African Journal of Biotechnology Vol. 7 (2), pp. 139-146, 18 January, 2008
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB08.718
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Estimation of out-crossing rate in a natural breeding population of *Warburgia ugandensis* using AFLP marker

A. Muchugi[1]*, G. M. Muluvi[1], A. J. Simons[2], F. N. Wachira[3] and R. H. Jamnadass[2]

[1]Department of Biochemistry and Biotechnology, Kenyatta University, PO. Box 43844 00100 Nairobi, Kenya.
[2]World Agroforestry Centre (ICRAF), P.O. Box 30677 00100 Nairobi, Kenya.
[3]Department of Biochemistry and Molecular Biology, Egerton University, PO Box 536 Egerton, Kenya.

Accepted 29 November, 2007

Warburgia ugandensis **Sprague (Canellaceae) occurs in East and Central Africa, and the species is of great medicinal importance to the local communities where it occurs. As the wild populations diminish, planted stands will in future be used as the source of medicinal products as well as germplasm. This study investigated the levels of out-crossing rates to provide knowledge for proper planning in future cultivation programmes. The mating parameters estimated using the mixed mating model (software MLTR) showed the species to be predominantly out-crossing (89%) with significant levels of selfing. The multi-locus population out-crossing rate was higher than the single-locus population out-crossing rate (t_m- t_s= 0.023; SE = 0.010), implying that there was less likelihood of mating between relatives (bi-parental inbreeding). Low values were also obtained for the correlation of paternity, $r_{p(s)}$ = 0.028 (SE = 0.040) and correlation of selfing among family, r_s = 0.016 (SE = 0.015). For most loci, allele frequencies of pollen and ovule contributions to the progeny genotypes were significantly different (P <0.05). These results indicate that with proper sampling, the populations being established for conservation, breeding and planting purposes will be able to sustain high genetic diversity found in the wild populations.**

Key words: AFLP, mating system, *warburgia ugandensis.*

INTRODUCTION

The World Health Organisation estimates that about 80% of the local communities in sub-Saharan Africa rely on traditional medicines while global trend in the use of herbal medicine has also been on rise (Lange, 2002). The tropical tree, *Warburgia ugandensis* Sprague is one of such highly valued species within the traditional health systems of the communities where it naturally occurs (Beentje, 1994; Kokwaro, 1976). The curative efficacy of the species extracts is ascribed to its antibacterial and antifungal medicinal properties (Haraguchi, 1998; Mashimbye et al., 1999; Olila et al., 2001). *W. ugandensis* has a great potential for commercial market development. Already extracts from a species in the same genus, *W. salutaris*, are being processed commercially and marketed at highly competitive prices in South Africa (Botha et al., 2004). The plant material used in herbal remedies is nearly exclusively harvested from the wild and this threatens the species survival. Planting of the species on farms is therefore being encouraged to sustain the herbal production as well as for conservation purposes. To develop sustainable management strategies for conservation and utilization of the species, there is need for detailed understanding of its reproductive biology.

Plant mating patterns vary with reproductive biology and spatial structure of a species and these two factors influence the levels and dynamics of genetic diversity (Loveless, 1992; Hamrick and Murawski, 1990). Consequently, an understanding of the basic processes of tree reproductive biology (sexual systems, incompatibility mechanisms, flowering patterns and pollination processes) and how they combine to produce observed patterns

*Corresponding author. E-mail: a.muchugi@cgiar.org. Tel: +254 733946793. Fax: +254 7224001.

Table 1. Estimates of out-crossing rates in *W. ugandensis* generated by the multi-locus mixed mating program (MLTR) using 80 AFLP markers of 186 progenies arrays.

No. of families	No. of offspring	F	t_m	t_s	t_m-t_s	r_t	r_s	$r_{p(m)}$	$r_{p(s)}$	$r_{p(m)}$-$r_{p(s)}$
13	186	-0.200 (0.000)	0.889 (0.029)	0.866 (0.031)	0.023 (0.014)	0.007 (0.099)	1.200 (0.002)	0.253 (0.030)	0.028 (0.040)	-0.225 (0.028)

Standard error in parentheses was obtained from 100 bootstraps of the data. F is the fixation index; t_m = multi-locus out-crossing rate; t_s = single locus out-crossing rate; t_m-t_s= bi-parental inbreeding; r_p= multi-locus correlation of out-crossed paternity within progeny arrays; r_s= correlation of selfing; r_s=correlation of selfing among loci.

of gene flow and genetic variation is important in effective resource management and maintenance of evolutionary flexibility, and in designing of species based conservation strategies and planting programs (Boshier, 2000). Apart from studying the species sexual systems, various genetic parameters derived from molecular marker studies may be used to provide estimates of the mating system (Ritland, 1983).

Continued forest degradation of the *W. ugandensis* habitat may lead to population fragmentation which will affect the mating system of the remnant populations by promoting reproductive isolation through a reduction in the effective population size and alterations of pollen dispersal patterns (Saunders et al., 1991; Young et al., 1996). Reproductive isolation and mating of closely related individuals would increase frequency of inbreeding (Templeton et al., 1990; Young et al., 1996); hence affect the species genetic diversity. However, domestication of *W. ugandensis* is taking place in many areas neighbouring the remnant populations and there is the likelihood of genetic exchange between the planted populations and the remnant populations. Information on the biological characteristics of the species will therefore provide a sound basis in the conservation strategies planting programs, especially if the established stands will in future be of use in germplasm collection. This study aimed to estimate the predominant type of mating system prevalent in *W. ugandensis* using AFLP markers.

MATERIAL AND METHODS

The study area was located in Marmarnet North Forest of Laikipia District in Kenya, a natural forest under the management of the Forestry Department of the Kenya Government. The forest centre is located at 00^0 07′N, 036^0 25′E at an elevation of 2100 m a.s.l. *W. ugandensis* is one main species in the forest, while the other two major trees are *Cordia africana* and *Podocarpus* spp. Twenty mature trees of *W. ugandensis* were randomly selected within two plots of about 200 m X 200 m in size which were 500 m apart. Mature fruits were then collected from each of the twenty mother trees and kept separately. Seeds were extracted from these fruits, cleaned and then bulked per mother tree. About 50 healthy seeds from each of the mother trees were then raised under glass house conditions in individual batches. After eight months of establishment, 20 leaves were randomly sampled from each maternal plant plot, dried in silica gel and stored at –20°C. Genomic DNA was then extracted from these leaf samples using a CTAB method according to Doyle and Doyle (1987). Genomic DNA was also isolated from maternal trees. Amplified fragment length polymorphism (AFLP, Vos et al., 1995) analysis was carried out according to "Plant Mapping Protocol" using an AFLP Kit Module [Applied Biosystems (ABI), USA]. Selective amplification was conducted using three MseI and EcoRI primer combinations [EcoRI-ACA/MseI-CAA, EcoRI-AGC/MseI-CAT and EcoRI-AGC/MseI-CAG] selected from an earlier studied genetic structure studied within the genus (Muchugi et al., submitted). The capillary system (ABI PRISM 3730^{TM}) was used to resolve selective amplification products. From the ABI PRISM 3730, the sample data were directed to the GeneMapper^{TM} software for tabulation. The results were displayed as electrograms and allele frequency data of product presence /absence.

Data analysis

The data generated by the GeneMapper^{TM} software were analysed using the multi-locus, mixed mating program (MLTR) Version 3.0 (Ritland, 2002), an improved version of the MLT (Ritland, 1990) computer program. The program is based on multi-locus, mixed mating model and estimation procedure of Ritland and Jain (1981), which assumes that progenies are derived from either random mating (out-crossing) or self-fertilisation. From the progeny array data, the program simultaneously estimated the multi-locus out-crossing rates (t_m) by Newton–Raphson (NR) method, the mean single locus out-crossing rate (t_s), the correlation of out-crossed paternity (r_p), correlation of selfing among families (r_s), fixation index of maternal plant (F). The pollen and ovule gene frequencies were simultaneously estimated using the Expectation-Maximization (EM) method. This is an iteration of the NR method that involves calculating a score for each observation then over all observations, dividing the mean score by the mean squared score and adding this quantity on the current parameter value. Maternal genotypes for each family were inferred by a modification of Brown and Allard (1970). A parent was chosen at random in proportion to the probability of parentage. Variances of the above quantities were estimated using the bootstrap method where the progeny arrays were re-sampled within families using 100 bootstraps.

RESULTS

The selected AFLP primer combinations (EcoRI-ACA/MseI-CAA, EcoRI-AGC/MseI-CAT and EcoRI-AGC/MseI-CAG) gave a total of 80 polymorphic products for 186 progenies arrays from thirteen mother trees. Results of the analysis of the data using the multi-locus, mixed mating program (MLTR) Version 3.0 (Ritland, 2002) are shown in Table 1. The single-locus inbreeding

coefficient of maternal parents, F, was -0.200, a negative value, indicating heterozygotes were in excess of homozygotes. The multi-locus population out-crossing rate (t_m) was quite high (0.889; SE = 0.030) indicating that *W. ugandensis* is predominantly out-crossed (88.9%) with a low level of selfing (11.1%). The multi-locus population out-crossing rate was higher than the single-locus population-out-crossing rate (t_m- t_s = 0.023; SE = 0.010), implying that there was less likelihood of mating between relatives (bi-parental inbreeding). This was in agreement with the low values obtained for the correlation of paternity (fraction of siblings that share the same father), $r_{p(s)}$ = 0.028 (SE = 0.040) and correlation of selfing among family, r_s = 0.016 (SE = 0.015). Multi-locus correlated paternity was greater than single locus correlated paternity [$r_{p(m)}$ - $r_{p(s)}$= -0.225 (SE = 0.028)]. For most loci, allele frequencies of pollen and ovule contributions to the progeny genotypes were significantly different (P <0.05) (Table 2).

DISCUSSION

The estimate of out-crossing rates in this study revealed that *W. ugandensis* has a mixed mating system, which is predominantly out-crossing with a possibility of about 11% selfing. At the inflorescence level, the low selfing rates contrast the expectations of species with bisexual flowers where majority of the seeds are thought to result from self- fertilisation (Murawski et al., 1994). *W. ugandensis* has bisexual flowers with the stigma placed at the same height, almost covered by the abundant anthers, which implies that self-fertilisation may be prevalent unless other self-incompatibility systems are active. Since heterozygotes are in excess, the level of selfing expected to be quite varied among populations. This is consistent with the fact that no mating between relatives is inferred from this data (t_s-t_m) hence minimal chance of selfing due to bi-parental inbreeding.

Studies on biological characteristics of some tropical trees species have revealed high levels of out-crossing (Bawa and Ashton, 1991; Murawski, 1995). Assessment of mating systems of tropical tree species utilising different molecular markers have shown high out-crossing rates: *Eucalyptus grandis*, (t_m = 0.84; Eldridge et al., 1993), *Platypodium elegan, (*t_m = 0.92; Hamrick and Murawski, 1990), *Shorea congestiflora* (t_m=0.87; Murawski et al., 1994), among others. While using the AFLP marker, Gaitto et al. (1997) and Muluvi et al. (2004) similarly found high levels of out-crossing rates in Eucalyptus urophylla (t_m = 0.87) and *Moringa oleifera* (t_m = 0.74), respectively. The predominance of out-crossing explains why among other factors, these long-lived tree species retain high genetic variation within populations (Hamrick et al., 1992; Loveless and Hamrick, 1984).

Possible reasons for the significant differences between the gene frequencies of ovules and incoming pollen as explained in Murawski and Hamrick (1992) could be as a result of the immigrant pollen coming from outside the sample population or from un-represented sample of maternal trees. These results are in agreement with the low values obtained for the correlation of paternity (fraction of siblings that share the same father), $r_{p(s)}$ = 0.028 (SE = 0.040) and correlation of selfing among family, r_s = 0.016 (SE = 0.015). Since the r_s value is positive it implies that any two-selfed progenies samples from the same parent are not the result of two independent selfing events. Such correlation may arise when movement of pollinators causes a within flower selfing. This type of correlated selfing is expected to be high among seeds within a single fruit and therefore the need for a completely randomised sampling if progenies. The low r_p value also means that there was a frequent and successful multiple mating as most offsprings are derived from non-related crosses. This maybe ascribed to the pollinator behaviour whereby the pollinator visits mostly distant plants, and synchrony in flowering among plants. Multi-locus correlated paternity was found to be greater than single-locus correlated paternity [$r_{p(m)}$ - $r_{p(s)}$= -0.225 (SE = 0.028)], implying that there was no effect of population sub-structuring on male similarity between out-crosses.

The fixation index, F in the progeny was higher than expected based on the estimate of t_m. Taking t_m = 0.889, the expected fixation index [F= (1- t_m)/ (1+ t_m)] is 0.059 while the estimated F was - 0.200. A lower F value than the expected F suggests less inbreeding than expected in the progeny population involved in the study. Since the mating system in *W. ugandensis* involves some selfing, an excess of heterozygotes as suggested by the negative value of F is expected if the populations are in mating-system equilibrium (Furnier and Adams, 1986). This factor and the high out-crossing rates obtained may explain the high genetic diversity values observed in *W. ugandensis* populations (Muchugi et al. unpublished). For conservation purposes, these findings imply that genetic diversity would be sustained within planted populations if *W. ugandensis* stands were established from properly sampled genetic resources. Therefore, such planted stands can be targets of germplasm collections in future, without compromising the species genetic diversity.

Floral biology is often correlated with the pollination mechanism and affects the breeding system in a species (Waser and Price, 1983). Although important aspects relating to mating systems such as pollination details (pollinators and their time and frequency of visits on the plants) or anthesis were not monitored in our study, some inferences could be made from the floral structures. *W. ugandensis* flowers were found not to have a strong scent, but had dish-shaped flowers with brightly coloured inner petals which might suggest possibility of insect pollination. Warburgia flowers are small though and offer small rewards and would suit small bees (e.g. *Trigon*a) and other insects. In insect pollinated species, there is

Table 2. Ovule and pollen gene frequencies estimates generated by the multi-locus mixed mating program (MLTR) Version 3.0 (Ritland, 2002) from 80 AFLP markers. Standard error in parentheses.

Locus	Allele Designation	Gene frequency	
		Pollen Pool	Ovule pool
Ale1	Dominant	0.441 (0.106)	0.429 (0.099)
Ale1	Recessive	0.559 (0.106)	0.571 (0.099)
Ale2	Dominant	0.042 (0.014)	0.000 (0.000)
Ale2	Recessive	0.958 (0.014)	1.000 (0.000)
Ale3	Dominant	0.199 (0.049)	0.250 (0.018)
Ale3	Recessive	0.801 (0.049)	0.750 (0.018)
Ale4	Dominant	0.196 (0.050)	0.179 (0.029)
Ale4	Recessive	0.804 (0.050)	0.821 (0.029)
Ale5	Dominant	0.260 (0.073)	0.393 (0.044)
Ale5	Recessive	0.740 (0.072)	0.607 (0.044)
Ale6	Dominant	0.118 (0.028)	0.036 (0.013)
Ale6	Recessive	0.882 (0.028)	0.964 (0.013)
Ale7	Dominant	0.414 (0.054)	0.429 (0.004)
Ale7	Recessive	0.586 (0.054)	0.571 (0.003)
Ale8	Dominant	0.733 (0.074)	0.714 (0.029)
Ale8	Recessive	0.267 (0.074)	0.286 (0.029)
Ale9	Dominant	0.183 (0.077)	0.321 (0.052)
Ale9	Recessive	0.817 (0.077)	0.679 (0.052)
Ale10	Dominant	0.081 (0.044)	0.143 (0.032)
Ale11	Recessive	0.987 (0.008)	0.964 (0.023)
Ale10	Recessive	0.919 (0.044)	0.857 (0.032)
Ale11	Dominant	0.013 (0.008)	0.036 (0.023)
Ale12	Dominant	0.111 (0.060)	0.214 (0.041)
Ale12	Recessive	0.889 (0.060)	0.786 (0.041)
Ale13	Dominant	0.145 (0.025)	0.000 (0.000)
Ale13	Recessive	0.855 (0.025)	0.000 (0.000)
Ale14	Dominant	0.733 (0.074)	0.714 (0.029)
Ale14	Recessive	0.267 (0.074)	0.286 (0.029)
Ale15	Dominant	0.121 (0.026)	0.071 (0.005)
Ale15	Recessive	0.879 (0.026)	0.929 (0.005)
Ale16	Dominant	0.701 (0.072)	0.607 (0.027)
Ale16	Recessive	0.299 (0.072)	0.393 (0.027)
Ale17	Dominant	0.374 (0.038)	0.071 (0.021)
Ale17	Recessive	0.626 (0.038)	0.929 (0.021)
Ale18	Dominant	0.299 (0.042)	0.107 (0.020)
Ale18	Recessive	0.701 (0.042)	0.893 (0.020)
Ale19	Dominant	0.590 (0.064)	0.357 (0.035)
Ale19	Recessive	0.410 (0.064)	0.643 (0.035)
Ale20	Recessive	0.964 (0.015)	1.000 (0.000)
Ale20	Recessive	0.964 (0.015)	1.000 (0.000)
Ale21	Dominant	0.131 (0.025)	0.071 (0.000)
Ale21	Recessive	0.869 (0.025)	0.929 (0.000)
Ale22	Dominant	0.573 (0.082)	0.464 (0.055)
Ale22	Recessive	0.427 (0.082)	0.536 (0.055)
Ale23	Dominant	0.748 (0.064)	0.571 (0.021)
Ale23	Recessive	0.252 (0.064)	0.429 (0.021)
Ale24	Dominant	0.648 (0.096)	0.500 (0.145)
Ale24	Recessive	0.352 (0.096)	0.500 (0.145)

Table 2. Contd.

Ale25	Dominant	0.687 (0.055)	0.536 (0.008)
Ale25	Recessive	0.313 (0.055)	0.464 (0.008)
Ale26	Dominant	0.083 (0.035)	0.107 (0.030)
Ale26	Recessive	0.917 (0.035)	0.893 (0.030)
Ale27	Dominant	0.669 (0.065)	0.464 (0.030)
Ale27	Recessive	0.331 (0.065)	0.536 (0.030)
Ale28	Dominant	0.182 (0.075)	0.179 (0.058)
Ale28	Recessive	0.818 (0.075)	0.821 (0.058)
Ale29	Dominant	0.314 (0.058)	0.286 (0.020)
Ale29	Recessive	0.686 (0.058)	0.714 (0.020)
Ale30	Dominant	0.296 (0.052)	0.071 (0.042)
Ale30	Recessive	0.704 (0.052)	0.929 (0.042)
Ale31	Dominant	0.189 (0.036)	0.036 (0.011)
Ale31	Recessive	0.811 (0.036)	0.964 (0.011)
Ale32	Dominant	0.091 (0.021)	0.036 (0.005)
Ale32	Recessive	0.909 (0.021)	0.964 (0.005)
Ale33	Dominant	0.423 (0.036)	0.036 (0.012)
Ale33	Recessive	0.577 (0.036)	0.964 (0.012)
Ale34	Dominant	0.090 (0.030)	0.036 (0.032)
Ale34	Recessive	0.910 (0.030)	0.964 (0.032)
Ale35	Dominant	0.025 (0.020)	0.036 (0.017)
Ale35	Recessive	0.975 (0.020)	0.964 (0.017)
Ale36	Dominant	0.031 (0.018)	0.036 (0.017)
Ale36	Recessive	0.969 (0.018)	0.964 (0.017)
Ale37	Dominant	0.538 (0.048)	0.179 (0.035)
Ale37	Recessive	0.462 (0.048)	0.821 (0.035)
Ale38	Dominant	0.636 (0.036)	0.214 (0.000)
Ale38	Recessive	0.364 (0.036)	0.786 (0.001)
Ale39	Dominant	0.066 (0.016)	0.000 (0.000)
Ale40	Dominant	0.332 (0.054)	0.179 (0.024)
Ale40	Recessive	0.668 (0.054)	0.821 (0.024)
Ale41	Dominant	0.117 (0.037)	0.107 (0.024)
Ale41	Recessive	0.883 (0.037)	0.893 (0.024)
Ale42	Dominant	0.048 (0.016)	0.000 (0.000)
Ale42	Recessive	0.952 (0.016)	1.000 (0.000)
Ale43	Dominant	0.495 (0.045)	0.143 (0.021)
Ale43	Recessive	0.505 (0.045)	0.857 (0.021)
Ale44	Dominant	0.019 (0.014)	0.036 (0.034)
Ale44	Recessive	0.981 (0.014)	0.964 (0.034)
Ale45	Dominant	0.038 (0.022)	0.036 (0.016)
Ale45	Recessive	0.962 (0.022)	0.964 (0.016)
Ale46	Dominant	0.091 (0.026)	0.107 (0.011)
Ale46	Recessive	0.909 (0.026)	0.893 (0.011)
Ale47	Dominant	0.097 (0.018)	0.000 (0.000)
Ale47	Recessive	0.903 (0.018)	1.000 (0.000)
Ale48	Dominant	0.326 (0.039)	0.036 (0.015)
Ale48	Recessive	0.674 (0.039)	0.964 (0.015)
Ale49	Dominant	0.403 (0.040)	0.036 (0.009)
Ale49	Recessive	0.597 (0.040)	0.964 (0.009)
Ale50	Dominant	0.496 (0.038)	0.000 (0.000)
Ale50	Recessive	0.504 (0.038)	1.000 (0.000)
Ale51	Dominant	0.144 (0.025)	0.036 (0.000)

Table 2. Contd.

Ale51	Recessive	0.856 (0.025)	0.964 (0.000)
Ale52	Dominant	0.115 (0.025)	0.000 (0.000)
Ale52	Recessive	0.885 (0.025)	1.000 (0.000)
Ale53	Dominant	0.300 (0.063)	0.107 (0.050)
Ale53	Recessive	0.700 (0.063)	0.893 (0.050)
Ale54	Dominant	0.193 (0.026)	0.000 (0.000)
Ale54	Recessive	0.807 (0.026)	1.000 (0.000)
Ale55	Dominant	0.307 (0.038)	0.107 (0.016)
Ale55	Recessive	0.693 (0.038)	0.893 (0.016)
Ale56	Dominant	0.651 (0.040)	0.036 (0.014)
Ale56	Recessive	0.349 (0.040)	0.964 (0.014)
Ale57	Dominant	0.371 (0.037)	0.036 (0.004)
Ale57	Recessive	0.629 (0.037)	0.964 (0.003)
Ale58	Dominant	0.409 (0.095)	0.214 (0.106)
Ale58	Recessive	0.591 (0.095)	0.786 (0.106)
Ale59	Dominant	0.568 (0.049)	0.071 (0.031)
Ale59	Recessive	0.432 (0.049)	0.929 (0.031
Ale60	Dominant	0.681 (0.069)	0.286 (0.093)
Ale60	Recessive	0.319 (0.069)	0.714 (0.093)
Ale61	Dominant	0.666 (0.071)	0.214 (0.096)
Ale61	Recessive	0.334 (0.071)	0.786 (0.096)
Ale62	Dominant	0.274 (0.042)	0.107 (0.021)
Ale62	Recessive	0.726 (0.042)	0.893 (0.021)
Ale63	Dominant	0.053 (0.032)	0.071 (0.028)
Ale63	Recessive	0.947 (0.032)	0.929 (0.028)
Ale64	Dominant	0.070 (0.024)	0.036 (0.023)
Ale64	Recessive	0.930 (0.024)	0.964 (0.023)
Ale65	Dominant	0.568 (0.048)	0.071 (0.031)
Ale65	Recessive	0.432 (0.048)	0.929 (0.031)
Ale66	Dominant	0.127 (0.072)	0.143 (0.073)
Ale66	Recessive	0.873 (0.072)	0.857 (0.073)
Ale67	Dominant	0.107 (0.027)	0.107 (0.012)
Ale67	Recessive	0.893 (0.027)	0.893 (0.012)
Ale68	Dominant	0.196 (0.034)	0.179 (0.005)
Ale68	Recessive	0.804 (0.034)	0.821 (0.005)
Ale69	Dominant	0.025 (0.013)	0.036 (0.015)
Ale69	Recessive	0.975 (0.013)	0.964 (0.015)
Ale70	Dominant	0.145 (0.026)	0.000 (0.000)
Ale70	Recessive	0.855 (0.026)	1.000 (0.000)
Ale71	Dominant	0.128 (0.026)	0.143 (0.000)
Ale71	Recessive	0.872 (0.026)	0.857 (0.001)
Ale72	Dominant	0.310 (0.036)	0.036 (0.009)
Ale72	Recessive	0.690 (0.036)	0.964 (0.009)
Ale73	Dominant	0.412 (0.049)	0.071 (0.028)
Ale73	Recessive	0.588 (0.049)	0.929 (0.028)
Ale74	Dominant	0.073 (0.019)	0.000 (0.000)
Ale74	Recessive	0.927 (0.019)	1.000 (0.000)
Ale75	Dominant	0.617 (0.062)	0.214 (0.071)
Ale75	Recessive	0.383 (0.062)	0.786 (0.071)
Ale76	Dominant	0.668 (0.076)	0.429 (0.079)
Ale76	Recessive	0.332 (0.076)	0.571 (0.079)
Ale77	Dominant	0.163 (0.073)	0.179 (0.064)

Table 2. Contd.

Ale77	Recessive	0.837 (0.073)	0.821 (0.064)
Ale78	Dominant	0.019 (0.012)	0.036 (0.021)
Ale78	Recessive	0.981 (0.012)	0.964 (0.021)
Ale79	Recessive	0.916 (0.030)	0.929 (0.011)
Ale80	Dominant	0.051 (0.020)	0.036 (0.005)
Ale80	Recessive	0.949 (0.020)	0.964 (0.005

limited pollen movement and therefore plants tend to have reduced amount of variability within populations but increased differentiation among populations (Loveless and Hamrick, 1984). However, populations of *W. ugandensis* were found to have high genetic variation within populations (66%) (Muchugi et al., unpublished) contradicting the inferred insect pollination. However for species with bisexual flowers, and no selfing occurs, high genetic variation is retained within populations (Hamrick et al., 1992) which may explain the values observed in *W. ugandensis* populations. As wild animals (monkeys and elephants) eat the *W. ugandensis* fruits and disperse the seeds, this may also have contributed to the high genetic diversity observed in the populations studied as species whose seeds are ingested by animals have high genetic diversity values (Hamrick et al., 1992). The small movement of pollen and the wider movement of seeds may also explain the pollen ovule gene frequency differences.

The efficacy of the AFLP marker to resolve questions on the types of mating system in a species is well addressed in Gaitto et al. (1997). Scoring a large number of polymorphic markers in the progeny array overcomes the problem of the lower information obtained per loci which is associated with the dominant nature of AFLP markers when compared to co-dominant markers. The automated sequencers utilising the fluorescent-labelled primers in capillary system add to the robustness of the AFLP technique by allowing analysis of large number of individuals within a short time. Similarly, the technique allows numerous polymorphisms to be detected accurately which would rather have been difficult in manual scoring of polyacrylamide gels.

ACKNOWLEDGEMENTS

This work formed part of Alice Muchugi's PhD thesis at Kenyatta University, Nairobi. We are grateful to Kenya Forest Department officers at Marmarnet Forest, Laikipia, for their assistance in fruit/flowering data collection. Funding was provided by International Foundation for Agricultural Research (IFAR), International Foundation for Science (IFS), European Commission and the core financial support from USAID to the World Agroforestry Centre (ICRAF).

REFERENCES

Bawa KS, Ashton PS (1991). Conservation of rare trees in tropical rain forests: a genetic perspective. In: Falk DA and Holsisinger KE (eds.) Genetics and Conservation of Rare Plants Oxford University Press, New York, pp. 62-71.

Beentje HJ (1994). Kenya trees, shrubs and lianas. National Museums of Kenya, Nairobi, Kenya.

Boshier DH (2000). Mating systems. In: Young A, Boshier D and Boyle T (eds.). Forest Conservation Genetics: principles and practice, CABI Publishing, Wallingford, Oxon OX10 8DE, United Kingdom, pp. 63-80.

Botha J, Witkowskie ETF, Shackleton CM (2004). The impact of commercial harvesting on *Warburgia salutaris* ('pepper-bark tree') in Mpumalanga, South Africa. Biodivers. Conserv. 13: 1675-1698.

Brown ADH, Allard RW (1970) Estimation of the mating system in open pollinated maize populations using isozyme polymorphisms. Genetics, 66: 133-145.

Doyle JJ, Doyle JL (1987) CTAB DNA extraction in plants. Phytochem. Bull., 19: 11-15.

Eldridge KG, Davidson J, Harwood CE, van Wyk G (1993). *Eucalyptus domestication and breeding*. Clarendon Press, Oxford.

Furnier GR, Adams WT (1986). Mating systems in natural populations of Jeffrey pine. Am. J. Bot., 3: 1002-1008.

Gaitto FA, Bramucci M, Grattapaglia D (1997) Estimation of outcrossing rate in a breeding population of *Eucalyptus urophylla* with dominant RAPD and AFLP markers. Theor. Appl. Genet. 95: 842-849.

Hamrick JL, Godt MW, Sherman-Broyles SL (1992) Factors influencing genetic diversity in woody plants species. New Forests, 6: 95-124.

Hamrick JL, Murawski DA (1990). The breeding structure of tropical tree populations. Plant Species Biol., 5: 157-165.

Haraguchi H (1998). Mode of action of antimicrobial substances in plants. Recent Research Developments in Agricultural and Food Chemistry. 2: 259-268.

Kokwaro JO (1976). Medicinal plants of East Africa. East African Literature Bureau. Nairobi, Kenya.

Lange D (2002). The role of east and southeast Europe in the medicinal and aromatic plants' trade. Med. Plants Conserv. 8: 14-18.

Loveless MD (1992). Isozyme variation in tropical trees: Patterns of genetic organisation. New Forests, 6: 67-94.

Loveless MD, Hamrick JL (1984). Ecological determinants of genetic structure in plant populations. Annu. Rev. Plant Ecol. Syst. 15: 65-95.

Mashimbye MJ, Maumela MC, Drewes SE (1999). A drimane sesquiterpinoid lactone from *Warburgia salutaris*. Phytochemistry, 51: 435-438.

Muluvi GM, Sprent JI, Odee D, Powell W (2004) Estimates of outcrossing rates in *Moringa oleifera* using amplified fragment length polymorphism (AFLP). Afr. J. Biotechnol. 3(2): 146-151.

Murawski DA (1995). Reproductive biology and genetics of tropical trees from a canopy perspective. In: Lowman MD, Nadkarni NM(eds.) Forest Canopies. Academic Press, San Diego, pp. 457-493.

Murawski DA, Dayanandan B, Bawa KS (1994). Outcrossing rates of two endemic Shorea species from Sri Lankan tropical rain forests. Biotropica, 26: 23-29.

Murawski DA, Hamrick JL (1992). Mating system of *Cavanillesis platanifolia* under extremes of flowering tree density: A test of predic-

tions. Biotropica, 24: 99-101.
Olila D, Olwa-Odyek B, Opuda-Asibo J (2001). Antibacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis*, gandan medicinal plants. Afr. Health Sci., 1(2): 66-72.
Ritland K (1983). Estimation of mating systems. In: Tanksley SD, Orton TJ (eds) Isozymes in Plant Genetics and Breeding. Part A. Elsevier Sciences Publishers bv, Amsterdam, Netherlands, pp. 289-302.
Ritland K (1990). A series of FORTRAN computer programs for estimating plant mating systems. Heredity 81: 235-237.
Ritland K (2002). Extensions of models for the estimation of mating systems using n independent loci. Heredity, 88: 221-228.
Saunders DA, Hobbs RJ, Margules CR (1991). Biological consequences of ecosystem fragmentation: A review. Conserv. Biol. 5: 18-35.
Ritland K, Jain S (1981) A model for the estimation of outcrossing rate and gene frequencies using *n* independent loci. Heredity, 47: 35-52.
Templeton AR, Shaw K, Routman E, Davis SK (1990). The genetic consequences of habitat fragmentation. Ann. Missouri Bot. Garden 77: 13-27.
Waser NM, Price MV (1983). Optimal and actual outcrossing in plants, and the nature of plant-pollinator interaction. In: Jones CE and Little RJ (eds) Handbook of Experimental Pollination Biology, Van Nostrand-Reinhold. New York, USA, pp. 341-359.
Young A, Boyle T, Brown T (1996). The population genetic consequences of habitat fragmentation for plants. Trends Ecol. Evol. 11: 413-418.