RESEARCH ARTICLE

GENETIC DIVERSITY OF SCORODOPHLOEUS FISCHERI IN THE COASTAL FORESTS OF TANZANIA

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Tanzanian coastal forests are diverse in endemic plant species, *Scorodophloeus fischeri* being among them. It was aimed to investigate the genetic diversity of *S. fischeri* within and among populations. The total genomic DNA was extracted from young leaves of *S. fischeri* using CTAB procedures and then determined genetic diversity using PCR-RAPD markers. 73.19% of 97 scorable bands were polymorphic. UPGMA showed three clusters, each being a distinct population. Partitioning of genetic variability indicated that 60% occurred within and 40% among *S. fischeri* populations. This implies that more polymorphic loci existed within *S. fischeri* populations than among populations. The genetic differentiation (Gst =0.252) but low gene flow among populations (Nm = 2.301). It was concluded that there is higher genetic diversity at molecular level within *S. fischeri* than among populations of this species. *S. fischeri* experienced different complexities of micro-environmental conditions within populations. Human activities created a significant barrier of gene flow among populations and each forest warrants conservation.

Key words: *Scorodophloeus fischeri*, Genetic diversity, Variability, Differentiation, Geneflow, RAPD

INTRODUCTION

Tanzanian coastal forests are diverse in endemic plant species (Burgess et *al.* 2000), *Scorodophloeus fischeri* being one among them. *Scorodophloeus fischeri* (Taub.) J. Leon. is a tropical African hardwood plant, which belongs to the family *Leguminosae* (*Caesalpinaceae*). It is a dioecious plant with white petals, purple sepals, slightly buttressed at the base and with unequal leaflets (Wingfield 1974). *S. fischeri is* ecologically associated with *Brachylaena huillensis, Tessmannia burttii, Afzelia quanzensis, Manilkara sulcata, Newtonia paucijuga* in the East African coastal forests. Globally *S. fischeri* has a very limited geographical distribution because it is confined to the East African coastal forests, hence a coastal endemic (Cowan and Polhill 1981). *S. fischeri* is an economically valuable tree in the coastal areas in Tanzania as it produces strong and durable wood products due to its compacted tissues. There has been a growing demand for wood products such as charcoal, fuel wood and poles in Dar es Salaam and the surrounding coastal towns where the S. fischeri is the victim of exploitation for such products. *S. fischeri is* currently not a domesticated tree and all products are extracted from the natural forests, in which its regeneration is very low (Mligo *et al.,* 2009). It produces heavy seeds with very short viability, is subject to desiccation or, if it germinates, it does so only in favourable moist conditions (Hart 1990).

Due to its low regeneration, exploitation and fragmentation due to land use types, its habitats and populations are affected in coastal forests. However, the effects of exploitation and population fragmentation on genetic diversity have not been emphasized in many of the previous ecological studies. The genetic diversity of *S. fischeri* occurring in different forest fragments may be correctly assumed to vary due to physical barriers caused by human activities. These activities may result in population fragmentation and possibly genetic erosion resulting from inbreeding and introgression. Similarly, the very small S. *fischeri* populations in the severely fragmented Kazimzumbwi and Pande Forests may not constitute a minimum viable population (MVP); hence such populations may be isolated from one another and become susceptible to major catastrophic events. Its populations contain a distinct set of genetic instructions on how the species might react and adapt to natural threats (Chaisurisri *et al.,* 2004). If any of a few individuals of the populations remain, then there are a few survival tactics that the species can deploy in the face of threats such as global warming, climatic changes, diseases, drought and the effects of human activities (Nakasaka *et al.,* 1997). One of prerequisites in population conservation genetics is to determine the levels of genetic variability within and among the targeted/affected populations in forests. This study therefore aimed to determine the level of genetic diversity and genetic differentiation within and among *S. fischeri* populations in coastal forests in Tanzania.

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MATERIAL AND METHODS

Location of the study area

The study area consisted of Pande, Kazimzumbwi and Zaraninge Forests (Figure 1). They are found between 38° 30' to 39° 6'E and 5° 40' to 7° 0S' (Burgess and Clarke, 2000). To the east, the study area borders the Indian Ocean and to its north is Tanga region; Lindi is located to the south and Morogoro to the west.
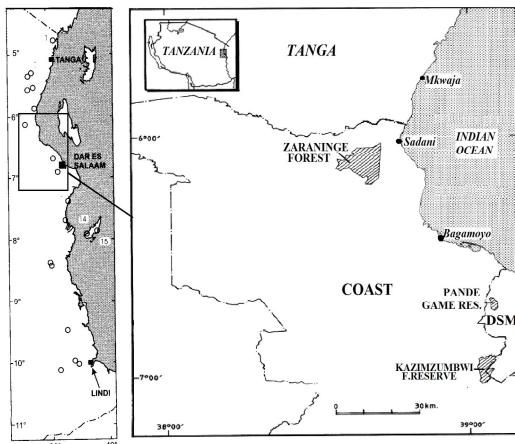

Fig. 1. Location of Zaraninge, Pande and Kazimzumbwi Forests in Tanzania

Sampling of Plant Material and DNA Isolation

Leaf samples of *Scorodophloeus fischeri* were collected from Pande, Zaraninge and Kazimzumbwi Forests and each forest was considered a population. The first five young leaves were collected from 10 individuals of each population of *Scorodophloeus fischeri* (Figure 2) and preserved with silica gel in a snap-top plastic container (Milligan's (1994). DNA isolation was carried out following the protocol by Edwards *et al.* (1991). 0.2g of the leaf sample was placed into a sterile motor followed by liquid nitrogen after which it was ground with Kontes pestle to a fine powder and then transferred to a sterile eppendorfs tube. 33µl containing 10% SDS and 500µl extraction buffer 1M Tris (pH 7.5), 5m NaCl, 0.5M EDTA, 7µl of mercaptol ethanol) were then added to the eppendorfs tube with the leaf samples, vortexed for 10 seconds and then incubated at 650C for 65minutes. 160µl of potassium acetate was added and vortexed for 10 minutes and the tubes were placed in ice for 10 minutes. The tubes were later removed from the ice and centrifuged at 10,000rpm for 10 minutes. 500µl of the supernatant was transferred into a fresh sterile eppendorfs tube and an equal volume of chilled isopropanol was added and mixed thoroughly. The mixture was centrifuged at 10,000 rpm for 10 minutes to pellet the nucleic acids and then the supernatant was poured off. The pellets were washed by 500µl of 70% ethanol through centrifuging at 10,000 rpm for 5 minutes followed by draining the ethanol by inverting the tubes. In so doing the DNA pellets were re-

suspended in 200µl of TE buffer (10mM Tris (pH 7.5). 1mM EDTA). 2 µl of RNAse (10mgml-1) was then added

Fig. 2. *S. fischeri* **Population well represented at all Size Classes**

into each sample and then incubated at 350C for 15 minutes. 2 volumes (400 µl) of cold absolute ethanol (100%) were added to each sample and then centrifuged at 10,000 rpm for 10 minutes to re-precipitate the DNA pellets. The pellets were dried in a vacuum and then re-suspended in 1000 µl of TE buffer (10mM Tris (pH 7.5), 1mM EDTA) and then stored at -200C for PCR reactions. Finally, the DNA quality and concentration were determined by UV spectrophotometry.

DNA Amplification

DNA concentration ranged from 50-400ng/µl, which was then diluted by PCR grade water (sigma W4502) at a ratioof 1 µl to 9 µl. The polymerase chain reactions (PCR) were carried out in a 25 µl volume reaction mix, which contained 200 µM of each of deoxynucleotide (dNTPs), 2.5units of pure Taq DNA polymerase enzyme, 1x enzyme buffer, 1.5 mM MgCl2, 2µM primer (Operon Technologies Inc., Alameida, California, USA), PCR reaction buffer (10 mmol/L Tris-HCl, pH 9.0, 50 mmol/L KCl; Perkin Elmer), 1 µl of genomic DNA genomic DNA and 22 µl grade water. The amplification was carried out in a DNA thermo cycler (TECHNE-TC–412-155913-2). Amplification conditions were set as, 1 cycle of 15 min at 94.5ºC (initial denaturation), 35 cycles of 1 minutes at 94ºC (denaturation), 1.3 minutes at 37ºC (annealing) and 1.3 min at 72ºC (extension). A final 5 min final extension (72ºC) was allowed to ensure full extension of all amplified products following Williams *et al.* (1990). Amplification products were stained by 0.5µg/ml of ethidium bromide and separated on 1.2% agarose gel in

0.5xTBE, observed under ultra violet light and then photographed.

Screening of RAPD PCR Primers

A total of 40 primers (Operon Technologies, Alameda, California, USA) were screened using two samples from each population. Twelve primers that produced the largest number of polymorphism, with clear and reproducible fragment patterns over multiple amplifications, were selected and used for amplification of all samples from three populations (Williams *et al.*, 1990). Such primers include, KFP4 (5'-CGGAGAGTAC-3'), KFP5 (5'-CCT GGC GAG C-3'), KFP10 (5'-ACG GTC CGC C-3'), KFP 21 (5'-GTA GGC GTC G-3'), KFP 22 (5'-TAC GCA CAC C-3'), KFP 23 (5'-GCT CGT CAA C-3'), KFP 24 (5'-ACT CGT AGC C-3'), KFP 27 (5'-TCC TCG CGG C-3'), KFP 33 (5'-TGA AGG TCC C-3'), KFP 34 (5'-GTC GGT GCA A-3'), KFP 35 (5'-CGT AGC CCC G-3') and KFP 36 (5'-TGC AGG CTT C-3').

Data analysis

Having done with all the lab work as detailed above, the RAPD bands were scored as present (1) or absent (0) and entered into an MS excel as a data matrix sheet. Only the clearest visible and strongest bands above 300bp were scored. The matrix was then used to calculate genetic diversity according to Nei's unbiased statistic using POPGENE 1.31 (Yeh *et al.,* 1987). The polymorphic bands were identified and quantified as number and percentage polymorphism in *S. fischeri* populations (Nei 1987). The phenotypic frequency in *S. fischeri* populations was calculated based on Shannon's diversity (Ho) where pi is the phenotypic frequency (Kings and Schaal 1989).

The diversity indices were partitioned into within and among a population's genetic variability (Table 3). Hpop was the measure of diversity within *S. fischeri* populations and Hsp the measure of the population samples of the same species populations. A portion of the diversity present within population was calculated using the formula Hpop/Hsp and a portion of the diversity present between populations was calculated using the formula Hsp-Hpop)/Hsp. The distance values were based on the proportion of different bands between possible pairs of genotypes. The degree of genetic isolation among populations was estimated as the number of migrants per generation (Nm). Nm was estimated from Gst (Slatkin and Barton 1989) as follows:-

$$Nm = 0.5\left(\frac{1-Gst}{Gst}\right)\text{w}$$

Where, Nm = Estimated gene flow between populations
Gst = Gene differentiation between populations

RESULTS

The level of polymorphism detected by using RAPDS

A considerable amount of polymorphisms were detected by RAPD primers among *S. fischeri* genotypes with an amount of DNA above the detection limit (25ng/ul) (Table1). The sizes of amplification products ranged from 100-1600bps with scorable regions being from 300-1400bp. Amplification of 33 *S. fischeri* DNA samples with twelve primers produced about 97 bands in a range of between 5-11 bands, giving an average of 8 bands per primer (Table 1). Of the total of 97 scored bands, 71 (73.19%) were polymorphic and the primers, coded as KPF22, KPF23, KPF33, KPF34 and KPF36, altogether contributed more than 50% of all the polymorphisms (Table 1). On the other hand, about twenty-seven (26.81%) of the total scorable bands were monomorphic among the individuals of *S. fischeri*.

The Genetic Variability within Populations of *S. fischeri*

The RAPD survey of the three natural populations of *S. fischeri* in the coastal forests of Kazimzumbwi (Kz), Pande (P) and Zaraninge (Z) indicated a substantial degree of genetic variation (Table 2). The estimate of the percentage of polymorphic loci showed more of a variability within populations than among *S. fischeri* populations. Among the three populations, Kazimzumbwi population exhibited the most genetic variability of 44 (corresponding to 46.81%) among individuals within a population whereas Zaraninge exhibited the lowest variability of 32 (34.04%). However, the accessions were grouped into four major clusters, excluding accessions K8 and K9 that were regarded as outliers (Figure 3).
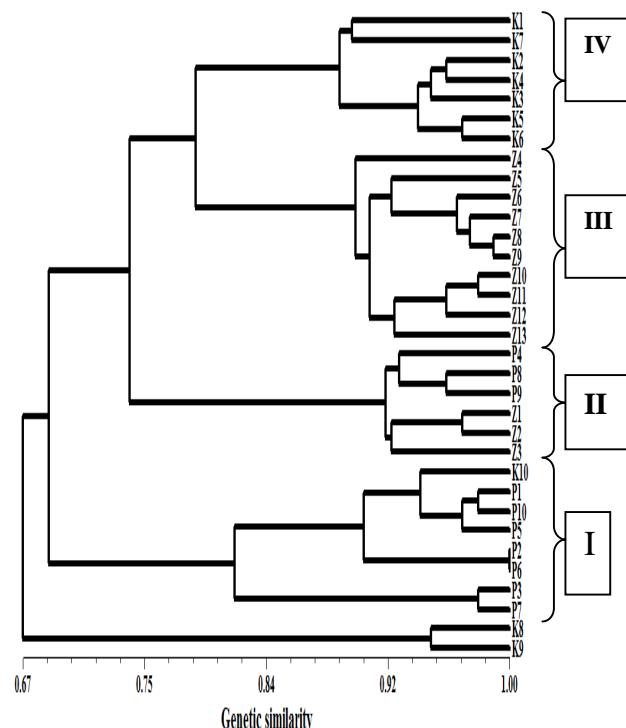

Fig. 3. UPGMA dendrogram showing more than 67% similarity among individuals of *S. fischeri* populations

Cluster I, comprised accessions from Pande Forest in which K10 from Kazimzumbwi Forest might have been included by chance. Cluster III was accessions from

Table 1. Number of bands, primers and the level of polymorphism per primers

Primer	Sequence	No. of bands	Polymorphic bands	% polymorphism
KFP 4	5'-CGG AGA GTA C-3'	8	4	50.00
KFP 5	5'-CCT GGC GAG C-3'	7	3	42.86
KFP 10	5'-ACG GTC CGC C-3'	6	4	66.67
KFP 21	5'-GTA GGC GTC G-3'	7	5	71.43
KFP 22	5'-TAC GCA CAC C-3'	11	10	90.91
KFP 23	5'-GCT CGT CAA C-3'	10	8	80.00
KFP 24	5'-ACT CGT AGC C-3'	6	3	50.00
KFP 27	5'-TCC TCG CGG C-3'	6	6	100.00
KFP 33	5'-TGA AGG TCC C-3'	10	8	80.00
KFP 34	5'-GTC GGT GCA A-3'	9	6	66.67
KFP 35	5'-CGT AGC CCC G-3'	5	4	80.00
KFP 36	5'-TGC AGG CTT C-3'	12	10	83.33
Total		97	71	
Range		5 -11	3 - 10	
Average		8.08	5.9 (73.19%)	73.19

Table 2. Estimates of Genetic diversity (Ho) within *S. fischeri* populations

Primers	Kazimzumbwi (KZ)	Pande (P)	Zaraninge (Z)
KPF 21	0.3000	0.2000	0.2300
KPF 22	0.4400	0.0310	0.0890
KPF 23	0.3300	0.0770	0.0650
KPF 24	0.1300	0.0290	0.0540
KPF 27	0.0060	0.1400	0.2000
KPF 33	0.0070	0.4393	0.4990
KPF 34	0.2300	0.2000	0.2000
KPF 35	0.0000	0.0000	0.2600
KPF 36	0.3900	0.1727	0.0450
KPF 10	0.0420	0.1900	0.0800
KPF 4	0.0100	0.0000	0.0000
KPF 5	0.0360	0.0000	0.0000
No. and % Polymorphism	44 (46.81%)	37 (39.36%)	32 (34.04%)
Mean	0.16008	0.1225	0.1435
sem	0.048	0.037	0.041
AMOVA	*F = 0.2096, P = 0.812, not significant*		

Table 3. Partitioning of genetic diversity within and among *S. fischeri* populations

Primers	H_{pop}	H_{sp}	H_{pop}/H_{sp}	$(H_{sp}-H_{pop})/H_{sp}$
KPF 21	0.2433	0.4100	0.5935	0.4065
KPF 22	0.1867	0.2600	0.7179	0.2821
KPF 23	0.1573	0.2100	0.7492	0.2508
KPF 24	0.0710	0.1200	0.5917	0.4083
KPF 27	0.1153	0.2400	0.4806	0.5194
KPF 33	0.3151	0.4759	0.6621	0.3379
KPF 34	0.2100	0.4400	0.4773	0.5227
KPF 35	0.0867	0.1300	0.6667	0.3333
KPF 36	0.2026	0.4400	0.4604	0.5396
KPF 10	0.1040	0.2800	0.3714	0.6286
KPF 4	0.0033	0.0066	0.5051	0.4949
KPF 5	0.0120	0.0130	0.9231	0.0769
Mean	0.142	0.251	0.599	0.400
sem.	0.027	0.047	0.044	0.044
			t = 3.19, df=22, P<0.0042; significant	

Table 4. The total genetic diversity and Gene flow among *Scorodophloeus fischeri* populations

Primer	Ht	Hs	Gst	Nm*
KFP 4	0.000	0.000	0.000	0.000
KFP 5	0.013	0.012	0.015	0.621
KFP 10	0.281	0.104	0.393	0.322
KFP 21	0.411	0.244	0.413	1.899
KFP 22	0.261	0.187	0.280	1.784
KFP 23	0.214	0.157	0.201	4.149
KFP 24	0.120	0.070	0.115	3.712
KFP 27	0.238	0.115	0.193	4.991
KFP 33	0.448	0.294	0.336	1.006
KFP 34	0.337	0.195	0.366	7.713
KFP 35	0.132	0.088	0.180	0.919
KFP 36	0.444	0.197	0.536	0.498
Mean	0.24	0.14	0.25	2.30
Sem	0.04	0.02	0.04	0.68

Nm* = Estimate of gene flow from Gst; Ht = Diversity in overall collections; Gst = Gene differentiation

Zaraninge Forest and Cluster II was a mixture of accessions from Zaraninge and Pande Forests (Figure 3). It could be interpreted that these individuals may have developed similar polymorphic fragments that were different from the mother populations. Cluster IV was accessions from Kazimzumbwi Forest. From the dendrogram, accessions from different fragments were clustered separately, with only K8 and K9 outliers, which may have evolved differently from the rest of the population.

Partitioning of Genetic Variability within and among *S. fischeri* Populations

The examined polymorphic loci showed that 60% variability had occurred within *S. fischeri* populations and 40% variability among populations. The genetic variability within *S. fischeri* populations varied with primers. Primers 5 (KPF 5; 92.31%) detected most of the variability within populations whereas KPF 10 (62.86%) detected most of the variability among populations (Table 3). The total genetic diversity (HT) per primer ranged from 0.094 to 0.388 (0.241 $\pm$ 0.04) with high gene differentiation (GST = 0.25 $\pm$ 0.04). Gene flow among genotypes was low (mean Nm=2.301$\pm$ 0.68) (Table 4).

DISCUSSION

Level of polymorphism in Scorodophloeus fischeri populations

The level of polymorphism that was detected by amplification of arbitrary RAPD primers within and among *S. fischeri populations* was extensive. The polymorphic assay procedures showed that *S. fischeri* populations in coastal forests are isolated. The percentage of polymorphism in *S. fischeri* was generally very high (73.19%), implying high genetic diversity within *S. fischeri* populations. *S. fischeri* is genetically diverse at the local scale with a variety of genetic combinations, making it adaptive to different micro-habitat conditions which are maintained by the monsoonal climatic conditions as well as natural selection. This polymorphism is balanced because of more than two morphs being maintained within *S. fischeri* populations. This has resulted in a high degree of genetic diversity being maintained within *S. fischeri* populations in a particular locality. Changes in micro-habitat conditions have influenced *S. fischeri* populations such that a particular geographical locality favours a gene combination that makes it adapt accordingly. However, with the current pressure caused by human activities it is not easy for *S. fischeri* populations to adjust genetically and adapt to changes. Since accessions within populations are genetically different, exhausting any one particular forest population erodes the genetic diversity in the coastal forest since the existing populations are isolated and genetically different. The current exploitation is causing an enormous reduction in sizes of *S. fischeri* populations, which later might result in a population genetic bottleneck. Inbreeding in such a small population is inevitable and will consequently result in loss of fitness among individuals of the *S. fischeri* populations.

Genetic diversity among *Scorodophloeus fischeri* Populations

The clusters in the UPGMA dendrogram (Figure 3), are a function of genetic distance between populations due to forest fragmentation. These were typically groups of individuals from separate populations with one consisting of mixed individuals from two populations which might have clustered together due to the great similarity in the morphs of their DNA fragments and which had come from populations that had benefited from recent gene flow. Although individuals from different populations had separated into distinct clusters, their genetic differences were lower than individuals within populations. The existence of higher genetic variation among individuals of *S. fischeri* within populations rather than individuals among populations is due to differences in the micro-environment within a population (forest). *S. fischeri* performs well in shaded habitats that are moist with diffuse light such as in Zaraninge Forest, particularly on the raised hill tops where soils are moist with a high level of organic matter (Mligo *et al.,* 2009). It also grows well on the hill tops and deep slopes with dry sandy soils in Pande and Kazimzumbwi Forests (Mligo, 2010). The heterogeneity in landscapes and micro-habitat conditions combined with forest fragmentation due to human activities, might have caused synergistic genetic variability among populations.

The Genetic Variability within and among *Scorodophloeus fischeri* Populations

Genetic variations among populations were lower than within populations. This finding agrees with the earlier hypothesis that genetic diversity within *S. fischeri* populations is significantly higher than among populations. High genetic diversity within *S. fischeri* populations implies that the species has plenty of potential and scope for evolution to occur. According to Hamrick (1990), most variability is exhibited by populations of crossbreeding woody perennials. Having more variability within *S. fischeri* populations implies a substantial amount of crossbreeding among individuals. However, the results are contrary to what was reported by Chalmers *et al.* (1992) that genetic diversity occurs between populations. These results suggest a random mating within populations with limited gene flow among sub-populations due to variations in micro-habitats, genetic drift and fragmentation. High genetic diversity within populations is in agreement with the conclusion that out-breeding of woody plant species retains considerable variability within populations (Hamrick 1990). *S. fischeri* population in Kazimzumbwi Forest was more diverse than populations of the same in other forests. This population has been exposed to severer micro-habitat changes than populations in other forests. Climatic data by Burgess *et al.* (2000) showed that the average annual rainfall decreases towards the southern part of the coastal belt. These variations contribute to the adaptation of species to various micro-habitats so that varieties of gene combinations have evolved differently at the local scale (Burgess *et al.,* 2000). However, low genetic diversity among *S. fischeri* populations is due to environmental conditions in these

forests having the same pattern since the history of evolution of these populations. *S. fischeri* populations have consistently been affected by the prevailing conditions in the coastal belt but have adapted differently in their localities. The patterns of genetic variation among *S. fischeri* populations are the outcome of a combination of the past colonization and gene flow due to successful dispersal in the coastal forests before human interference. It appears that low genetic diversity among *S. fischeri* populations is an indication of its previous continuous distribution before being isolated by fragmentation in the coastal landscape. The increasing amount of exploitation, apart from reducing population sizes, has also caused habitat fragmentation. Fragmented habitats increase spatial isolation among populations and limit gene flow among *S. fischeri* sub-populations. This is supported by Young et al. (1996) that a reduction in the proportion of immigrant genes (limited gene flow), due to the isolation of fragments by distance, in combination with allelic drift in small populations increases genetic differentiation among disturbed sub-populations.

The genetic diversity HT = 0.241 can be regarded as the diverse genetic base among *S. fischeri* populations. The gene differentiation (GST=0.252) is similar to the value reported by Okun et *al.* (2008) which was GST=0.264, and this implies significant variations among populations, i.e. 25.2% is attributed to variations among local populations. High gene differentiation among *S. fischeri* populations implies wide gene combinations mong genotypes within populations. Ken *et al.* (1993) reported a gene flow value of Nm = 20.8, and concluded that it was high and that the sub-populations at different altitudes were connected. A gene flow value of Nm = 2.301 was estimated inferring that there was limited gene flow among *S. fischeri*. This suggests that *S. fischeri* populations in different forest fragments are disconnected based on the loci analyzed. This may be explained by the recent origin of some habitat fragmentations caused by human activities, whereby *S. fischeri* populations showed genetic patterns of isolation-by-distance as forest fragments, and that, the micro-climatic complexities within each forest fragment might have contributed to slight variations in the genetic combination of individuals in the sub-populations. As regards to the restricted gene flow across shorter distances, the level of genetic differentiation among populations increases as a function of the spatial distance (Wright 1943). Austerlitz *et al.* (2000) reported that forest trees have lower gene differentiation than annual plants. This is due to the lower colonization and reproduction rates of trees than annual and herbaceous plants (Austerlitz et al. 2004). The situation is more complex for *S. fischeri* as it has large heavy seeds which combined with habitat fragmentation limit dispersal and cause this wood plant to exhibit a genetic structure at limited spatial scale. Consequently, *S. fischeri* populations are disjunct and genetically different, although they are in fact relicts of a single large and previously continuously distributed population. This means populations had different frequencies of dominant genetic markers and hence should be regarded as units of conservation significance. With increased human pressure

S. fischeri continuity is unlikely to be restored and the genetic variability among populations will keep on increasing, while the random mating of individuals within sub-populations of a particular population will continue to increase its genetic diversity.

It can be concluded that the recent development in technology for analyzing of molecular population genetics has provided a potential means for assessing the effects of human activities on genetic diversity in *S. fischeri* populations in coastal forests. Although DNA extraction was problematic due to the high level of oil in this species which produced poor amplification, it was possible to improvise and modify the protocol to get a clear amplification product. The RAPD markers used in this study sufficiently helped the researcher to observe the genetic variability within and among *S. fischeri* populations. There have been wide genetic differences among populations in coastal forests. This proves that coastal forest fragmentation due to human activities has a significant effect on the genetic diversity of populations and extinction is likely to be a consequence of exploitation. Fragmentation of coastal forests and the exploitation of trees in the ecological context have reduced population sizes and make *S. fischeri more* vulnerable to random loss of genetic variability as the sub-populations increasingly become reproductively isolated.

The low genetic variability among *S. fischeri* populations fits in with the ecological characteristics of coastal forests. The studied populations are part of several existing sub-ominant populations in the evergreen coastal forests in Tanzania. The height of the trees, heavy seed production, limited viability and habitat fragmentation have all contributed to localized seed dispersal leading to the patchy spatial distribution of populations. However, the distribution of *S. fischeri* over a wide variety of micro-habitat conditions in coastal forests suggests that these trees have developed a reasonably low level of genetic variations among populations that will migrate in response to changes in future vegetation communities when conservation becomes intensive. The low level of genetic variation found in the isolated populations will make it difficult for them to exist in the event of extreme catastrophic conditions. However, adaptation is more likely to be restricted in the forest edge of the distribution because these populations will be the first to experience gene flow from nearby pre-adapted populations. Habitat restoration is required, using the readily available propagules of *S. fischeri* from nearby populations to reduce the current great loss of genetic variability within populations. A great number of individuals of the populations from natural relatives would increase the chance of random hybridization in order to restore and establish patterns of genetic variation between and within *S. fischeri* populations. This would help to conserve the existing S. fischeri phenotypes, their genetic integrity and maintain local adaptations. Conservation targets should consider protecting any existing coastal forest fragments because the individuals of *S. fischeri* from different fragments are genetically different and gene flow is limited.

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