Comparing genetic diversity in agroforestry systems with natural forest: a case study of the important timber tree *Vitex fischeri* in central Kenya

Ard G. Lengkeek[1,2,*], Alice Muchugi Mwangi[1], Caroline A.C. Agufa[1], Joseph O. Ahenda[3] and Ian K. Dawson[1]

[1]*World Agroforestry Centre (ICRAF), P.O. Box 30677, Nairobi, Kenya;* [2]*Wageningen University and Research centre, Biosystematics Group, P.O. Box 8010, 6700 ED Wageningen, The Netherlands;* [3]*Kenya Forest Seed Centre, Muguga, P.O. Box 20412, Nairobi, Kenya;* *Author for correspondence (e-mail: Ard. Lengkeek@zonnet.nl; phone: +31-317-483170; fax: +31-317-484917)*

Abstract

It is possible that current tree domestication practices undertaken by farmers reduce the genetic base of tree resources on farms, raising concerns regarding the productivity, sustainability and conservation value of agroforestry ecosystems. Here, we assessed possible changes in genetic variation during domestication in the important and heavily utilised timber species, *Vitex fischeri* Gürke (syn. *Vitex keniensis*), by comparing geographically proximate forest and farm material in central Kenya. Employing RAPD analysis, a total of 104 polymorphic markers revealed by five arbitrary primers were scored in a total of 65 individuals, 32 from forest and 33 from farmland. Despite concerns of possible genetic erosion, forest and farm stands did not differ significantly in levels of genetic variation, with H values of 0.278 and 0.269, respectively. However, Mantel tests did reveal greater geographically related associative genetic structure among individuals in farm rather than forest material, with r_M values of 0.217 and 0.114, respectively. A more detailed analysis of structure suggested this could be due to local variation in origin of some on-farm trees. Implications of data for the genetic management of *V. fischeri* stands during farmer-led tree domestication activities are discussed. At present, there appears little reason to reject on-farm *V. fischeri* as a source of germplasm for future on-farm planting or for conservation purposes, although this situation may change and will require monitoring.

Introduction

Farmers in the tropics plant trees in pursuit of their livelihood goals of income generation, household food security and risk management, endeavouring to make optimum use of available land, labour and capital (Arnold and Dewees 1995). In the tropics, Simons et al. (2000) cite examples where the numbers of trees planted in farmer's fields now approaches or exceeds those established in formal plantations. Simons et al. (2000) predict a situation where human populations in many areas increase to the extent that many natural sources of important tree products are exhausted, followed by a lag phase before farmers compensate by increased cultivation of trees for these products on-farm. In order to exit this lag phase, suitable existing on-farm sources of germplasm are essential to effect extended planting programmes. In some tropical areas where farmers

have an active tree planting culture, farmer cultivation already provides important reservoirs of tree biodiversity (Kindt 2002; Lengkeek et al. 2005a). Bringing more inter- and intra-specific diversity into efficient usage on-farm is a survival mechanism used by farmers (Tapia and De la Torre 1998; SGRP 2000) and an important approach for conducting tree domestication activities (Kindt and Lengkeek 1999; Weber et al. 2001; Lengkeek et al. 2005a).

At an intraspecific level, the proportion and structure of variation maintained on-farm in tree species during the development of tropical agroforestry systems is largely uncharacterised. Such knowledge is however of key importance for supporting or developing appropriate utilisation and conservation strategies. The long-term viability of individual tree species in farm landscapes depends upon a wide genetic base providing the capacity to adapt to environmental fluctuations or changing farmer requirements, such as a change in species use or planting niche. Moreover, since most tree species are out-breeding, a wide genetic base provides the ability to withstand potential inbreeding depression through future generations of farmer propagation (Simons et al. 1994; Simons 1996; Brodie et al. 1997; Boshier 2000). A number of authors have however indicated that farmers and nursery managers often collect germplasm from a relatively narrow range of maternal parents during propagation (Kindt 1997; Weber et al. 1997; Holding and Omondi 1998; Lengkeek 2003; Lengkeek et al. 2005b), suggesting that on-farm stands may be at risk from genetic erosion.

In this study, we use *Vitex fischeri* Gürke (syn. *Vitex keniensis*, see Ahenda 1999; Lengkeek 2003) as a model species to test for concerns regarding genetic erosion during the development of tropical agroforestry systems. This long-living tetraploid species is mainly bee pollinated, with seed dispersed by hornbills and possibly other animals (Ahenda 1999). Our aim was to provide information to help guide genetic management strategies that benefit farmers during farmer-led tree domestication activities on *V. fischeri* and to provide indicators for the management of other species. *Vitex fischeri*, known locally as Meru oak or Muuru, is an important commercial species in the central highlands of Kenya. Recent surveys ranked the species as the first or second most important indigenous timber around Mount Kenya and

Meru, with demand exceeding the capacity of natural stands and commercial plantations (Betser et al. 2000; MOA 2000; Lengkeek 2003). The species is extensively cut illegally from forest, and with the natural distribution of the species also shrinking due to general habitat destruction, it is listed as locally vulnerable (under its synonym *Vitex keniensis*) by CITES (Ahenda 1999; KWS 1999). As a result of demand, some saw-millers in parts of central Kenya have shifted sourcing of timber to on-farm stands (Holding and Carsan 2001). In such areas, farmers have become increasingly interested in planting and on-farm management of the species for purposes of commercial sale. Little, however, is known about the influence of these activities on genetic variation within the species, and whether on-farm stands are suitable sources of material for further planting activities.

Our specific objective in the current study was to assess possible losses in genetic variation in farm stands of *V. fischeri* by a comparison with proximate forest material, using the technique of random amplified polymorphic DNA (RAPD) analysis (Williams et al. 1990). RAPDs have been employed widely to assess genetic variation in a range of African tree species (Lowe et al. 2000; Agufa 2002; Bouvet et al. 2004; Jamnadass et al. 2005). The technique provides a large number of predominantly nuclear-encoded markers and can be applied to previously unstudied taxa since DNA sequence information is not required (Williams et al. 1990). Although applied widely, RAPD analysis suffers from a number of limitations, primarily associated with dominance and the potential non-homology of apparently similar character states (Black 1993). The impact of these limitations can however be reduced by scoring an appropriate number of RAPD fragments and using appropriate techniques for analysis (Lynch and Milligan 1994; Jenczewski et al. 1999).

Materials and methods

Sampling of material, DNA isolation and RAPD analysis

Our primary concern during sampling was to allow valid comparison between forest and farm categories of *V. fischeri*. To minimise the effect of

geographical variables on this comparison, an area in central Kenya where forest and farm material are distinct but in close proximity was chosen for analysis. The area chosen covers the eastern foothills of Mount Kenya and the southern slopes of the Nyambene Hills. Detailed sample locations for each tree, recorded using a GPS receiver, are shown in Figure 1. Due to a number of practical limitations on collection determined by the detailed distribution of forested and cultivated land in the area, the overall dimensions of the sampled area were somewhat greater for forest than farm material, though efforts were made to minimise this difference. Practical considerations related to
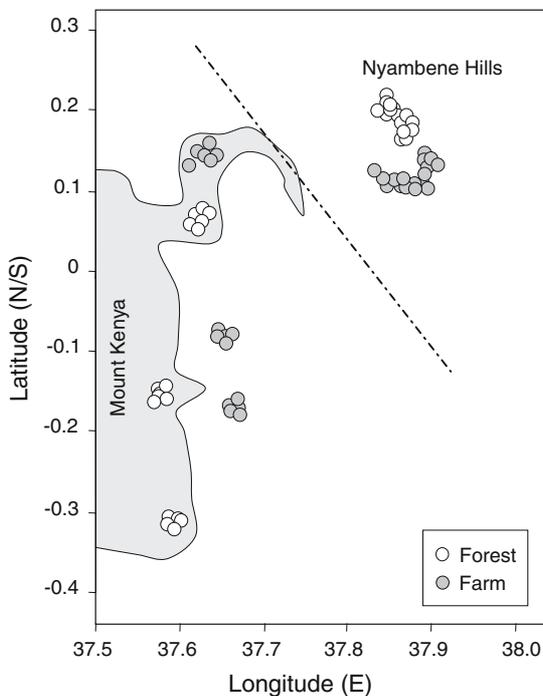

Figure 1. Geographic coordinates of 65 individuals of *V. fischeri* sampled from natural forest and farmland in central Kenya. The dotted line indicates a split into 'Mount Kenya' and 'Nyambene Hills' sub-locations for a detailed analysis of geographically related genetic structure. This division was positioned so that approximately equal numbers of individuals in forest and farm categories lie in each sub-location. The approximate eastern boundary of the Forest Reserves that surround Mount Kenya is also shown. Although forest within Reserves has protected status, this status is not always enforced and this explains the presence of some farms within the Reserve area. Forest material at the 'Nyambene Hills' sub-location is not within a formal Reserve.

access routes also resulted in rather clumped distributions of sampled individuals, though this pattern was similar for forest and farm categories. For both categories of material, a range of tree diameters was sampled, with mean extrapolated age estimates somewhat greater for forest than farm material. For both categories, however, the majority of trees sampled were expected to be sexually mature.

In the case of natural forest, sampled trees were chosen at random and separated by a minimum distance of 100 m. The strategy for sampling farm trees involved the collection of single individuals from a series of separate small farms and did not have a minimum distance requirement. Most of these farms had been involved in earlier participatory research trials (Lengkeek and Carsan 2004) and had been subject to on-farm tree inventories by van Oijen (2002), which revealed a relatively high density of *V. fischeri* in the area (597 individuals on the 35 farms surveyed, average farm area 1.7 ha). Trees on a few additional farms were identified for sampling during the collection process. According to van Oijen (2002), the origin of *V. fischeri* on farms in the area represents a variety of sources, including local tree nurseries (~25%), transplanted wild seedlings from local forest, natural regenerants and (rarely) forest remnants; it was not possible in the present survey to specifically determine which of these sources individual trees sampled from farms belonged to. For forest and farm categories, leaf samples were collected from individual trees during survey work in 2000 or 2001 and silica gel employed to dry and preserve material. In total, leaf material from 65 individuals was assessed, 32 from forest and 33 from farmland.

Total genomic DNA preparations were undertaken using a modification of the CTAB method of Doyle and Doyle (1987) and RAPD analysis carried out according to Dawson et al. (1995). Polymorphisms were scored as presence (1) or absence (0) after resolution on 2% agarose gels and sized against *lambda* DNA cut with *Eco*RI and *Hin*dIII. Initially, 18 arbitrary primers were screened on a test panel of eight individuals. Five primers that revealed clear polymorphisms were chosen for analysis of all 65 trees (primer sequences are available upon request from Ard Lengkeek).

Data analysis

Although *V. fischeri* is believed to have a mixed mating system, it is thought to be predominantly outcrossing in natural stands (Ahenda 1999), and we therefore assumed Hardy-Weinberg equilibrium during data analysis. The genetic diversity (H) of forest and farm categories was calculated according to Nei's (1978) unbiased measure, using allele frequency data estimated assuming the species acts as a true autotetraploid (Ahenda 1999). To assess possible geographically related genetic structuring in forest and farm categories, the standardised Mantel Statistic (r_M; Mantel 1967; Legendre and Legendre 1998) was employed. Analysis was undertaken by comparing a Euclidean distance matrix of RAPD phenotypes with a geographic distance matrix of individuals based on latitude/longitude coordinates, employing the PC-ORD software package (McCune and Mefford 1999). A positive r_M value indicates that geographically proximate individuals are on average more similar genetically than distant individuals, while a negative value indicates the opposite. PC-ORD was used to assign significance to r_M values based on a randomisation (Monte Carlo) test (5,000 permutations). To further assess possible genetic sub-structuring within forest and farm categories, a principal coordinate analysis (PCoA; Legendre and Legendre 1998) of individual RAPD phenotypes based on Sorensen's (1948) similarity coefficient was undertaken with the PAST 0.82 software package (Hammer et al. 2002).

Results

The five arbitrary primers employed in this study revealed a total of 104 clear polymorphisms that could be scored for all 65 individuals surveyed, with a mean product presence frequency across all RAPD markers of 0.279. Estimates of Nei's unbiased genetic diversity were 0.278 ($N = 32$) and 0.269 ($N = 33$) for forest and farm categories, respectively. Mantel tests revealed a greater degree of geographically based associative genetic structure among individuals from farm than forest (r_M values of 0.217 and 0.114, respectively), with association significant in both cases (p values of 0.001 and 0.022, respectively). A more detailed analysis of geographically related genetic

Table 1. Values of the standardised Mantel statistic (r_M; Legendre and Legendre 1998) for forest and farm categories of *V. fischeri* in central Kenya, based on 104 RAPD markers. Significance (p) values based on a Monte Carlo randomisation test (5,000 permutations) are also shown. N denotes the number of individuals tested. For purposes of a more detailed analysis, r_M values were also calculated by sub-locations. Nei's (1978) diversity values (H) for forest and farm categories (overall) are also shown. For further information see Materials and methods and Figure 1.

Designation	N	r_M (p)	H
Forest	32	0.114 (0.022)	0.278
Farm	33	0.217 (0.001)	0.269
Structuring by sub-location			
'Nyambene Hills' forest	15	0.102 (0.165)	–
'Nyambene Hills' farm	17	−0.060 (0.275)	–
'Mount Kenya' forest	17	0.228 (0.009)	–
'Mount Kenya' farm	16	0.179 (0.030)	–

structuring was undertaken by splitting forest and farm material into 'Mount Kenya' and 'Nyambene Hills' sub-locations (Figure 1). In this case, r_M values were higher for forest than farm material for each sub-location, though only in the case of 'Mount Kenya' were values significant (Table 1), probably due to the greater distances covered at this sub-location during sampling (Figure 1). Taken with the higher r_M value for the farm category as a whole, these values suggest that a degree of genetic differentiation may exist between farm individuals from sub-locations. PCoA supports this, with a degree of differentiation observed for farm material by sub-location, especially along the second principal axis (Figure 2). However, the differentiation revealed in PCoA for the first two principal axes was a relatively small 13% of total variation, indicating that overall differentiation in the current study was low, as might be expected for trees sampled across a relatively small geographic area.

Discussion

A number of authors have indicated that farmers and nursery managers in the tropics often collect germplasm from a relatively narrow range of maternal parents during propagation in agroforestry systems (Kindt 1997; Weber et al. 1997; Holding and Omondi 1998; Lengkeek 2003; Lengkeek et al. 2005b), raising concerns regarding
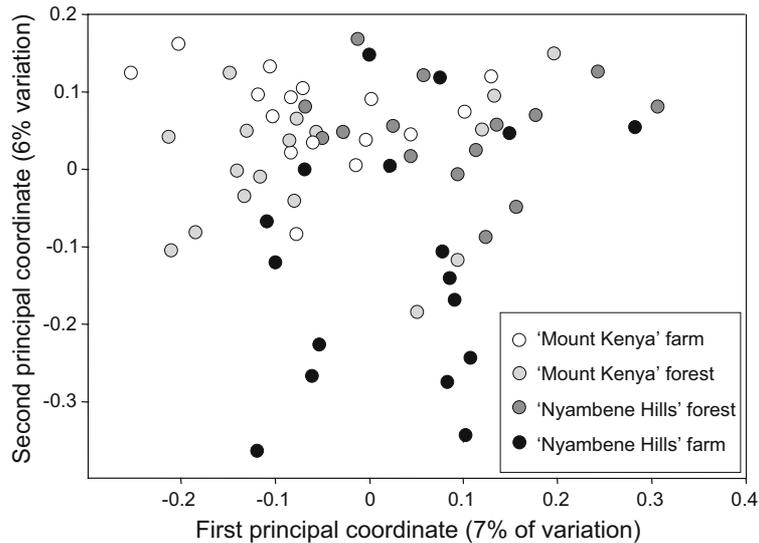
Figure 2. Principal coordinate analysis based on Sorensen's (1948) similarity coefficient for 65 individuals of *V. fischeri* sampled from central Kenya employing 104 RAPD markers revealed by five arbitrary primers. Some differentiation between farm individuals from 'Mount Kenya' and 'Nyambene Hills' sub-locations is observed (see Figure 1 and Results for further information).

the vigour, sustainability and conservation value of on-farm tree stands. In a survey undertaken on a wide range of tree species found in tree nurseries in the Meru region of central Kenya, Lengkeek (2003) indicated that on average germplasm collected from six trees of a given species was used to raise nursery lots. Furthermore, Lengkeek (2003) showed that after initial farm introductions most germplasm for subsequent planting rounds was harvested from trees on the same farm or, less frequently, from neighbouring farms, likely further exacerbating genetic bottlenecks. In the study presented here, we used the important and heavily exploited timber tree *V. fischeri* as a model to test for concerns of genetic erosion in central Kenya by employing RAPD analysis to assess the genetic composition of geographically proximate forest and farm material. Our study showed that, despite the above concerns, genetic variation of forest and farm categories of *V. fischeri* did not differ markedly, suggesting that to date local farmer-led domestication activities have had little effect on diversity levels in the species. The minor differentiation found within farm material, however, is consistent with possible variation in the local origin of some farm trees.

In addition, the current study was concerned with genetic markers that are expected to be neutral in relation to tree phenotype. No indication was found that sampled farmers have undertaken phenotypic selection on *V. fischeri*. This corresponded with research on nursery managers practises in east Africa, including Meru nursery managers raising *V. fischeri*, showing that selection in timber trees is extremely rare (Lengkeek 2003). Nevertheless further work on adaptive traits due to on farm planting could be considered.

Genetic diversity studies in the tropics on matched stands of forest and farm trees are very rare. Placing our work in a proper context is therefore difficult. A number of authors have examined both natural stands of tropical trees and cultivated material sampled either within (e.g. Muchugi-Mwangi 2001), or outside (e.g. Chamberlain 1998; Muluvi et al. 1999), the native range of the species in question. However, the absence of geographically comparative sampling makes these studies of limited utility for understanding human impacts on genetic variation. More recently, however, Hollingsworth et al. (2005) and Kelly et al. (2004) have studied geographically matched natural and agroforestry ecosystems using *Inga edulis* and *Vitellaria paradoxa*, respectively, as model trees. In the case of *I. edulis*, Hollingsworth et al. (2005) examined matched natural and planted stands at five locations in the Peruvian Amazon, finding limited but significant bottlenecks in on-farm material. In the

case of *V. paradoxa*, Kelly et al. (2004) compared matched forest and field/fallow tree stands subjected to different human management intensities at a single location in southern Mali, finding little difference in diversity between stand categories but some difference in geographically related genetic structuring. Our study therefore corresponds most closely with that on *V. paradoxa*. The difference between both African studies and that on *I. edulis* may be explained by the different origins of agroforestry material and the intensity of human management in each case. Whereas all on-farm *I. edulis* material was of planted origin, in the case of *V. fischeri* trees present within agroforestry systems in central Kenya may be planted or naturally regenerated, while in the case of *V. paradoxa* most field/fallow material could represent natural regenerants. In addition, whereas *Inga edulis* has been cultivated as a fruit tree in Peru over millennia and therefore subject to extensive human management, forest clearance in central Kenya is a more recent activity, with active interest in *V. fischeri* management on small-scale farms correspondingly recent. In the case of *V. paradoxa*, extensive management in field/fallow sites also appears to be relatively recent compared to *I. edulis* (Bouvet et al. 2004).

In addition to the above, the maintenance of genetic variation in on-farm material of *V. fischeri* in central Kenya may reflect a number of other factors that could delay diversity loss. First, the relatively high density of *V. fischeri* trees on-farm in central Kenya (van Oijen 2002) means that effective population sizes of farm stands are expected to be high compared to other tree species. This may delay a decline in diversity levels by promoting outcrossing (Lengkeek 2003). Second, effective population sizes are expected theoretically to be relatively high for *V. fischeri* because it is an autotetraploid, in contrast to most other (diploid) tree species. Third, although the juvenile phase of *V. fischeri* is relatively short (approximately eight years) compared to potential longevity (several hundred years), this length of juvenile phase provides multiple opportunities for on-farm germplasm introduction to take place before original immigrants can set seed. A key factor in avoiding genetic bottlenecks in tree species is delayed reproduction, as this allows a large increase in the number of initial founders of a given population before subsequent colonisation begins (Austerlitz et al. 2000). Fourth, although none of the farm trees

sampled in the current survey were thought to be remnants from natural forest, a small number of remnants are observed in proximity to farm sample sites (Ard Lengkeek, unpublished observations). Although these remnants may contribute only a small number of seed or wildings to on-farm stands, they may also contribute pollen to on-farm trees and therefore delay diversity loss. In some areas, pollen flow from forest to farm trees may also be possible.

Conclusion

Despite concerns of genetic erosion our observations on *V. fischeri* suggest the utility of on-farm stands as sources of germplasm for future farmer planting exercises and for conservation purposes. In the future, therefore, emphasis for sourcing *V. fischeri* for on-farm planting may be placed on farmers' own collection activities rather than developing external seed sources, a situation that provides for greater self-sufficiency. In addition, on-farm stands appear to be suitable candidates for providing germplasm for replanting/enrichment programmes in natural forest, a situation that is also relevant for a number of other tree species that are heavily exploited in natural forest in central Kenya (Ian Dawson, personal observations). Our results must, however, be interpreted with caution when extrapolating to other tree species, since specific factors may have contributed to maintaining on-farm genetic variation in *V. fischeri*. Our data may be considered as baseline information for further studies on *V. fischeri* and other species. In the future, natural stands of *V. fischeri* in central Kenya are likely to continue to contract and further changes in germplasm sourcing for on-farm planting may be expected. Future monitoring of on-farm and forest stands of *V. fischeri* will thus be important.

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