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Effect of habitat fragmentation on the genetic structure of the narrow endemic *Brongniartia vazquezii*

Jorge González-Astorga[1]* and Juan Núñez-Farfán[2]

[1]*Jardín Botánico Clavijero, Instituto de Ecología, A.C., km 2.5 Antigua Carretera a Coatepec, Xalapa 91000, Veracruz and* [2]*Departamento de Ecología Evolutiva, Instituto de Ecología, UNAM, Apartado Postal 70275, C.P. 04510 D.F., México*

ABSTRACT

The monoecious, animal-pollinated shrub *Brongniartia vazquezii* is an endemic and endangered species of the tropical dry forests of Central Mexico. Deforestation of the tropical dry forest has fragmented the habitat and resulted in the isolation of the only four extant populations of *B. vazquezii*. In this study, we assessed the genetic consequences of habitat fragmentation by comparing the genetic variability (allelic richness and expected mean heterozygosity, H_e), gene flow and population differentiation in both adult (before fragmentation) and seedling (after fragmentation) populations. Six polymorphic enzymatic loci were used to estimate genetic parameters. *F*-statistics revealed a deficiency of heterozygous plants in both adults and seedlings in all populations. This probably results from geitonogamous selfing. Genetic diversity was lower in adult than in seedling populations ($H_e = 0.201$ and 0.357, respectively). Significant genetic differentiation among populations and of similar magnitude was detected for the adult and seedling populations ($F_{st} = 0.1013$ and 0.0705, respectively). Average gene flow between pairs of reproductive populations was high ($Nm = 2.23$). The genetic structure of adult and seedling populations of *B. vazquezii* suggests that habitat fragmentation has not reduced, as yet, allelic richness and genetic diversity, nor has it increased genetic differentiation. Furthermore, high levels of gene flow were found, suggesting that habitat fragmentation has broken the former population structure.

Keywords: Brongniartia vazquezii, conservation genetics, endemic and narrow species, gene flow, genetic structure, habitat fragmentation, Mexico.

INTRODUCTION

Population genetics theory has provided the framework to explore the potential effects of habitat fragmentation on the genetic structure of populations (Young *et al.*, 1996). Populations in fragmented habitats are expected to become differentiated due to founder effects, genetic drift and increased inbreeding, and a reduction in gene flow among populations (Templeton *et al.*, 1990).

* Author to whom all correspondence should be addressed. e-mail: astorga@ecologia.edu.mx

A reduction in population size due to habitat fragmentation may result in loss of allelic richness or gene diversity (Lande, 1999). This can occur through population bottlenecks at the time of disturbance and genetic drift afterwards (Barrett and Kohn, 1991; Ellstrand and Elam, 1993). Habitat fragmentation in continuous, panmictic populations may create a metapopulation structure and limited gene flow (Lande and Barrowclough, 1987; Lande, 1999). Metapopulations may experience frequent local extinctions and recolonization (Harrison and Hastings, 1996; Booy *et al.*, 2000). Besides creating a metapopulation structure, habitat fragmentation may exacerbate local extinction, because it often alters demographic parameters and environmental factors (Lande, 1999). Thus the study of the genetic consequences of habitat fragmentation in plant populations has implications for species conservation (Young *et al.*, 1996). In this study, we tried to measure the impact of recent habitat fragmentation on the population structure of a narrowly distributed plant species.

Species with narrow geographic distributions may be more vulnerable to (local) extinction if reductions in population genetic variation produced by habitat disturbance – both natural and by humans – and reproductive bottlenecks occur (Wright, 1933; Simberloff, 1988; Barrett and Kohn, 1991; Kirkpatrick and Jarne, 2000). Rare, endemic and endangered plant species are often found to occur in small and disjunct populations and sustain reduced genetic variability due to increased habitat fragmentation and inbreeding (Simberloff, 1988; Barrett and Kohn, 1991; Godt *et al.*, 1996; Sun, 1996; Allphin *et al.*, 1998). Since heterozygosity often correlates with fitness, a substantial loss of fitness in a population is expected as a consequence of a large and sudden reduction in population size and increased inbreeding (Lande, 1999).

In populations in equilibrium between drift and migration, genetic differentiation among populations is expected to increase with the geographic distance (Slatkin, 1994). Thus, in fragmented populations, gene flow can be diminished and divergence is promoted (but see Young *et al.*, 1996). The aim of the present study was to assess, in the tropical shrub *Brongniartia vazquezii*, if habitat fragmentation produces: (1) a reduction in genetic diversity (allelic richness and gene diversity), (2) an increment in inbreeding, (3) a reduction in gene flow and (4) a consequent increment in population differentiation. For this, genetic analyses were performed in the individuals established before disturbance (hereafter adults) and in individuals derived from seeds of the previous year (hereafter seedling population).

Brongniartia vazquezii (Fabaceae: Faboideae) is a shrub with a restricted geographic distribution in the tropical dry forests of the Balsas River Basin (Dorado, 1989). It is a monoecious plant that blooms massively (50.5 ± 9.7 flowers per plant) during the dry season (May–June). Its small purple flowers (1.37 ± 0.19 cm long) are visited by bumblebees (*Bombus* sp.) and honeybees (*Apis mellifera*). Recent deforestation of this region of Mexico has produced isolated fragments of tropical dry forest. Almost 60% of the original area of tropical dry forests in Mexico has been destroyed (Trejo and Hernández, 1996). *B. vazquezii* is endemic and was described recently (Dorado, 1989). It occurs at low population densities (< 50 individuals per 0.1 ha; J. González-Astorga, unpublished data) compared with other shrubs of the same family, with densities of 200–300 individuals per 0.1 ha (e.g. *Brongniartia montalvoana*, *Acacia cochliacantha*, *A. bilimekii*, *A. farnesiana*; Dorado and Arias, 1992; Sousa and Delgado, 1998). The small population sizes and patchy distribution of *B. vazquezii* allow us to determine the effect of isolation produced by humans on the genetic structure of this plant species.

METHODS

Study sites

The study was conducted during 1997–98 at the Sierra de Huautla Natural Preserve (CEAMISH-UAEM), which is located in the State of Morelos (99°05′ to 99°08′W; 18°29′ to 18°32′N) in the central region of Mexico. The vegetation is tropical dry forest (Rzedowski, 1978), highly fragmented as a result of recent human activities (roads, cultivated fields, ranching, etc.). The physiognomy of the forest is produced by a marked climatic seasonality. Most plants lose their leaves during 5–7 months of the dry season. The climate is warm sub-humid (the driest of the sub-humid climates; García, 1988). The precipitation/temperature ratio is less than 43.2, and less than 5% of the rainfall occurs in winter. Mean annual temperature and precipitation for the study site are 24.5°C and 1039 mm, respectively (García, 1988).

Sample collection

Tissue sampling was done in the four extant populations of *B. vazquezii* in the Valle de Vázquez, Morelos, Mexico. Populations are separated by distances of 1.3–7.5 km. The sampled populations of *B. vazquezii* encompassed the total geographic range of the species (Table 1). Full-expanded young leaves were collected from 40 reproductive individuals in each population (adult populations). Simultaneously, seeds collected in the field were germinated in environmentally controlled conditions (humidity 50%, temperature 25°C and constant light) (seedling populations). Leaves of seedlings randomly chosen (*c*. 40 individuals per population) were used for electrophoretic analysis as in the case of adult individuals.

Electrophoresis

Multi-locus genotypes of 40 individuals from each population were obtained through horizontal starch gel electrophoresis (12% w/v). Allozymic variation was scored in six loci for each individual plant. The loci were: aminopeptidase (E.C. 3.4.11.1, locus *Amp*), esterase (E.C. 3.1.1, locus *Est*), phosphoglucoisomerase (E.C. 5.3.1.9, locus *Pgi*), phosphoglucomutase (E.C. 5.2.2, locus *Pgm*) and malate-dehydrogenase (E.C. 1.1.1.37, loci *Mdh1* and *Mdh2*). The extraction buffer (tris-HCl pH 7.5, sucrose, PVP-40, mercaptoethanol, ascorbic acid, diethyldithiocarbamate, bovine serum albumin, sodium metabisulphite and sodium tetraborate; Wendel and Weeden, 1989) was added to solubilize and stabilize the enzyme extracts, which were stored on filter paper wicks at −70°C until used for analyses. The buffers (gel and electrode) used were histidine pH 5.7 and citric acid (Soltis *et al.*, 1983). Electrophoresis was performed at 4°C for 3 h (constant current of 70 mA and voltage of 200 V). Electrophoretic analyses were performed for adults and seedlings of each population.

Statistical methods

The bands from each enzyme system were assigned to alleles and genotypes based on theoretical expectations and observed banding patterns. The TFPGA 1.3 package (Miller,

Table 1. Intrapopulation genetic variation for adult and seedling populations of *Brongniartia vazquezii* in the Sierra of Huautla Natural Preserve, Morelos, Mexico

	Latitude and longitude	Altitude (m a.s.l.)	A	P	N_i	H_o	H_e
Population 1	18°32′N, 99°05′W	1783					
Adults			2.16	83.33	40.0	0.116	0.224
Seedlings			2.50	100	37.8	0.236	0.392
Population 2	18°29′N, 99°08′W	865					
Adults			2.16	83.33	40.0	0.037	0.217
Seedlings			2.66	100	36.8	0.101	0.306
Population 3	18°30′N, 99°07′W	832					
Adults			1.83	50.00	40.0	0.088	0.170
Seedlings			2.66	100	36.5	0.174	0.288
Population 4	18°32′N, 99°06′W	1200					
Adults			2.00	66.66	40.0	0.104	0.193
Seedlings			2.66	100	35.1	0.244	0.344
Mean[a]							
Adults			2.03	70.83	40.0	0.086	0.201
			(0.016)	(15.95)	(0.000)	(0.017)	(0.012)
Seedlings			2.62	100	36.55	0.188	0.357
			(0.080)	(0.00)	(0.557)	(0.033)	(0.023)

Abbreviations: A = mean number of alleles per locus; P = percentage of polymorphic loci; N_i = average sample size; H_o and H_e = observed and expected mean heterozygosity, respectively.
[a] Standard deviations in parentheses.

1997) was used to obtain the genetic estimators from the data. Genotypic frequencies were obtained and used to calculate observed mean heterozygosity (H_o) and allelic frequencies. Allelic frequencies at each population were used to estimate the mean number of alleles per locus (A), the average proportion of polymorphic loci (P) and expected mean heterozygosity (H_e), based on Hardy-Weinberg expectations (Hartl and Clark, 1989). Significance of estimators was obtained by Monte Carlo methods (Weir, 1990; Guo and Thompson, 1992).

The partitioning of genetic diversity was done using F-statistics (Wright, 1965, 1978). To determine whether F_{is} and F_{it} estimations for each locus were significantly different from zero, chi-square statistics ($\chi^2 = F[2N]\,[k-1]$) were obtained, with $k(k-1)/2$ degrees of freedom, where N is the sample size and k is the number of alleles (Weir, 1990). To determine the significance of the F_{st} statistic for each locus, chi-square statistics were used: $\chi^2 = (2N)\ F_{st}\ (k-1)$, with $(k-1)(s-1)$ degrees of freedom, where s is the number of subpopulations (Workman and Niswander, 1970). The 95% confidence intervals of the F-statistics were obtained by bootstrapping over loci for the multi-locus estimate, and jackknifing over populations for the single-locus estimates (Weir and Cockerham, 1984;

Weir, 1990). The average gene flow among populations (*Nm*) was estimated from F_{st} values, as $F_{st} = 1/(4Nm\alpha + 1)$, where $\alpha = (n/n-1)^2$ and n is the number of subpopulations (Crow and Aoki, 1984). This method considers the migration between pairs of subpopulations with restricted dispersal, and near to equilibrium, based on a continent–island population structure model (Slatkin, 1993). *Nm* is interpreted as the number of migrants per generation among two given populations. The significance of this relationship was evaluated by Mantel's (1967) test, since typical statistical methods cannot be used because values of *Nm* from different pairs of populations are not independent (Sokal and Rohlf, 1995).

RESULTS

Genetic variation

We found no effect of habitat fragmentation on the allelic richness of populations. The number of alleles per locus was 2.03 ± 0.16 (mean ± standard deviation) and 2.62 ± 0.08 for adults and seedlings of *B. vazquezii* (Table 1), respectively. In fact, the seedling populations had, on average, higher allelic richness than the parents ($t_{46} = 2.15$, $P = 0.0368$), suggesting high gene flow among populations.

Allelic frequencies for the six loci (*Amp*, *Est*, *Pgi* and *Pgm*, one locus each; *Mdh*, two loci) scored for each individual plant are given in the Appendix. In the adults, the percentage of polymorphic loci per population varied from 50.0% (population 3) to 83.33% (populations 1 and 2), with an average of 70.86%. In contrast, the percentage of polymorphic loci was 100% in all seedling populations. Observed mean heterozygosity was 0.086 ± 0.017 (range 0.037–0.116) and 0.188 ± 0.033 (range 0.101–0.244) for the adult and seedling populations, respectively (Mann-Whitney test, $U = 16$, $n_1 = n_2 = 4$, $P < 0.05$; Table 1). Similarly, expected mean heterozygosity was higher for the seedling than for the adult population (0.375 ± 0.023 *vs* 0.201 ± 0.012) (Mann-Whitney test, $U = 16$, $n_1 = n_2 = 4$, $P < 0.05$; Table 1).

Test for Hardy-Weinberg equilibrium

Using allelic frequencies (see Appendix), the Monte Carlo tests showed that genotypic frequencies deviated from Hardy-Weinberg expectations in both adult and seedling populations, indicating inbreeding (Table 2). For adult plants, all populations exhibited significant deviations, except population 1 at *Mdh1* and population 4 at *Est* and *Mdh2*. For the seedlings, all populations showed significant deviations of the fixation index, except for locus *Est* in populations 1 and 4, *Mdh* in population 1, *Mdh2* in populations 3 and 4 and *Amp* in population 4 (Table 2).

Genetic structure

Wright's *F*-statistics, F_{it} and F_{is}, were positive and significantly different from zero for all loci ($P < 0.01$) in both adult and seedling populations, indicating inbreeding (Table 3). Similarly, all loci showed values of F_{st} that were positive and significantly different from zero ($P < 0.05$), except for locus *Amp* in both the adult and seedling populations. The F_{st} values

Table 2. Probabilities of the Hardy-Weinberg test of equilibrium for adult and seedling populations of *Brongniartia vazquezii*, using the conventional Monte Carlo method (10 batches per analysis, 1000 permutations per batch, 10,000 total permutations), on each of six loci.

	Locus					
	Est	*Pgi*	*Mdh1*	*Mdh2*	*Pgm*	*Amp*
Adults						
Population 1	0.0495 (0.0119)	0.0019 (0.0013)	1.0000 (0.0000)	0.00001 (0.0000)	0.0038 (0.0025)	*
Population 2	0.00001 (0.0000)	0.00001 (0.0000)	0.0122 (0.0024)	0.00001 (0.0000)	*	0.00001 (0.0000)
Population 3	0.00001 (0.0000)	0.0006 (0.0008)	*	*	0.0114 (0.0024)	*
Population 4	1.0000 (0.0000)	*	0.00001 (0.0000)	1.0000 (0.0000)	0.0018 (0.0015)	*
Total	0.00001 (0.0000)	0.00001 (0.0000)	0.00001 (0.0000)	0.00001 (0.0000)	0.00001 (0.0000)	0.00001 (0.0000)
Seedlings						
Population 1	0.9226 (0.0070)	0.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0062 (0.0037)	0.0028 (0.0012)
Population 2	0.0001 (0.0003)	0.0000 (0.0000)	0.0372 (0.0073)	0.0000 (0.0000)	0.0115 (0.0032)	0.0000 (0.0000)
Population 3	0.0004 (0.0005)	0.0028 (0.0018)	0.0001 (0.0003)	1.0000 (0.0000)	0.0210 (0.0042)	0.0093 (0.0022)
Population 4	0.2999 (0.1558)	0.0360 (0.0040)	0.0000 (0.0000)	0.2001 (0.0137)	0.0017 (0.0009)	0.1363 (0.0140)
Total	0.0001 (0.0003)	0.00001 (0.0000)	0.00001 (0.0000)	0.00001 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)

* Locus fixed for one allele.

were relatively similar among loci in both populations (range: adults, 0.0685–0.1574; seedlings, 0.0165–0.1537), suggesting that genetic drift or inbreeding has been the dominant differentiating process. The average magnitude of genetic differentiation among adult populations ($F_{st} = 0.101$) was slightly higher than for seedling populations ($F_{st} = 0.070$). However, although both estimates were significantly different from zero, there were no differences between them (Table 3) according to the 95% confidence intervals.

On average, 10% of the genetic variation in *B. vazquezii* is due to differences among populations (Table 3). High and similar values of local inbreeding were found for both adult and seedling populations ($F_{is} = 0.533$ and 0.437 respectively), probably as a result of selfing (Table 3).

Table 3. Wright's *F*-statistics for *Brongniartia vazquezii*, for adult and seedling populations, in the Sierra of Huautla Natural Preserve, Morelos, Mexico

Loci	F_{it}	F_{st}	F_{is}
Est			
Adults	0.7127*****	0.1225****	0.6726*****
Seedlings	0.5684*****	0.0868**	0.5273*****
Pgi			
Adults	0.5941*****	0.1121***	0.5428*****
Seedlings	0.4794*****	0.0817*	0.4331****
Mdh1			
Adults	0.6112*****	0.1033**	0.5664*****
Seedlings	0.5024*****	0.0765*	0.4612****
Mdh2			
Adults	0.5501*****	0.0819*	0.5100*****
Seedlings	0.4271****	0.0295*	0.4096***
Pgm			
Adults	0.5958*****	0.1085**	0.5467*****
Seedlings	0.4713****	0.0681*	0.4326***
Amp			
Adults	0.5686*****	0.0975	0.5219*****
Seedlings	0.4708***	0.0807	0.4243*****
Mean			
Adults	0.5830*****	0.1013**	0.5329*****
Seedlings	0.4792***	0.0705*	0.4374***
Standard deviation			
Adults	0.1164	0.0283	0.1200
Seedlings	0.0959	0.0430	0.0865
95% confidence intervals			
Adults	0.4593–0.7876	0.0685–0.1574	0.4158–0.7544
Seedlings	0.3314–0.6383	0.0165–0.1537	0.3113–0.5810

* $P < 0.05$; ** $P < 0.025$; *** $P < 0.01$; **** $P < 0.005$; ***** $P < 0.001$.

Gene flow

Indirect estimation of gene flow (*Nm*) for *B. vazquezii* indicated that habitat fragmentation has not reduced gene flow. An average estimate of 2.23 ± 0.46 migrant individuals per generation between pairs of populations was obtained (Table 4). The lowest value was obtained between populations 3 and 4 ($Nm = 1.36$) separated by about 4.3 km, and the

Table 4. Number of individual migrants per generation (*Nm*; above diagonal) and geographical distance (m; below diagonal) between pairs of populations of *Brongniartia vazquezii*

Population	1	2	3	4
1		2.62	2.31	2.40
2	7500		2.54	2.17
3	5600	1850		1.36
4	1300	6100	4350	

Note: Data from adult plant populations.

highest value between populations 1 and 2 ($Nm = 2.62$) separated by about 7.5 km. The correlation between the matrices of *Nm* values and the geographical distance between pairs of populations does not support the idea that genetic differentiation has occurred as expected under the 'isolation by distance' model, as demonstrated by Mantel's test ($r = -0.0129$, $P = 0.514$; Table 4).

DISCUSSION

Despite the short geographic distances that separate the extant populations of *B. vazquezii*, low but significant genetic differentiation and high inbreeding were detected. Although loss of genetic diversity and a reduction in gene flow are expected in fragmented or highly structured populations (Young *et al.*, 1996), our results do not support these expectations.

Species with restricted geographical range, like *B. vazquezii*, do not necessarily have low genetic diversity. High gene diversity has been reported for the rare Hawaiian fern *Adenophorus periens* (Ranker, 1994), the endangered *Caesalpinia echinata* (Cardoso *et al.*, 1998), the narrow endemic fern *Polystichum otomasui* (Maki and Asada, 1998), an endangered pine (Delgado *et al.*, 1999), the rare Mexican pinyon pine (Ledig *et al.*, 1999), the endemic *Agave victoriae-reginae* (Martínez-Palacios *et al.*, 1999), in three endemics from Florida (*Eryngium cuneifolium*, *Hypericum cumulicola* and *Liatris ohlingerae*; Dolan *et al.*, 1999), in the annual endemic of Florida *Warea carteri* (Evans *et al.*, 2000) and in the endemics *Iris cristata* and *I. lacustris* (Hannan and Orick, 2000). In *B. vazquezii*, seedlings had higher heterozygosity than adult populations. Thus, despite being narrowly distributed and fragmented, *B. vazquezii* shows no signs of a reduction in genetic diversity, suggesting that gene flow may be mixing a formerly structured population (see Levin and Kerster, 1974; Young *et al.*, 1996)

Gene flow among populations is the only explanation for finding alleles in low frequencies at the seedling stage and their absence in the adult population (e.g. allele c of *Pgi* in population 2 can only derive from populations 1 or 3; Appendix). Accordingly, values of *Nm* suggest high gene flow among populations (cf. Table 4). However, this result is not unexpected given the short distances that separate the populations. Furthermore, the reduction in gene flow among fragmented populations might be lower if the plant populations are wind-dispersed or animal-pollinated, as in the case of *B. vazquezii*. For instance, fragmented populations of *Acer saccharum* showed increased gene flow compared

with intact or pre-fragmented populations (Foré *et al.*, 1992; Young *et al.*, 1993). As this result is counterintuitive, it may be a consequence of the breakdown of the former population structure, which, in turn, can produce negative effects by putting in contact two differentiated gene pools through outbreeding depression or hybridization between locally adapted populations (Young *et al.*, 1996; Holsinger *et al.*, 1999; Lande, 1999). Yet, positive effects of this phenomenon may occur if it enhances the replenishment of genetic variation lost from some populations by drift. It is not possible to affirm to what extent these two possibilities apply to *B. vazquezii*, but these hypotheses should be addressed in future studies of the genetic consequences of habitat fragmentation.

Homogeneity in F_{st} values for all loci analysed in *B. vazquezii* indicate that genetic drift or inbreeding may be responsible for the deficiency of heterozygous individuals both at the subpopulation and population levels. Similarly, the fixation indices (F_{is} and F_{it}) in adult and seedling populations indicate that there is high inbreeding in *B. vazquezii*. Inbreeding in *B. vazquezii* may result from geitonogamous pollinations by bumblebees (*Bombus* sp.; J.G.-A., personal observation) or through mating among relatives. Even though bumblebees can move pollen from moderate to long distances, promoting gene flow, *B. vazquezii* blooms massively and hence self-pollination occurs. Further studies on the reproductive biology of *B. vazquezii* are needed to account for the high inbreeding found in this species and to measure the intensity of selection on the mating system (e.g. inbreeding depression). Despite differentiation being similar in magnitude in adult and seedling populations, natural selection cannot be ruled out as the process responsible for population structure in *B. vazquezii*.

The present results indicate that the populations of *B. vazquezii* established before and after forest disturbance contain similar genetic variability. Thus the main short-term threat this species faces is anthropogenic (Lande, 1999). A simple means of preserving genetic variation of this and other endemic species in this region is to stop deforestation and to enlarge the Preserve Sierra de Huautla, Morelos.

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APPENDIX

Allelic frequencies of six enzymatic loci in individuals, adults and seedlings, of four populations of *B. vazquezii*, in the Sierra de Huautla Natural Preserve, Morelos

		Population			
Locus	Allele	1	2	3	4
Est					
Adults	a	0.7125	0.8000	0.5375	0.8875
	b	0.2000	0.1250	0.2875	0.0500
	c	0.0875	0.0750	0.1750	0.0625
Seedlings	a	0.5286	0.6945	0.4848	0.6970
	b	0.3285	0.1944	0.3334	0.1364
	c	0.1429	0.1111	0.1818	0.1666
Pgi					
Adults	a	0.9250	0.8500	0.7750	1.0000
	b	0.0500	0.1500	0.1125	0.0000
	c	0.0250	0.0000	0.1125	0.0000
Seedlings	a	0.6750	0.7632	0.6892	0.8784
	b	0.2375	0.2237	0.1757	0.0811
	c	0.0875	0.0131	0.1351	0.0405
Mdh1					
Adults	a	0.9750	0.9750	1.0000	0.7875
	b	0.0250	0.0250	0.0000	0.0625
	c	0.0000	0.0000	0.0000	0.1500
Seedlings	a	0.8975	0.8919	0.9054	0.7436
	b	0.0769	0.0946	0.0676	0.1026
	c	0.0256	0.0135	0.0270	0.1538
Mdh2					
Adults	a	0.6750	0.7875	1.0000	0.9500
	b	0.3250	0.0125	0.0000	0.0500
	c	0.0000	0.2000	0.0000	0.0000
Seedlings	a	0.4865	0.7568	0.9736	0.8281
	b	0.5135	0.0405	0.0132	0.0781
	c	0.0000	0.2027	0.0132	0.0938
Pgm					
Adults	a	0.8375	1.0000	0.9750	0.8500
	b	0.1625	0.0000	0.0250	0.1500
Seedlings	a	0.7500	0.9744	0.9054	0.7237
	b	0.2500	0.0256	0.0946	0.2763
Amp					
Adults	a	1.0000	0.8000	1.0000	1.0000
	b	0.0000	0.2000	0.0000	0.0000
Seedlings	a	0.8750	0.7500	0.8649	0.8438
	b	0.1250	0.2500	0.1351	0.1562
No. of alleles per locus (mean ± *s*)					
Adults		2.16 ± 0.75	2.16 ± 0.75	1.83 ± 0.98	2.00 ± 0.89
Seedlings		2.50 ± 0.54	2.66 ± 0.51	2.66 ± 0.61	2.66 ± 0.51