Allozyme diversity in *Leavenworthia* populations with different inbreeding levels

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Levels of allozyme diversity were studied in five species of the annual plant genus *Leavenworthia*. The breeding systems of the populations were also characterized in terms of measures of autogamous seed-set and self-fertility. The populations that appeared more inbreeding by these criteria also tended to have lower within-population gene diversity (H_S) values, but there was nevertheless considerable allozyme variability in two of the four sets of inbred populations studied. Comparing the outcrosser *L. stylosa* and the related inbreeders *L. uniflora* and *L. torulosa* the reduction in diversity associated with inbreeding was very high, as no variants were seen within populations of the inbreeders. In *L. crassa* and *L. alabamica*, the reduction was lower, but correcting for the fact that this comparison is over less extreme selfing rate differences, it is estimated that the difference between extreme inbreeding and outcrossing populations of *L. crassa* considerably exceeds twofold. These results are compared with published studies within closely related taxa with different inbreeding levels. The effect of inbreeding on allozyme diversity is consistently larger than has been estimated from comparisons of unrelated species.

Keywords: allozymes, genetic diversity, inbreeding, *Leavenworthia*.

Introduction

An important problem in population genetics is the maintenance of genetic variability within and between natural and other populations (e.g. Kimura, 1983; Kreitman, 1991). Differences in diversity patterns, such as the lowered neutral DNA diversity at loci in regions of unusually low recombination in *Drosophila* species, may illuminate factors that play important roles in determining genetic variability (e.g. Langley, 1990; Begun & Aquadro, 1992). One situation in which diversity is often low is in inbreeding populations (Schoen & Brown, 1991; Jarne & Staedler, 1995; Hamrick & Godt, 1996), and it is important to understand what causes this highly repeatable pattern.

It is sometimes stated that inbreeding does not lead to loss of genetic diversity in species but merely redistributes it between individuals and between populations (e.g. Fenster & Ritland, 1992), but this is true only in the evolutionary short-term. Over evolutionary time, several factors operate in inbreeding populations that may reduce neutral diversity compared with outbreeders. In very inbred populations, each individual carries two identical genomes (i.e. is highly homozygous). This reduces effective population sizes (N_e values; complete inbreeding halves N_e, see Brown, 1979 and Pollak, 1987). Under the classical theory of neutral molecular evolution, allelic diversity depends on N_e values (Kimura, 1983). A very inbreeding population thus behaves similarly to one of half its actual size, as far as molecular variation is concerned, and is expected to have half the neutral diversity levels of otherwise similar outcrossing populations. In addition, bottlenecks in population size may be more extreme in inbreeders (Schoen & Brown, 1991), in which a single seed can found a new population. Also, the lack of effective recombination (i.e. the rarity of production of recombinant genotypes) in inbreeding populations may lead to phenomena similar to those seen in low-recombination regions of genomes (see above). This increases the importance of selective sweeps of advantageous mutations (Hedrick, 1980) and 'background selection' caused by elimination of deleterious mutations, which also leads to the evolution of lower levels of neutral diversity in such populations (reviewed in Charlesworth *et al.*, 1993).

In comparing genetic diversity from populations differing in their levels of inbreeding, the effect of

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breeding system on diversity should ideally be studied in a set of related taxa differing in little else than the breeding system variations that are the focus of interest. Plants offer opportunities to make such comparisons. An effect of breeding system differences on allozyme diversity is quite well-documented in plants (Schoen & Brown, 1991; Hamrick & Godt, 1996), and animals (Jarne & Staedler, 1995), but the data are not at present completely satisfactory as there have been few paired comparisons involving inbreeder/outbreeder species that are similar in their other characteristics, such as life history (Wolff, 1991). In the few studies comparing species within genera, however, the patterns appear robust (Charlesworth *et al.*, 1997).

Allozyme variants may be selectively maintained (e.g. Hudson *et al.*, 1994), so theories for neutral differences may not apply to them. Loci with variants maintained by heterozygote advantage are expected to lose diversity under inbreeding and, at high selfing rates, such polymorphism is unlikely to be maintained (Kimura & Ohta, 1971). It is, nevertheless, worthwhile to study them, as they offer a relatively technically simple source of diversity information, and the contrast to DNA sequence data can be valuable (Karl & Avise, 1992; Pogson & Zouros, 1994). Some data on plants of the mustard genus, *Leavenworthia*, are reported here. This genus is a classic example of breeding system evolution (Lloyd, 1965; Solbrig & Rollins, 1977; Lyons & Antonovics 1991). The general aim of the work was to estimate genetic diversity from populations with different levels of inbreeding, to test whether inbreeding populations have lower diversity than outbreeding ones, and to estimate the magnitude of any such difference, for comparison with the results on DNA sequence diversity (Liu *et al.*, 1998).

Materials and methods

The genus Leavenworthia

Leavenworthia is a small genus of eight diploid winter annual species in the Brassicaceae, and is known to have breeding systems ranging from self-incompatible populations to highly selfing. *Leavenworthia stylosa* and some populations of *L. crassa* and *L. alabamica* are self-incompatible. Some populations of both *L. crassa* and *L. alabamica* are self-compatible, with varying frequencies of self-fertilization, whereas *L. uniflora*, *L. torulosa* and *L. exigua* are uniformly self-compatible and highly inbreeding (Lloyd, 1965; Solbrig & Rollins, 1977). Four independent comparisons between more outcrossing and more inbreeding populations are

thus possible. Two are between *L. stylosa* and its sister species *L. uniflora* and *L. torulosa*, in the group with $n = 15$ chromosomes, another involves comparisons between different populations of *L. crassa*, in a different section of the genus having $n = 11$ chromosomes, and the fourth is between different *L. alabamica* populations (also $n = 11$). The natural habitat of the species is 'cedar glade' outcrops in the southeastern United States, which form about 5% of the habitat in the region where the species occurs (Quarterman, 1950), so populations are scattered and separated by unsuitable habitat (see Lloyd, 1965). Natural pollinators are nonsocial bees (but high proportions of honeybees were observed on flowers of *L. crassa* and *L. alabamica* in cultivated fields), and seeds are large and have no dispersal mechanism (Lloyd, 1965). Both pollen and seed flow between glades are therefore thought to be rare. Infrequent migration is consistent with the observations that apparently suitable habitat is often unoccupied, and that nearby glades may be occupied by different *L. crassa* races, but seeds may sometimes be transferred by streams or flooding (Lloyd, 1965).

Population samples

Population samples were studied from three populations of the $n = 15$ species, *L. stylosa*, two populations of *L. uniflora* and one of *L. torulosa* (Table 1). From the three $n = 11$ species, six populations of *L. crassa*, two *L. alabamica* populations and one of *L. exigua* were studied (Table 1). Some plants were collected by Dr E. E. Lyons, from populations of known breeding system (Lyons & Antonovics, 1991), and seed from an additional population of *L. stylosa* was collected by Dr T. E. Hemmerly (Middle Tennessee State University). Other plants were collected in the field in spring 1995 with fruits already developing, and brought to the University of Chicago greenhouses where they were grown until their seeds could be collected. Plants were grown from seeds from the different populations (see Charlesworth *et al.*, 1994), and breeding systems for populations for which this information was not already available were estimated by measuring self-incompatibility (by hand self-pollination), and/or by autogamous fruit-set in an insect-free greenhouse at the University of Chicago.

Allozyme electrophoresis

Allozyme genotypes of the greenhouse-grown plants of four of the species were determined by cellulose acetate electrophoresis (Hebert & Beaton, 1989).

Table 1 Autogamy and self-compatibility results of plants grown from seeds from *Leavenworthia* populations of six species. Autogamy rates are proportions of marked flowers that set fruits, based on 10 or more marked flowers on each plant, and self-compatibility values are fruit set per hand-pollinated flower, based on five or more flowers per plant

Population number, source	State, locality collected	Autogamy rate	Self-compatibility	Estimated selfing rate
L. stylosa				
95007	TN, Long Hunter State Park	Very low	Self-incompatible	≈ 0
Hem1‡	TN, Cedars of Lebanon State Park	Very low	Self-incompatible	≈ 0
9113§	TN, Davidson Co.	Very low	Self-incompatible	≈ 0
L. uniflora				
9108§	AL, Morgan Co., Decatur	High‡‡	Highly self-compatible	High
95011¶	AL, Morgan Co., Speake, Highway 157, glade L	0.947 ± 0.026	Highly self-compatible	High
L. torulosa				
95008	KY, Bullitt Co., Rt. 480/1442	0.663 ± 0.036	Highly self-compatible	High
L. crassa				
95005	AL, Morgan Co., Cedar Plains Rd., Massey	0.315 ± 0.043	0.600 ± 0.075	Intermediate†
95010	AL, Morgan Co., Cedar Plains Rd., Massey	0.321 ± 0.074	0.278 ± 0.242	Intermediate†
95002	AL, Morgan Co., small glade off State Rd. 86, S. of Speake	0.363 ± 0.145	0.471 ± 0.192	High
95003	AL, Morgan Co., big glade off State Rd. 86, S. of Speake	0.546 ± 0.103	0.958 ± 0.042	High
95004	AL, Morgan Co., Landersville, glade population	0.915 ± 0.053	Not tested	High
9107§	AL, Morgan Co., Cedar Plains Rd., Massey	Not tested	Not tested	0.95††
L. alabamica				
95006	TN, Cedars of Lebanon St Pk	0.019 ± 0.009	0.099 ± 0.028	≈ 0, self-incompatible†
95009	AL, Landersville, 1st population	0.325 ± 0.081	Highly self-compatible	High
L. exigua				
95001	AL, Guntersville	0.675 ± 0.11	Highly self-compatible	High

†Some plants self-incompatible.
‡Seeds supplied by Dr T.E. Hemmerly.
§Seeds supplied by Dr E.E. Lyons.
¶Seeds supplied by Dr G. Hilton.
††Estimated by Dr E.E. Lyons (pers. comm.).
‡‡The autogamy rate for this population was not estimated quantitatively, but was observed to be high.

Tris-glycine buffer pH 8.5 was used for both the polymorphic enzymes. Gels were run at 109 V. For phosphoglucoisomerase (*Pgi*), runs were 15 min, and for phosphoglucomutase (*Pgm*) 30 min. Good resolution of bands was obtained only for these two enzymes. There were two Pgi systems (A, B) and three Pgm (A–C). Using pollen, which does not have plastids, it was determined that PgiA corresponds to the cytosolic Pgi (usually denoted by *PgiC*, see Ford *et al.*, 1995). Other enzymes stained include acid phosphatase, glutamic-oxaloacetic transaminase, and 6-phosphogluconate, isocitrate, malate and shikimate dehydrogenases, but although variability was seen, the band patterns were not clear enough for genotypes to be scored reliably, despite testing several different buffer systems and running conditions. Starch gels were also tried, with several buffer systems, but good bands for other enzymes were not obtained. Single-locus inheritance of the *Pgi* and *Pgm* variants was checked by raising families from crosses between *L. stylosa* plants of known genotypes.

Analysis

Genotype frequencies of samples from each population were estimated from progeny plants from as many maternal plants as possible. For some populations, where the numbers of parents were small, more than one progeny per maternal plant was used. To minimize bias, four offspring per parent were grown, but a few families were smaller. This sampling is not ideal, and is likely to underestimate genetic diversity, as rare variants present in the populations may be absent from a small sample of maternal plants, but it was imposed by the small numbers of seeds that germinated from some localities. However, the main interest for the present study is in comparing genetic diversity within different populations and, with five loci studied, the sample sizes should be suitable for this purpose (Pons & Petit, 1995). Fixation indices may, however, be inaccurately estimated, given the small samples from some of the populations studied. The alternative approach, of scoring progeny genotypes and estimating those of the maternal parents, was rejected as it does not, in general, provide reliable estimates of maternal genotypes, when the estimates are compared with genotypes known from directly scoring maternal plants (D. Charlesworth, unpubl. data). Inbreeding coefficients (*F*) were estimated within each population using a computer program written for this purpose, and diversity values and fixation indices were estimated using FSTAT version

2.0 (Goudet, 1995). Within-population diversity was estimated as expected gene diversity (H_e, see Nei, 1987) and will be referred to here as H_S; H_T will be used for total species-wide diversity, which was estimated whenever more than a single population of a species was studied. Standard errors of the diversity measures are estimated in the FSTAT program by the method of Nei & Roychoudhury (1974), and those for G_{ST} (Nei, 1987) were estimated by jackknifing over populations.

Results

Autogamy and self-fertility

The autogamy results are summarized in Table 1. All plants of *L. stylosa* were non-autogamous and self-incompatible. All *L. uniflora* and *L. torulosa* plants were highly autogamous, but these taxa differed significantly in their autogamy rates ($t_{18} = 7.04$, $P < 0.001$). The one *L. exigua* population (from Guntersville, AL) has an autogamy rate of 0.675 ± 0.11. These results are as expected for these species (Solbrig & Rollins, 1977). In *L. crassa* and *L. alabamica*, autogamy was variable, again as expected for these species (Lloyd, 1965). The two *L. alabamica* populations studied differed significantly ($t_{39} = 5.83$, $P < 0.001$). *Leavenworthia crassa* showed highly significant autogamy differences, both within and between populations. Contingency table analyses of the numbers of fruits set by marked flowers gave results significant at the $P < 0.0001$ level for all five populations tested, showing that individual plants varied within populations, and heterogeneity between populations was significant at the same level. Population 95004 had highly significantly higher mean autogamy than the other four populations (for the comparison with the next most autogamous population, 95003, $t_{24} = 3.19$, $P = 0.004$), and 95003 differed marginally significantly from the least autogamous one 95005 ($t_{63} = 2.3$, $P = 0.025$) but not from populations with lesser differences in autogamy (95010 and 95002).

Self-compatibility (fruits per hand self-pollination) was estimated on a subset of the plants; in particular, non-autogamous individuals were tested for self-fertility. Self-incompatible plants were found at low frequency in the *L. crassa* populations 95005 (17% of 29 plants tested) and 95010 (21% of 19 plants), so these populations are not merely intermediate in autogamy and selfing rates, but appear to be polymorphic for self-incompatibility. In these two populations, autogamy was highly significantly correlated with self-fertility, but there was no correlation within the other populations. Self-fertility and autogamy of

different populations did not rank in the same order (Table 1). In *L. alabamica*, 63% of the 19 population 95006 plants tested were self-incompatible, whereas individuals that were self-compatible also showed some autogamy.

Allozyme inheritance in L. stylosa

Variants were seen for all five allozyme loci that could be scored. The variability will be quantified below. The inheritance of the variants was studied in a set of nine families involving seven plants, chosen because they differed at three of the loci (systems PgiB and PgmA were not variable in this set of plants). The other three loci segregated as expected given their phenotypes, assuming single-locus control of these variants. One plant was heterozygous for both the *PgiC* and *PgmC* loci, so it was possible to test for linkage between these loci (in crosses with two other plants), and linkage between *PgmB* and *PgmC* could be tested in two families. Despite small family sizes (average family size 17 plants), each of the four families yielded all the four possible genotypes. There is thus enough information to show that these loci are not closely linked in *L. stylosa*.

Allozyme diversity in Leavenworthia species

Leavenworthia stylosa was polymorphic for all five allozyme loci scored, in two of the three populations, and diversity was high (Table 2). *Leavenworthia uniflora* and *L. torulosa* could be scored for only four loci. There was no diversity within either population, but there were fixed differences between the populations for two of the loci (*PgiC* and *PgmC*); the few plants scored from *L. uniflora* population 9108 were identical in genotype to those from 95011. Seeds from several *L. crassa* populations collected failed to germinate. The five *L. crassa* populations that could be studied ranged from intermediate to high autogamy. In both *L. crassa* and *L. alabamica*, four of the five loci scored were polymorphic in the species as a whole (*PgiB* was monomorphic), but only three individual populations of *L. crassa* were polymorphic for all four loci.

Overall, there is thus a clear relationship between high autogamy and low allozyme diversity, with *L. crassa* showing values intermediate between *L. stylosa* and the inbreeders *L. uniflora* and *L. torulosa*. Within *L. crassa*, the same relationship holds, with an overall statistically significant linear regression ($P < 0.02$). Population 95005, which had the

Table 2 Allozyme diversity in some *Leavenworthia* populations in which breeding systems have been estimated. Estimates and standard errors are given

Species and population	Breeding system	Polymorphic loci (%)	Mean number of alleles	Number of plants	H_S or H_T†	F	G_{ST}	D_{ST}
L. stylosa								
95007	SI	80	3.2	30	0.372 ± 0.331	0.331		
Hem1	SI	100	3.6	10	0.592 ± 0.104	0.443		
9113	SI	100	3.0	43	0.495 ± 0.178	0.648		
Total for species		100	—	—	$H_T = 0.524 \pm 0.240$	—	0.268 ± 0.087	0.140
L. uniflora								
95011	Inbreeder	0	1.0	13	0	—		
L. torulosa								
95008	Inbreeder	0	1.0	31	0	—		
L. crassa								
95005	Intermediate	80	3.0	51	0.433 ± 0.249	0.571		
95010	Inbreeder	80	2.4	19	0.347 ± 0.197	0.675		
95002	Inbreeder	80	2.2	8	0.303 ± 0.264	0.531		
95003	Inbreeder	40	1.6	10	0.232 ± 0.321	0.792		
95004	Inbreeder	20	1.2	8	0.092 ± 0.204	0.709		
Total for species		80	—	—	$H_T = 0.424 \pm 0.266$	—	0.213 ± 0.047	0.090
L. alabamica								
95006	Mostly SI	80	3.4	36	0.471 ± 0.267	0.182		
95009	Inbreeder	80	3.6	23	0.406 ± 0.277	0.699		
Total for species		80	—	—	$H_T = 0.561 \pm 0.322$	—	0.352 ± 0.104	0.197

†H_T values are given only for species for which multiple populations were studied.

lowest autogamy and is polymorphic for self-incompatibility, also had the highest diversity values, whereas the most autogamous population, 95004, had low diversity and fewer alleles per locus than the other populations of this species. In Fig. 1, the results from *L. crassa* are summarized and compared with the only other published data sets that include multiple different populations from single species. In *L. alabamica*, however, both the more inbreeding and the self-incompatible population had high diversity. In this species, there was also very high between-population diversity.

The allozyme data yield higher inbreeding coefficients for the more inbreeding populations than for those that are self-incompatible, as expected, but the *F*-values are high even in self-incompatible populations (observed heterozygosity was lower than the Hardy–Weinberg expectations in all populations, at least for some loci). The reason for this is uncertain, although similar values have been seen in other plant populations (Brown, 1979). Null alleles are one possibility. No evidence for null homozygotes was seen in data found by the present study, but the number of parents involved in the study crosses was not large, and as the species used in the genetic tests is self-incompatible there were no progeny from selfing, so null alleles might remain undetected. Local differentiation in allele frequencies is another possibility (Ennos & Clegg, 1982), but is unlikely as frequencies would have to be very different in different subpopulations. Allele frequencies differ substantially between different populations of the same species, as reflected in the quite large values of D_{ST}, but these differences are over distances of many km. Given the high *F*-values, it is clearly not worthwhile to attempt to estimate the selfing rate, *S*, of the populations, using the well-known relationship $F = S/(2 - S)$ (Haldane, 1924). Overall, however, there is a relationship with the levels of inbreeding, as assessed by autogamy and self-compatibility. In *L. alabamica F* is much higher for the inbreeding than the self-incompatible population. In the set of moderately to highly inbreeding *L. crassa* populations, the same trend is seen (Fig. 1), but is not statistically significant. G_{ST} for the outcrossing *L. stylosa* (0.268, with 95% confidence interval 0.156–0.481) was low, but cannot be compared with results for inbreeders, as only single populations of *L. uniflora* and *L. torulosa* were studied, and, for *L. crassa*, only one partially outcrossing population was surveyed, because of poor germination of seeds from most outcrossing populations.

Discussion

These results support earlier findings showing extreme selfing rate differences in *Leavenworthia*, even between different populations of *L. crassa* or *L. alabamica* (Lloyd, 1965). All the evidence is consistent in showing that *L. stylosa* and some popu-
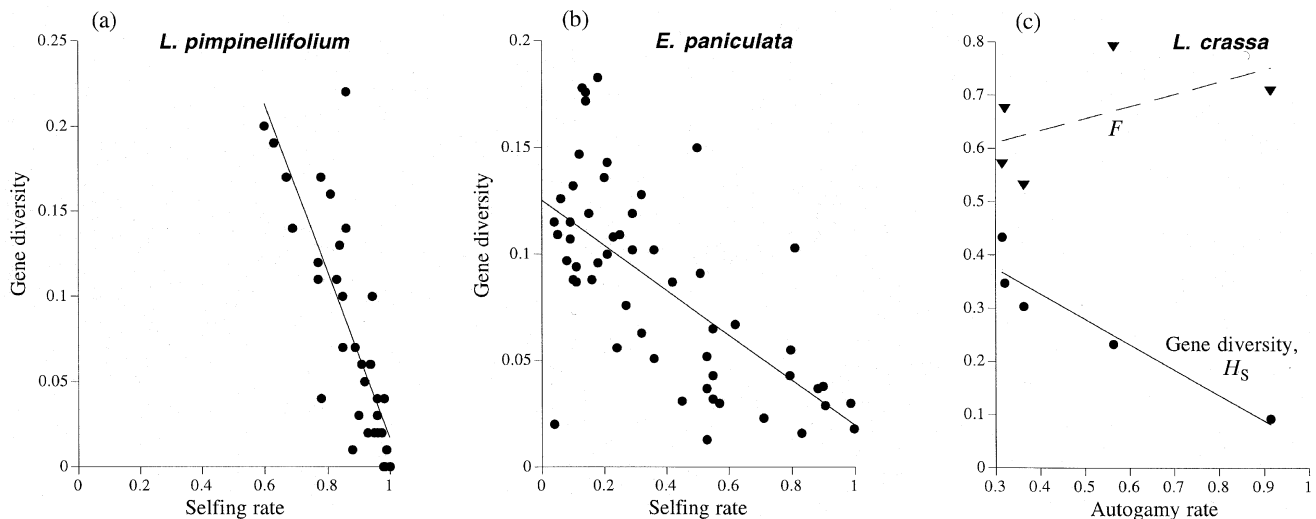

Fig. 1 The relationship between allozyme diversity and levels of inbreeding in three species. (a) Selfing rates and gene diversity values for different populations of *Lycopersicon pimpinellifolium* (re-analysis of data in Rick *et al.*, 1977); (b) selfing rates and gene diversity values for different populations of *Eichhornia paniculata* (unpubl. data from the same populations as in Barrett & Husband, 1990); (c) gene diversity values and inbreeding coefficients in relation to autogamy rates in *Leavenworthia crassa*.

lations of *L. crassa* and *L. alabamica* are highly outcrossing, whereas other populations of *L. crassa* and *L. alabamica* are inbreeders, as are *L. uniflora*, *L. torulosa* and *L. exigua*. Autogamy measured in the greenhouse is very variable from year to year (Lloyd, 1965), presumably because of environmental differences, but the clear correlations with self-fertility measurements show that real differences exist between plants within populations, and between different *Leavenworthia* species. Self-fertility and autogamy of *L. crassa* populations did not rank in the same order, showing that autogamy is not an inevitable consequence of self-fertility.

The allozyme electrophoresis results also support these conclusions and agree with those of Solbrig (1972), who showed that band variability is higher in outbreeding than inbred *Leavenworthia* populations, but did not quantify allozyme diversity, and Lyons & Antonovics (1991), who found a higher frequency of polymorphic loci in inbreeding than outbreeding populations of *L. crassa*. Although the rankings of autogamy and gene diversity do not agree perfectly in the data from *L. crassa*, the only deviation from such agreement is the reversed order of the two most autogamous populations, both with low diversity. In *L. alabamica*, however, both populations had high diversity, even though one population (95009) is clearly more self-compatible and more autogamous than the other (95006), and has a higher inbreeding coefficient. No consistent effect of breeding system was seen on between-population differentiation.

The results are thus also consistent with the patterns of allozyme diversity seen generally in inbreeding and outcrossing plants. Overall, within-population allozyme diversity in inbreeders averages about half that of outcrossers (e.g. Schoen & Brown, 1991; Hamrick & Godt, 1996), but a greater proportion of the diversity of selfing plants is between populations. G_{ST} values for inbreeding and outcrossing dicotyledonous plants average 0.587 and 0.184, respectively, and breeding system is a major factor influencing G_{ST} and F_{ST} values of plant species (Hamrick & Godt, 1996). The few within-genus comparisons available give similar results, although selfers often have less than half the within-population diversity of related outcrossers (Fenster & Ritland, 1992; Wyatt *et al.*, 1992; Charlesworth *et al.*, 1997) and they appear also to show lower total diversity. These results also agree well with those reviewed in the past for morphological and quantitative genetic diversity, as well as diversity for genetic markers (Baker, 1953; Brown, 1979). Much of the G_{ST} difference could be explained by the lower

within-population diversity in inbreeding species (0.165 for dicotyledonous selfers vs. 0.091 for outcrossers, see Hamrick & Godt, 1996); differences in D_{ST} are less pronounced, although there is still a tendency for inbreeders to have higher values (Charlesworth *et al.*, 1997).

The reasons for lower within-population diversity in inbreeding compared with outbreeding populations are not yet fully understood, and it is difficult to discriminate between the different possibilities outlined above. Comparing *L. stylosa* with its inbreeding relatives *L. uniflora* and *L. torulosa*, the effect of loss of self-incompatibility on allozyme diversity (measured as gene diversity) is clearly greater than twofold (but the effect of inbreeding on total species allozyme diversity is not known, because only single populations of the inbreeding species were studied). In *L. crassa*, the most inbreeding population averaged about 21% of the diversity values of the most outcrossing population studied here, the diversity of which was only slightly lower than the average H_S value of the three *L. stylosa* populations (0.486). Given that the most highly outcrossing *L. crassa* populations studied here are moderately inbreeding, this again suggests a considerably more than twofold effect for the diversity difference between highly inbred and outcrossing populations. These results are in agreement with data from the few other comparisons between closely related populations (Rick *et al.*, 1977; Barrett & Husband, 1990; Fenster & Ritland, 1992); all these studies find an effect of inbreeding greater than that estimated from comparisons of unrelated species (Hamrick & Godt, 1996). This large effect argues for an effect of inbreeding on neutral diversity additional to the direct effect of homozygosity on effective population size.

Hitch-hiking events (selective sweeps) are a possible cause of low allozyme diversity in inbreeders, but do not seem likely to explain the data obtained by the present study. When populations lose self-incompatibility and evolve selfing, spread of alleles causing increased selfing must have occurred, and selective sweeps would have been possible during this process. Yet, in both *L. crassa* and *L. alabamica*, there is considerable allozyme diversity within inbreeding populations. There must therefore have been enough outcrossing during the spread of the alleles causing selfing to allow recombination to occur between heterozygous genomes, and sufficient time for these exchanges to make the allozyme loci independent of the locus or loci that control the breeding system difference. This is quite plausible, given that neither of these taxa includes extremely

inbreeding populations, and very high inbreeding is needed for large effects of selective sweeps (Hedrick, 1980). Even in *L. uniflora* and *L. torulosa*, there is probably enough outcrossing for selective sweeps to be unlikely to explain their complete lack of diversity.

Another possible explanation for the presence of diversity in *L. crassa* and *L. alabamica* is that some form of balancing selection maintains allozyme diversity, even in highly inbreeding populations. Because polymorphisms maintained by overdominance are expected to be lost under sufficiently high inbreeding (Kimura & Ohta, 1971), some other form of selection may be involved. Alternatively, given the complete absence of allozyme diversity within *L. uniflora* and *L. torulosa* populations, these species (but not the inbreeding populations of *L. crassa* and *L. alabamica*) may exceed the threshold inbreeding level at which the polymorphisms are lost.

Highly inbreeding populations are also expected to evolve reduced diversity because of background selection (Charlesworth *et al.*, 1993). Under this hypothesis, the non-negligible allozyme diversity in some present-day inbreeding populations is explicable in terms of recent evolution of selfing, because equilibrium levels of genetic diversity will not be established until many generations have passed under a changed breeding system. As both *L. crassa* and *L. alabamica* are polymorphic for self-incompatibility (see above), a recent origin is probable.

To gain further insight into the causes of low genetic diversity of inbreeders, it will be very helpful to have data on DNA sequence variability from several loci. DNA sequence analysis can provide data on silent variants, such as synonymous and intron sites in loci, which are probably close to neutral. It will be important to know whether similar patterns of low diversity in inbreeders are seen for neutral variants. If allozymes and DNA variants are not concordant, this might support the suggestion that the allozyme diversity is maintained by balancing selection. Furthermore, even assuming selective neutrality, differences between genotypes for allozymes probably do not increase linearly with genealogical times (Marshall & Brown, 1975), and cannot therefore be used in tests, such as Tajima's test (Tajima, 1989), which can detect events such as selective sweeps that affect diversity, and may thus be able to reveal the causes of any differences found.

Although some sequence diversity estimates exist for plant populations that have also been studied for their allozyme diversity (mostly from RFLP studies, e.g. Zhang *et al.*, 1993; Raybould *et al.*, 1996), the diversity is not often expressed in terms of comparable measures such as estimated diversity per site. There are, moreover, as yet no quantitative comparisons of sequence diversity involving populations with different inbreeding levels, although a general concordance between the two kinds of data, with low diversity in inbreeding species, has been established in *Lycopersicon* (Miller & Tanksley, 1990). Some preliminary data show a large difference between inbreeding and outcrossing *Leavenworthia* populations, and no diversity within inbreeding populations of *L. uniflora* and *L. crassa*, at least at some loci (Liu *et al.*, 1998). For *L. crassa*, this contrasts with the allozyme data presented here.

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