Genetic variation for selfing rate and the dependence of selfing rate on mating history in *Brassica napus* (rape seed)

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Significant genotypic variation for selfing rate was found in the summer-annual rape seed variety Topas. The 'broad-sense' heritability of selfing rate was estimated as 0.80 and the 'narrow-sense' heritability as 0.41. Outcrossed plants had a significantly higher selfing rate than selfed plants. The relationship between plant size and selfing rate was ambiguous. The evolution of selfing rates is discussed.

Keywords: *Brassica napus*, evolution, genetic variation, heritability, mating history, selfing rate.

Introduction

The rate of self-pollination is of great importance for the evolution of self-compatible plant species. The selfing rate affects the mutation–selection balance of recessive deleterious alleles in populations (Haldane, 1932; Charlesworth *et al.*, 1990) and resource allocation between male and female functions (Charnov, 1982). The evolution of the selfing rate has received much attention in recent years with a number of population genetic and ecological models used to explain intermediary selfing rates as possible evolutionary stable strategies (reviewed by Uyenoyama *et al.*, 1993). The evolution of selfing rate is dependent on the amount of genetic variation and whether variation in selfing rate is correlated to plant size or other fitness traits.

Most population genetic models assume that the selfing rate of an individual heterozygous for a selfing rate determining locus will be intermediate between the selfing rates of the two homozygotes (codominance). However, if heterozygotes have a higher selfing rate than both homozygotes, populations with stable intermediate selfing rates can be obtained under less restrictive conditions (Damgaard *et al.*, 1992). Assuming that heterozygotes for a selfing rate determining locus have a higher selfing rate than both homozygotes, then outcrossed individuals have a higher selfing rate than selfed individuals because outcrossed individuals are more likely to be heterozygous. Thus, if the selfing rate of a population is observed to be dependent on the

number of consecutive selfing events such that outcrossed individuals have a higher selfing rate than selfed individuals, than an intermediate selfing rate as a stable mating strategy will be obtained more 'easily' than if the selfing rate were independent of the mating history, assuming that the selfing rate is mainly genetically controlled.

In this paper we estimated the genetic variation for selfing rate in eight selfed rape seed lines and looked for possible correlations between selfing rate and other fitness characters. Furthermore, the selfed lines were compared with their outcrossed half-sibs to study the effect of mating history on selfing rate.

Materials and methods

Study system

The rape seed lines came from a population of the summer-annual variety Topas. Rape seeds normally have 200–300 nectar-producing yellow flowers with six anthers and a single central stigma with about 30 ovules. The fruits (pods) are green, assimilate carbohydrates and each produces about 20 seeds. Rape seed is self-compatible and the stigma is receptive while the anthers dehisce. Topas typically has a selfing rate of about 70 per cent with some geographical variation and it varies with flowering time, i.e. the early flowers have a higher selfing rate than those from the middle or late in the flowering season (Becker *et al.*, 1992).

The eight lines used in this study were derived from the offspring of eight maternal plants that had a rare

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SDH isozyme pattern with a frequency of about 0.01 in the population (Becker *et al.*, 1992). The rare isozyme pattern enabled us to distinguish between self-pollinated and foreign-pollinated offspring following open-pollination under field condition in 1989. The isozyme patterns among the progenies of artificially selfed individuals and among double haploid lines (H. Becker, personal communication) were compatible with simple Mendelian inheritance at two *SDH* loci, which will be assumed in the following. The maternal plants being homozygous for rare alleles were assumed to result from selfing events. Three foreign-pollinated and three self-pollinated plants from each maternal plant were transplanted randomly, although making sure that no transplants were close together, at the three-to-four leaf stage into a normally grown rape seed field at approximately the same growth stage at Påskehøjgård, Denmark, in 1991.

Measurements and analyses

At the end of the growing season the plants were harvested, the number of side branches was counted, the dry above-ground biomass without the seeds was weighed, the total number of flowers was determined by summing the remaining flower stalks and fruits, and the seeds were weighed.

The seeds were germinated and after approximately a week the seedlings were used for electrophoresis. From each selfed plant, 10 seeds were examined for selfing events by the procedure outlined in Becker *et al.* (1992), and from each outcrossed plant 25 seeds were examined. More seeds were examined from outcrossed plants because the experimental error in determining the selfing rate was higher, as on average only one in four of the selfed genotypes could be scored due to segregation of the rare allele and the estimated number of selfed genotypes was therefore four times the number of selfed genotypes scored. If the estimated selfing rate was above 1, it was set to 1, which made the hypothesis testing more conservative. Eight plants (two selfed and six outcrossed) had poor germination and were not included in the analyses. As the experimental error among selfed and outcrossed individuals is expected not to be equal and the within-line variation therefore not homogeneous, we used a paired *t*-test instead of an ANOVA. In the paired *t*-test a selfed plant was paired randomly to an outcrossed plant from the same line. The conclusions were robust to permutations of the pairs.

The comparisons among the selfing rate and different plant fitness characters were done only for selfed plants as the experimental error in determining the selfing rate is less among those plants. The among-line

variation of selfing rate among the selfed individuals was an estimate of genotypic variation (σ_G^2) for selfing rate (Falconer, 1989) whereas the residual variance (σ_e^2) was an estimate of the variance due to environment and the experimental error due to sampling of seeds in determining the selfing rate of the individual plant. The 'broad-sense' heritability was estimated as $\sigma_G^2/(\sigma_G^2 + \sigma_e^2)$. The 'narrow-sense' heritability was estimated as twice the regression coefficient between the selfing rates of the maternal plants and the means within the outcrossed lines, which were assumed to be half-sib groups (Falconer, 1989). The analyses were done using the software package SYSTAT.

Results

Thirteen of the 18 outcrossed individuals had an estimated selfing rate of above 1 (Table 1) which means that the experimental error in determining the selfing rate of outcrossed individuals is too high to give a good picture of the selfing rate among outcrossed individuals. The outcrossed individuals had a significantly higher selfing rate than the selfed individuals (Table 2),

Table 1 Selfing rate and number of flowers of selfed and outcrossed plants

Line	Selfed plants		Outcrossed plants	
	Selfing rate	Flowers	Selfing rate	Flowers
1	0.60	143	1.00	206
1	0.47	270	—	212
1	0.60	139	0.36	414
2	0.70	211	0.44	325
2	0.75	228	—	303
2	0.67	198	—	215
3	0.60	293	1.00	315
3	0.65	274	1.00	237
3	—	294	—	277
4	0.47	372	0.53	420
4	0.56	373	0.80	560
4	—	352	—	302
5	0.70	294	1.00	441
5	0.70	312	1.00	190
5	0.60	321	1.00	327
6	0.30	270	1.00	375
6	0.00	277	1.00	204
6	0.33	311	1.00	410
7	0.67	223	—	236
7	0.78	354	1.00	252
7	0.89	203	0.95	243
8	0.86	330	1.00	530
8	1.00	370	1.00	283
8	0.80	382	1.00	705

Table 2 A paired *t*-test between outcrossed and selfed plants paired randomly within lines (the pairs were the same as in Table 1)

d.f.	Mean difference	s.d.	<i>t</i>	<i>P</i>
17	0.274	0.313	3.722	0.002

Mean of all outcrossed plants = 0.893, mean of all selfed plants = 0.623.

the difference in means among the 18 pairs used in the analysis being 0.27. There were no significant correlations between selfing rate and either total number of flowers, above-ground biomass, weight of seeds or number of side branches among the 22 selfed plants in the experiment (Table 3).

Among the selfed plants there was a significant effect of lines on selfing rate and 80 per cent of the variance was explained by differences among lines (Table 4). The regression coefficient between the selfing rate of the maternal plants and the means within the outcrossed lines was 0.20 ($n=8$, $P=0.63$; data not shown).

Discussion

The selfing rate in outcrossed plants was significantly higher than in selfed plants. Some of the explanation for a higher selfing rate among outcrossed plants may lie in the fact that selfed plants resulting from inbreeding depression are smaller and have fewer flowers. The residuals of numbers of flower after correcting for the means of lines showed that outcrossed plants had significantly more flowers than selfed plants (Kruskal-Wallis, $P=0.003$; Damgaard & Loeschcke, 1994). Against this hypothesis, however, we did not find any correlation between selfing rate and either number of flowers or above-ground biomass among the selfed plants.

A similar situation, that outcrossing plants have a higher selfing rate than selfed plants, has previously been reported in *Vicia faba*, where several studies have demonstrated a higher selfing rate of outcrossed plants compared with selfed plants (Drayner, 1956; Link, 1990). More studies are needed in rape seed and other species, preferably in a design with less experimental variation, to understand the exact nature of the influence of the mating history on the mating system. Such a dependency will quantitatively as well as qualitatively affect the evolution of selfing rates and should be incorporated into evolutionary models on mating system dynamics.

We found genotypic variation for selfing rate in Topas, which is in agreement with other studies that

Table 3 Pearson correlation coefficients and significance values between the selfing rates of the selfed plants and some plant characters ($n=22$)

	<i>r</i>	<i>P</i>
Total number of flowers	0.068	0.76
Above-ground biomass	0.182	0.41
Weight of seeds	0	—
Number of side branches	−0.003	0.99

have shown such genotypic variation in rape seed varieties (Lewis & Woods, 1991; Rudloff, 1992). The 'broad-sense' heritability was estimated as 0.80, a surprisingly high value as the error variance was confounded by the effect of environment and the experimental error from sampling, but it is in close agreement with Lewis & Woods (1991) who reported a 'broad-sense' heritability of 0.79 in a summer-annual rape seed variety. The 'narrow-sense' heritability was estimated as 0.41, but this estimate is very uncertain because of the large experimental error in determining the selfing rates of outcrossed plants. Lewis & Woods (1991) reported a 'narrow-sense' heritability of 0.16. Recurrent selection during three generations for high and low selfing rate in rape seed has previously been shown to lead to lines with very high and lines with very low selfing rates (Rudloff, 1992). Hence the amount of selfing in rape seed responds quickly to selection.

Unfortunately, the genetic variation for factors controlling the selfing rate has only been measured in a few species (Barret & Eckert, 1990). In *Senecio vulgaris* a 'narrow-sense' heritability of selfing rate was reported as 0.43 (Campbell & Abbott, 1976). In *Vicia faba* the heritability of selfing rate was found to be 'high' (Link, 1988) and in *Corchorus olitorius* a 'broad-sense' heritability of selfing rate was reported as 0.2 by Basak & Paria (1989). From these few studies emerges a rather surprising picture of the genetic basis of selfing rate as having a rather high amount of genetic variation within populations, although the genetic variation in agricultural species may partly result from recent crossing events. The high genetic variation suggests that the selfing rate is rather firmly controlled by developmental events and not, which is commonly believed, more or less controlled by the stochastic behaviour of pollinators and the general environment.

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Table 4 Analysis of variance of selfing rate and the estimated variance components for the selfed individuals

	d.f.	MS	F	P	σ^2
Among lines	7	0.122 ($\sigma_e^2 + n_0 \sigma_G^2$)	12.394	<0.001	0.041
Error	14	0.010 (σ_e^2)			0.010

n_0 , number of replicates in an unbalanced design = 2.74 (Snedecor & Cochran, 1989).

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