farmers were sampled perhaps the results would have been different.

The maintenance of remnant palms from previous generations of swiddens may be helping to preserve genetic diversity within the indigenous metapopulation, by reducing founder effects and loss of alleles. Since changes in the genetic composition of a population require a generational turnover, maintaining remnant palms from previous swidden generations would potentially lessen the negative consequences of founder effects. In addition, these remnant palms are quite possibly an important cohesive factor in metapopulations; for instance, Chase et al. (1996) found that isolated trees could act as stepping stones, facilitating gene flow among larger populations. Intermittently throughout the secondary forest and fallows sampled, peach palms from previous swidden generations extended up through the canopy (≈ 2 to 3 palms per hectare) in search of light in the midst of encroaching vegetation.

The indigenous farmers of the particular area under investigation have been reported to clear and reuse the same plot of land after fallowing periods lasting 20 years

(Denevan and Treacy 1987). Given the current shortage of land available to each family, these farmers are very rarely able to wait any longer for fallowed land to ‘replenish’ itself (Manuel Nibeco, October 2003, personal communication). This period of time is short enough to prevent the complete extinction of peach palms planted during previously cultivated swiddens (Clement 1990), especially considering that during the first five years these swidden-fallows are managed by the farmers for the long term growth of the fruit trees in the weeding out of competing secondary vegetation (Denevan and Treacy 1987). Some of the farmers claimed that they clear the vegetation around their palms when harvesting fruit; more or less every year throughout the entire fallowing period.

In addition, management of the common Amazonian agroforestry intercrop manioc (*Manihot esculenta*) and peach palm differed in a slight yet significant manner between the indigenous and colonist farmers. Indigenous farmers in the northwest Amazon are known to predominately cultivate highly toxic manioc varieties (McKey and Beckerman 1993), and the farmers indicated they were used to produce their traditional bread. They also claimed that these varieties mature relatively slowly, preserving well in the soil for 5 years or more. On the other hand, the colonist farmers all grew a non-cyanogenous variety of manioc (to be eaten boiled, unprocessed), which produces yields in less than three years. The soil would then be exhausted for root crop cultivation and management of the entire swidden would taper off into fallow. Thus the choice of manioc varieties determines the amount of years a swidden will be regularly cleared of undesirable secondary vegetation, which relates to the amount of time peach palm will accumulate biomass in an open environment— the extra years conferring an advantage for the palms once the fallow period begins.

The older palms that persist in indigenous fallows are spared during subsequent forest clearings and survive the burning of slash to sprout up from their bases (a trait characteristic of *Bactris gasipaes*); fueled by the nutrient flush they soon are producing fruit again. Thus the supportive effect of generational overlap on the maintenance of genetic variability and structure within peach palm populations is facilitated by the longevity of highly managed palms.

Comparing Genetic Diversity and Heterozygosity Estimates

Considering the historical-ecological context of the two study locations, observed heterozygosity and levels of genetic variability (Tables 4-2 and 4-3) provide a broad evaluation of the effects of migration and the preservation of remnant individuals in maintaining diversity in peach palm. It is important to note that the 165 indigenous palms represent a sample size that is 25% less than that of the 221 colonist palms, yet there were still more alleles per locus observed in the indigenous metapopulation (Table 4-2). The additional private alleles in Table 4-3 might have been obtained (introduced) through the increased rates of seed immigration from afar (Figure 4-1), although extensive sampling in source areas would be needed in order to prove this. Yet alleles are extremely sensitive to population bottlenecks; therefore, the preservation of remnant individuals might be playing a role in maintaining these private alleles, albeit at extremely low frequencies.

Evidence (or the lack thereof) of genotypic disequilibrium is important in making inferences about the history of a population and the evolutionary forces governing allele frequencies at these loci. In this case, a lack of strong evidence for genotypic associations within the peach palm populations sampled corresponds with the fact that migration among peach palm populations is occurring among gene pools that are

somewhat closely related, as we would expect strong zygotic associations if this were not the case (Barton and Gale 1993).

General Discussion

The allelic components of peach palm's diversity, such as those sampled from the farms surveyed for this study, fluctuate over time. Through the migration of genetic material, farmers seem to be managing general levels of diversity within a metapopulation matrix, more than they are managing individual genes in isolation (Louette 2000). This study offers insight into developing strategies to preserve the evolutionary processes that actively create and maintain general levels of genetic diversity on-farm. If participatory domestication projects are able to preserve the actual genes underlying the more useful phenotypes (like the ongoing program with peach palm in Peru; see Weber et al. 2001), then it is important that there is a degree of movement of this 'improved' genetic material within and between regions to maintain general levels of 'useful' diversity. The problem then lies in preventing the frequency of these targeted genes from becoming excessively diluted on-farm and within the metapopulation over the long run.

It is difficult to generalize about farmer behavior and assume that this kind of genetic migration (crucial to the metapopulation's dynamics) is taking place often enough, throughout the entire geographical distribution of peach palm, to prevent a degree of genetic erosion in the species as a whole. In Peru, the flow of peach palm seed is highly decentralized and sporadic, oftentimes dependent solely upon individual farmers' perceptions about the use and value of specific palm types. And since both the number of populations and their sizes are never held perfectly constant, the effects of migration within and among metapopulations are likely to be highly variable and site-

specific. Therefore, formal institutionalized support is needed to develop a network capable of guiding an appropriate degree of exchange of genetic material. It is believed that in developing markets for improved peach palm seed, this exchange will be facilitated (Clement et al. 2004a). Yet it would be helpful to monitor changes in measures of genetic diversity in recipient populations over time, to gauge an appropriate scale for this migration to take place on a case-by-case basis.

Finally, it is important to keep in mind when reviewing any study of tropical crop genetic resources, whose centers of diversity are often located in lesser-developed regions of the world, that they are often managed in the context of immediate subsistence irrespective of long-term genetic consequences. Subsistence farmers tend to do what they need to feed their families in the short run. In the context of genetic resources this often translates into making a conscious decision to use limited time and resources to cultivate only the most productive and appealing varieties of a given crop at the expense of all others, thereby eroding the underlying genetic diversity. While it is true that even in the darkest reaches of Amazonian backwaters one might find an occasional crop connoisseur growing a wide variety of types, he or she will always be the exception.

Conclusion

Based on the molecular results presented, and the contrasting management of palm germplasm observed between the two groups of farmers, what conclusions can realistically be made? The palm populations sampled have a long evolutionary history, which is not addressed when asking farmers about recent management of the genetic resource. Nonetheless, the amount of neutral genetic variation and heterozygosity observed in both the indigenous and colonist metapopulations was high.

These relatively high levels of diversity were not expected, considering the seed selection practices of the farmers and the high inbreeding within both metapopulations. Although these particular microsatellite loci are only representative of neutral genetic variation, founder effects and selection in the 10,000 year process of peach palm domestication are expected to have reduced overall allelic diversity. Yet these neutral marker loci are extremely sensitive to gene flow (migration). These results suggest long-distance seed migration events in the relatively recent past have attributed to the genetic variability and heterozygosity observed at present in the indigenous populations. Since migration is taking place on a reduced scale in the colonist metapopulation, the same is true to a lesser extent. In turn, the extremely low genetic differentiation observed within and between the two metapopulations suggests that seed exchange (migration) has been extensive throughout the region over long periods of time. It cannot be ruled out that an unforeseen potential of the species for pollination among populations might have played a role here as well, over larger than expected distances by nonspecialist insect vectors.

In addition, the preservation of remnant palms has the potential to maintain alleles introduced into the indigenous metapopulation for longer periods of time than would be possible otherwise. Barring any sudden change in traditional agroforestry management strategies, this factor should continue to play a role in the composition of the indigenous metapopulation's genetic diversity, yet the frequency and extent of future genetic migration within and among regions will remain crucial.

Epilogue: Participatory Domestication of Peach Palm in Peru

ICRAF (International Centre for Research in Agroforestry) and INIA (Instituto Nacional de Investigación Agraria) initiated a participatory domestication project for *Bactris gasipaes* in Peru in 1997. Farmers in the Yurimaguas region wanted to conserve

the phenotypic diversity of their palms, and produce seeds for sale to other regions and countries where there was a demand for their spineless type to establish heart-of-palm plantations. This region is known to possess the most economically valuable landrace of the species, Pampa Hermosa, with a high frequency of spineless trunks and leaves, and rapid growth for heart-of-palm production (Mora Urpí and Echeverría 1999). There is high phenotypic variability and relatively high genotypic variability in this particular landrace (Rodrigues 2001), so it would benefit the farmers if this project produced seed with relatively uniform trait characters. This is always the trade off with the domestication of crops, and lesser-known crops in particular; those genetic resources which fall outside of the domesticated type perceived as the most economically promising will always erode.

Researchers asked 142 farmers from 16 different communities to select their best spineless palms based on preferred fruit characteristics, expecting to create dual-purpose populations (fruit for food security; heart-of-palm seeds for export). They then collected at least 300 seed from each of these palms to establish on-farm progeny trials in the Yurimaguas and Pucallpa regions (Weber et al. 2001). Each trial replication consisted of two progeny plants of the 400 selected mother palms. The trials were rogued after 5 years, leaving the best plant from each progeny for the production of selected seed. To maintain as much variability as possible at every step in the domestication process, no selection among progenies took place.

The heritability of fruit traits in peach palm is unknown at present and those for heart-of-palm growth and yield are low (Clement 1995). Nonetheless, genetic gain under this scenario of selection might be improved if the trials were rogued among progenies as

well, rather than only within. Especially since the principal objective of both the farmers and the researchers was to collect and conserve valuable phenotypes, and they did not presume their project would conserve the underlying molecular diversity. They focused instead on collecting palms that the farmers themselves identified as useful to them, rather than collecting a wide range of randomly selected palms or palms identified through molecular analysis as rare and valuable for genetic preservation.

The farmers are the owners of their replications, and they received periodic stipends to finance the maintenance and fertilization of the trials. They certainly expect INIA to help them export seed to Brazil and other Latin American heart-of-palm producers, but it is not yet clear that INIA is organized for this task.

APPENDIX A
EXACT ORIGINS OF POPULATIONS ANALYZED

Table A-1. Exact origins of the 221 colonist (Tamshiyacu-Tahuayo) samples

# of palm samples	Farm	Community	Lat.	Long.
14	Farm 1	Nuevo Tarapaca	04° 05' S	73° 06' W
14	Farm 2	Nuevo Tarapaca	04° 05' S	73° 06' W
14	Farm 3	Nuevo Tarapaca	04° 05' S	73° 06' W
28	Farm 4	San Carlos	04° 13' S	73° 11' W
27	Farm 5	San Carlos	04° 13' S	73° 11' W
26	Farm 6	San Carlos	04° 13' S	73° 11' W
30	Farm 7	Nuevo Triunfo	04° 08' S	73° 09' W
30	Farm 8	Nuevo Triunfo	04° 08' S	73° 09' W
22	Farm 9	Nuevo San Juan	04° 05' S	73° 04' W
16	Farm 10	Nuevo San Juan	04° 05' S	73° 04' W

Table A-2. Exact origins of the 165 indigenous (Yahuasyacu-Ampiyacu) samples

# of palm samples	Farm	Community	Lat.	Long.
14	Farm 1	Brillo Nuevo	03° 15' S	71° 55' W
16	Farm 2	Brillo Nuevo	03° 15' S	71° 55' W
25	Farm 3	Brillo Nuevo	03° 15' S	71° 55' W
11	Farm 4	Puerto Isango	03° 20' S	71° 55' W
13	Farm 5	Puerto Isango	03° 20' S	71° 55' W
5	Farm 6	Pucaurquillo	03° 24' S	71° 50' W
19	Farm 7	Pucaurquillo	03° 24' S	71° 50' W
34	Farm 8	Pucaurquillo	03° 24' S	71° 50' W
13	Farm 9	Santa Lucia de Pro	03° 24' S	71° 48' W
15	Farm 10	Santa Lucia de Pro	03° 24' S	71° 48' W

Table A-3. Exact origins of the 8 populations from 5 landraces sampled from El Dorado collection

Collection #	Landrace	Pop.	Location collected	Lat.	Long.
171-177	Putumayo	1	Mazán-Río Napo	03° 43' S	73° 04' W
180-182, 185	Pampa Hermosa	2	Barranquita-Río Huallaga	06° 15' S	75° 45' W
223, 228, 235	Pampa Hermosa	3	Lagunas-Río Huallaga	05° 45' S	76° 05' W
194	Pastaza	4	Lago Agrio-Río Napo	00° 05' N	76° 50' W
199	Pastaza	4	Chuchufindi-Río Napo	00° 05' S	76° 02' W
244, 246-248	Pastaza	5	Río Napo	00° 50' S	77° 15' W
201, 203-204, 206-210	Tigre	6	Intuto-Río Tigre	04° 00' S	74° 25' W
213, 215-216, 219-222	Tigre	7	Quebrada Intuto	04° 00' S	74° 45' W
333	Vaupés	8	Río Papuri	00° 40' N	70° 10' W
334	Vaupés	8	Río Papuri	00° 40' N	70° 15' W

Collection #'s 194 and 199 were combined to form population 4 and #'s 333 and 334 were combined to form population 8 in order to include these individuals in *F*-statistic estimates.

APPENDIX B
DNA EXTRACTION PROTOCOL

Extraction Protocol Adapted from Doyle and Doyle (1987)

1. CTAB buffer prepared daily
 - a. Polyvinylpyrrolidone (PVP) added to b-mercaptoethanol (b-merc) in CTAB solution and stirred to dissolve right before starting extractions. Used 0.5 ml CTAB + 0.02 g PVP + 2.5 μ l b-merc per plant sample.
2. 15 mg of silica-dried plant tissue weighed out
3. Tissue ground with mortar and pestles
 - a. Pestles were stored in 10% bleach solution and rinsed well with DI purified water before they were used on a new plant sample
 - b. Grinding was done with a pinch of autoclaved sand
4. 500 μ L of CTAB buffer added to ground tissue sample in separate eppendorf tubes
5. Sample tubes incubated at 55° C for 1 h
6. 500 μ L of 24:1 chloroform:iso-amyl alcohol added to sample tubes which were then vortexed (mixed)
7. Tubes centrifuged for 10 minutes at maximum speed (13000 rpm).
 - a. Following centrifugation, there were three layers; top: aqueous phase, middle: debris and proteins, bottom: chloroform
8. Pipetted off the aqueous phase taking care not to suck up any of the middle or chloroform phases. Pipetting slowly helped with this
9. Placed each of the aqueous phases into new labeled eppendorf tubes
10. Estimated the volume of the aqueous phases
11. Added 0.08 volumes of cold 7.5 M ammonium acetate into the new tubes
12. Added 0.54 volumes (using the combined volume of aqueous phase and added AmAc) of cold isopropanol (= 2-propanol) into the new tubes
13. Mixed (vortexed) tubes well

14. Placed tubes in freezer for 15 min
15. Centrifuged tubes for 3 min at maximum speed
16. Poured off the liquid in each tube, being careful not to lose the DNA pellets
17. Added 700 μ L of cold 70% Ethanol to each tube and mixed
18. Centrifuged tubes for 1 min at maximum speed
19. Poured off the liquid in each tube, being careful not to lose the DNA pellet
20. Added 700 μ L of cold 95% Ethanol to each tube and mixed
21. Centrifuged tubes for 1 min at maximum speed
22. Poured off the liquid in each tube, being careful not to lose the DNA pellets
23. Dried the pellets in the speed vacuum centrifuge for 20 min
24. Re-suspended DNA sample pellets with 100 μ L of TE buffer overnight in refrigerator

Stock Solutions

CTAB: for 1L of CTAB buffer

100 mL of 1 M Tris, pH 8.0
280 mL of 5 M NaCl
40 mL of 0.5 M EDTA
20 g of CTAB (Cetyltrimethyl ammonium bromide)

TE buffer: for 1L

10 mM 10 ml of 1 M Tris, pH 8.0
1 mM 2 ml of 0.5 M EDTA

1 M Tris, pH 8.0: for 1 L

121.1 g Tris (Fisher Cat#: BP152-5)
700 ml ddH₂O
Dissolved Tris and brought to 900 ml.
pH brought to 8.0 with concentrated HCl (~50ml)
Brought to 1 L

0.5 M EDTA pH 8.0: for 1 L

186.12 g of EDTA
750 ml ddH₂O
Added about 20 g of NaOH pellets
Slowly added more NaOH until pH was 8.0

5 M NaCl: for 1 L

292.2 g of NaCl
700 ml ddH₂O
Dissolved and brought to 1 L

APPENDIX C
PAIRWISE GENETIC DIFFERENTIATION AMONG POPULATIONS AND TESTS
OF SIGNIFICANCE WITH BONFERRONI CORRECTIONS

Table C-1. Pairwise genetic differentiation within colonist metapopulation

POP	1	2	3	4	5	6	7	8	9	10
1	0	0.0192	0.0179	0.0578	0.0586	0.0388	0.0449	0.0331	0.0052	0.0205
2	n.s.	0	0.0096	0.0579	0.0379	0.0236	0.0174	0.0258	-0.009	0.0035
3	n.s.	n.s.	0	0.0613	0.0344	0.0245	0.0115	0.0109	0.0051	0.0136
4	0.0045	0.009	0.009	0	0.0497	0.0451	0.0547	0.0489	0.0491	0.0513
5	0.0225	n.s.	n.s.	0.0045	0	0.0158	0.0501	0.0430	0.0301	0.0085
6	n.s.	n.s.	n.s.	0.0045	n.s.	0	0.0245	0.0335	0.0240	0.0133
7	n.s.	n.s.	n.s.	0.0045	0.0045	n.s.	0	0.0054	0.0250	0.0202
8	n.s.	n.s.	n.s.	0.0045	0.0045	0.045	n.s.	0	0.0213	0.0277
9	n.s.	n.s.	n.s.	0.0045	n.s.	n.s.	n.s.	n.s.	0	0.0012
10	n.s.	n.s.	n.s.	0.009	n.s.	n.s.	n.s.	n.s.	n.s.	0

Pairwise θ -values (Weir and Cockerham 1984) averaged over loci on top half of matrix, P-values (Bonferroni corrections) on bottom. Bold pairwise θ -values correspond to significant P-values ($P < 0.05$) listed in bottom half of matrix; n.s. indicates non-significance.

Table C-2. Pairwise genetic differentiation within indigenous metapopulation

POP	1	2	3	4	5	6	7	8	9	10
1	0	0.017	0.0131	0.0384	0.0038	0.071	0.0368	0.0091	0.0447	0.0246
2	n.s.	0	-0.0047	0.0575	-0.0167	0.0084	0.0083	0.0033	0.0091	0.0264
3	n.s.	n.s.	0	0.0665	0.0068	0.0395	0.0246	0.0021	0.0296	0.0307
4	n.s.	0.0315	0.018	0	0.0215	0.0652	0.0323	0.0369	0.0372	0.0211
5	n.s.	n.s.	n.s.	n.s.	0	-0.0065	-0.0149	-0.0042	0.0061	0.0115
6	n.s.	n.s.	n.s.	n.s.	n.s.	0	0.0037	0.0164	0.0043	0.0423
7	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0	0.0115	0.0222	0.0202
8	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0	0.0128	0.0083
9	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0	0.0072
10	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0

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BIOGRAPHICAL SKETCH

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