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Conservation of *Acacia tortilis* subsp. *raddiana* Populations in Southern Sinai, Egypt I- Genetic Diversity and Structure

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ABSTRACT

Acacia tortilis (Fabaceae) provides food and shelter for desert animals and is a major source of livestock food and firewood for the native Bedouin people in Southern Sinai, Egypt. High mortality of established individuals and low juvenile recruitment has been reported in recent years. As a result, this once common tree has experienced local extinction and is now a species of conservation concern in the region. Sixteen allozyme loci were used to examine regional genetic diversity within and among twelve natural populations of *A. tortilis* selected to represent its geographic range in two large wadis in Southern Sinai, Egypt. The results showed that regional genetic diversity for *A. tortilis* (H_e = 0.213) is high compared not only to estimates for other tropical acacias but for plants generally. The majority (96%) of the genetic variation occurs within populations indicating that historically this species experienced high rates of gene flow over the geographic scale sampled. A UPGMA phenogram didn't match genetic distance among populations with their geographic relationships. High historical rates of gene flow and the recent fragmentation of once more continuous populations coupled with the longevity of these trees could explain the results obtained.
Key words: *Acacia tortilis*, Sinai, conservation genetics, genetic diversity.

INTRODUCTION

Acacia is a large genus with about 1350 woody species (Maslin *et al*., 2003). Most are shrubs or small trees of dry savannas and arid regions of Australia, Africa, India, and the Americas. A few occur in cool, moist areas, both in temperate regions and in tropical highlands. A third small group of acacias is native to the lowland wet tropics. A few (e.g. *A. mangium*) are planted extensively outside their natural range for timber, pulpwood, tannin, fuel wood and erosion control (Wickens *et al*., 1995). Others are little known but have attributes that suggest they could be more widely utilized to improve the wellbeing of people in developing countries.

There are about twenty five species of *Acacia* growing in dry areas of the Middle East - ten occur in Egypt - of which *Acacia tortilis* is the most widespread, drought-resistant, and heat-tolerant (Halevy and Orshan, 1972; Zohary, 1973; and Boulos, 1999). It reaches its northern limit in the Dead Sea area (Judean and Negev deserts including Arava valley). It is known from Sudan and other countries in East Africa and south west Arabia (Zohary, 1973). *Acacia tortilis* is a polyploid species (Fagg, 1991) within which four subspecies are recognized in the arid and semi-arid lands of Africa and the Near East (Wickens *et al.*, 1995). Two of the four *A. tortilis* subspecies are found in Egypt; *A. tortilis* subspecies *tortilis* extends south to Somalia and east to Arabia, and *A. tortilis* subspecies *raddiana* extends south to Sudan, Senegal, Somalia, and Kenya, and north and east to Israel, Jordan, and Saudi Arabia. Both subspecies are known from the Sinai but *raddiana* is the only subspecies in the study area (Halevy and Orshan, 1972).

In Egypt, *A. tortilis* subspecies *raddiana* grows in desert wadis and sandy plains, usually in water catchment areas, and is found in the Sinai, Red Sea coast, Eastern Desert, and Gebel Elba (Boulos, 1999). It is widely distributed throughout Southern Sinai's wadis and plains where it dominates mainly rocky wadi-bed habitats near the foothills and sometimes the slopes of metamorphic mountains (Moustafa *et al.*, 1998). Wadi Feiran and its tributaries, W. Mandar, W. Lithi, W. Solaf, W. El-Nasb, Ladid area, and the El-Qaa plain are its main localities in the Sinai.

In recent decades, drought and human interference including over-grazing, over-cutting, over-collecting, and habitat destruction have threatened this species in the Sinai by increasing the mortality of mature trees and reducing natural recruitment to the point that it is difficult to find juveniles. Ward and Rohner (1997) estimated that up to 60% of the total mortality of *A. tortilis* in the Negev desert is caused by anthropogenic disturbances. As a multipurpose tree, *A. tortilis* has been cut and used as firewood, structural support, and charcoal. Branches are occasionally pruned, especially during the dry summer, for green fodder for goats and sheep. Camels browse the lower branches of even relatively tall trees. An extraordinarily high level of seed predation by bruchid beetles is an additional threat to *A. tortilis* in the Sinai making the recruitment of young seedlings relatively rare (Rohner and Ward 1999; Moustafa *et al*., 2000). Infestation rates vary from 39-95% in the Sinai (unpublished data on the studied populations) and reaches 80-95% or more in the Negev desert (Ward and Rohner, 1997; Rohner and Ward, 1999). Mature pods (containing both sound and infested seeds) that fall beneath trees are a favorite food of goats and sheep as well as desert gazelles, which usually find shelter in the shadow of trees during the heat of the summer (Abd El-Wahab, 1995; Moustafa *et al.*, 2000). Ungulates play an important role in seed dispersal since

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passage through the gut greatly enhances germination (Rohner and Ward, 1999; Moustafa *et al*., 2000). Conversely, seed consumption prevents the accumulation of a persistent seed bank while the few seedlings that succeed in germinating are subjected to grazing.

Research on *A. tortilis* is needed in view of the considerable socioeconomic benefits which could result from its cultivation and/or conservation. Halevy and Orshan (1972) studied the distribution of *A. tortilis* subspecies *raddiana* and subspecies *tortilis* and *A. pachycera* in the Negev and Sinai. They concluded that the frequency of *A. tortilis* subspecies *raddiana* within its natural range is distinctly affected by its relatively high demand for water and the indifference to soil type and lithology when growing in wadi beds. It depends more on the size of the wadi and its catchment area affecting the amount of water runoff. Abd El-Wahab (1995) studied the ecology of *A. tortilis* in Southern Sinai determining that its presence is highly dependent on soil moisture content and the presence of boulders on the soil surface. Ward and Rohner (1997) investigated aquifer depletion and road building as the major purported anthropogenic sources of acacia mortality in the Negev desert and concluded that a major cause of mortality is road-building that blocks wadis preventing floodwater during winter storms from reaching the lower parts of wadis. Moustafa *et al.* (1998; 1999) highlighted the threats affecting *Acacia* populations in low wadis and plains in southern Sinai including W. Mandar, W. Lithi, W. Solaf, W. El-Nasb, Ladid area, and El-Qaa plain. They concluded that unmanaged anthropogenic effects (e.g. grazing, cutting for different purposes, quarrying) are the main threats to the tree there. These anthropogenic factors also indirectly aggravate the general aridity prevailing in the area.

Studying the effects of large mammalian herbivores on the establishment of young *A. tortilis* subspecies *raddiana* and *A. tortilis* subspecies *tortilis* in the Arava Valley, Rohner and Ward (1999) concluded that large mammalian herbivores are essential components of arid *Acacia* savannas and that wild and domestic ungulates must be included in any conservation plans involving *A. tortilis*. Moustafa *et al.* (2000) demonstrated a positive response to *in situ* conservation through fencing selected natural populations in Wadi Mandar and Wadi Lithi emphasizing the value of incorporating indigenous people in conservation efforts as an essential element for success. They also demonstrated the potentiality of establishing plantation areas from seeds contained in goat faeces.

Shrestha *et al.* (2002) used random amplified polymorphic DNA (RAPD) to assess patterns of genetic variation within and among 12 populations of *A. tortilis* subspecies *raddiana* from the Arava (Syrian-African Rift) valley and western Negev. A high level of genetic polymorphism was recorded within populations with 59.4% of total genetic variance occurring among populations. Contrary to Halevy and Orshan (1972), Shrestha *et al.* (2002) suggest that there may have been two invasions of *A. tortilis* subspecies *raddiana* into their study area, the first across the northern Sinai and Gaza into western Negev and the second across southern Sinai.

Acacias may play a dual role contributing to industrial forestry plantations, for timber, pulpwood and tannin, as well as at the Bedouin community level to provide fuel wood, land rehabilitation and as a component of agro-forestry systems. Understanding the genetic composition of a species is basic for the appropriate utilization of the species' genetic resources, either for genetic improvement under cultivation or for the conservation and management of natural populations. Our goals in this study are to measure and critically evaluate genetic variation within and among *Acacia tortilis* subspecies *raddiana* populations and to determine implications of the genetic analyses for the conservation of this species in the Sinai.

MATERIALS AND METHODS

Study area

The Wadi Mandar and Wadi Lithi basins are among the most prominent wadi systems in Southeastern Sinai (Figure 1). The Wadi Mandar basin contains a group of plains and tributaries surrounded by granitic hills of medium height (75-100m). The nature of the soil surface is a mix of gravel and cobbles except at the wadi entrance as well as the end of tributaries where the soil surface is sandy. Total plant cover is 3-5% on the foothills and the end of tributaries and less than 1% elsewhere. The only dominant species is *A. tortilis* whose height ranges from 70 cm to 2 m in the middle and entrance of the wadi and 3-4 m or more on the foothills and upstream. Few individuals of *Zilla spinosa, Fagonia mollis, Lycium shawii,* and *Artemisia judaica* are scattered in the wadi. *Acacia tortilis* at Wadi Mandar is subjected to great human interference due to the presence of about 68 Bedouin families inhabiting the wadi entrance. Two quarries of white granite are present 5 km from the Bedouin community.

Wadi Lithi is heavily stressed with grazing by sheep and goats. W. Hamer El-Atshan, W. Hamer El-Rayan, Roknet Amra, Umm Ormot, Umm Gratt, Umm Merkha, Talae'et Rashid, El-Menaizla, and Umm Zaraba are the most prominent tributaries of the basin. The vegetation cover generally ranges from 5-10 % with some few exceptions like at Roknet Amra (10-15%). The wadi is characterized by high number of associated species including; *Aerva javonica, Blepharis ciliaris, Fagonia arabica, Fagonia mollis, Fagonia scabra, Iphiona scabra, Launaea spinosa, Lavandula pubescens, Lycium shawii, Pulicaria crispa, Senna italica, Solenostemma arghel, and Zilla spinosa*.

Cutting *Acacia* trees for fuel wood and construction is widespread especially near the Bedouin community and the quarries. Tourism is another threat through the over-collection of branches (along with all the vegetation) for trekking and camp cooking and barbecuing.

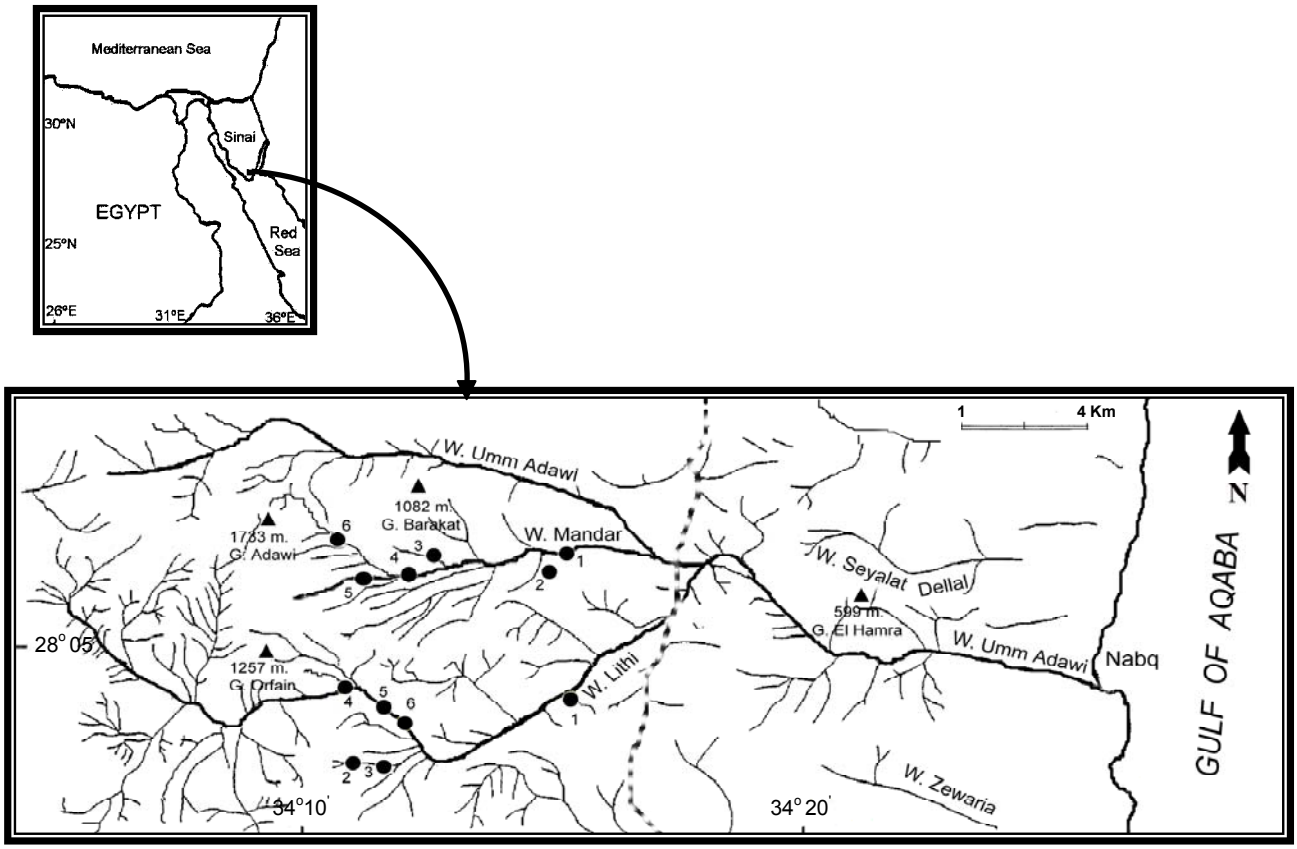

Figure (1): Location map for the study area. The smaller scale map shows the location of the study area in the Sinai.

The upstream part of Wadi Lithi is a tourist site where surface water is present, surrounded by granite boulders with a shaded area. These characteristics encourage safari tourism which results in soil compaction and clearing as a result of 4-wheel drive vehicles and to the over-collection of firewood.

Population samples and seed collection and germination

Twelve *A. tortilis* populations were sampled representing the geographic distribution of the species in the study area; six populations from Wadi Mandar and six populations from Wadi Lithi (Fig. 1). Three populations were represented by more than 20 individuals (families), three by 13-17, and six by 3-8. The number of sampled individuals depended on the actual population size as well as seed availability. Seeds analyzed by electrophoresis were chosen at random from bulked samples representing many pods from each tree.

Seeds of *A. tortilis*, like those of most acacias, germinate poorly unless the impervious seed coat is pierced or scarified so that the endosperm and embryo can take up water. To increase germination, open-pollinated seeds were treated with 95% sulfuric acid for 25-30 min and then rinsed thoroughly with water. They were sown on Fafard mix no.3B (Canadian Sphagnum Peat, Vermiculite, Perlite, and Processed Pine Bark.) and placed in a greenhouse at the University of Georgia Plant Growth Facility. Seedlings were allowed to grow to 5-10 cm in height.

Isozyme analysis

Whole plant tissues were crushed manually with pre-cooled mortar and pestle using the extraction buffer of Wendel and Parks (1982) and a pinch of sea sand with no elaborate protein purification or concentration steps. Crude enzyme extracts were absorbed onto sample wicks made from No.3 Whatman filter paper and stored in microtest plates at -70^{o} C. Up to three seedlings from each maternal tree were crushed (Table 1). Starch-gel electrophoresis (10% starch gels) was used to resolve allozyme electromorphs.

Gels were stained for nine enzyme systems to resolve 16 allozyme loci: Menadione reductase (Mnr-1), Phosphoglucoisomerase (Pgi-1), Phosphoglucomutase (Pgm-1, and Pgm-2), Fluorescent esterase (Fe-1, and Fe-2), Triose-phosphate isomerase (Tpi-1, Tpi-2, Tpi-3, and Tpi-4), Alcohol dehydrogenase (Adh-1), Diaphorase (Dia-1, and Dia-2), Isocitrate dehydrogenase (Idh-1, and Idh-2), and Malate dehydrogenase (Mdh-1). Three buffer systems of Soltis *et al.* (1983) were used to resolve the enzymes; System 6 (Mnr, Pgi, Pgm, Tpi, Fe, Adh), a modified system

Table (1): Allele frequencies at 11 polymorphic loci for 12 Sinai populations of *Acacia tortilis.*

Locus	Allele	Population											
		Wadi Mandar						Wadi Lithi					
		M1	M2	M3	M4	M5	M6	L1	L2	L3	L4	L5	L6
Mnr	1	0.000	0.062	0.029	0.000	0.033	0.062	0.108	0.107	0.021	0.200	0.043	0.222
	2	0.962	0.771	0.784	0.889	0.762	0.719	0.730	0.571	0.646	0.667	0.700	0.722
	3	0.038	0.167	0.186	0.111	0.205	0.219	0.162	0.321	0.333	0.133	0.257	0.056
Pgi-2	1	0.000	0.000	0.000	0.056	0.057	0.082	0.028	0.000	0.000	0.000	0.040	0.000
	2	0.167	0.104	0.150	0.056	0.221	0.158	0.306	0.321	0.188	0.133	0.135	0.111
	3	0.833	0.896	0.850	0.889	0.721	0.760	0.667	0.679	0.813	0.867	0.824	0.889
Pgm-2	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.111
	2	0.326	0.250	0.364	0.278	0.263	0.212	0.357	0.464	0.413	0.467	0.468	0.333
	3	0.674	0.750	0.636	0.556	0.728	0.773	0.643	0.536	0.522	0.533	0.516	0.500
	4	0.000	0.000	0.000	0.167	0.009	0.015	0.000	0.000	0.065	0.000	0.016	0.056
Fe-1	1	0.779	0.646	0.784	0.611	0.667	0.547	0.568	0.700	0.625	0.667	0.514	0.833
	2	0.221	0.354	0.216	0.389	0.333	0.453	0.432	0.300	0.375	0.333	0.487	0.167
Fe-3	1	0.329	0.435	0.302	0.222	0.270	0.345	0.371	0.367	0.391	0.192	0.309	0.222
	2	0.658	0.522	0.581	0.778	0.672	0.534	0.586	0.533	0.478	0.654	0.515	0.778
	3	0.012	0.044	0.116	0.000	0.057	0.122	0.043	0.100	0.130	0.154	0.177	0.000
Tpi-2	1	1.000	1.000	1.000	0.889	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.000	0.000	0.000	0.111	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Tpi-4	1	1.000	1.000	1.000	0.944	1.000	1.000	0.973	1.000	1.000	1.000	1.000	1.000
	2	0.000	0.000	0.000	0.056	0.000	0.000	0.027	0.000	0.000	0.000	0.000	0.000
Dia-1	1	0.833	0.854	0.955	0.722	0.922	0.855	0.917	0.800	0.875	0.767	0.868	0.667
	2	0.167	0.146	0.046	0.278	0.078	0.145	0.083	0.200	0.125	0.233	0.132	0.333
Dia-2	1	0.000	0.000	0.015	0.214	0.094	0.081	0.014	0.071	0.132	0.133	0.203	0.125
	2	0.727	0.761	0.970	0.786	0.906	0.912	0.943	0.929	0.842	0.800	0.757	0.875
	3	0.273	0.239	0.015	0.000	0.000	0.007	0.043	0.000	0.026	0.067	0.040	0.000
Idh-2	1	0.129	0.130	0.200	0.000	0.241	0.147	0.344	0.136	0.294	0.167	0.300	0.556
	2	0.790	0.674	0.471	0.812	0.517	0.560	0.438	0.682	0.382	0.542	0.514	0.333
	3	0.081	0.196	0.329	0.188	0.241	0.293	0.219	0.182	0.324	0.292	0.186	0.111
Mdh	1	0.046	0.062	0.000	0.000	0.000	0.007	0.000	0.000	0.042	0.000	0.000	0.056
	2	0.012	0.062	0.019	0.056	0.040	0.027	0.068	0.067	0.042	0.000	0.040	0.000
	3	0.930	0.875	0.907	0.944	0.873	0.932	0.932	0.933	0.917	1.000	0.960	0.944
	4	0.012	0.000	0.074	0.000	0.087	0.034	0.000	0.000	0.000	0.000	0.000	0.000

8 (Dia), and system 4 (Idh). The morpholine citrate (MC) buffer system of Conkle *et al.* (1982) was used to resolve Mdh.

Enzymes were visualized using standard colorimetric staining methods (Vallejos, 1983). Stain recipes were taken from Soltis *et al.* (1983) except for Mnr and Dia, which were taken from Cheliak and Pitel (1984). For each enzyme system, the most anodally migrating band was designated as locus 1, the next faster was locus 2, and so on. Likewise, within each locus the fastest migrating allele was designated allele 1, and each successively slower band was numbered 2, 3, etc.

Data analysis

To estimate genetic variation within populations standard measures of genetic diversity for each population were calculated including the mean number of alleles per locus (*A*) and per polymorphic locus (*AP*), the effective number of alleles per locus (A_e) the percentage of polymorphic loci (*P*), and mean observed (H_o) and expected heterozygosity (H_e) at the species and within population levels (Nei, 1973). Wright's fixation index (Wright, 1922) was used to calculate deviations from Hardy-Weinberg equilibrium for each polymorphic locus within populations. Chi-square tests were also used to test heterogeneity in allele frequencies among populations (Workman and Niswander, 1970). Total genetic diversity (H_T), diversity within populations (H_S), and diversity among populations (G_{ST}, Nei, 1973; 1977) were calculated for each polymorphic locus using a computer program written by M.D. Loveless and A. Schnabel. Allele frequency data were

used to calculate Nei's (1972) genetic distance between populations. An UPGMA dendrogram was produced by POPGENE version 1.31 (Yeh *et al.* 1999) and was viewed with the TREEVIEW program (Page 1996).

RESULTS

Loci and alleles scored

Even though *A. tortilis* is a polyploid, inheritance patterns were consistent with diploid inheritance. Enzyme electrophoresis resulted in clear staining for nine enzyme systems encoded by sixteen putative loci (Table 1). Of the sixteen loci assayed, five (Pgm-1, Tpi-1, Tpi-3, Adh, and Idh-1) were monomorphic in all populations. A locus was considered polymorphic if two or more alleles were detected, regardless of their frequencies. Mdh was the most variable locus over populations with two or three alleles in all populations except population M1 and M6 which have four alleles, and population L4 which was fixed for the common allele. At six loci (Mnr, Tpi-2, Tpi-4, Dia-1, Dia-2, and Mdh) the frequency of the common allele was more than 90% in at least one population.

At all but one locus (Idh-2 in L6), the common allele was the same in every population. Two rare alleles, Pgm-2(1) and Tpi-2(2), occurred in one population only (L6 and M4, respectively). Another rare allele, Tpi-4(2), was seen in a single population in each wadi at a low frequency (0.056 in M4 and 0.027 in L1). Although a rare allele of Mdh (4) occurred in four populations in Wadi Mandar (M1, M3, M5, and M6), it was absent in Wadi Lithi. The total number of alleles at the sixteen loci was 36 ranging from 28 in populations L4 and L6 to 32 in populations M6 with a mean of 29.8.

Genetic diversity

Regional genetic diversity in *A. tortilis* was quantified using standard measures of genetic variation to estimate allelic diversity, levels of polymorphism, and heterozygosity (Table 2). They were estimated separately for Wadi Lithi and Wadi Mandar and for populations from both wadis collectively. At the species level, 68.8% of loci were polymorphic. Polymorphism was slightly higher in Wadi Mandar for both pooled and populations mean values (68.8% and 58.3%, respectively) than in Wadi Lithi (62.5% and 56.3%, respectively). The average number of alleles per locus (*A*) within each population ranged from 1.81 (M1, M2, and L6) to 2.06 (M6). While the overall population mean for *A* was almost the same (1.88) as that at Wadi Mandar (1.89) and Wadi Lithi (1.85), the pooled *A* value at the species level was higher (2.31) than at Wadi Mandar (2.19) and Wadi Lithi (2.13). The average number of alleles per polymorphic locus (*AP)* ranged from 2.27 (M4) to 2.89 (M6) with an overall population mean of 2.53. The populations mean of *AP* was equal in Wadi Mandar (2.53) and Wadi Lithi (2.52). The pooled *AP* value was somewhat higher at the species level (2.91) than at Wadi Lithi (2.80) and Wadi Mandar (2.73). The effective mean number of alleles per locus (A_e) ranged from 1.22 (M1) to 1.45 (L3) with a mean of

Table (2): Genetic diversity at the species, wadi, and population level for *Acacia tortilis* in southern Sinai. *T* = number of trees from which seeds were obtained, *N* = the number of seedlings assayed, *P* = % polymorphic loci, *AP* = mean number of alleles per polymorphic locus, *A* = mean number of alleles per locus, A_e = effective number of alleles per locus, H_o = observed heterozygosity, and H_e = unbiased heterozygosity expected under Hardy-Weinberg assumptions.

Population	*T*	*N*	*P*	*AP*	*A*	A_e	H_o (SD)	H_e (SD)
M1	17	43	56.3	2.44	1.81	1.22	0.153 (0.053)	0.143 (0.047)
M2	8	24	56.3	2.44	1.81	1.34	0.216 (0.068)	0.201 (0.051)
M3	21	54	56.3	2.56	1.88	1.30	0.176 (0.047)	0.161 (0.056)
M4	3	9	68.8	2.27	1.88	1.36	0.262 (0.120)	0.198 (0.046)
M5	21	63	56.3	2.67	1.94	1.37	0.222 (0.044)	0.201 (0.055)
M6	28	74	56.3	2.89	2.06	1.38	0.245 (0.039)	0.204 (0.057)
Population mean (SD)			*58.3 (5.01)*	*2.53(0.24)*	*1.89 (0.10)*	*1.33 (0.06)*	*0.211 (0.028)*	*0.185 (0.021)*
Pooled		*267*	*68.75*	*2.73*	*2.19*	*1.36*	–	*0.201*
L1	16	37	62.5	2.50	1.94	1.41	0.280 (0.053)	0.210 (0.059)
L2	5	15	56.3	2.33	1.75	1.41	0.272 (0.087)	0.214 (0.059)
L3	8	24	56.3	2.67	1.94	1.45	0.259 (0.071)	0.221 (0.062)
L4	5	15	50.0	2.50	1.75	1.40	0.247 (0.085)	0.210 (0.060)
L5	13	38	56.3	2.67	1.94	1.44	0.272 (0.053)	0.219 (0.062)
L6	3	9	56.3	2.44	1.81	1.38	0.252 (0.112)	0.200 (0.056)
Population mean (SD)			*56.3 (5.05)*	*2.52 (0.13)*	*1.85 (0.09)*	*1.42 (0.03)*	*0.264 (0.032)*	*0.212 (0.024)*
Pooled		*138*	*62.5*	*2.80*	*2.13*	*1.45*	–	*0.229*
*Mean (*SD)			*57.3 (*3.56)	*2.53 (*0.17*)*	*1.88 (*0.09)	*1.37 (*0.06)	*0.238 (*0.021)	*0.198 (*0.016)
Species pooled			*68.8*	*2.91*	*2.31*	*1.39*	–	*0.213*

1.39. Both pooled and population mean values were slightly higher at Wadi Lithi (1.45 and 1.42) than at the species level (1.39 and 1.37) and Wadi Mandar (1.36 and 1.33). Observed heterozygosity (H_o) ranged from 0.153 to 0.280 (mean = 0.238) and was higher than Hardy-Weinberg expectations in all populations (Table 2) suggesting an excess of heterozygotes within populations throughout the species. The population mean was higher at Wadi Lithi than that at Wadi Mandar. Expected heterozygosity within the 12 populations (H_e) ranged from 0.143 to 0.221 (mean = 0.198). The H_e values of pooled and population mean were higher at Wadi Lithi (0.229 and 0.212) and at the species level (0.213 and 0.198) than that at Wadi Mandar (0.201 and 0.185).

Genetic structure

Deviations from Hardy-Weinberg expectations (F_{IS}) were negative at all loci except Dia-2 (0.255) and Idh-2 (0.379) at Wadi Mandar, Idh-2 (0.166) at Wadi Lithi, and Dia-2 (0.075) and Idh-2 (0.299) at the species level (Table 3). Mean within population inbreeding coefficients (F_{IS}) were -0.108 ± 0.258 at Wadi Mandar populations, -0.211 ± 0.237 at Wadi Lithi populations, and -0.146 ± 0.237 at the species level. These negative values are due to an excess of heterozygote individuals in the studied populations.

Heterogeneity in allele frequencies among populations was highly significant (Table 3) for all polymorphic loci at Wadi Mander and across both wadis. However, significant heterogeneity in allele frequencies at Wadi Lithi only occurred at two of the 10 polymorphic loci (Mnr and Pgm-2).

Although populations differed significantly in allele frequencies, the proportion of total genetic variation found among populations (G_{ST}) was relatively low. While it ranged from 0.014 (Mdh) to 0.108 (Tpi-2) with a mean of 0.041 at Wadi Mandar, it ranged from 0.014 (Pgm-2) to 0.044 (Dia-2) with a mean of 0.029 at Wadi Lithi. At the species level, G_{ST} ranged from 0.02 (Mdh) to 0.11 (Tpi-2) with a mean of 0.044 indicating that about 95.6% of the allozyme variation occurred within populations (Table 3). Very little genetic differentiation was recorded between the two wadis (G_{STW} = 0.006) which means that most of the genetic differentiation is among populations within wadis (G_{STP} = 0.038).

Nei's (1972) genetic identities (I) and distances (D) were estimated between population pairs across the two wadis (Table 4). The lowest genetic identity value for any two populations compared in this study was 0.960 between L6 and M2, M4 and M6. The highest was 0.995 between M5 and M3. The average genetic identity didn't change much between the two wadis; it was 0.987 ± 0.005 for Wadi Mandar and 0.982 ± 0.010 for Wadi Lithi. The mean value for all populations was 0.983 ± 0.009. The lowest average identity value was recorded for L6 (0.967) and the highest for M2 (0.994). The UPGMA phenogram (Figure 2) based on Nei's genetic distances (D) didn't match the geographical relationships among populations of *A. tortilis* within the two wadis.

Discussion

The amount and distribution of genetic diversity within tree species is influenced by factors such as size, longevity, fecundity, breeding systems and geographic distribution (Hamrick and Godt 1989). Wind-pollinated outcrossing species with wide geographic ranges and long generation times, such as conifers, tend to have high variation, most of which occurs within populations. In contrast, annual herbaceous species that are primarily selfing have less genetic variation, with a much higher proportion occurring among their populations (Hamrick and Godt, 1989; Hamrick *et al.*, 1992).

While regional genetic diversity of *Acacia tortilis* subsp. *raddiana* herein is high (P = 68.8 %, H_{es} = 0.213)

Table (3): Levels of inbreeding within populations and genetic differentiation among populations, F_{IS} = inbreeding within populations, and G_{ST} = proportion of gene diversity among populations. χ^2 = Chi Square for allele frequency heterogeneity.

	Wadi Mandar			Wadi Lithi			Species level		
Locus	F_{IS}	G_{ST}	χ^2 (*df*)	F_{IS}	G_{ST}	χ^2 (*df*)	F_{IS}	G_{ST}	χ^2 (*df*)
Mnr	-0.165	0.031	22.40 (10)**	-0.262	0.029	21.38 (10)**	-0.206	0.040	64.69 (22)****
Pgi-2	-0.231	0.020	24.72 (10)***	-0.315	0.039	15.60 (10)	-0.260	0.028	47.37 (22)****
Pgm-2	-0.371	0.018	38.19 (10)****	-0.420	0.014	37.79 (15)****	-0.394	0.041	137.84 (44)****
Fe-1	-0.554	0.040	21.16 (5)****	-0.704	0.032	8.65 (5)	-0.608	0.041	32.87 (11)****
Fe-3	-0.115	0.017	20.68 (10)**	-0.198	0.025	14.42 (10)	-0.145	0.021	38.17 (22)****
Tpi-2	-0.125	0.108	57.55 (5)****	0.000	0.000	0.00 (0)	-0.125	0.109	88.22 (11)****
Tpi-4	-0.059	0.054	28.72 (5)****	-0.028	0.020	5.50 (5)	-0.038	0.033	26.69 (11)***
Dia-1	-0.165	0.029	13.17 (5)**	-0.161	0.036	9.93 (5)	-0.163	0.035	25.08 (11)***
Dia-2	0.255	0.079	82.91 (10)****	-0.129	0.044	16.38 (10)	0.075	0.067	114.3 (22)****
Idh-2	0.379	0.038	27.71 (10)****	0.166	0.034	15.34 (10)	0.299	0.048	59.94 (22)****
Mdh	-0.040	0.014	33.78 (15)****	-0.064	0.015	13.43 (10)	-0.045	0.020	68.01 (33)****
Avg. (SD)	-0.108 (0.258)	0.041 (0.029)	-	-0.211 (0.237)	0.029 (0.0130)	-	-0.146 (0.237)	0.044 (0.025)	-

Significance level: * P < 0.05, ** P <0.025, *** P <0.01, and **** P <0.005.

Table (4): Estimates of Nei's (1972) mean genetic distance (lower triangle) and identity (upper triangle) between 12 populations of *Acacia tortilis*.

Population	M1	M2	M3	M4	M5	M6	L1	L2	L3	L4	L5	L6
M1	-	0.994	0.985	0.984	0.986	0.981	0.979	0.982	0.970	0.983	0.974	0.965
M2	0.006	-	0.988	0.982	0.990	0.993	0.986	0.985	0.981	0.983	0.982	**0.960**
M3	0.015	0.012	-	0.978	**0.995**	0.990	0.989	0.987	0.991	0.991	0.985	0.972
M4	0.016	0.018	0.022	-	0.983	0.984	0.972	0.978	0.970	0.986	0.978	**0.960**
M5	0.014	0.011	0.005	0.017	-	0.995	0.994	0.988	0.988	0.989	0.988	0.974
M6	0.019	0.007	0.010	0.017	0.005	-	0.991	0.986	0.988	0.987	0.989	**0.960**
L1	0.022	0.015	0.011	0.029	0.006	0.010	-	0.986	0.991	0.985	0.991	0.975
L2	0.019	0.015	0.013	0.022	0.013	0.014	0.014	-	0.987	0.988	0.987	0.962
L3	0.031	0.019	0.009	0.031	0.012	0.012	0.009	0.013	-	0.986	0.993	0.969
L4	0.018	0.017	0.009	0.015	0.011	0.013	0.015	0.012	0.014	-	0.990	0.976
L5	0.026	0.018	0.015	0.022	0.012	0.011	0.009	0.013	0.007	0.011	-	0.968
L6	0.035	0.041	0.028	0.041	0.027	0.041	0.025	0.039	0.031	0.024	0.032	-
Average identity	*0.987*	*0.994*	*0.986*	*0.981*	*0.989*	*0.989*	*0.985*	*0.984*	*0.983*	*0.986*	*0.986*	*0.967*
Average distance	*0.020*	*0.016*	*0.014*	*0.023*	*0.012*	*0.015*	*0.015*	*0.017*	*0.017*	*0.014*	*0.016*	*0.033*

relative to other plant species, (P = 50.5%, H_{es} = 0.149, Hamrick and Godt, 1989), these values are moderate compared to other acacia species (e.g. Coates, 1988; Moran *et al.*, 1989; McGranahan *et al.*, 1997; and Varghese *et al.*, 1999). From a RAPD analysis of genetic diversity of *A. tortilis* subsp. *raddiana* populations from the Negev desert, Shrestha *et al.* (2002) showed that 90.7% of the loci were polymorphic. However, genetic diversity can vary markedly depending on the molecular marker employed (Moran *et al.*, 2000). In *A. mangium* more variation was detected with restriction fragment length polymorphism (RFLP) markers than with allozymes and the highest variation was detected by microsatellites (Butcher *et al.*, 1998; Moran *et al.*, 2000). Similarly, in *Eucalyptus nitens*, RFLP variation was much higher than allozyme variation (Byrne *et al,.* 1998). According to the observed concordance of geographic patterns of differentiation across marker types, Glaubitz and Moran (2000) argue that broad-scale marker-based assessment of genetic resources for conservation is best done with allozymes which remain the most cost effective and efficient available markers.

A small proportion (4.4%) of the genetic variation in *A. tortilis* from the Sinai, in the present study, occurs among populations. Values for other acacias (e.g. Coates, 1988; Moran *et al.*, 1989; McGranahan *et al.*, 1997; and Varghese *et al.*, 1999) range widely but most are higher than the value got from this study. Furthermore, differentiation among the Sinai populations is much lower than at value (59.4%) found among populations of *A. tortilis* growing in the Negev and Arava valley deserts (Shrestha *et al.*, 2002). However, much of the interpopulation variation they observed was among geographic regions (G_{ST} = 0.367) while G_{ST} among populations within regions was 0.227 only which is still much higher than that observed in the Sinai populations. To explain their results Shrestha *et al.* (2002) proposed that *A. tortilis* in the two geographic regions had resulted from separate colonization events. Most acacias are primarily insect-pollinated but it is not known how much pollen dispersal there is between populations and what role it plays in their genetic differentiation (Moran, 1992). The results herein may also reflect the historical population structure of *A. tortilis* in the study area. Previously, *A. tortilis* probably existed as a continuous array of subpopulations between which high levels of gene flow could have occurred. Due to the relatively small distances between populations in this study compared to studies done on other acacias, we might expect less genetic differentiation. As a result, comparisons are difficult and should be done cautiously.

Genetic distance (D) between populations may reflect recent fragmentation and the longevity of the surviving trees. The genetic distance values observed are similar to those found between conspecific populations of many plant species (Crawford, 1983). The low degree of differentiation was reflected in mismatching between the UPGMA phenogram and the geographic relationships among populations of *A. tortilis* within the two wadis. Recent fragmentation and low effective sizes for many of the surviving populations may also explain the UPGMA mismatching.

Since most of the genetic diversity is contained within populations of *A. tortilis*, the priority of *in situ* conservation should be to conserve a few large well-distributed populations representing different geographic regions. Small fragmented populations are more prone to extinction from random environmental fluctuations and may lose genetic variability due to genetic drift. Based on the presence of rare alleles and higher genetic diversity, populations M4 and M6 should get the first priority for conservation at Wadi Mandar and L6 and L3 at Wadi Lithi. Although the UPGMA phenogram didn't correspond to the geographical relationships among populations, it implies that population L6 (the most distant) should get the top priority of conservation efforts at the two wadis due to its unique genetic composition. Also, it may be

desirable to establish *ex situ* conservation populations to ensure the preservation of genetic resources and to meet the objectives of commercial plantations (e.g. as fodder or fuel wood) and/or of different environments. Since this species is predominantly outcrossing (Zaghloul and Hamrick, unpublished data), sampling large numbers of pods from relatively few individuals from as many populations as possible will result in a genetically diverse sample, preserving the evolutionary potential of the species.

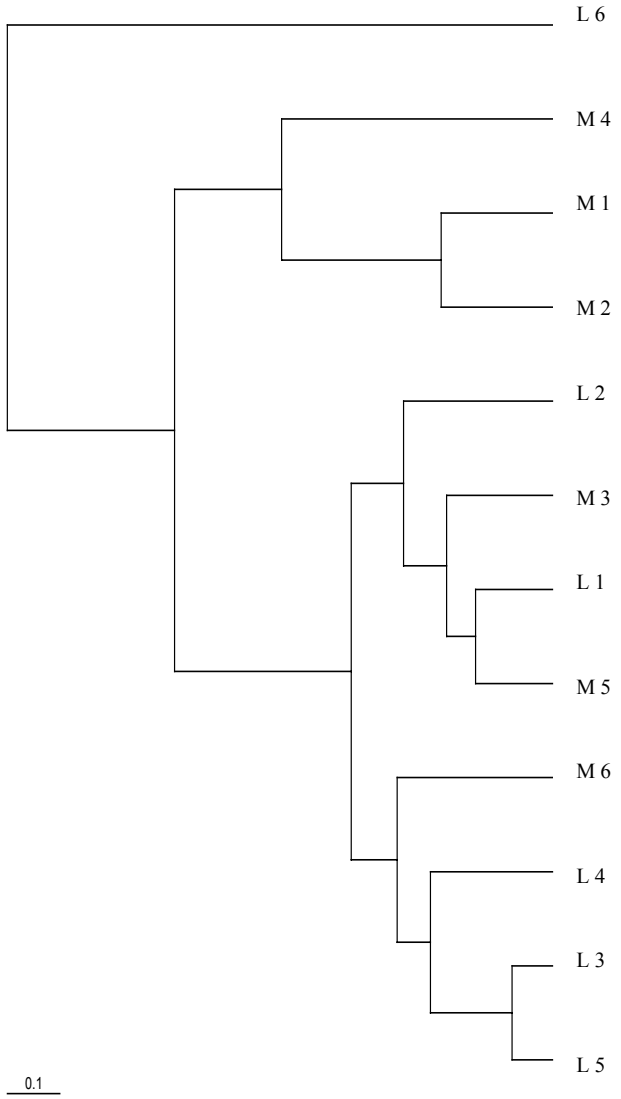

Figure (2): UPGMA clustering based on Nei's genetic distances (D) estimated for 12 populations of *Acacia tortilis* in Southern Sinai.

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صون مجتمعات السيال *Acacia tortilis* subsp. *raddiana* فى جنوب سيناء، مصر
1- التنوع والتركيب الوراثى

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الملخص العربـــى

توفر أشجار السيال الغذاء والمأوى للحيوانات فى الصحارى. كما يستخدمها السكان المحليون من البدو فى العديد من الاغراض الأخرى، منها على سبيل المثال إستخدامها كوقود وإستخدامها كدعامات فى بناء منازلهم. ولقد لوحظ فى السنوات الاخيرة إرتفاع معدلات الوفاة للأفراد الكبيرة وإنخفاض معدلات تثبيت أفراد صغيرة. وفى هذا إشارة إلى أن هذه الشجرة تتعرض للعديد من التهديدات التى قد تؤدى إلى إنقراض عشائرها، وهو ما جعل لها أهمية كبيرة فى برامج صون الأنواع فى المنطقة. لذا تهدف هذه الدراسة إلى تقدير التنوع والتركيب الوراثى لعشائر أشجار السيال فى جنوب سيناء والتى بدورها تساهم فى تحديد الأنشطة المطلوبة لبرامج صون هذا النبات. ولتحقيق هدف الدراسة تم رصد ستة عشر إنزيمات أليلية allozymes لإختبار التنوع الوراثى داخل وبين إثنا عشر عشيرةً ممثلة للنطاق الجغرافى لأشجار السيال *A. tortilis* فى واديان كبيران من أهم أودية جنوب سيناء.

ولقد أظهرت النتائج أن التنوع الوراثى لأشجار السيال (H_e = 0.213) كبيراً بالمقارنة ليس فقط بالتقديرات الأخرى لأشجار ال *Acacia* ولكن أيضاً للنباتات عموماً، وأن النسبة الأكبر (96%) فى هذا التنوع موجود داخل االعشائر مما يدل على أن هذا النوع كان له معدل تدفق جينى كبير فى النطاق الجغرافى المدروس. ومن ناحية أخرى لم يطابق UPGMA phenogram البعد الوراثى لعشائر السيال مع التوزيع الجغرافى لهذه العشائر.

تشير نتائج الدراسة من إرتفاع معدلات التدفق الجينى التاريخى بين عشائر السيال بالمنطقة مع الأخذ فى الإعتبار طول عمر هذه الاشجار إلى أن التفتت الحالى لعشائر السيال قد حدث مؤخرا مما يؤكد وجود ضغط تدميرى على هذه العشائر يهدد (فى حالة إستمراره) بقاء هذه العشائر بقاءً حيوياً.