THE MATING SYSTEM OF *SESBANIA SESBAN* (L.) MERR. (LEGUMINOSAE)

Girma Gebremariam [1], Sileshi Nemomissa [2], Abebe Demissie [3] and Jean Hanson [1]

[1] International Livestock Research Institute (ILRI), PO Box 5689
Addis Ababa, Ethiopia, E-mail: girmamariam@hotmail.com
[2] Department of Biology, Faculty of Science, Addis Ababa University
PO Box 3434, Addis Ababa, Ethiopia
[3] Biodiversity Conservation and Research Institute, PO Box 30726
Addis Ababa, Ethiopia

ABSTRACT: The mating system of *Sesbania sesban* was studied using one natural population and two cultivated populations. A large number of progeny (600) was assayed from each population for one polymorphic locus of Malate dehydrogenase (Mdh). The level of out-crossing in the studied populations ranged from 40–100%. The genotype frequencies were in agreement with those of the Hardy-Weinberg expected genotype proportions as in randomly mating populations at P < 0.05. The floral structure and the pollen shedding behaviour of the flowers of *S. sesban* could favour cross-pollination and/or self-pollination depending on the circumstances. Self-pollination usually occurs late in the flowering period when pollinators fail to visit the flowers. Such a "delayed selfing" facilitates out-crossing in the presence of appropriate pollinators under natural conditions, but confers a selective advantage where pollinators are limiting. The results indicated that *S. sesban* could be self-fertilized in the absence of pollinators or could out-cross with other heterozygous individuals in the neighboring population when suitable pollinators are available. *S. sesban* exhibits "facultative xenogamy" or "delayed selfing" and is a mixed mating species.

Key words/phrases: Allozymes, delayed selfing, Hardy-Weinberg equilibrium, malate dehydrogenase, mixed mating system

INTRODUCTION

Sesbania sesban has been used in alley farming as a source of green manure and as feed supplement to poor quality diets. It also serves as a source of pulp fibres, fuelwood and wood for construction. Leaves, roots, bark and seeds have been utilized for medicinal purposes for both human and livestock (Woodward, 1988; Evan and Macklin, 1990; Azene Bekele *et al.*, 1993; Weigand *et al.*, 1995). Despite its wide adaptation and multiple uses, little research has been conducted on the mating behaviour of this species.

The mating system plays an important role in determining the genetic structure of populations (Brown and Allard, 1970; Hamrick, 1989) and detailed knowledge on the mating systems of plant populations is essential to understand their genetic composition and evolutionary potential (Smyth and Hamrick, 1984). Such systems are expressed in plant species in a great variety of ways. Their genetic impact is measurable by different approaches, *i.e.*, the segregation of markers both at protein level (*e.g.*, allozymes/ isoenzymes) and molecular level in progeny arrays. Isoenzyme and RAPD (Random Amplified Polymorphic DNA) markers have been successfully used to study the mating systems of plants and understand their diversity (Brown *et al.*, 1985; Brown, 1990; Gjuric and Smith, 1996; Gabrielsen *et al.*, 1997; Gabrielsen and Brochmann, 1998). One to many polymorphic loci have been variously used as markers to estimate out-crossing rates in different species (Philips and Brown, 1977; Ellstrand *et al.*, 1978; Moran *et al.*, 1980; Elstrand and Foster, 1983; Smyth and Hamrick, 1984; O'Malley and Bawa, 1987; Wagner and Allard, 1991; Knapp and Teuber, 1993; Boshier *et al.*, 1995). Shaw and Brown (1982) suggested that it is more efficient to score more plants on the most polymorphic locus than fewer plants on more loci; except in a highly out-crossing population. When there is no limit in attaining more polymorphic loci, three or four loci will give more accurate estimates and more often this will provide the minimum possible variance (Ritland and Jain, 1981).

The objectives of the research are to provide information on plant mating system studies and to estimate the out-crossing level of *S. sesban* using genetic markers. This study used allozymes to assess polymorphism and estimate mating system parameters in *S. sesban* as inferred from the genotype data of the progeny arrays derived from three populations and attempts to explain its selfing behavior.

MATERIALS AND METHODS

Seed samples

The original seeds of three populations of *S. sesban* were obtained from the Forage Gene Bank of the International Livestock Research Institute (ILRI), which is held in trust under the auspices of the Food and Agricultural Organization of the United Nations (FAO). The Zambian (ZAM-R024) *S. sesban* population pod samples were collected from natural stands growing along the shore of Lake Bangweulu. Twenty families (individual trees) were randomly selected and 20 pods per tree were sampled and seeds from each tree were bulked and 30 seeds per tree were assayed. The other pod samples were collected from two populations of *S. sesban* at two sites in Ethiopia. Pod samples from one accession (cultivated population) of *S. sesban* var. nubica

(ILRI Accession No. 15019) were collected from Debre-Zeit. Since the number of trees was limited at Debre-Zeit only 10 trees were considered. However, the number of assayed seeds from each tree was doubled to be equal to the number of seeds of the other two populations. The third cultivated population from which samples were acquired (ILRI Accession No. 15022) was at Addis Ababa. Twenty trees were randomly selected using random table numbers and 20 pods per tree were harvested and the seeds from individual trees were mixed. Thirty seeds were assayed from each tree or family. Six hundred seeds were used per population. In all sites, pods were sampled from all parts of the canopy of each tree to ensure a representative sample. Information on the sample collection sites is provided in Table 1.

Seed germination and sample extraction

Seeds were gently scarified using sandpaper and set to germinate in an incubator at 25–29° C with 12 hours light and 12 hours darkness. The light source in the incubator was Osram Fluorescent lamps (L 8 w/20) with light intensity of 36 $\mu\in$ m^{-2}s^{-1} PAR. Enzymes were extracted from four days old seedlings. Individual seedlings were pulverized in an ice-cold pestle and mortar with extraction buffer. The buffer was prepared following the procedures of Hussain *et al*. (1988). Each sample was collected in a micro-centrifuge tube and centrifuged at 12000 rpm for 10 minutes. The supernatant from each tube was collected separately and then mixed with a drop of bromophenol blue (tracking dye) and used immediately for electrophoresis. The supernatant could also be stored at –20° C for later use.

Gel preparation, buffer and enzyme systems

Polyacrylamide gels and the buffers were prepared following the recipe in Hames (1990) and Hussain *et al*. (1988) with minor modifications (20 g polyacrylamide, 0.42 g bis-acrylamide and 15.88 g of Tris-HCl/ 100 ml were used instead of 22.2, 0.6 and 18.15 g, respectively). Preliminary experiment was carried out to screen for polymorphism (by assaying ten seeds from some of the families in the three populations) using a range of stains for common enzyme systems including Acid phosphatase (Acp; EC 3.1.3.2), Alcohol dehydrogenase (Adh; EC 1.1.1.1), Esterase (Est; EC 3.1.1.1), Malate dehydrogenase (Mdh; EC 1.1.1.40) and Peroxidase (Prx; EC 1.11.1.7). Malate dehydrogenase was selected finally for the study since it showed clear polymorphism. Large number of progenies (Table 2) were assayed on this locus to reliably determine the most likely maternal genotypes.

Table 1. Information on the seed collection sites of *Sesbania sesban*.

Population	Origin	Altitude (m)	Latitude	Longitude	Plants age	Area (m²)	Density	No. trees sampled	Remark
Addis Ababa [1]	Rwanda	2380	9° 00′ N	38° 45′ E	5 years	2000	1 plant /4 m²	20	Cultivated population
Debre-Zeit [2]	Zaire	1850	8° 44′ N	38° 58′ E	3 years	200	1 plant/ 2.3 m²	10	Cultivated population
Zambian [3]	Zambia	1120	11.20′ N/S	29.35′ E/W	Mixed age	Unknown	Unknown	20	Natural population

Note:

1. The *S. sesban* population that were grown in Addis Ababa were found being partially surrounded with other population of *S. sesban* that were planted in the same season. Most of the plants from these populations were producing flowers at the same time with that of the Addis population.

2. The Debre-Zeit population was more or less grown in semi-isolation and there were about 25 trees and some were not performing well. There were species of *Acacia, Eucalyptus* etc grown around.

3. The Zambian *S. sesban* pods were collected initially from 30 randomly selected trees of a narrow population along the shore. Only pods from 20 of the trees were used for the isozyme analysis. All trees were found submerged in water. Plants that were found growing in association include Aeschynomene, Phragmites, Albizia, Ficus and plenty of Reeds. It means that the population was fragmented by other species.

Sample loading and gel staining

The slab gel with pre-formed wells was mounted onto an electrophoretic apparatus with upper and lower buffer compartments. The apparatus was placed in a mini-cold room at 4° C. The compartments were filled with electrode buffer. A sample volume of 20 μl was loaded in each well using gel loader tips fitted onto a micropipette. A constant voltage of 225 V from an electrophoresis power supply unit (EPS 500/400) was used for 2–3 hours. Under this electrophoretic condition the tracking dye was expected to move 8 cm, a distance within which most of the enzyme loci could be resolved. Gels were stained for Mdh following the staining recipe from Pasture *et al.* (1988) and incubated at 37° C in the dark for 1 hour until blue bands appeared.

Data collection and analysis

The first locus at 3.2 cm was the only polymorhic locus of Mdh from which genotypic data were scored and used in the estimation of the mating system parameters in *S. sesban*. Homozygous individuals revealed one band which was either fast (FF) or slow (SS) and the heterozygotes showed 3 bands and were assigned FS (fast and slow). The middle band in the heterozygotes is considered to be a hybrid resulting from the joint action of the two alleles, F and S (Pasteur *et al.*, 1988).

The Multi-locus mating system (MLTR) programme of Ritland (1994) (for both single and multi-locus data) was employed to analyze the genotype data of the progeny arrays in order to estimate mating system parameters. The MLTR computer programme requires numeric assignments for the genotypes. Fast bands (FF) were assigned 11; slow bands (SS) 22 and the heterozygotes (FS) were designated 12. The paternal pollen and ovule allele frequencies and most likely maternal genotypes were determined from the analysis of the progeny genotype data. The same data set was also used to estimate the apparent outcrossing rate for the three populations. The standard deviations for outcrossing estimates were based on 100 bootstraps as recommended in the method of analysis of Newton-Raphson (Ritland, 1994).

The allele frequencies [MdhF (p) and MdhS (q)] were derived from the progeny genotype data and these values were used to calculate observed and expected genotypic frequencies. To find if there was any association between the observed genotype frequencies and the panmictic value of the Hardy-Weinberg equilibrium, Chi-square values were calculated.

RESULTS

Mating system parameters such as out-crossing rate, progeny heterogeneity, most likely maternal genotypes and pollen and ovule gene frequencies were determined for the samples from the 3 populations of *S. sesban* (Tables 2, 3 and 4).

Table 2. Number of progeny assayed, gene frequencies and out-crossing rates of the samples from the 3 populations.

Population	Number of progeny assayed	Gene frequencies (SD)				$\hat{t}$-value (SD)
		MdhF pollen	MdhS Pollen	MdhF ovule	MdhS ovule	
Addis population	20 X 30	0.089 (.03)	0.911 (.03)	0.075 (.05)	0.925 (.05)	1.105 (.20)
Debre-Zeit population	10 X 60	0.171 (.02)	0.829 (.02)	0.048 (.00)	0.952 (.00)	0.395 (.06)
Zambian population	20 X 30	0.213 (.07)	0.787 (.07)	0.125 (.05)	0.875 (.05)	0.428 (.11)

Key: MdhF, Malate dehydrogenase fast allele; MdhS, Malate dehydrogenase slow allele; SD, standard deviation; $\hat{t}$-value, out-crossing rate.

Table 3. Allele frequency, observed and expected genotype frequencies and Chi-square values for the samples from the 3 populations.

Populations	Allele frequency	Observed no. of individuals and their genotypes	Observed genotype frequency	Expected genotype frequency	Chi-square Values (χ^2)	Probability (P-value)
Addis Ababa	F or p = 0.088 3	FF = 5	0.0083	0.0078		
		FS = 96	0.1600	0.1610	0.0000 (df*=1)	1.000
	S or q = 0.9117	SS = 499	0.8317	0.8312		
Debre-Zeit	F or p = 0.036	FF = 0	0.0000	0. 0013		
		FS = 43	0.0717	0. 0690	0.0014 (df=1)	0.970
	S or p = 0.964	SS = 557	0.9283	0. 9297		
Zambia	F or p = 0.140	FF = 36	0.0600	0.0196		
		FS = 96	0.1600	0. 2408	0.1126 (df=1)	0.737
	S or q = 0.860	SS = 468	0.7800	0. 7396		

* df, degrees of freedom.

Table 4. Proportion of heterozygous progeny and most likely maternal genotypes of each family in the samples of the 3 populations of *S. sesban*.

Family (Tree)	Addis Ababa		Debre-Zeit		Zambia	
	Maternal genotype	Proportion of heterozygotes	Maternal genotype	Proportion of heterozygotes	Maternal genotype	Proportion of heterozygotes
1	22	.266	22	.133	22	.166
2	22	.033	22	.033	22	.066
3	22	.033	22	.150	21	.000
4	21	.566	22	.050	22	.133
5	21	.500	22	.100	22	.000
6	22	.066	22	.018	21	.000
7	22	.200	22	.117	21	.033
8	22	.300	22	.083	22	.266
9	22	.166	22	.050	22	.366
10	22	.000	22	.018	22	.400
11	22	.100	-	-	22	.366
12	22	.066	-	-	22	.133
13	22	.033	-	-	22	.366
14	22	.300	-	-	22	.333
15	21	.466	-	-	22	.033
16	22	.000	-	-	22	.000
17	22	.000	-	-	22	.033
18	22	.066	-	-	22	.033
19	22	.033	-	-	21	.333
20	22	.000	-	-	21	.133

Five enzyme systems were assayed in the preliminary experiment and it was only malate dehydrogenase that showed clear polymorphism in one of the loci and was used as a marker to study the mating system parameters in the samples of the three populations.

Out-crossing estimation ($\hat{t}$) was based on the genotype data of the progeny arrays scored on one polymorphic locus of Malate dehydrogenase. A second locus of Mdh (Mdh-2) that was closer (1.5 cm) to the origin was monomorphic for all the progenies assayed. Brown *et al.* (1975) reported that Mdh-2 was polymorphic only for those populations at more elevated sites, but monomorphic for populations of *Eucalyptus obliqua*. This locus also did not show polymorphism for the populations of *S. sesban* probably, because samples were collected from sites at altitudes less than what Brown *et al.* (1975) called as the elevated sites (altitude not given). The first locus (Mdh-1) at 3.2 cm from the origin was polymorphic for most of the seeds assayed from the 3 populations (Fig. 1).

Based on the single locus of Mdh, out-crossing rate ($\hat{t}$) was found to range between 0.395 and 1.105 for the three populations (Table 2). The $\hat{t}$ estimate for Debre-Zeit (0.395) and Zambian (0.428) population significantly deviate from unity at 95% confidence interval.
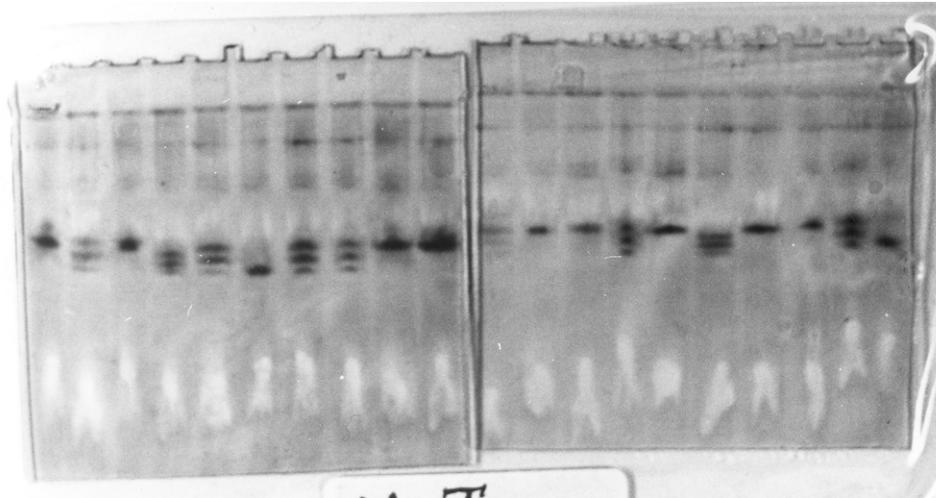
Fig. 1. The banding patterns of *Malate dehydrogenase* **for some individuals of the population of** *S. sesban.*

Data on gene frequencies (both p and q values) are given in Table 3. Observed and expected genotype frequencies were calculated from observed progeny genotypic data and allelic frequencies (Table 3). The Chi-square (χ^2, df = 1) = 0.000, 0.0014 and 0.1126 for samples of Addis Ababa, Debre-Zeit and Zambian populations, respectively, were calculated based on the same data set. The probability values corresponding to the calculated Chi-square values were greater than 0.05 (Table 3) indicating that the observed genotype frequencies are in agreement with the Hardy-Weinberg genotype proportions for the Mdh-1 locus.

In *S. sesban* the proportion of heterozygous genotypes present in the families of the 3 populations are variable from 0.033 to 0.566 as shown in Table 4. Population average heterozygosity were 0.160, 0.075 and 0.160 for Addis Ababa, Debre-Zeit and Zambian populations, respectively. These were obtained by dividing the total heterozygous genotypes in each population sample by the number of families assayed in each population.

DISCUSSION

The high out-crossing rate ($\hat{t}$ = 1.105) for the Addis Ababa population indicated that this species could undergo complete out-crossing when circumstances are favourable. Plants of the Addis Ababa population were grown close to other populations of *S. sesban* (Table 1). In the presence of

pollinators, gene flow between populations is inevitable and this may have accounted for the apparent high out-crossing rate. The abundance of pollinators and negative assortative matings may also be factors for the increased out-crossing rate. This compares with other fodder species, *Giliricidia sepium* (Papilionoideae), which is also strongly out-crossing ($\hat{t}$ =1.106) (Dawson and Chamberlain, 1996).

Out-crossing values greater than 1 could be the rule in randomly out-crossing species when different genotypes mate at higher frequency than expected (Ellstrand *et al.*, 1978). The effect of selfing (inbreeding effect) could increase the rate of out-crossing as a result of increased chances of cross zygote survival. In *S. sesban*, about 45.6% of the flowers aborted before forming pods and some seeds either do not germinate readily or germinate very poorly. Such seeds may be the result of self-fertilization. Complete or selective abortion of ovules was observed in *S. sesban, S. kinensis* and *S. geotzei* (Heering, 1994). This phenomenon was also reported by Stevens and Bougourd (1988), when they estimated $\hat{t}$ as 0.91 for *Allim schoenoprasum* L. that is, a clonal, self-compatible hermaphrodite plant with apparently ample opportunities for geitonogamous selfing. Barnes *et al.* (1972) reported that the frequency of ovule abortion is greater in zygotes and embryos resulting from self-pollination than from cross-pollination. Phillips and Brown (1977) found out that portions of seeds derived from self-fertilization do not germinate as readily as the seeds derived from out-crossing. The authors attributed this observation to heterosis, which they believe disfavors the evolution of a higher level of self-fertilization in populations of *Eucalyptus pauciflora*. Similarly, Moran *et al.*, (1980) reported that the level of selfing (10%) for *Pinus radiata* does not necessarily reflect how much selfing occurred during fertilization. The authors observed a considerable embryo abortion between fertilization and seed formation and many of the zygotes that die are progenies of self-fertilization.

The out-crossing value less than 1.0 in the Debre-Zeit and Zambian populations could be due to positive assortative mating, *i.e.*, when like genotypes mate among themselves more frequently than expected as a result of selfing. It is likely that related individuals could be spatially clumped in natural plant populations and that consanguineous mating of related neighbors is inevitable (Ellstrand and Foster, 1983). Flowering synchrony is common in plants that grow in close proximity to genetically related trees. This facilitates transfer of pollen among similar genotypes leading to a reduced out-crossing rate (Boshier and Lamb, 1997). The number of plants of the population at Debre-Zeit was limited and the plants were planted at 2 meters distance and partially isolated (surrounded by other species of plants). This kind of population structure re-enforces consanguineous mating. Smyth

and Hamrick (1984) reported that isolated or semi-isolated species were found to have highly variable out-crossing rate but averaged less than 50%. If genotypes are clumped in space and pollen flow is limited the apparent out-crossing rate is reduced relative to a randomly structured population.

The reduced out-crossing estimate for the Zambian population may also be attributed to population sub-structure as a result of interference from human activities and other species that were found growing among the stands of *S. sesban* (Table 1). This might have influenced the number and movement of pollinating insects. Smyth and Hamrick (1984) reported that *Carduus nutans* is predominantly out-crossed when pollen and pollinators were available.

The result of the single locus allozyme analysis indicated that *S. sesban* is a mixed mating species. The mixed mating model has several assumptions for the interpretation of estimates. The model considers progenies as the outcome of self-fertilization and random out-crossing. This will have its drawbacks when the model is applied to predominantly out-crossing species due to the heterogeneity of maternal genotype or in pollen allele frequencies (Brown *et al.*, 1989). One of the solutions to this problem is to use multi-locus estimation. However, in this study an option was considered where the experiment could be improved, *i.e.*, assaying large number of progeny per family (Table 2). The use of large samples for each family, at least, helps to reliably infer the maternal genotype, which supports the assumptions of the mixed mating model estimates.

Several studies have shown that mating systems may vary among populations in different years (Hamrick, 1982). The $\hat{t}$ estimates on one polymorphic locus for 2 cultivated populations of *Sorghum bicolor* were 0.28, 0.36 and 0.37 for three consecutive years (Ellstrand and Foster, 1983). The out-crossing value of *S. sesba*n is consistent with ranges recorded for other species with variable out-crossing rate. Dawson and Chamberlain (1996) reported a $\hat{t}$ estimate of 1.106 for *Giliricidia sepium* (Papilionoideae), which is strongly out-crossing. According to Levin *et al.* (1979), the out-crossing estimation for the 10 populations of *Oenothera organensis* varied from 0.74 to 1.27. For *Eucalyptus obliqua,* $\hat{t}$ was found to range from 0.42 to 1.05 with an overall mean of 0.76 for the 4 populations assayed (Brown *et al.*, 1975). In three populations of *E. pauciflora* $\hat{t}$ ranged from 0.30 to 0.85 with a mean of 0.63 overall (Philips and Brown, 1977). One variable locus (Alcohol dehydrogenase, Adh-1) was used to estimate the out-crossing rate in five populations of *Helianthus annuus* ($\hat{t}$ = 0.54–0.91).

The MdhS (the slow allele) occurs with high frequency in both the pollen pool and maternal ovules of the samples of the 3 populations. Despite its high frequency the proportions of the observed progeny arrays are in accordance with Hardy-Weinberg proportions for all the populations (Debre-Zeit, Addis Ababa and Zambia) at 95% significant level. The apparent variation in out-crossing rate for the populations of *S. sesban* could be due to delayed autonomous self-pollination. Delayed selfing was reported as a reproductive assurance in various species of plants (Lloyd 1979; 1992; Sakai, 1995) such as *Hibiscus laevis* (Klips and Snow, 1997), *Campanula* species (Faegri and Van der Pijil, 1979), *Lupinus nanus* (Juncosa and Webster, 1989) and *Mimulus guttans* (Dole, 1990; 1992). In such circumstances selfing could be viewed as a strategy to maintain an individual gene within a population or the reproductive output of populations with unpredictable pollinator behavior. This phenomenon has a selective advantage in case pollinators fail to visit flowers. Abundant pod development and normal seed set was observed on plants of *S. sesban* that were protected from pollinators in the greenhouse. Hand tripping was also found to initiate early self-pollination (self-fertilization) that would have been delayed under natural conditions to favor out-crossing.

Brown *et al.* (1989) concluded that entomophilous species display more variation in out-crossing rate both within and between species and could also vary greatly among and within populations. The large variation in out-crossing rate in *Lupinus nanus, L. sculentus* and *Collinsia sparsiflora* was attributed to fluctuations in activity of pollinators (Brown *et al.*, 1989). The high rate of out-crossing for the Addis Ababa population may also be due to the high activity of pollinators. Large crowd of bumblebees (*Bombus spp*) were observed actively hovering from flower to flower and/or from plant to plant in the peak flowering period. *Bombus canariensis*, which is closely related to *B. terrestris* is the probable pollinator of *Chamaesytisus palmensis* (Papilionoideae) and is large enough to easily manipulate the Papilonoideae flowers for either nectar or pollen (Webb and Shand, 1985).

CONCLUSION

The result of the assay of the progeny arrays from the three populations of *S. sesban* indicated that the species could undergo both inbreeding and out-crossing depending on circumstances. The values of the out-crossing rate (0.395–1.105) of the three *S. sesban* populations based on a single allozyme marker locus suggested that *S. sesban* is a mixed mating species.

Due to lack of polymorphism in the assayed enzyme systems, a single variable locus was used to estimate the out-crossing rate. However, due to non-robustness of single locus estimates the conclusion made here should be

viewed with caution. In pollinator dependent species, limitations of pollinators favour autogamy late in the flowering period (Cruden and Lyon, 1989). Species could also exhibit variable out-crossing rates across flowering periods due to variable pollinator service. The two forces, *i.e.*, to maintain heterozygosity for the continuity of evolution and to assure reproduction could account for such observed high and variable out-crossing rates in *S. sesban*. Future studies on the mating system of S. *sesban* may need to consider realities of the pollinator's behaviour.

ACKNOWLEDGEMENTS

REFERENCES

1. Azene Bekele Tessema, Birnie, A. and Tengans, B.O. (1993). Useful trees and shrubs for Ethiopia. Identification, propagation and management for agricultural and pastoral communities. Soil Conservation Unit, Swedih International Development Authority (SIDA).

2. Barnes, D.K., Bingham, E.T., Axtell, J.D. and Davis, W.H. (1972). The flower, sterility mechanisms and pollination control. **In**: *Alfalfa Science and Technology*, pp. 123-141, (Hanson, C.H., ed.). American Society of Agronomy.

3. Boshier, D.H., Chase, M.R. and Bawa, K.S. (1995). Population genetics of *Cordia alliodora* (Boraginaceae), A Neotropical tree, 2. Mating system. *Amer. J. Bot.* **82**:476–483.

4. Boshier, D.H. and Lamb, A.T. (1997). Mating system, population structure and gene flow. **In**: *Cordia alliodora*: *Genetics and Tree Improvement*. Tropical Forestry Papers No. 36. pp. 29–36, (Boshier, D.H. and Lamb, A.T., eds). Oxford Forestry Institute, University of Oxford.

5. Brown, A.H.D. (1990). Genetic characterization of plant mating systems. **In**: *Plant Population Genetics, Breeding and Genetic Resources*, pp. 145–162, (Brown, A.H.D., Clegg, M.T., Kahler. A.L. and Weir, B.S., eds). Sinaur Associates Inc. Publishers.

6. Brown. A.H.D. and Allard, R.W. (1970). Estimation of the mating system in open-pollinating maize populations using allozyme polymorphisms. *Genetics* **66**:133–145.

7. Brown, A.H.D., Barret, S.C.H. and Moran, G.F. (1985). Mating system estimation in forest trees: models, methods and meanings. **In**: *Population Genetics in Forestry*, (Gregoris H.R., ed.). Springer-verlag, Berlin.

8. Brown, A.H.D., Burdon, J.J. and Jarosz, A.M. (1989). Allozyme analysis of plant mating systems. **In**: *Isozymes in Plant Biology*, pp. 73-86, (Soltis, D.E. and Soltis, P.S., eds). Chapman and Hall, London.

9. Brown, A.H.D., Matheson, A.C. and Eldridge, K.G. (1975). Mating system of *Eucalyptus obliqua* L' Herit by using allozyme polymorphisms. *Aust. J. Bot.* **23**:931–949.

10. Cruden, R.W. and Lyon, D.L. (1989). Facultative xenogamy: examination of a mixed mating system. **In**: *Evolutionary Ecology of Plants*, pp. 171–207, (Bock, J. and Linhart Y.B., eds). Westview Press, Boulder, CO.

11. Dawson, I.K. and Chamberlain, J.R. (1996). Molecular analysis of genetic variation. **In**: *Gliricidia sepium*: *Genetic Resources for Farmers*, Tropical Forestry Papers No. 33, pp. 77-91, (Stewart, J.L. and Simons, A.J., eds). Oxford Forestry Institute, University of Oxford.

12. Dole, J.A. (1990). Role of corolla abscission in delayed self-pollination of *Mimulus guttatus* (Scrophulariaceae). *Amer. J. Bot.* **77**:1505–1507.

13. Dole, J.A. (1992). Reproductive assurance mechanisms in three taxa of the *Mimulus guttatus* complex (Scrophulariaceae). *Amer. J. Bot.* **79**:650–659.

14. Ellstrand, N.C. and Foster, K.W. (1983). Impact of population structure on the apparent out-crossing rate of grain sorghum (*Sorghum bicolor*). *Theor. Appl. Genet.* **66**:323–327.

15. Ellstrand, N.C., Torres, A.M. and Levin, D.A. (1978). Density and the rate of apparent out-crossing in *Helianthus annus* (Asteraceae). *Syst. Bot.* **3**:403-407.

16. Evans, D.O. and Macklin, B. (eds). (1990). *Perennial* Sesbania *Production and Use*. A manual of practical information for extension agents and development workers. Nitrogen Fixing Tree Assoc., Waimanalo, Hawaii, USA.

17. Faegri, K. and Van Der Pijl, L. (1979). *The Principles of Pollination Ecology*, 3rd. ed., Pergamon Press, Oxford, 244 pp.

18. Gabrielsen, T.M., Bachmann, K., Jakobsen, K.S. and Brochmann, C. (1997). Glacial survival does not matter: RAPD phylogeography of Nordic *Saxifraga oppositifolia*. *Mol. Ecol.* **6** (9):831–842.

19. Gabrielsen, T.M. and Brochmann, C. (1998). Sex after all: high levels of diversity detected in the arctic clonal plant *Saxifraga cernua* using RAPD markers. *Mol. Ecol.* **7**(12):1701–1708.

20. Gjuric, R. and Smith, S.R. (1996). Identification of cross-pollinated and self-pollinated progeny in Alfalfa through RAPD nulliplex loci analysis. *Crop Science* **36**:389–393.

21. Hames, B.D. (1990). One dimensional polyacrylamide gel electrophoresis of proteins. **In**: *Gel Electrophoresis of Protiens*, 2nd ed., pp. 1–147, (Hames, B.D. and Rickwood, D., eds). IRL Press, Oxford, England.

22. Hamrick, J.L. (1989). Isozymes and the analysis of genetic structure in plant populations. **In**: *Isozymes in Plant Biology*, pp. 87–105, (Soltis, D.E. and Soltis, P.S., eds). Chapman and Hall, London.

23. Hamrick, J.L. (1982). Plant population genetics and evolution. *Amer. J. Bot.* **69**:1685–1693.

24. Heering, J.H. (1994). The reproductive biology of three perennial *Sesbania* species (Leguminosae). *Euphytica* **74**:143–148.

25. Hussain, A., Bushuk, W., Ramirez, H. and Roca, W. (1988). *A practical guides for electrophoretic analysis of isozymes and proteins in cassava, field beans and forage legumes*. No. 40, working document,. Centro Internacional de Agricultura Tropical, CIAT, Cali, Colombia, 52 pp.

26. Juncosa, A.M. and Webster, B.D. (1989). Pollination in *Lupinus nanus* subsp. *latifilius* (Leguminosae). *Amer. J. Bot.* **76**:59–66.

27. Klips, R.A. and Snow, A.A. (1997). Delayed autonomous self-pollination in *Hibiscus laevis* (Malvaceae). *Amer. J. Bot.* **84**:48–53.

28. Knapp, E.E. and Teuber, L.R. (1993). Out-crossing rates of alfalfa populations differing in ease of floret tripping. *Crop Science* **33**:1181–1185.

29. Levin, D.A., Ritter, K. and Ellstrand, N.C. (1979). Protein polymorphisim in the narrow endemic *Oenothera organensis*. *Evolution* **33**:534–542.

30. Lloyd, D.G. (1979). Some reproductive factors affecting the selection of self-fertilization in plants. *Amer. Natur.* **113**:67–79.

31. Lloyd, D.G. (1992). Self- and cross- fertilization in plants. II. The selection of self-fertilization. *International J. Pl. Sci.* **153**:370–380.

32. Moran, G.F., Bell, J.C. and Matheson, A.C. (1980). The genetic structure and levels of inbreeding in *Pinus radiata* D. Don seed orchard. *Silvae Gent.* **29**:190–193.

33. O'Malley, D.M. and Bawa, K.S. (1987). Mating system of a tropical rain forest tree species. *Amer. J. Bot.* **74**:1143–1149.

34. Pasteur, N., Pasteur, G., Bonhomme, F., Catalan, J. and Britton-Davidian, J. (1988). Staining protocols. **In**: *Practical Allozyme Genetics,* pp. 95-150, (Wiseman, A., ed.). Ellis Horwood series in Gene Technology.

35. Philips, M.A. and Brown, A.H.D. (1977). Mating systems and hybridity in *Eucalyptus paucifflora*. *Aust. J. Biol. Sci.* **30**:337–344.

36. Ritland, K. (1994). Multilocus Mating System Program (MLTR), a revised version 0.9, written and copyrighted by Kermit Ritland. University of Toronto.

37. Ritland, K. and Jain, S. (1981). A model for the estimation of out-crossing rate and gene frequencies using **n** independent loci. *Heredity* **47**:35–52.

38. Sakai, S. (1995). Evolutionary stable selfing rates of hermaphroditic plants with competing and delayed selfing modes with allocation to attractive structures. *Evolution* **49**:557–564.

39. Shaw, D.V. and Brown, A.H.D. (1982). The optimum number of loci for estimating out-crossing rate in plant populations. *Theor. Appl. Genet.* **61**:321–325.

40. Smyth, C.A. and Hamrick, J.L. (1984). Variation in estimates of out-crossing in Musk Thistle population. *J. Heredity* **75**:303–307.

41. Stevens, J.P. and Bougourd, S.M. (1988). Inbreeding depression and the out-crossing rate in natural populations of *Allium schoenoprasum* L. *Heredity* **60**:257–261.

42. Wagner, D.B. and Allard, R.W. (1991). Pollen migration in the predominantly self-fertilizing plants: barley. *J. Heredity* **82**:302-304.

43. Webb, C.J. and Shand, J.E. (1985). Reproductive biology of tree Lucerne, *Chamaecystisus Palmensis* (Leguminosae). *New Zealand J. Bot.* **23**:597–600.

44. Weigand, R.O., Reed, J.D., Said, A.N. and Ummuna, V.N. (1995). Proanthocyanidins (condensed tannins) and the use of leaves from *Sesbania sesban* and *S. goetzei* as protein supplements. *Animal Feed Science and Technology* **54**:175–192.

45. Woodward, A. (1988). Chemical composition of browse in relation to relative consumption of species and nitrogen metabolism of livestock in Southern Ethiopia. Ph. D. Thesis, 195 pp. Cornell University, Ithaca, N.Y., USA.