Fitness of Three Ballota Species (Lamiacea) Growing in Saint Catherine Protectorate, Egypt.

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ABSTRACT

Ballota undulata, B. kaiseri, and *B. saxatilis* are very rare species grow in small-sized populations. As small populations tend to be subject to an increased probability of stochastic extinction due to demographic, environmental and genetic factors, this study aimed to figure out if *Ballota* populations suffer reduced fitness and its correlation or association with genetic diversity and mating parameters. Also, it aimed to investigate if there is significant difference in fitness between the three species of *Ballota*. Correlations and association between fitness, population size, genetic variation, and mating system parameters were tested using Spearman correlation and simple regression analyses. Stepwise regression analyses were used to identify useful predictors for fitness. The results revealed that *Ballota* populations' fitness is generally low to very low with no significant difference between the three *Ballota* species. There was no detected relationship between *B. undulata* fitness or population size and observed or expected heterozygosity. While there was no evidence for reduced fitness in *Ballota* populations, the study indicated that fitness is negatively associated with single locus outcrossing rate (t_s) implying that *Ballota* populations are locally adapted. Population size was associated only with the number of alleles (A) and number of alleles per polymorphic locus (A_P).

Keywords: *Ballota*, Sinai, endangered species, fitness, restoration.

INTRODUCTION

In recent decades, as a result of habitat destruction and fragmentation, many species have undergone severe reductions in population sizes (Saunders *et al.*, 1991; Jennersten *et al.*, 1992; Aizen and Feinsinger, 1994). Reduced fitness because of a loss of genetic variation is often considered factor that may increase the probability of extinction of small, isolated populations (Menges, 1992; Widén, 1993; Van Treuren *et al.*, 1993a; Heschel and Paige, 1995). Therefore, the influence of inbreeding and loss of genetic diversity on fitness has become a major topic in conservation biology (Barrett and Kohn, 1991; Hedrick and Miller, 1992). In plant populations limited dispersal of both seeds and pollen often leads to population substructure that makes selfing or inbreeding with close relatives more likely (Wright, 1943; Campbell and Waser, 1987; Dudash, 1990; Waser, 1993). In small isolated populations the limited number of mating partners and reduced levels of genetic variation as a result of genetic drift may further increase the likelihood of inbreeding (Lacy, 1987; Ellstrand and Elam, 1993). Plants in large populations and populations that have experienced reductions in the number of individuals only recently may show reduced fitness after inbreeding, whereas individuals of populations that have been small during several generations may exhibit a much lower response to inbreeding because of purging of the genetic load (Van Treuren *et al.*, 1993b).

Germination differences among populations could well reflect inbreeding effects, and germination differences among individuals have important fitness consequences (Menges, 1995). Population differences in seed germination percentages and populations' responses to environmental conditions have been documented for many species (Farmer *et al.* 1986; Wu *et al.* 1987; Meyer *et al.* 1989), but to consider the possible effects of fragmentation-caused reduced genetic variation, these germination differences must be correlated with population size and isolation.

In this study, we address three questions; 1) do small, isolated *Ballota* populations suffer reduced fitness (seed set, germination, and/or establishment), 2) is there any correlation or association between fitness and both genetic diversity and mating parameters in *Ballota* populations, and 3) are there significant differences in fitness between the three species of *Ballota*.

MATERIALS AND METHODS

Sampling and seed set estimation

Seeds were collected from 337 individuals representing 14 *Ballota undulata* (Sieb. ex Fresen.) Bentham (= *Marrubium undulatum* Sieb. ex Fresen) (297 plants) populations, one *B. kaiseri* Täckh. (21 plants), and another *B. saxatilis* C. Frest (19 plants) populations in 10 localities in Saint Catherine Protectorate, south Sinai, Egypt (Fig.1). Seeds were stored under laboratory conditions in paper bags until they were taken out of their dried calyx where they were stored in plastic bags in the dark until germination. UArbHe, KArHe, and SArHe are three populations represent the three studied *Ballota* species growing in the same habitat conditions in the same locality (Abu Hemat Gorge in Wadi El-Arbaie'en). In these populations environmental component is ignored in consideration of differences might be figured out in seed setting, germination, and/or establishment. In other words, any difference in fitness characters in these populations would be primarily due to genetic variation.

For an herbaceous species survives in its natural habitat under heavy stresses, especially sever grazing (like *Ballota*), it is practically almost impossible to get a direct measurement for the plant seed production without a pretty high ambiguity. However, an estimation of seed production (seed set) in *Ballota* could be calculated by the following equation:

Seed production per plant = seeds per fruit X no. of flowers per verticellaster X no. of verticellaster per branch X no. of branches per plant.

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Figure (1): Location of sampled *Ballota* populations

All the independent variables in this equation except seeds per fruit are traits basically been affected by sever grazing in the area and environmental stochasticity. Number of seeds per fruit is the only independent variable that could be accurately estimated and used as indicator for the *Ballota* plants (or populations) seed production. So, sound seeds were counted in five replicates of ten ripe fruits from individuals in each population to estimate the mean number of seeds per fruit as a reference for the actual seed set. The mean numbers of seeds per fruit for each individual were used to calculate the mean and standard deviation for each population.

Germination and establishment

Seeds were germinated on cotton pads in Petri dishes in a growth chamber under $15^{o}C/5^{o}C$ day/night (12h) regime. After germinating, the Petri dishes were moved into a greenhouse for five to seven days. Then, the seedlings were transplanted to Fafard #3B mix. Seedlings were considered to be established when they formed leaves and survived for at least a month after transplanting. The mean germination and establishment percent were calculated and used to calculate the population mean and standard deviation.

Early-stage fitness index

Seed production, germination, and establishment percentages are the main and the most important factors in life history of higher plant affecting its early survivorship especially in harsh desert environments. Since fitness is the probability of a plant to survive until the reproductive age and gives offspring, it is a net function of the total number of seeds that the mother plant produces, the probability of germination and producing seedlings, and the probability of these seedlings to grow ahead of seedling (juvenile) stage and get established (adult).Therefore, seed set, germination, and establishment percentages could be used to construct the early-stage fitness index as follows:

Fitness = seed set X germination% X establishment%

Data Analyses

Statistical analyses of fitness parameters and fitness index were performed using Minitab release 13 computer program. One-way ANOVA or Kruskal-Wallis was used to test for significant differences between treatments, populations, and species. Kruskal-Wallis test was used when an ANOVA could not be used due to a violation of its assumptions, normality and/or variance homogeneity. Correlations between fitness parameters and genetic variation and mating system parameters (Data from Zaghloul, 2003) were tested using a Spearman correlation analysis. Association between population size

and genetic variation, mating system, and fitness characters were analyzed with simple regression analysis. Stepwise regression analyses were used to identify a useful subset of the predictors (genetic and mating system parameters) for the investigated independent parameter (fitness characters, and fitness index).

RESULTS

Seed Set

Although the fruit in Lamiaceae is a tetranucule when completely developed, most of the *Ballota* fruits had either one seed or none except very few cases where the fruit had two or three seeds. The number of developed nutlets (seeds)/fruit was very highly significantly different ($P \le 0.001$) between *Ballota undulate* populations (Table 1) and ranged from 0.32 (UToboq) to 0.82 (UFaraa) with a mean over all populations of 0.64. It was significantly higher than zero in all populations except UArbHe and UAsbai. In UToboq and UTalHu the mean of seed set was significantly lower than one seed per fruit. Seed set was significantly and positively correlated with germination percentage in *B. undulata* populations (Table 2).

Although Spearman correlation (Table 2) showed that there is no association between seed set and any of the genetic or mating system parameters in *Ballota undulata* populations, the simple regression (Table 3) associated seed set with both Parental F (+ve) and single locus outcrossing (t_s, -ve). The stepwise regression equation (Table 4) indicated the single locus outcrossing rate (t_s, -ve), number of alleles per polymorphic loci (A_P, +ve), polymorphic loci (A_P, +ve), and biparental inbreeding ($t_m - t_s$, -ve) are the most useful variables for predicting seed set in *B. undulata* populations.

Seed set was not significantly different (Table 5) between *Ballota undulata*, *B. kaiseri*, and *B. saxatilis* populations living under the same ecological conditions (i.e. in Abu Hemat gorge).

Table (1): Population size, means and standard deviations of fitness characters in *Ballota undulata* populations.* $P \le 0.05$,*** $P \le 0.001$
  *Abbr.=Abbreviation

Species	Population	Abbr.	Pop. size	Seed set (SD)	Germination% (SD)	Establishment % (SD)	Fitness index (SD)
	W. El-Arbaie'en (the mouth)	UArbMo	42	0.673 (0.283)	38.244 (25.717)	83.049 (26.878)	0.187 (0.175)
	W. El-Arbaie'en (Abu Hemat Gorge)	UArbHe	17	0.596 (0.373)	42.936 (26.019)	71.999 (35.890)	0.206 (0.253)
	W. El-Arbaie'en (Ramadan Garden)	UArbRa	13	0.730 (0.253)	32.935 (27.640)	50.419 (31.680)	0.124 (0.104)
	W. El-Arbaie'en (El-Deir Garden)	UArbDi	41	0.606 (0.205)	29.865 (26.586)	64.089 (39.889)	0.132 (0.142)
	W. El-Arbaie'en (El-Sarw Garden)	UArbSa	25	0.684 (0.202)	32.656 (18.579)	76.412 (30.406)	0.153 (0.112)
Ballota undulata	W. El-Ahmar	UAhmar	17	0.462 (0.170)	16.743 (10.563)	81.699 (25.605)	0.059 (0.050)
	W. El-Shraiq (mouth of W. El-Fara'a)	UFaraa	38	0.817 (0.355)	35.279 (19.846)	65.886 (28.586)	0.199 (0.141)
	Shaq Mousa	UShaMo	13	0.636 (0.214)	26.068 (19.997)	82.629 (29.127)	0.124 (0.087)
	W. El-Tofahah	UTofah	17	0.747 (0.196)	24.079 (24.160)	51.288 (42.886)	0.144 (0.178)
	W. El-Deir	UDier	15	0.792 (0.273)	40.861 (26.610)	68.207 (35.890)	0.213 (0.188)
	W. El-Asbaei'a	UAsbai	16	0.525 (0.355)	29.780 (26.202)	67.051 (43.390)	0.078 (0.151)
	W. Toboq	UToboq	13	0.318 (0.103)	13.811 (7.111)	77.343 (36.895)	0.036 (0.030)
	Mt. Mousa (Stairs way)	UMousa	16	0.818 (0.277)	53.589 (19.743)	88.866 (13.035)	0.354 (0.239)
	W. El-Tala'a (Hussien El- Hashash Garden)	UTalHu	14	0.543 (0.195)	16.649 (22.176)	68.313 (40.596)	0.087 (0.121)
Total	*14 populations*		297	**0.642 (0.147)***	**30.964 (11.118)***	**71.232 (11.437)***	**0.150 (0.081)***

Table (2): Spearman rank correlations between different fitness-related characters and population size, population genetics, and mating system parameters. Rho value is corrected for ties, * $P \le 0.05$

	Fitness index		Population size		Seed set		Germination		Establishment	
	Rho	t	Rho	T	Rho	t	Rho	t	Rho	t
Populating size	0.3796	1.3687	-	-	-	-	-	-	-	-
Seed set	-	-	0.1865	0.6723	-	-	-	-	-	-
Germination	-	-	0.3263	1.1766	0.6659	2.4011*	-	-	-	-
Establishment	-	-	-0.0133	-0.0480	-0.1473	-0.5309	0.1077	0.3883	-	-
P	0.0844	0.3045	0.4489	1.6187	-0.1889	-0.6811	0.1667	0.6010	0.0933	0.3365
A_p	0.6968	2.5124*	0.4020	1.4495	0.4895	1.7650	0.6902	2.4885*	0.1654	0.5963
A	0.3743	1.3496	0.5089	1.8350	0.1041	0.37753	0.4164	1.5014	0.1262	0.4552
A_e	0.3278	1.1819	0.1331	0.4799	0.0687	0.2476	0.3123	1.1260	0.0620	0.2236
H_o	0.1648	0.5943	0.1465	0.5283	-0.1604	-0.5784	0.1209	0.4358	0.2088	0.7528
H_e	0.0989	0.3565	0.0644	0.2321	-0.1297	-0.4675	0.1165	0.4110	-0.0154	-0.0555
Parental F	0.3454	1.2454	0.3133	1.1298	0.5369	1.9357	0.2376	0.8568	-0.2068	-0.7457
t_m	0.0682	0.2459	0.0333	0.1202	-0.4312	-1.5549	0.0484	0.1745	0.1584	0.5712
t_s	-0.4945	-1.7830	0.2286	0.8244	-0.7363	-2.6546	-0.5077	-1.8305	0.1868	0.6736
t_m-t_s	0.4022	1.4501	-0.4218	-1.5207	0.1473	0.5309	0.3846	1.3868	0.2044	0.7370
Correlation of P	-0.0821	-0.2962	0.1683	0.6068	-0.3638	-1.3116	-0.0446	-0.1608	0.4999	1.8024

Fitness of *Ballota sp.* Growing in Saint Catherine Protectorate, Egypt.

Table (3): Simple linear regression results and their ANOVA. * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$

Character	Parameter	Equation	S	R-Sq	R-Sq (adj)
Seed set	Ap	Seed set = -0.786 + 0.568 Ap	0.131	0.238	0.175
	A	Seed set = 0.511 + 0.058 A	0.1495	0.006	0.000
	A_e	Seed set = 0.62 + 0.017 A_e	0.1499	0.000	0.000
	H_o	Seed set = 0.722 - 0.54 H_o	0.1491	0.011	0.000
	H_e	Seed set = 0.722 - 0.45 H_e	0.1493	0.008	0.000
	Parental F	Seed set = 0.756 + 0.283 F	0.1109	0.453**	0.408
	t_m	Seed set = 0.809 - 0.174 t_m	0.1454	0.059	0.000
	t_s	Seed set = 1.633 – 1.261 t_s	0.105	0.510**	0.469
	t_m-t_s	Seed set = 0.633 + 0.033 t_m-t_s	0.15	0.002	0.000
	Correlation of P	Seed set = 0.949 - 0.327 r_P	0.1482	0.023	0.000
Germination	Ap	Germination = -99.0 + 51.8 Ap	9.455	0.332*	0.277
	A	Germination = -15.4 + 21.0 A	10.73	0.140	0.069
	A_e	Germination = -68.0 + 76.7 A_e	10.93	0.108	0.034
	H_o	Germination = 14.5 + 108 H_o	11.13	0.075	0.000
	H_e	Germination = 14.4 + 91 H_e	11.26	0.054	0.000
	Parental F	Germination = 36.2 + 12.7 F	10.65	0.153	0.083
	t_m	Germination = 23.3 + 7.8 t_m	11.45	0.020	0.000
	t_s	Germination = 90.1 – 74.9 t_s	9.666	0.302*	0.244
	t_m-t_s	Germination = 27.0 + 20.6 t_m-t_s	10.75	0.136	0.064
	Correlation of P	Germination = 40.4 – 9.9 r_P	11.55	0.004	0.000
Establishment	Ap	Establishment = 29.0 + 16.8 Ap	11.71	0.033	0.000
	A	Establishment = 41.7 + 13.4 A	11.58	0.054	0.000
	A_e	Establishment = 4.0 + 52.1 A_e	11.62	0.047	0.000
	H_o	Establishment = 56.2 + 99 H_o	11.54	0.060	0.000
	H_e	Establishment = 60.4 + 59 H_e	11.77	0.022	0.000
	Parental F	Establishment = 68.4 – 6.78 F	11.66	0.041	0.000
	t_m	Establishment = 65.4 + 5.9 t_m	11.84	0.011	0.000
	t_s	Establishment = 53.7 + 22.2 t_s	11.75	0.025	0.000
	t_m-t_s	Establishment = 70.8 + 2.4 t_m-t_s	11.89	0.002	0.000
	Correlation of P	Establishment = 11.0 + 63.5 r_P	11.09	0.140	0.068
Fitness index	Ap	Fitness = -0.746 + 0.365 Ap	0.078	0.265	0.204
	A	Fitness = -0.116 + 0.129 A	0.087	0.085	0.009
	A_e	Fitness = -0.508 + 0.524 A_e	0.087	0.081	0.005
	H_o	Fitness = 0.083 + 0.561 H_o	0.09	0.033	0.000
	H_e	Fitness = 0.079 + 0.493 H_e	0.09	0.025	0.000
	Parental F	Fitness = 0.214 + 0.109 F	0.083	0.180	0.112
	t_m	Fitness = 0.173 - 0.004 t_m	0.091	0.000	0.000
	t_s	Fitness = 0.661 - 0.624 t_s	0.074	0.338*	0.282
	t_m-t_s	Fitness = 0.150 + 0.100 t_m-t_s	0.089	0.052	0.000
	Correlation of P	Fitness = 0.137 + 0.034 r_P	0.091	0.001	0.000
Population size	Ap	Ap = 2.30 + 0.008 pop. size	0.083	0.613**	0.570
	A	A = 1.87 + 0.014 pop. size	0.128	0.651**	0.612
	A_e	A_e = 1.26 + 0.001 pop. size	0.44	0.050	0.000
	H_o	H_o = 0.134 + 0.001 pop. size	0.022	0.087	0.000
	H_e	H_e = 0.168 + 0.000 pop. size	0.024	0.051	0.000
	Parental F	Parental F = -0.432 + 0.005 pop. size	0.288	0.042	0.000
	t_m	t_m = 0.828 + 0.002 pop. size	0.050	0.267	0.185
	t_s	t_s = 0.711 + 0.003 pop. size	0.076	0.211	0.123
	t_m-t_s	t_m-t_s = 0.117 + 0.001 pop. size	0.056	0.025	0.000
	Correlation of P	r_P = 0.922 + 0.001 pop. size	0.070	0.045	0.000

Seed Germination

The mean germination percent of *B. undulata* populations ranged from 13.8% (UToboq) to 53.59% (UMousa) with a mean of 30.96% (Table 1). The difference between populations was very highly significant ($P \leq 0.001$). Although all the populations except UMousa are not significantly higher than zero, the UMousa population was significantly higher than 14%.

All populations are significantly less than 94%. Germination was found to be significantly ($P \leq 0.05$) and positively correlated with seed set and number of alleles analysis revealed that germination of *B. undulata* is significantly ($P \leq 0.05$) associated with both A_P and single locus outcrossing rate (t_s) (Table 3). Also the stepwise regression analysis indicated both parameters (A_P and t_s) as the most useful predictors for germination (Table 4).

Although the mean germination percent was higher in *Ballota saxatilis* population at Abu Hemat gorge (SArHe, 39.4%) than *B. undulata* (UArbHe, 42.9) and *B. kaiseri* (KArHe, 33.7%), this difference was not significant and all the three germination percentages were not significantly higher than zero (Table 5)**.**

Establishment

Establishment percent has the least significant ($P \leq 0.05$) difference among *B. undulata* populations in the three fitness characters. It ranged from 50.42% (UArbRa) to 88.87% (UMousa) with a mean of 71.23%. It was not significantly higher than zero in six populations (UArbRa, UArbDi, UTofah, UDier, UAsbai, and UTalHu) out of fourteen *B. undulata* populations (Table 1). The UMousa population had the highest establishment percent as well as the lowest standard deviation and hence it was the only population with an establishment percent significantly higher than 63%.

Establishment percent has no correlation (Table 2) or association (Table 3) with any genetic or mating system parameter or any of other fitness characters. So, there was not any useful predictors indicated by stepwise multiple regression (Table 4).

Ballota undulata, B. kaiseri, and *B. saxatilis* are highly significantly ($P \leq 0.01$) different in establishment (Table 5) with *B. undulata* having the lowest percent (71.1%), been followed by *B. kaiseri* (80.20%), and *B. saxatilis* (96.5%). While both *B. undulata* and *B. kaiseri* establishment percentages have high standard deviations which made these percentages not significantly higher than zero, *B. saxatilis* has a relatively very low standard deviation made the establishment percent significantly higher than 78.8%.

Early-stage fitness

The early stage fitness index was very highly significantly ($P \leq 0.001$) different between *B. undulata* populations (Table 1). UToboq was the population has the lowest fitness (0.036) which is significantly lower than 0.01, while UMousa population has the highest fitness (0.354). The mean fitness for all populations was 0.15. All the populations had a fitness index not significantly higher than zero.

While the Spearman correlation revealed that fitness index is positively ($P \leq 0.05$) correlated with number of alleles per polymorphic locus (Table 2), the simple regression analysis associated it with single locus outcrossing rate (t_s) negatively (Table 3). These two parameters (A_P and t_s) were indicated in the stepwise regression to be the useful predictors for fitness index (Table 4). A_P (+ve) and t_s (-ve) affect the fitness index through their effect on both seed set and germination

Although there was a highly significant ($P \leq 0.01$) difference between *Ballota undulata* (UArbHe), *B kaiseri* (KArHe), and *B. saxatilis* (SArHe) in

Table (4): Stepwise regression

Character	Stepwise Equation	S	R-Sq	R-Sq (adj)
Seed set	Seed set = -0.0333 − 1.35 t_s + 0.71 A_P - 0.24 t_m-t_s	0.068	0.829	0.777
Germination	Germination = -39.42 + 51 A_P − 74 t_s	7.35	0.630	0.563
Establishment	No variables entered or removed			
Fitness index	Fitness = -0.2491 − 0.62 t_s + 0.36 A_P	0.060	0.598	0.525

Table (5): Difference between the *Ballota undulata, B. kaiseri,* and *B. saxatilis* populations growing in Abu Hemat gorge. Kruskal-Wallis test was used to test of significant difference between the three species except when ANOVA was validated(§). Data represented as Mean (SD) ** $P \leq 0.01$, *** $P \leq 0.00$.

Parameter	*Ballota undulate*	*Ballota kaiseri*	*Ballota saxatilis*
Population size	17	21	19
Volume (cm^3)*	10,328 (9,162)	57,110 (87,611)	94,965 (103,886)
Shoot length (cm) §*	22.19 (7.59)	31.89 (13.95)	35.24 (11.77)
Seed set (seeds/fruit)§	0.596 (0.374)	0.588 (0.263)	0.539 (0.232)
Germination§	42.94 (26.02)	33.69 (20.44)	39.37 (21.05)
Establishment	71.10 (35.89)	80.20 (37.55)	96.51 (9.02)
Fitness index	0.21 (0.25)	0.203 (0.220)	0.197 (0.153)

establishment percent, and a non significant yet large difference in germination percent there was no significant difference in fitness index between the three populations and they were all not significantly higher than zero.

Population size

Simple regression analysis significantly associated ($P \leq 0.005$) the number of alleles per polymorphic locus (A_p) and number of alleles (A) with *B. undulata* population size while Spearman test had not correlated it with any parameter.

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DISCUSSION

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Small populations tend to be subject to an increased probability of stochastic extinction, due to demographic, environmental and genetic factors (Shaffer, 1981; and 1987). It has been argued that genetic problems may often be secondary to the demographic problems of small populations and that extinction may occur before genetic problems become evident (Lande and Barrowclough, 1987; Lande, 1988; and Menges, 1991).However, Schemske *et al.* (1994) showed how within-population dynamics are affected by ecological and genetic attributes. The ecological and genetic characteristics of populations may influence vital rates (birth, growth, and death) and the resulting population size in turn impacts the genetic and demographic composition of the population. Population genetic processes can influence vital rates through changes in the number and organization of alleles within and among individuals.

Theory predicts that high levels of inbreeding and genetic drift will occur in small populations, and that these processes will, respectively, lead to increased homozygosity and the random loss of alleles. In species with a naturally restricted distribution, heterozygote deficits have been found in many plant populations (e.g. Godt *et al.*, 1996). In *Ballota undulata* there was little decrease in observed heterozygosity (Zaghloul *et al.*, 2006). A decrease in observed heterozygosity through inbreeding and loss of genetic diversity seems less likely in *Ballota* as a long-lived perennial. Also, mating system analysis revealed that *Ballota* is highly outcrossing plant (Zaghloul, 2003). Furthermore, it is probable that the small isolated populations of *Ballota* seen today are a rather recent occurrence.

In this study there is no detected relationship between *Ballota undulata* fitness and heterozygosity suggesting that inbreeding and genetic drift have not yet had the chance to act to reduce diversity in small populations. Also, we found no correlation between population size and observed or expected heterozygosity. Positive correlations have frequently been found between individual heterozygosity and fitness components (Mitton and Grant, 1984; Allendorf and Leary, 1986; Zouros, 1987; Bush *et al.*, 1987; and Wolff and Haeck, 1990). Koehn *et al.* (1988) and Booth *et al.* (1990) reported that such relationships could not be demonstrated. The relationship between heterozygosity

and fitness traits seems to be restricted to growth rate and developmental homeostasis of some characters and is only occasionally reported for other fitness characters (Ouborg and Van Treuren, 1995).

The only association was found between population size and gene diversity was between population size and both the number of alleles (A) and number of alleles per polymorphic locus (A_P) as might be expected. The relationship between population size and the amount of genetic variation within populations has been extensively studied and it has frequently been found that small plant populations exhibit lower allelic diversity (Moran and Hopper, 1983; Lesica *et al.*, 1988; Billington Hopper, 1983; Lesica *et al.*, 1988; Billington, 1991; and Van Treuren *et al.*, 1991).c

A causal relationship between the population size of *Ballota undulata*, and offspring fitness was not observed, this was not unexpected since although the fitness traits (seed set, germination, establishment, and fitness index) were significantly different among populations, none of these differences was related to population size. Less attention has been given in the literature to the possible relationship between population size and fitness (Menges, 1992; Widén, 1993; Ellstrand and Elam, 1993; and Hauser and Loeschcke, 1994). Ouborg and Van Treuren (1995) found no relationship between the proportion of nonviable seeds and population size for *Salvia pratensis*. In contrast, lower seed set was found in small compared to large populations of *Dianthus deltoids* (Jennersten, 1988), *Eupatorium resinosum* (Byers and Meagher, 1992), *Fagus sylvatica* (Nilsson and Wästljung, 1987) and *Senecio integrifolius* (Widén, 1993).

Estimated seed set for *Ballota* was very low. Most of the *Ballota* fruits had either one seed or none except very few cases where the fruit had two or three seeds. The mean number of seeds per fruit was 0.64 while it should be 4 if the fruit is completely developed as a member of Lamiaceae. For a rare and highly outcrossing species, seed production depends not only on the pollinator but also on the mating type. It remains unclear if seed production in small populations of *B. undulata* is reduced by pollinator limitation, or by the absence of cross-compatible mates. Specialized plant-pollinator systems are sensitive to disturbances of any kind, and the pollinator as well as the plant may be influenced if either of the two changes in abundance (e.g. Bronstein *et al.*, 1990). Severe bottlenecks and lack of recruitment decrease population sizes but also affect the number of mating types. Cross-pollination in small populations is also less successful because of possible asynchronous flowering of compatible mates (Luijten *et al.*, 2000). A computer model by Byers and Meagher (1992) showed that populations smaller than 25 individuals were unable to maintain a high diversity of mating types. More than 70% of *B. undulata* as well as all *B. kaiseri*, and *B. saxatilis* populations have fewer individuals than this. As it was shown in mating system analysis (Zaghloul, 2003), *Ballota* are highly outcrossing plants with

occurrence of some selfing suggesting that these plants are self-compatible with some mechanism that promotes outcrossing. It is likely that reproduction in these small Populations may therefore be seriously limited by the presence of this outcrossing promoting mechanism.

Seed set, germination, and establishment were not correlated with *B. undulata* population size. Perhaps this is a result of having all the studied populations being considered small (maximum 42 individuals). The current small isolated populations may be are remnants of large and continuous populations that have declined in recent history. As a result, genetic erosion (the combined negative effects of isolation, inbreeding, and genetic drift) may not have appeared yet. Hauser and Loeschcke (1994) and Ouborg and Van Treuren (1995) have found no relationship between population size and offspring performance in early life stages. Menges (1992) found a positive correlation between percentage germination and population size in *Silene regia*, while no relationship was found for the rare perennial *Senecio integrifolius* (Widén, 1993).

The loss of genetic diversity (due to genetic drift) could lead to reduction of fitness and ultimately to reduced population viability (Frankel and Soulé, 1981; and Soulé, 1986). *Ballota* populations' fitness is generally low to very low (not significantly higher than zero) and there is no significant difference between *B. undulata, B. kaiseri,* and *B. saxatilis*. This low fitness is primarily due to low seed set and low germination percent. Further seed viability studies should be conducted to determine if this low germination percent is due to low seed viability or due to strong innate dormancy. If it's due to strong innate dormancy, or in other words carry over mechanisms which prevent germination even in response to conditions maximize seedling survival, then it could be an advantage to accumulate a persistent soil seed bank (Meyer *et al.,* 1995) that can buffer the dramatic changes in genetic diversity due to small population sizes (Mahy *et al.*, 1999).

The results revealed that *B. undulata* fitness index is positively ($P \leq 0.05$) correlated only with number of alleles per polymorphic locus (A_P) (Table 2). Variation in allozymes is widely accepted as being selectively neutral, but variation in fitness characters (seed set, germination, and establishment) is generally under both polygenic control and selection. Therefore, loss of allozyme variation does not necessarily imply loss of fitness but may indicate that low frequency alleles at loci affecting fitness may also be lost by chance (e.g. Keohn *et al.*, 1988; and Booth *et al.*, 1990). Whether or not low genetic diversity in a particular population is accompanied by low fitness, is strongly dependent on the history of that population (Ouborg and Van Treuren, 1995). The knowledge of the history of the populations is of vital importance both for formulating expectations and for interpretation of results. Unfortunately, detailed information about the history of the *Ballota* populations investigated in the study area is lacking, but they have been small-sized for at least the last 15 years (personal observation).

There was no evidence for reduced fitness in *Ballota* small populations. Similar results were obtained for *Scabiosa columbaria* (Van Treuren *et al.,* 1993a), *Lychnis flos-cuculi* (Hauser and Loeschcke, 1994), and *Salvia pratensis* (Ouborg and Van Treuren, 1995). The UMousa population has the highest seed set, germination percent, establishment percent, and hence is exceptionally (relatively) fit over all other studied populations which makes it the first candidate as seed source in restoration efforts. The mating system analysis result (Zaghloul, 2003) showed that UMousa population has the lowest t_m and t_s among *B. undulata* populations. If this result is combined with the present information that the *Ballota* fitness is negatively associated with single locus outcrossing rate (t_s), it may imply that *Ballota* populations are locally adapted and interpopulation crossing should be avoided as much as possible when restoration efforts are considered. Increased fitness of offspring from interpopulation crosses has been found in a number of studies and the artificial increase of gene flow among rare plant populations has been suggested as a management tool (Oostermeijer *et al.*, 1995). However, large-distance gene transfer is not always beneficial. If genes in a local population have been adapted to the genetic environment defined by other genes (intrinsic coadaptation) or if different populations have become locally adapted, gene flow might results in outbreeding depression (Templeton, 1986; Waser and Price, 1989; Lynch, 1991; Waser, 1993; and Fischer and Matthies, 1997).

There were significant difference between *Ballota undulata, B. kaiseri,* and *B. saxatilis* in volume and shoot length with *B. saxatilis* being the highest in both characters been followed by *B. kaiseri* and *B. undulata* (Table 5). There was no significant difference between the behavior and response of *B. undulata, B. kaiseri,* and *B. saxatilis* in fitness characters (seed setting, germination behavior) and fitness index. Although *B. saxatilis* has higher germination and establishment percentages, it has a lower fitness index and seed set (Table 5). Therefore, the three species should be treated equally when management efforts are taken.

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درجة اللياقة لثلاث أنواع من جنس نبات البالوتا التى تنمو بمحية سانت كاترين، مصر

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الملخص عربي

تناولت الدراسه المقدمه ثلاثه أنواع من جنس نبات البالوتا التابع للعائلة الشفوية: الغصة *Ballota undulata* والمسيسة *B. saxatilis* والشيرما *B. kaiseri* والذي يتميز بندرة تواجده وكذلك حجم العشائر الصغيرة نظراً لتعرضه للضغوط التي أدت إلى إنخفاض عدد وحجم عشائر هذا الجنس إلى درجة تهدده بالانقراض. وقد أستهدف البحث معرفة ما إذا كانت عشائر نبات البالوتا تعاني انخفاض في درجة اللياقة (Fitness) ومدي إرتباطها بالتنوع الوراثي وعوامل التزاوج. وتعرض البحث الي دراسة الفرق المعنوي في اللياقة بين الأنواع الثلاثة. وتم اختبار مدى الإرتباط بين درجة اللياقة وحجم العشيرة والتباين الوراثي ونظام التزاوج باستخدام معامل الارتباط والإرتداد البسيط وتم استخدام معامل الارتداد المتدرج (Stepwise regression) لتعريف أحسن التوقعات عن لياقة النباتات. وأوضحت النتائج أن درجة لياقة عشائر نبات البالوتا تتراوح عامة بين قليل الي قليل جداً ولا توجد اي فروق معنويه بين الثلاث أنواع لنبات البالوتا. وكذلك أوضحت الدراسة عدم وجود أي علاقه بين درجة اللياقة أو حجم العشيرة وكذلك إختلاف الأمشاج المشاهد observed والمتوقع expected في نوع الغصة بالرغم من عدم وجود دليل علي إنخفاض درجة اللياقة في عشائر نبات الغصة، فقد أوضحت الدراسة أن درجة اللياقة تتحد بدرجة سالبة مع معدل التزاوج الخارجي للمواقع الجينية وأن حجم العشيرة لنبات الغصه يرتبط فقط مع عدد الأليلات (Alleles) وكذلك عدد الأليلات للمواقع الجينية متعددة المظاهر.