Theor Appl Genet (1990) 79:427–430

Quantitatively determined self-incompatibility

2. Outcrossing in *Borago officinalis*

C. R. Leach [1], O. Mayo [2,]* and R. Bürger [3]

[1] Genetics Department, The University of Adelaide, North Terrace, Adelaide 5000, Australia
[2] Biometry Section, Waite Agricultural Research Institute, Glen Osmond 5064, Australia
[3] Institute of Mathematics, University of Vienna, A-1090 Vienna, Austria

Received October 1, 1989; Accepted October 20, 1989
Communicated by H. F. Linskens

Summary. It has been claimed that Borage (*Borago officinalis* L.) has a multifactorial self-incompatibility system. Such systems may have a high level of ineffective pollination, and we show that this is the case in borage. The ranking of seed set from highest to lowest is as follows: bee-pollination; natural pollination in the absence of bees; artificial cross-pollination between unrelated plants; artificial cross-pollination between related plants; artificial self-pollination. In diallel crosses, significant parental effects were detected but no consistent patterns of seed set, which suggest a simple self-incompatibility system, were detected. The level of outcrossing with natural pollination was very variable but greater than 50%. Thus, there appears to be no straightforward self-incompatibility system in borage.

Key words: *Borago officinalis* – Inbreeding depression – Outcrossing – Self-incompatibility

Introduction

In the first paper in this series (Mayo and Leach 1989), we identified some of the properties of hypothetical multi-locus self-incompatibility systems. Considering gametophytic and sporophytic variants of a ten-locus diallelic system, we showed that in small populations, mutation rates necessary to maintain a given level of variability are high, as already determined theoretically for oligogenic self-incompatibility systems. However, we found the extent of ineffective pollination to be very much greater than in oligogenic systems. The Appendix finalizes some of the concepts of the multi-locus diallelic system, but this remains completely speculative.

We have, therefore, examined outcrossing in borage (*Borago officinalis*), which has been reported to have a multifactorial system of self-incompatibility (Crowe 1971), in order to try to determine whether any of the properties of its reproductive system could indeed be explained by a multifactorial model of self-incompatibility.

Materials and methods

We have established three small populations of borage, and have obtained seed from a number of other sources (Table 1).

A reliable, repeatable technique of artificial pollination had to be developed. This was done on a diallel cross of the original 12 garden escape plants, results of which are not reported here.

The crossing procedure ws as follows. Immature heads were emasculated by removal of petals and attached anther, and covered with small bags made of porous glassine material. After 3–5 days they were pollinated with a sample of freshly collected pollen from the male parent, and bags were replaced over the flowers.

Results

Since the postulated method of avoidance of inbreeding was embryo abortion (Crowe 1971), seed set out of a usual maximum of four could be used as a model for the effectiveness of such avoidance. However, it could also simply be a manifestation of inbreeding depression, if outcrossing were determined not by self-incompatibility but rather by, e.g., cryptic protandry or protogyny.

The first question to be answered is the actual level of seed set with natural pollination by bees. Accordingly, on a number of occasions we randomly sampled flowers of

---

* Present address: CSIRO Division of Animal Production, Prospect 2149, Australia

Table 1. Establishment of initial populations and other seed sources

1	Garden escape population	12 original plants now dead 50 progeny from crosses between these plants
2	Seed merchant 1 (Yates)	1st planting 53 plants 2nd planting 44 plants
3	Seed merchant 2 (Henderson)	1st planting 41 plants 2nd planting 43 plants
4	Welsh Plant Breeding Station	
5	UK Seed Merchant 1 (Arne)	
6	Montpellier Botanic Gardens	
7	UK Seed Merchant 2 (Fothergill)	
8	Oxford Botanic Gardens	
9	UK Seed Merchant 3 (Suttons)	

Table 2. Seed set in natural open pollination in different environments

Population	Environment	Seed set (mean ± SD)	Sample size
1	Garden 1 (single plant)	0.91 ± 1.10	23
	(single plant)	1.64 ± 1.17	59
	(several plants)	2.26 ± 0.85	89
3,3 × 2	Garden 2	1.56 ± 1.29	642
4	Glasshouse	1.27 ± 0.75	232
5	Glasshouse	1.16 ± 1.01	56
6	Glasshouse	1.33 ± 0.94	104
7	Glasshouse	1.04 ± 0.87	139
8	Glasshouse	1.18 ± 0.88	74
9	Glasshouse	1.06 ± 1.01	70

Table 3. Diallel crosses carried out

Cross 1	6 × 6 diallel. Four sibling plants from a single capsule of cross 7 × 4 (of the original plants 1, 2, ... 12) and two sibling plants from the reciprocal 4 × 7 were crossed in all combinations including selfs. 7 × 4–1, 2, 3, 4, 4 × 7–1, 2
Cross 2	7 × 7 diallel. Two sibling plants from one capsule of 6 × 4, two sibling plants from one capsule of the reciprocal 4 × 6, one from a different capsule of 4 × 6, and two sibling plants of one capsule of 5 × 4. 6 × 4(3)–1, 2, 4 × 6(1)–1, 4 × 6(1)–2, 5 × 4–1, 2, 4 × 6(2)–1
Cross 3	9 × 9 diallel. Three, four and two sibling plants from three different capsules of 6 × 4. 6 × 4(3)–1, 2, 3, 6 × 4(2)–1, 2, 3, 4, 6 × 4(3)–1, 2
Cross 4	4 × 4 diallel. Two plants from seed from Yates Seeds, two plants from seed from Henderson's Seeds. Y4, H37, H54, Y19

Table 4. Seed set following selfing or crossing in the four diallel experiments. (Mean ± SD; no. of selfs or crosses in brackets)

Experiment	Self	Cross
1	0.693 ± 0.253 (6)	0.837 ± 0.528 (30)
2	0.610 ± 0.407 (5)	0.638 ± 0.524 (32)
3	0.583 ± 0.409 (8)	0.913 ± 0.603 (61)
4	0.523 ± 0.382 (4)	1.086 ± 0.765 (12)

Table 5. Mean seed set in diallel crosses

Plant	1	2	3	4	5	6	7	8	9
Diallel 1 [overall mean seed set 0.775 ± 0.060 (373)]									
1	0.75	0.15	1.65	1.35	1.65	0.38			
2	0.35	0.65	0.35	0.15	0.87	0.75			
3	1.55	0.55	0.55	0.35	0.85	0.75			
4	1.15	0.97	0.15	0.35	0.75	0.71			
5	1.35	1.35	1.75	0.47	1.11	1.63			
6	1.32	0.55	0.05	0.25	0.95	0.75			
Diallel 2 [overall mean seed set 0.565 ± 0.059 (329)]									
1	1.00	0.09	0.25	0.00	0.00	0.00	1.00		
2	0.00	–	–	–	0.60	0.76	–		
3	0.37	0.82	0.05	1.30	0.83	0.80	1.09		
4	0.00	1.73	0.35	0.40	1.63	1.71	–		
5	0.50	1.30	0.51	0.60	1.00	0.50	1.10		
6	0.33	1.70	0.55	0.10	0.82	0.60	0.44		
Diallel 3 (mean seed set 0.898 ± 0.218)									
1	0.40	0.39	0.50	0.60	0.90	0.50	0.70	0.12	0.60
2	1.00	0.22	2.12	1.00	1.67	–	1.60	–	–
3	1.00	0.82	1.00	1.37	0.75	2.00	0.71	0.50	1.00
4	1.40	1.00	0.12	1.30	1.00	0.00	0.00	–	1.20
5	0.17	0.00	0.67	0.75	0.22	1.00	0.50	1.00	1.00
6	0.78	2.26	1.44	1.56	0.70	0.50	0.64	0.67	2.30
7	0.00	1.30	1.67	1.00	2.17	0.80	0.80	1.00	0.50
8	0.27	–	–	–	–	–	–	–	–
9	2.20	0.50	1.50	0.40	1.12	0.14	0.20	1.80	0.22
Diallel 4 [mean seed set 0.840 ± 0.072 (232)]									
1	0.78	0.64	1.33	0.93					
2	1.80	0.88	2.75	2.33					
3	1.24	0.82	0.38	0.79					
4	0.60	0.00	0.60	0.05					

plants grown in gardens. The results are shown in Table 2.

Next, we set out to determine the level of seed set in crossed plants when they were artificially either selfed or crossed. Seed set in selfed or crossed offspring of selfed or crossed plants, or subsequent crosses and selfs of more extremely inbred plants, might determine whether depression in seed set resulted from the occurrence of deleterious (homozygous) recessive traits or from the action of the outbreeding system. However, the initial question was the relative success of selfing or crossing, which could be assessed from diallel crosses. Table 3 shows the design of these experiments. Tables 4 and 5 show the results. There were significant differences between female parents in seed set and also between male parents. In addition, there were significant differences among female-male combinations. (These tests are not presented, but the analyses were carried out in GLIM (McCullagh and Nelder 1983), treating seed number as a binomially distributed variate.)

We also considered that it would be important to determine the extent of natural outcrossing and so set up a garden experiment with alternating genotypes for di-

Table 6. Segregation ratios for alleles of the diaphorase structural gene together with estimates of the outcrossing rate

Genotype of parent	Progeny				Outcrossing rate
	3	34	4	Total	
3	29	7		36	0.39±0.08
	54	13		67	0.39±0.06
	46	27		73	0.74±0.05
	42	13		55	0.47±0.07
	23	15		38	0.79±0.07
34	12	23	9	44	
	13	23	10	46	
	17	25	6	48	
4		25	29	54	0.93±0.03
		9	13	22	0.82±0.08
		17	15	32	1.06
		11	16	27	0.79±0.07
		55	36	91	1.21
		13	8	21	0.76±0.09

aphorase, one of the genes found to show variability (C. R. Leach and O. Mayo, in preparation).

The layout was as follows:

N 3 4 3 4 3 4 3 4 3 4 34
4 34 4 3 4 34 4 3 4 3 4

The plants were in 20-cm pots placed no more than 5 cm apart in either direction.

Our aim was to bag the first 50 flowers to open on all plants. This was not possible for all plants, since seasonal factors (extreme heat and drying winds) caused two plants to set no seed and two to set very few seed. Also, one plant appeared to be male sterile.

Results for the plants for which progenies could be raised are shown in Table 6. The degree of outcrossing was estimated very simply as twice the frequency of heterozygotes arising in the progeny of the homozygotes. The progeny of the heterozygotes could not be used for this purpose; the genotypes were included to test for disturbed segregation.

(If the female is genotype $A_1 A_1$ and the frequencies of alleles A_1 and A_2 in the non-self pollen are p and q respectively, then the proportion of heterozygotes in the offspring is simply fq, where f is the frequency of cross-pollination. In the present case, $q \approx 25/42$, but we have used $q = 1/2$ because of uncertainties about the pollination effectiveness of some plants. This underestimates the degree of outcrossing.)

The homozygote: heterozygote ratios are significantly different between the three homozygous parents and the four homozygous parents. Thus, we cannot pool the data for the two groups. Further, the four homozygotes, while homogeneous in their ratios, have two values greater than 1.

We can, however, pool the data for the three homozygous parents, which are also homogeneous. (All χ^2 tests have used a 1% significance level since so many tests have been performed.) We obtain $\hat{f} = 0.56 \pm 0.03$. (For the four homozygotes, $\hat{f} > 1$.) Thus, we find natural outcrossing in borage is at the level of high intermediate frequency that is difficult to explain (Mayo and Leach 1987).

Discussion

From Table 5 we see that, among crosses, seed set is lower when the parents are more closely related. This phenomenon is not seen in every case, but where there are exceptions, e.g., 4 × 2 in cross 4, they tend to result from small numbers of pollinations late in the season (three only for 4 × 2; but plant 2 performed better as a female than the other plants in the cross).

All of these data are compatible with inbreeding depression as the only phenomenon necessary to describe the results. Bee pollination gives higher seed set than open pollination with absence of bees; open pollination gives higher seed set than artificial pollination; artificial crossing gives higher seed set than artificial selfing; the more similar the genotypes in the artificial crossing, the lower the seed set. That different plants perform differently as male and female parents could be the result of some self-incompatibility system, but in the diallel crosses of closely related plants, we see no evidence of systematic differences that would suggest self-incompatibility. The work of Ziehe and Roberts (1989) shows that a very large range of inbreeding depression is possible in partially selfing populations so that, without an understanding of the outbreeding mechanism (and of course its rate), it is impossible to associate the inbreeding observed with the predictions of the models of Ziehe and Roberts.

Acknowledgements. We thank Karen Goodwins for careful technical assistance. We thank Dr. M. Cornish for supplying borage seed. This work was partly supported by Australian Research Council Grant No. A187 1 6246.

Appendix

Probability of selfing in an n-gene diallelic self-incompatibility system

Suppose we have n gene loci with two alleles each and scale their effect on ability to pollinate as ± 1. Then we can describe the genotype of a female plant as $k_1 \times (-1, -1)$, $k_2 \times (+1, -1)$, $k_3 \times (+1, +1)$ with $k_1 + k_2 + k_3 = n$. Then the female's genotypic value is $F = -2k_1 + 2k_3$. A male gamete consists of $m \times (-1)$ and $(n-m) \times (+1)$ gametes, where $m = k_1 + m_2$, $n - m = k_3 + (k_2 - m_2)$ and $0 \leq m_2 \leq k_2$. The male's genotypic value is $M = 2(-m + n - m) = 2n - 4m$. Then $|M - F| = 2|n - 2m + k_1 - k_3| = 2|k_2 - 2m_2|$.

We set $|M - F| > 2nc$ for some fixed c, $0 < c < 1$, as the criterion for successful pollination.

Assuming Mendelian segregation we observe that

Pr[randomly formed gamete is of type $(m, n-m)$ | female $=(k_1, k_2, k_3)$]

$$= \begin{cases} 0 & \text{if } m > k_1 + k_2, \text{ i.e., } m_2 > k_2 \\ 0 & \text{if } m < k_1, \text{ i.e., } m_2 < 0 \\ 2^{-k_2}\binom{k_2}{m_2} & \text{if } k_1 \leq m \leq k_1 + k_2 \text{ i.e., } 0 \leq m_2 \leq k_2. \end{cases}$$

Here and below $\binom{n}{k} = \frac{n!}{k!(n-k)!}$ denotes the binomial symbol.

Now denote by $p(k_2)$ the frequency of females heterozygous at exactly k_2 loci, regardless of the distribution of homozygotes and by $p(k_1, k_2, k_3)$ the frequency of females of type (k_1, k_2, k_3). Then, according to the above pollination criterion

Pr[randomly drawn gamete $(m, n-m)$ is compatible with (k_1, k_2, k_3) female] = Pr[gamete is $(m, n-m)$ | female is (k_1, k_2, k_3)]

$$\times\, p(k_1, k_2, k_3) \times \begin{cases} 0 & \text{if } c \geq |k_2 - 2m_2|/n \\ 1 & \text{if } c < |k_2 - 2m_2|/n. \end{cases}$$

If we sum over all possible females and all gametes and set

$$\Pr[\text{selfing}] = \begin{cases} 0, & \text{if } 1 \leq c \\ A_1, & \text{if } \frac{n-1}{n} \leq c < 1 \\ \vdots & \\ A_j, & \text{if } \frac{n-j}{n} \leq c < \frac{n-j+1}{n} \\ \vdots & \\ A_n, & \text{if } 0 \leq c \leq \frac{1}{n} \end{cases}$$

then we can write

$$A_1 = \sum_{\substack{0 \leq k_2 \leq n \\ 0 \leq m_2 \leq k_2 \\ |k_2 - 2m_2| = n}} 2^{-k_2}\binom{k_2}{m_2} p(k_2)$$

and

$$A_j = A_{j-1} + \sum_{\substack{0 \leq k_2 \leq n \\ 0 \leq m_2 \leq k_2 \\ |k_2 - 2m_2| = n-j+1}} 2^{-k_2}\binom{k_2}{m_2} p(k_2)$$

with the summation range as shown in order that the pollination criterion $|M - F| = 2|k_2 - 2m_2| > 2nc$ is satisfied.

Now $|k_2 - 2m_2| = n - j + 1$ if and only if $m_2 = \frac{1}{2}(k_2 \pm (n+1-j))$ which yields in both cases the same $\binom{k_2}{m_2}$ value and implies

$$\sum_{\substack{0 \leq k_2 \leq n \\ 0 \leq m_2 \leq k_2 \\ |k_2 - 2m_2| = n-j+1}} 2^{-k_2}\binom{k_2}{m_2} p(k_2) =$$

$$= \sum_{\substack{n-j+1 \leq k_2 \leq n \\ k_2 - (n-j+1) \text{ is even}}} 2^{-k_2+1}\binom{k_2}{k_2-(n-j+1)} p(k_2).$$

Introducing the new summation variable l via $k_2 = n - j + 1 + 2l$ we obtain

$$A_j = A_{j-1} + \sum_{l=0}^{[(j-1)/2]} 2^{-(n-j+2l)}\binom{n-j+1+2l}{l} p(n-j+1-2l),$$

where $[(j-1)/2]$ denotes the greatest integer $\leq (j-1)/2$. Now each A_j for $j \geq 2$ can be calculated recursively. Introducing the new variable $k = i - 2l - 1$ and collecting coefficients of $p(n-k)$ we find

$$A_j = \sum_{k=0}^{j-1} \left\{ \sum_{i=0}^{[(i-k-1)/2]} \binom{n-k}{i} \right\} 2^{-(n-k-1)} p(n-k)$$

and this is the probability of selfing if $\frac{n-j}{n} \leq c < \frac{n-j+1}{n}$. Hence

$$A_1 = 2^{-(n-1)} p(n)$$
$$A_2 = 2^{-(n-1)} p(n) + 2^{-(n-2)} p(n-1)$$
$$A_3 = 2^{-(n-1)} (n+1) p(n) + 2^{-(n-2)} p(n-1) + 2^{(n-3)} p(n-2)$$
$$\vdots$$

For any given set of initial conditions, we can then derive the proportion of selfing to be expected. However, given that the system is entirely hypothetical, we have not pursued this course. The analysis demonstrates the complexity of a simple system, given that all effects have been assumed equal, linkage has been assumed absent, etc.

References

Crowe LK (1971) The polygenic control of outbreeding in *Borago officinalis* L. Heredity 27:111–118

McCullagh P, Nelder JA (1983) Generalized linear models Chapman and Hall, London

Mayo O, Leach CR (1987) Stability of self-incompatibility systems. Theor Appl Genet 74:789–792

Mayo O, Leach CR (1989) Quantitatively determined self-incompatibility. 1. Theoretical considerations. Theor Appl Genet 77:375–378

Ziehe M, Roberts JH (1989) Inbreeding depression due to overdominance in partially self-fertilizing plant populations. Genetics 121:861–868