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Inbreeding depression and dominance-suppression competition after inbreeding in rapeseed (*Brassica napus*)

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Abstract Rapeseed plants, of the summer annual variety Topas, that had been selfed twice consecutively were compared to outcrossed half-sibs for inbreeding depression in a rapeseed population at mating equilibrium. The effect of dominance-suppression competition was included in the effect of inbreeding. Both female- and male-fitness characters showed significant inbreeding depression. Biomass decreased 17% with inbreeding and was highly correlated with seed weight. The total number of flowers decreased 15% with inbreeding. There was a significant effect of lines. The possible importance of experimental design in studies that estimate inbreeding depression is discussed.

Key words *Brassica napus* · Inbreeding · Inbreeding depression · Line variation · Competition

Introduction

The effect of inbreeding on phenotypic characters in plants has been studied for more than 100 years, and generally it is found that predominantly outcrossing populations show a decrease in fitness-relevant characters under inbreeding. This observation has had a large influence on plant breeding methods and population genetic thoery, where inbreeding depression has become and explanatory tool in understanding many evolutionary processes. The consequence of inbreeding on the genetic structure of a population is a decrease in the frequency of heterozygotes compared to random mating, an increase of inter-population genetic variance and, for small populations, a decrease in intra-population genetic variance due to genetic drift.

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The genetic basis of inbreeding depression is poorly understood, although three not-mutually-exclusive hypotheses have been proposed (e.g., Wright 1977). The dominance hypothesis: heterozygotes have a higher "phenotypic value" because recessive deleterious alleles are not expressed; the overdominance hypothesis: a heterozygote at a locus has a higher phenotypic value than either homozygote; and the pseudo-overdominance hypothesis: two recessive deleterious alleles in the "trans-phase" at two linked loci resemble overdominance in a genetic analysis. Charlesworth and Charlesworth (1987) reviewed inbreeding depression experiments and suggested that inbreeding depression is due mainly to recessive deleterious alleles.

An estimate of the amount of inbreeding depression in rapeseed and its variation among lines is of course beneficial for the breeding of rapeseed, but inbreeding depression data are also valuable in understanding the evolution of breeding systems and the importance of genetic load in small populations. "Normally", inbreeding depression is measured by comparing different inbred classes to a base population, where each inbreed class and the base population are grown in separate plots. Such an experimental design underestimates inbreeding depression compared to field conditions if inter-plant competition is of any significance, because a highly-inbred plant next to an outbred plant may experience more competition (dominance-suppression competition) than if it is next to an inbred plant.

Here we study the amount of inbreeding depression in the summer annual rapeseed variety Topas by comparing consecutively-selfed plants to outcrossed half-sibs in eight lines. To account for a possible effect of dominance-suppression competition the experiment was done by transplanting plants into a rapeseed population at mating equilibrium, i.e., the proportion of plants that have been outcrossed, selfed once, selfed twice etc., was assumed to be at equilibrium.

Materials and methods

Study system

The summer annual "double-low" variety Topas from Svalöf AB, Sweden was chosen as material. Rapeseed is a self-compatible allo-tetraploid from *Brassica oleracea* and *Brassica campestris*, both of which are self-incompatible (Song et al. 1988). Topas plants have 200–300 nectar-producing hermaphroditic yellow flowers, and a selfing rate of about 70% with some environmental variation (Becker et al. 1992).

The maternal plants of the eight lines came from a mixed-mated population of Topas, which was grown under normal field conditions in 1989. The maternal plants were homozygotic for a rare SDH isoenzyme allele with a frequency of about 0.01 (Becker et al. 1992), which indicated that the maternal plants were the result of selfing events. Seeds were collected from the maternal plants and grown in pots. The genotypes of the plants in the pots were determined by isozyme electrophoresis when the plants had about three leaves. If the plant was homozygotic for tha rare SDH allele, the plant was classified as selfed; if it was a heterozygote, it was classified as outcrossed. Since the maternal plants were expected to be the result of a selfing event, this means that the plants classified as selfed were the result of two consecutive selfing events. Six plants from each of the eight lines, three selfed and three outcrossed, were randomly transplanted, at approximately the same growth stage, into a 40 m × 40 m rapeseed field (Topas) grown under normal agricultural conditions at Påskehøjgaard near Århus, Denmark in 1991.

Measurements

At the end of the season the 48 plants were harvested and the fruits (pods), the remaining flower stalks, and the branches were counted. The total number of flowers during the season was found by adding the number of fruits and the number of remaining flower stalks. The dry above-ground biomass was measured, and the total number of seeds per plant were weighed.

The design of the experiment was a two-factor (line, and level of inbreeding) completely randomized design. One of the assumptions in the analysis of variance, a homogeneous error variance, is expected not to be satisfied in a comparison between selfed full-sibs and outcrossed half-sibs, and non-parametric statistics were therefore used in the analysis of the data. Inbreeding depression was tested by ranking the residuals after the effect of line was subtracted, using a Kruskal-Wallis one-way analysis of variance. Similarly the effect of line was tested by ranking the residuals after the effect of inbreeding was subtracted. The analysis were done using "Systat 5.1".

Results

Selfed versus outcrossed had a significant effect on the above-ground biomass, the number of fruits, the number of flowers, and the number of branches (Table 1). The weight of seeds was highly correlated with biomass ($r = 0.933$, $P = 0.001$) and showed a similar decrease in mean, but the difference was not significant. The mean of the traits generally decreased with inbreeding except for number of branches which increased with inbreeding (Table 1).

There was significant variation among the eight lines for all plant characters (Table 1). The means of biomass for outcrossed families ranged from 25.36 g to 57.44 g, whereas the selfed family means range from 15.42 g to 42.41 g. Selfed and outcrossed line means were positively correlated ($r = 0.834$; $P = 0.01$), indicating that a line which had a relatively high biomass when outcrossed also had a relatively high biomass when selfed. Similar results (data not shown) were found for the other plant characters.

Table 1 The effect of outcrossed versus twice selfed plants, means of outcrossed and selfed, and the relative decrease in percent, with the level of significance indicated (Kruskal-Wallis, 1 *df*). In the last column, the effect of the line for each character is indicated by its *P* value (Kruskal-Wallis, 7 *df*)

Plant character	Outcrossed	Selfed	Decrease	Line
Biomass (g)	34.6	28.9	17%*	<0.001
Seed weight (g)	5.39	4.65	14%^{ns}	<0.001
Fruits (no.)	230	196	15%*	0.003
Flowers (no.)	332	283	15%*	0.003
Branches (no.)	5.83	6.29	−7%*	0.005

* Significant with $0.01 < P < 0.05$; ^{ns} non-significant

Partitioning the variance in biomass between among-line variance and residual varaince showed that most of the variance was of the among-line type (outcrossed: 58%; selfed: 90%). The total variance was highest among the outcrossed plants (206.82 compared to 98.34 g), but this was due mainly to residual variance within the lines.

Discussion

There was a significant negative effect of inbreeding on above-ground biomass and the number of fruits; both were highly correlated with total seed weight and, therefore, yield. Pollen production in Topas is determined mainly by the number of flowers on the plant (Damgaard and Loeschcke 1994), and the number of flowers showed a decrease with inbreeding comparable to the decrease in biomass. Therefore, inbreeding depression had approximately the same negative effect on male investment (pollen production) and female investment (seed production).

The design of this inbreeding depression experiment differed in two ways from most other inbreeding experiments, e.g., that of Brandle and McVetty (1989). Normally the outcrossed class (or base population) is a random sample from a population or a cross between two lines. In a mixed-mating species such as rapeseed, where on average 70% of the random sample has been selfed at least once, the random sample method is problematic. Another general problem is that the expected variance among lines is higher for inbred lines than the variance among the plants in the random sample. Thus the assumption of a homogeneous variance among lines in the analysis of variance is known not to be valid (Lynch 1988). In the design used here, where the outcrossed class was made by outcrossing selfed individuals and compared to their selfed-half-sibs (the selfed class has thus been selfed two consecutive times), the difference in among-line variance was expected to be less than in the "normal" design. In fact the variance in this experiment was highest for the outcrossed class, but this was due mainly to within-line variance (residual variance), which also was expected to be larger among half-sibs than among full-sibs.

Another difference in the design used in our experiment compared to the design normally used was that the individual plants were tested in a population of rapeseed at mating equilibrium, i.e., the proportion of plants that have been outcrossed, selfed once, selfed twice, etc., was assumed to be at equilibrium. When the plants are grown in a population in mating equilibrium instead of separate plots, a possible effect of dominance-suppression competition (or asymmetric competition) is confounded within the experiment. An effect of asymmetric competition on inbreeding depression was found in *Impatiens capensis*, where asymmetric competition of outcrossed individuals on selfed individuals increased inbreeding depression (Schmitt and Ehrhardt 1990). A similar effect has been found in *Plantago cornopus*, where inbred plants were less vigorous when grown together with outcrossed plants, than if they were grown together with other inbred plants Koelewijn (1993).

Brandle and McVetty (1989) found a significant negative effect of inbreeding on yield in three out of seven Summer annual rapeseed varieties, with a maximum decrease in yield of 22%. Shuster and Michael (1976) found a negative effect of inbreeding on yield in a Winter annual rapeseed variety, where the decrease after two consecutive selfing events was 34%, reaching 55% after eight consecutive selfing events.

The significant differences among lines found in Topas are in agreement with the findings of Brandle and McVetty (1989), who also observed significant differences among lines in all seven varieties. Such differences among lines support the hypothesis that inbreeding depression is due mainly to recessive deleterious alleles rather than single-locus overdominance (Charlesworth and Charlesworth 1987), since the differences among lines can be explained by genetic sampling of partly recessive deleterious alleles, but not by reducing heterozygosity which should be similar among lines. In this study, where we compared the selfed class to their outcrossed half-sibs, some of the among-line variance could be due to maternal effects, which is supported by the positive correlation between the outcrossed class and the selfed class among lines.

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