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Quantitatively determined self-incompatibility

3. Genetical variability in *Borago officinalis*

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Summary. It is shown by simulation that a hypothetical multilocus, quantitatively determined self-incompatibility system, whether gametophytic or sporophytic, should maintain variability in small populations at a higher level than would panmixia. Studies of more than 20 isozyme loci show that borage has almost no variability.

Key words: *Borago officinalis* – Isozyme – Polymorphism – Self-incompatibility

Introduction

In general, an outbreeding population of a given size must maintain a higher level of heterozygosity than an inbreeding population, and it should also maintain higher numbers of effectively neutral alleles at each locus, although the actual effects of any particular breeding system are hard to predict (e.g., Hedrick 1990). Since self-incompatibility is an outbreeding mechanism, the degree of self-incompatibility might be expected to be positively correlated with the level of heterozygosity or major gene variability. Table 1 shows the level of heterozygosity in a number of plant species classified according to their breeding system. The much more extensive, but broadly classified, tabulation of Hamrick and Godt (1989) shows that self-pollinated species average about 12% gene diversity (very similar to heterozygosity) and about 1.7 effective alleles per locus, whereas for outcrossed species the comparable figures are 16% and 2.2.

In previous papers, we have considered the maintenance of a hypothetical multilocus self-incompatibility system (Mayo and Leach 1989) and the effect of outcrossing and selfing on fertility in borage (Leach et al. 1990), which has been hypothesized to have such a system (Crowe 1971). Leach et al. (1990) showed that there was significant inbreeding depression, but did not investigate its origin, which might have involved more heterozygous advantage than would be expected in a completely outbred species (Ziehe and Roberds 1989).

We now consider two further aspects of the hypothetical defined system and the real, largely unknown, system. First, we consider some aspects of loss of variability in the model system and, hence, should such a system be more or less typical of quantitative systems, what level of variability might be expected. Secondly, we present results showing the extent of variability at a large number of enzymic structural gene loci in borage.

Table 1. Heterozygosity, H_e, in some natural plant populations (modified from Mayo 1987)

Species	H_e		Attributes
Phlox drummondii Hook	0.04	[a]	Self-incompatible
P. cuspidata Schaele	0.01	[a]	Self-compatible (facultative inbreeder)
Stephanomesia exigua	0.09		Outbreeder
Lupinus subcarnosus	0.36	[a]	Edaphically restricted
L. texensis	0.10	[a]	Edaphically restricted but less than *L. subcarnosus*
Hymenopappus scabiosaeus var. *carymbosus*	0.20		Not edaphically restricted
H. artemisiaefolius	0.21		Not edaphically restricted but different range from *H. scabiosaeus*
Conocephalum conicum	0.17 [b]		Haploid (a liverwort)

[a] Significantly different at 5% level
[b] Equivalent heterozygosity = 1-diploid expected homozygosity

Table 2. Variability, averaged over ten loci, of the sporophytic self-incompatibility genes. (Results over 200 generations, after 1,800 generations to equilibrate the population)

N	Breakdown	Stringency	$\bar{q}$ ($q_0=0.5$)	$\bar{H}_e$
100	0.1	0.1	0.5198±0.0034	0.2658±0.0008
		0.25	0.4557±0.0039	0.2735±0.0012
250		0.1	0.5317±0.0026	0.2577±0.0005
		0.25	0.4972±0.0021	0.2497±0.0004

Table 3. Time, T, to fixation for a diallelic gene with initial gene frequency $q=0.5$, $N=100$, stringency $=c=0.25$ at the unlinked sporophytic self-incompatibility system. (Ten replicates of each set of starting conditions)

Breakdown	$\bar{T}$
0.1	249.8± 57.6
0.2	354.9± 74.4
0.3	329.0±106.5
0.4	239.0± 59.9
0.5	173.0± 21.0

Table 4. Time, T, to fixation for a diallelic gene with initial gene frequency $q=0.5$, $N=100$, $c=0.1$, recombination rate ϕ between this gene and one of the ten genes in the gametophytic self-incompatibility system. (Five replicates of each set of starting conditions, except for the last two cases where there were six replicates)

ϕ	Breakdown	$\bar{T}$
0.5	0.1	203.0± 39.5
	0.5	407.0±153.3
0.01	0.1	220.4±103.4
	0.5	468.2±163.5
0.001	0.1	375.0±192.3
	0.5	428.0±230.1
0	0.1	254.7±103.2
	0.5	667.1±345.2

Variability in a hypothetical ten-locus system

The systems considered were described by Mayo and Leach (1989) and Mayo (1990). In essence, the ability of a pollen grain to effect pollination is determined by the genetical difference, summed over ten loci, between pollen grain (or male parent in the sporophytic variant) and female parent. The more stringent the system, the greater the difference required. Also, different levels of system breakdown of failure were incorporated.

Table 2 shows the variability maintained at the ten self-incompatibility loci at equilibrium, with mutation supplying variation at all ten loci.

We next considered the loss of variability for a neutral diallelic locus, either linked or unlinked to one of the genes in the self-incompatibility system. Ewens (1979) has shown that for an initial gene frequency of q, the expected mean time to fixation is given by $T=-4N(q\ln q=(1-q)\ln(1\text{-}q))$. For $N=100$ and $q=0.5$, $T=277$. Table 3 shows some results for an unlinked gene, Table 4 for a linked gene.

Structural gene variability in Borago officinalis

To assess the level of genetical variability in the populations of borage which we have established (Leach et al. 1990), we examined 18 enzyme systems, as shown in Table 5 and described below.

Materials and methods

Plants

Sources of borage have been extended a little beyond those described by Leach et al. (1990). We obtained seed from one local garden escape population, three local seed merchants, and ten seed merchants or botanical gardens in the UK and France.

Electrophoresis

Seeds were planted directly into potting soil. Approximately 20 mg of (preferably) first cotyledons or later very young leaf tissue was prepared for horizontal, starch-gel electrophoresis by crushing in 50 µl of Hepes lysis buffer and absorbing onto 9 mm × 3 mm wicks of Whatman 3 MM paper. One of the following three buffer systems was used in each case: system A – electrode buffer 25 m*M* LiOH, 180 m*M* H_3BO_3 (pH 8.3), gel 9 parts 70 m*M* TRIS, 7 m*M* citric acid (pH 8.3), and 1 part electrode buffer; system B – electrode 0.3 *M* H_3BO_3, 0.1 *M* NaOH (pH 8.7), gel 14 m*M* TRIS, 4 m*M* citric acid (pH 7.4); system C – electrode 37.5 m*M* TRIS, 12.5 m*M* citric acid (pH 7.0), gel 12.5 m*M* TRIS, 4 m*M* citric acid (pH 7.0).

For system C, electrophoresis was carried out for 5 h and for systems A and B until the borate front had migrated 9 cm from the slots.

Each gel was cut horizontally into two slices and the anodal portion of the gel was assayed for the following enzymes:

ACO, ALD, DIA, FUM, GPI, GDH, GOT, LAP, MDR	system A
ACP, CAT, PER, EST, SKD	system B
G6PD, MDH, PGM, 6PGD	system C

(See Table 5 for more detail.)

PER was assayed on the cathodal portion of gels run on system B with slots in the middle of the gel.

SOD stained on gels primarily assayed for MDH, SKD, or FUM.

Results

With the exception of DIA (see Tables 5 and 6), no reliably scorable variation was detected in any of the material tested. Table 7 details scoring of progeny from controlled crosses and selfs of DIA-typed plants, showing that segregations agree with single-factor Mendelian expectations. In the case of GOT-1, -2 and -3, plants were

Table 5. Isozyme systems tested

Name	Abbreviation	Number of loci detected
Acid phosphatase	ACP	2
Aconitase	ACO	1
Aldolase	ALD	1
Catalase	CAT	1
Diaphorase	DIA	1
Esterase	EST	2
Fumarase	FUM	1
Glucose phosphoisomerase	GPI	2
Glucose 6 phosphodehydrogenase	G6PD	1
Glutamate dehydrogenase	GDH	1
Glutamate oxaloacetate transaminase	GOT	3
Leucine amino peptidase	LAP	1
Malate dehydrogenase	MDH	1
Menedione reductase	MDR	1
Peroxidase	PER	1
Phosphoglucomutase	PGM	>1
6 Phosphogluconate dehydrogenase	6PGD	2
Superoxide dismutase	SOD	>3
Shikimate dehydrogenase	SKD	1

Table 6. Borage isozyme work – summary

DIA-1					
	Phenotypes	3-3	3-4	4-4	
Australia	Yates	86	9	2	97
	Henderson	63	13	3	79
	Hortico	18	–	–	18
p(3)=0.9191 p(4)=0.0909					
British & European	Suttons	28	2	–	30
	Fothergills	17	2	–	19
	Arne Herb	16	1	–	17
	Welsh Plant Breeding Inst.	8	–	–	8
	Montpellier	24	1	–	25
	Unwins	8	–	–	8
	Nulting	8	–	–	8
	Oxford Bot. Gdns.	8	–	–	8
	Suffox	7	–	–	7
	Johnsons	8	–	–	8
		132	6	0	138
p(3)=0.9783 p(4)=0.0217					

DIA-2 highly unreliable, not clearly heritable

initially scored as being polymorphic, but these typings were found to be variable with time. Some 100 plants tested four times over a 6-week growth period failed to provide consistent typings. Also, the phenotypes of progeny from crosses between putatively genotyped plants were not consistent with classical genetic expectations.

Table 7. Mendelian segregation at the diaphorase (DIA) locus

	3-3	3-4	4-4	χ^2_2(1:2:1)
YB2⊕(3-4)	4	12	4	0.8
YB2 × YB49 (3-4)	4	9	10	3.87
YB2 × YB38 (3-4)	0	13	5	6.33*
YB2 × YB41 (3-4)	12	17	4	3.91
YB38 × YB41	2	12	7	3.0
YB38⊕	4	5	5	1.2
YB41⊕	2	19	6	5.67
HB37⊕(3-4)	7	12	11	2.27
HB54 × YB4	9	5	0	1.14
YB4 × HB54	12	12	0	0
3-3 × 3-4				

* $P<0.05$. All sample sizes are relatively small. Low seed set per cross and incomplete germination invariably meant that at least four crosses were necessary to recover one typed plant. This being the case, there is an overall good fit between observed and expected

Discussion

Tables 3 and 4 allow one to conclude that the particular multilocus self-incompatibility system modelled here does not appear likely to alter the level of allelic variability in a small population from that to be expected under random mating. If, therefore, borage is normally outcrossed, allelic variability should be similar to that observed for other outbreeding annuals.

Our results (Tables 6 and 7) are not in conformity with this expectation. Given the wide range of sources of seed, we cannot attribute this to sampling of a spatially heterogeneous, locally invariant population (cf, e.g., Turner et al. 1982).

As noted by Leach et al. (1990), the results from borage are not in conformity with any systematic protection from inbreeding, but this is not to say that cell recognition mechanisms of the type now being elucidated (Walker and Ren 1990) have not influenced the incidence of cross- or self-pollination. What one can say is that predominant outbreeding, inbreeding depression, normal Mendelian segregation, and a very low level of heterozygosity coexist in borage.

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