

Fitness of F₁ hybrids between weedy *Brassica rapa* and oilseed rape (*B. napus*)

THURE P. HAUSER*†, RUTH G. SHAW‡ & HANNE ØSTERGÅRD§

†Botanical Institute, University of Copenhagen, Øster Farimagsgade 2D, 1353 Copenhagen K, Denmark,

‡Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN 55108, U.S.A. and §Plant Biology and Biogeochemistry Department, Risø National Laboratory, 4000 Roskilde, Denmark

As part of an ongoing study of the hybridization biology of cultivated oilseed rape (*Brassica napus*) and weedy *B. rapa*, we studied the fitness of hybrids between three weedy *B. rapa* populations and three varieties of *B. napus*. Reciprocal pollinations were performed, and the resulting offspring were scored for seed development, survival in the field, pod- and seed-set. Seeds from heterospecific crosses developed within pods in lower proportions than seeds from conspecific crosses. Hybrid offspring survived in the field as frequently as conspecific offspring, and produced many more pods that contained fewer seeds. Combining the fitness components into a multiplicative estimate, we found the hybrids to be intermediate to their parents, and significantly more fit than *B. rapa*. Significant genotypic differences were detected between offspring produced by different parental plants, populations and varieties for some of the fitness components scored. Our results on hybrid fitness are discussed with respect to the possibility that transgenes in oilseed rape may introgress spontaneously into weedy *B. rapa*.

Keywords: Brassicaceae, controlled crosses, genotypic variation, interspecific hybridization.

Introduction

In the breeding of crops, controlled introgression of advantageous genes from related taxa into the crop gene pool has been used successfully for many years. Recently, biotechnology has overcome many of the restrictions that sexual incompatibility imposes on the availability of genes for introgression. Many crop gene pools already contain, or will in the near future, genes from distantly related organisms (transgenes), and these genes may subsequently spread to related weedy or wild plants via spontaneous hybridization and introgression. This has raised concern among plant biologists and environmentalists (e.g. Ellstrand & Hoffman, 1990; Raybould & Gray, 1993, 1994; Snow & Palma, 1997), fearing that the incorporation of transgenes into wild plants may have negative consequences on the environment.

Transfer of a gene or chromosomal segment from one species to another involves its passage through a number of individuals of consecutive hybrid and backcross generations. Obviously, if the hybrid and backcross individuals do not survive, introgression will not take place. Less clear is the effect of a

partial fitness barrier, where some hybrids survive but suffer from a reduced fitness. Theoretical results show that neutral alleles may pass through a species barrier even when the fitness of hybrids is rather depressed, and that alleles with different selective values pass through the barrier with different speeds (Barton & Hewitt, 1985). In the case of transgenes, the speed and dynamics of introgression will thus be determined in part by the effect of the particular transgene, and by the fitness of the different generations of hybrid and backcross plants.

Hybridization is impossible between most pairs of species, as assumed by the biological species concept, and where possible, the hybrids often suffer from a low survival and reproduction (e.g. Stebbins, 1958; Barton & Hewitt, 1985). Nevertheless, interspecific hybrids are found in many taxa, especially so in plants (reviewed by Arnold, 1997), and hybrids may sometimes be as fit or more fit than their parents (e.g. Arriola & Ellstrand, 1997; reviewed by Arnold, 1997).

Even if hybrids are on average less fit, they may be so variable that some still survive and reproduce, and thereby act as an avenue for introgression. Part of this variation in fitness (and hybridization ability) may be genotypically determined, as reported for

*Correspondence. E-mail: thureh@bot.ku.dk

many crops and related species (e.g. beans: Mejia-Jimenez *et al.*, 1994; tomato: Sacks *et al.*, 1997; *Brassica*: Meng & Lu, 1993; see also Baranger *et al.*, 1995). To evaluate the possibility of introgression between two species, it is thus important to consider not only the average crossability and fitness, but also how these attributes vary among different genotypes.

Oilseed rape (*Brassica napus*) is a likely candidate for a crop species that could exchange genes with its many weedy relatives. Among these, *B. rapa* (= *B. campestris*) is generally considered the most compatible (reviewed by Scheffler & Dale, 1994). Recent studies have shown that hybrid seeds can be found in frequencies up to 13% in weedy *B. rapa* populations (Jørgensen & Andersen, 1994; Landbo *et al.*, 1996), that hybrid seeds are produced by both species in open-pollinated field trials (Jørgensen & Andersen, 1994), and that F₁ hybrids in field trials also set seed, some of which are from backcrosses to *B. rapa* (Jørgensen *et al.*, 1996; Mikkelsen, 1996; Mikkelsen *et al.*, 1996).

To understand better the likelihood and dynamics of hybridization and introgression from *B. napus* to *B. rapa*, we hybridized the species and estimated several components of fitness of the offspring. Here, we present results for the first generation of hybrids (F₁), and a subsequent paper presents those for the F₂/first backcross generations. As we used three different *B. rapa* populations and three *B. napus* varieties for the crosses, we also examined the genotypic variation in fitness of hybrid offspring produced by different parental plants, populations and varieties.

Materials and methods

Plant material

Brassica rapa (abbreviated Br, diploid, $2n = 20$) is a common weed in Denmark, growing in oilseed rape fields, along roadsides, in fallow and other disturbed areas. *Brassica rapa* is self-incompatible, and pollinated by insects. When ripe, the pods split open to disperse the seeds. Oilseed rape, *B. napus* (abbreviated Bn, $2n = 38$) is grown intensively in Denmark, mainly as a winter crop. *Brassica napus* has an intermediate selfing rate (Becker *et al.*, 1992), and the flowers are pollinated by insects and wind. The plants are bred to keep the pods closed upon ripening.

The parental plants for the experiment came from three weedy *B. rapa* populations: Br25, Br45 and Br54, and three spring varieties of *B. napus*: Drakkar, Topas and Westar (nontransgenic

material). To avoid including spontaneously formed hybrids, the *B. rapa* parents were all checked by morphology, isozyme profile and chromosome number (for methods, see Jørgensen & Andersen, 1994).

From each *B. rapa* population we used 12 maternal and six paternal plants, and from each *B. napus* variety six maternal and six paternal plants. Maternal and paternal plants were kept isolated to avoid unintentional pollinations, and grown at $\approx 18^\circ\text{C}$ for 16 h/ 15°C for 8 h.

Pollinations

Our crossing design was factorial at the level of populations and varieties, including all 36 possible combinations of these, but not at the level of individuals, where each maternal plant was pollinated with only a subset of the paternal plants from each population and variety.

Each *B. rapa* maternal plant received pollen from two randomly chosen donors from each of the *B. rapa* populations (one flower pollinated per donor) and *B. napus* varieties (two flowers pollinated per donor). Each *B. napus* maternal plant received pollen from two randomly chosen donors from each of the *B. rapa* populations and *B. napus* varieties (one flower per donor). Eighteen flowers were thus pollinated on each *B. rapa* plant and 12 on each *B. napus* plant; 864 flowers were pollinated in total (from 648 unique combinations of a maternal and a paternal plant). Individual pollen donors were used for an even number of pollinations in a given population/variety combination.

Flowers on the maternal plants were emasculated prior to anthesis, and pollinated by rubbing two to three anthers onto the stigmas. All flowers that were not pollinated were removed from the maternal plants. At maturity, pods were harvested and the seeds were sorted and counted as fully developed, germinated within pod, or aborted (small and shrunk). Fully developed seed were then used in a field trial.

Field trial

Mature seeds were available from 357 maternal-paternal plant combinations (after pooling of Br (female) \times Bn (male) seeds from the same combinations). For each combination, nine seeds were sown into three 5×5 cm sphagnum pots filled with standard soil (three seeds per pot; if fewer seeds were available, they were distributed over the pots).

A total of 2700 seeds was sown into 1035 pots (Br × Br: 311; Br × Bn: 463; Bn × Br: 141; Bn × Bn: 120). In May 1995, the pots were planted into the field in a randomized design of three blocks, each containing one of the three replicate pots. The pots were evenly spaced in rows, with a density of 25 pots/m². Because of dormancy of *B. rapa* seed and dry weather, only a proportion of the sown seeds were alive as plantlets at day 17, when counted (Br × Br: 0.17; Br × Bn: 0.31; Bn × Br: 0.52; Bn × Bn: 0.70). At this date, the plants were thinned to one per pot.

During the season, the field was weeded, irrigated, and sprayed with insecticide a few times, but not fertilized. Plants were again counted at harvest (mid-August to mid-September). The numbers of developed pods per plant were counted (if they appeared to contain at least one developed seed). Eight developed pods per plant were randomly chosen and the numbers of fully developed seeds within were counted.

Data analysis

For all the measured characters (see Table 1), differences between the crossing types (Br × Br, Br × Bn, Bn × Br and Bn × Bn) were tested by a one-way ANOVA (SAS, 1990).

The following fitness components of the offspring were analysed in more detail: the proportion of fully developed seeds in maternal pods, the proportion of plantlets alive at day 17 that survived to harvest, the number of pods per offspring plant, and the number of seeds per pod. These components were further combined into a fitness estimate: the proportion of fully developed seeds (i.e. zygote survival) for a maternal–paternal plant combination was multiplied by the survival and fertility components for each of the offspring plants derived from that combination. Germination and initial survival (until day 17) were not included in the fitness estimate to avoid the bias that would then be introduced by the pronounced dormancy of *B. rapa* (Landbo & Jørgensen, 1997).

The effect of the parental populations, varieties and plants on the fitness of their hybrid offspring was analysed by the following model:

$$Y_{ijklmn} = \mu + brpop_i + bnpop_j + type_k + brpar_{l(ik)} + bnpar_{m(jk)} + (brpop \times bnpop)_{ij} + (brpop \times type)_{ik} + (bnpop \times type)_{jk} + (brpop \times bnpop \times type)_{ijk} + \varepsilon_{ijklmn},$$

where Y_{ijklmn} are the individual fitness observations of the hybrid offspring, μ the overall mean, $brpop_i$ the effect of the i th parental *B. rapa* population ($i = 1, 2, 3$; the effects of a given population as maternal and paternal donor are combined), $bnpop_j$ the corresponding effect of the j th *B. napus* variety ($j = 1, 2,$

Table 1 Means of the characters scored on maternal plants and offspring for the different crossing types (with SE and sample sizes in parentheses). Significant differences between crossing types are indicated by different letters (Tukey’s test), where the ranking of letters corresponds to the ranking of means. Characters included in the multiplicative combination of fitness components are indicated by asterisks

	Br(fem.) × Br(male)	Br × Bn	Bn × Br	Bn × Bn
Proportion of pollinated flowers developing into pods on maternal plants	0.51 (0.03, 216) ^{ab}	0.56 (0.02, 432) ^a	0.44 (0.05, 108) ^{ab}	0.38 (0.05, 108) ^b
Number of fully developed seeds per pod on maternal plants	16.4 (0.7, 110) ^a	12.5 (0.4, 241) ^b	17.2 (0.8, 48) ^a	18.4 (1.1, 41) ^a
Proportion of fully developed seeds*	0.961 (0.006, 110) ^a	0.744 (0.016, 243) ^b	0.944 (0.014, 48) ^a	0.987 (0.005, 41) ^a
Survival from day 17 to harvest*	0.84 (0.04, 106) ^a	0.94 (0.02, 233) ^a	0.91 (0.03, 102) ^a	0.89 (0.03, 102) ^a
Number of pods per offspring plant*	135 (21, 89) ^b	1025 (50, 218) ^a	961 (81, 93) ^a	454 (29, 91) ^a
Number of seeds per pod on offspring plants*	7.13 (0.48, 83) ^b	3.51 (0.13, 214) ^c	3.48 (0.34, 91) ^c	18.30 (0.63, 90) ^a
Combined fitness components	917 (159, 106) ^c	2756 (182, 233) ^b	2767 (302, 102) ^b	7396 (644, 102) ^a

Bn, *Brassica napus*; Br, *Brassica rapa*; fem., female.

3), $type_k$ the effect of the direction of the cross (Br \times Bn or Bn \times Br), $brpar_{l(ik)}$ the effect of the l th *B. rapa* parental plant ($l = 1, \dots, 18$; nested within combinations of *B. rapa* populations and directions as different plants were used as maternal and paternal parents), $bnpar_{m(jk)}$ the corresponding effect of the m th *B. napus* parental plant ($m = 1, \dots, 12$), and ε_{ijklmn} the variation of individual observations around means and effects.

For the analyses, data on counts and the combination of fitness components were log-transformed and data on proportions transformed to the arcsine of their square root, after preliminary analyses of the normality and variance structure of the data. The Type III sums of squares of PROC GLM, SAS Statistical Software for Windows (SAS, 1990), were used for tests of the models. If main effects were found to be significant, comparisons of means were performed using Tukey's test (SAS, 1990).

Results

The crossing type (Br(female) \times Br(male), Br \times Bn, Bn \times Br and Bn \times Bn) affected all the measured characters on maternal plants and their offspring, except survival from day 17 to harvest (Table 1). Bn \times Bn crossings generally gave rise to offspring with the highest fitness for single components and their combination. Reciprocal differences between Br \times Bn and Bn \times Br crossings were detected for the

number of seeds and their proportional development on maternal plants. Hybrid offspring were significantly fitter than *B. rapa* with respect to the number of pods they set per plant, but less fit with respect to their development from zygote to seed (Br \times Bn hybrids only) and the number of seeds they set per pod (Table 1). For the combination of fitness components, *B. rapa* offspring were the least fit, hybrid offspring intermediate, and *B. napus* offspring the most fit (Table 1).

Parental populations and varieties gave rise to hybrid offspring that differed significantly in the number of seeds that their pods contained (Table 2) (further analysis of the significant $brpop \times type$ interaction showed that parental *B. rapa* populations only differed significantly in the Bn \times Br offspring, but that the ranking of populations was the same in Br \times Bn offspring (Br25 > Br45 > Br54)). No effect of parental populations or varieties was detected on the combined estimate of fitness for the hybrids (Table 2). This estimate, as analysed here, includes both the ones that died (aborted as seed, or died in the field), and the ones that survived and reproduced. If only the surviving plants are considered, hybrid offspring from different *B. rapa* populations differed significantly in their combined fitness ($brpop$: MS = 7.4, d.f. = 2, $P = 0.04$). The ranking of *B. rapa* populations was the same as for the number of seeds per pod, Br25 > Br45 > Br54, both when including and excluding dead hybrids.

Table 2 Effect of parental populations, varieties and plants on the fitness components of their hybrid (Br \times Bn and Bn \times Br) offspring. Variables $brpop$ and $bnpop$ indicate the effects of parental *Brassica rapa* populations and *B. napus* varieties, respectively, $brpar$ and $bnpar$ the corresponding effects of individual parental plants, and $type$ the effect of reciprocal crossing direction. Only results for components included in the combined fitness are shown

	Proportion of fully developed seeds		Survival from day 17 to harvest		Number of pods per offspring plant		Number of seeds per pod on offspring plants		Combined fitness components	
	MS	d.f.	MS	d.f.	MS	d.f.	MS	d.f.	MS	d.f.
$brpop$	0.038	2	0.48	2	1.8	2	1.430	2***	9.15	2
$bnpop$	0.066	2	0.19	2	3.4	2	1.435	2***	6.46	2
$type$	0.516	1**	0.15	1	0.9	1	0.006	1	0.19	1
$brpar (brpop \times type)$	0.247	47***	0.48	47***	2.5	46*	0.187	46*	4.82	46
$bnpar (bnpop \times type)$	0.084	27	0.24	27	5.9	27***	0.163	26	14.32	27***
$brpop \times bnpop$	0.060	4	0.36	4	0.3	4	0.017	4	1.88	4
$brpop \times type$	0.053	2	0.59	2	0.3	2	0.627	2**	1.25	2
$bnpop \times type$	0.015	2	0.26	2	3.6	2	0.002	2	7.18	2
$brpop \times bnpop \times type$	0.054	3	0.07	3	1.0	3	0.049	3	3.82	3
error	0.075	199	0.21	512	1.7	220	0.123	216	5.54	244

*0.01 < P < 0.05; **0.001 < P < 0.01; *** P < 0.001.

Individual *B. rapa* and *B. napus* parental plants gave rise to hybrid offspring that differed in proportional seed development, survival, numbers of pods and seeds, and in their combined fitness estimate (Table 2).

Discussion

Fitness components

The lower number of seeds per pod on maternal plants and the lower proportional seed development from Br(female) \times Bn(male) crosses than from the reciprocal crosses are in accordance with results from several other studies (seed number: reviewed by Nishiyama *et al.*, 1991 and Scheffler & Dale, 1994; proportional seed development: Röbbelen, 1966). They also match the common observation that hybridization and seed-set are more successful when the parent of the higher ploidy level (here *B. napus*) serves as the seed parent, and when an allo-diploid (*B. napus*) is pollinated by its diploid progenitor (Stebbins, 1958). In contrast, we have in another study found exactly the opposite reciprocal effect, that Br \times Bn seeds develop in higher proportions than Bn \times Br seeds when *B. rapa* and *B. napus* pollen are applied as mixtures (Hauser *et al.*, 1997). Under such conditions, conspecific and heterospecific pollen and seed may compete for fertilization and resources, respectively, which is not the case in the present and most other hybridization experiments where only single-species pollinations are performed.

Competition between hybrid and conspecific seed within pods may also affect the overall level of seed development of hybrids; in our mixed-pollination experiment (Hauser *et al.*, 1997) we found the proportional seed development of both Br \times Bn and Bn \times Br hybrids to be significantly lower than in the present experiment. The proportional seed development, as an estimate of zygote survival to seed, is therefore most likely overestimated in the present experiment relative to field conditions, where pollen is generally delivered as mixtures.

Whereas some hybrids had a lower fitness in the seed stage, they survived well in the field, and grew rather large (as indicated by the number of pods, pers. obs.). Other studies have similarly found *B. napus* \times *B. rapa* hybrids to grow well and have a high biomass accumulation (MacKay, 1973; Linder & Schmitt, 1995).

The large number of pods produced by hybrids in our experiment compensated for the low number of seeds they contained, so that the total number of

seeds produced per plant (product of the number of pods and average number of seeds per pod) was intermediate to that of the two parents (Br \times Br: 1085; Br \times Bn: 3810; Bn \times Br: 3389; Bn \times Bn: 8689). This result is very different from those reported by Jørgensen *et al.* (1996) and Mikkelsen (1996). In open-pollinated field experiments they found that hybrids (Bn \times Br) produced significantly fewer seeds per plant than weedy *B. rapa*. Different planting densities and pollen compositions could possibly explain these discrepancies. The density in our experiment was lower, and as hybrids are rather plastic in their branching, they became very big and probably suppressed their neighbouring *B. rapa* plants more than in the other studies. In our field experiment, pollen originated from *B. rapa*, *B. napus* and F₁ hybrids, suggesting that many zygotes on *B. rapa* were F₁ hybrids or backcrosses that have a low chance of developing into seeds (this study; Hauser *et al.*, 1998). In contrast, no *B. napus* plants were included in the experiment of Mikkelsen (1996) and the relative number of *B. rapa* plants was much larger in both the other experiments, suggesting that more zygotes on *B. rapa* were conspecific with a high chance of development.

However, neither planting density nor pollen composition seem to explain why the seed production of *B. rapa* in our second generation of crossings (Hauser *et al.*, 1998) was much higher, 4779 seeds on average per plant. Planting density and frequency of *B. rapa* relative to *B. napus* was the same as in the present study, but much higher proportions of 'hybrid' plants (F₂ and backcrosses) were present, suggesting that seed production of *B. rapa* should actually be lower in that experiment.

Despite these discrepancies, the seed production of F₁ plants in our experiment was as high or nearly as high as that reported for *B. rapa* in the other three experiments (F₁: 3389 (present experiment); *B. rapa*: 4779 (Hauser *et al.*, 1998); 3300 (Jørgensen *et al.*, 1996); or 2900 (Mikkelsen, 1996)).

Combination of fitness components

The relatively high fitness of hybrids that we estimated from the combination of fitness components was to a large degree determined by the high pod production of these plants, as discussed above.

Other fitness components, that were not included in this study, may influence the fitness realized by hybrids in the wild. Pollen fertility is one of these, and as is true of many hybrids (e.g. Stebbins, 1958), there is a reduction in pollen fertility; pollen of F₁ hybrids between *B. rapa* and *B. napus* is only *c.* 46%

fertile, whereas pollen of the parents is *c.* 91% and 98% fertile, respectively (measured on the parents for the second generation of crossings, Hauser *et al.*, 1998; see also Jørgensen & Andersen, 1994; Jørgensen *et al.*, 1996). Despite the lower proportional pollen viability, the total numbers of viable pollen grains produced by hybrid plants in our experiment was probably much higher than that produced by *B. rapa*, because hybrids produce many more flowers (indicated by the number of pods, Table 1, pers. obs.).

Other plant traits, like seed dormancy (discussed by Landbo & Jørgensen, 1997) and different environmental conditions, may influence fitness estimates and the fitness realized by hybrids in nature. Nevertheless, our results suggest that despite some inherent fitness disadvantages to F₁ hybrids, such as lower zygote survival and pollen fertility, they may sometimes and under some conditions be as fit or nearly as fit as their parents, because of their vegetative vigour.

Certain genomic properties of *B. rapa* and *B. napus* make it likely that their hybrids will be relatively fit. *Brassica napus* (genomic composition: AACC) is an amphidiploid derived from hybridization between *B. rapa* (AA) and *B. oleracea* (CC) some time in the past (U, 1935). In the *B. napus* × *B. rapa* hybrids (AAC) A genomes from the two species are brought together, and depending on the divergence between genes of the two genomes, we may expect a contribution of a higher fitness from heterosis (as found for biomass by MacKay, 1973) or a reduced fitness from genetic incompatibility and, for example, maladaptation. The interaction in the F₁ between the C genome (from *B. oleracea*) and the A genome from weedy *B. rapa* could be expected to contribute to a lower fitness, because these genomes diverged earlier than the two A genomes. Nevertheless, the C genome of *B. napus* has already functioned together with an A genome during the history of oilseed rape, and it may have adapted to this interaction (Song *et al.*, 1995). From these considerations, we may a priori expect F₁ hybrids between *B. rapa* and *B. napus* to be relatively fit compared to other interspecific hybrids (for a review of fitness of other hybrids, see Arnold, 1997).

Genotypic variation

As is commonly reported for agricultural plants and their relatives (references in Introduction), we found genotypic differences between populations, varieties and individual plants for some of the fitness components of their hybrid offspring (Table 2). These

differences could possibly be reflections of similar genotypic differences between populations/varieties/plants in the fitness of conspecific offspring, i.e. that fitter individuals give rise both to relatively fit conspecific and heterospecific offspring. Such a trend was not apparent in our material, however. In the conspecific offspring, only the number of seeds per pod was significantly affected by parental *B. rapa* populations (unpubl. obs.), but the ranking of populations was different from that for the seed-set of hybrid offspring.

In the analysis of the combined estimate of fitness, genotypic differences between parental *B. rapa* populations only appeared if we ignored hybrids that died (fitness of zero). This suggests that even if F₁ zygotes from different populations on average produce similar numbers of seeds in their lifetime, those that actually do survive and produce the most seeds are likely to originate from certain populations. These may then preferentially pass their genes on to the next generation. Interestingly, the population with the highest estimated fitness, Br25, is also the population in which we have so far detected the most spontaneous hybrid seeds (Landbo *et al.*, 1996).

Implications

Our results show that there is no strong hybridization barrier between oilseed rape and weedy *B. rapa*, and that the F₁ hybrids under some conditions may be as fit, or nearly as fit as their weedy parent. Spontaneous introgression of genes from oilseed rape to *B. rapa* thus seems to be rather likely, and may already have occurred for a long time, although unnoticed. Adding transgenes to the gene pool of oilseed rape is likely to result in their transfer to *B. rapa*. Further experiments and modelling are in progress to evaluate the effects of hybrid fitness on the transmission probability and speed of transgenes.

Acknowledgements

This work benefitted very much from the creative co-operation with Rikke B. Jørgensen, Bente Andersen, Elly Ibsen, Lars Landbo and Thomas Mikkelsen at Risø National Laboratory. The personnel at Risø are thanked for their help in counting the many pods and seeds, and Loren Rieseberg and Heiko Becker for their suggestions and comments on this study. The Second Biotechnological Programme in Denmark and Risø National Laboratory financed the project.

References

- ARNOLD, M. L. 1997. *Natural Hybridization and Evolution*. Oxford University Press, New York.
- ARRIOLA, P. E. AND ELLSTRAND, N. C. 1997. Fitness of interspecific hybrids in the genus *Sorghum*: persistence of crop genes in wild populations. *Ecol. Appl.*, **7**, 512–518.
- BARANGER, A., CHEVRE, A. M., EBER, F. AND RENARD, M. 1995. Effect of oilseed rape genotype on the spontaneous hybridization rate with a weedy species: an assessment of transgene dispersal. *Theor. Appl. Genet.*, **91**, 956–963.
- BARTON, N. H. AND HEWITT, G. M. 1985. Analysis of hybrid zones. *Ann. Rev. Ecol. Syst.*, **16**, 113–148.
- BECKER, H. C., DAMGAARD, C. AND KARLSSON, B. 1992. Environmental variation for outcrossing rate in rape-seed (*Brassica napus*). *Theor. Appl. Genet.*, **84**, 303–306.
- ELLSTRAND, N. C. AND HOFFMAN, C. A. 1990. Hybridization as an avenue of escape for engineered genes. *BioScience*, **40**, 438–442.
- HAUSER, T. P., JØRGENSEN, R. B. AND ØSTERGÅRD, H. 1997. Preferential exclusion of hybrids in mixed pollinations between oilseed rape (*Brassica napus*) and weedy *B. campestris*. *Am. J. Bot.*, **84**, 756–762.
- HAUSER, T. P., JØRGENSEN, R. B. AND ØSTERGÅRD, H. 1998. Fitness of backcross and F₂ hybrids between weedy *Brassica rapa* and oilseed rape (*B. napus*). *Heredity*, **81**, 436–443.
- JØRGENSEN, R. B. AND ANDERSEN, B. 1994. Spontaneous hybridization between oilseed rape (*Brassica napus*) and weedy *B. campestris* (*Brassicaceae*): a risk of growing genetically modified oilseed rape. *Am. J. Bot.*, **81**, 1620–1626.
- JØRGENSEN, R. B., ANDERSEN, B., LANDBO, L. AND MIKKELSEN, T. R. 1996. Spontaneous hybridization between oilseed rape (*Brassica napus*) and weedy relatives. *Acta Hort.*, **407**, 193–200.
- LANDBO, L. AND JØRGENSEN, R. B. 1997. Seed germination in weedy *Brassica campestris* and its hybrids with *B. napus*: implications for risk assessment of transgenic oilseed rape. *Euphytica*, **97**, 209–216.
- LANDBO, L., ANDERSEN, B. AND JØRGENSEN, R. B. 1996. Natural hybridization between oilseed rape and a wild relative: hybrids among seeds from weedy *B. campestris*. *Heredity*, **125**, 89–91.
- LINDER, C. R. AND SCHMITT, J. 1995. Potential persistence of escaped transgenes: performance of transgenic oil-modified *Brassica* seeds and seedlings. *Ecol. Appl.*, **5**, 1056–1068.
- MACKAY, G. R. 1973. Interspecific hybrids between forage rape (*Brassica napus* L.) and turnip (*Brassica campestris* L. ssp. *rapifera*) as alternatives to forage rape. 1. An exploratory study with single pair crosses. *Euphytica*, **22**, 495–499.
- MEJIA-JIMENEZ, A., MUNOZ, C., JACOBSEN, H. J., ROCA, W. M. AND SINGH, S. P. 1994. Interspecific hybridization between common and tepary beans: increased hybrid embryo growth, fertility, and efficiency of hybridization through recurrent and congruity backcrossing. *Theor. Appl. Genet.*, **88**, 324–331.
- MENG, J. AND LU, M. 1993. Genotype effects of *Brassica napus* on its reproductive behavior after pollination with *B. juncea*. *Theor. Appl. Genet.*, **87**, 238–242.
- MIKKELSEN, T. R. 1996. *Flow of Engineered Genes from Crops to Wild and Weedy Relatives - Is There an Environmental Risk? A Case Study on Oilseed Rape (Brassica napus) and Weedy Brassica campestris*. Ph.D. Diss., University of Copenhagen.
- MIKKELSEN, T. R., ANDERSEN, B. AND JØRGENSEN, R. B. 1996. The risk of crop transgene spread. *Nature*, **380**, 31.
- NISHIYAMA, I., SARASHIMA, M. AND MATSUZAWA, Y. 1991. Critical discussion on abortive interspecific crosses in *Brassica*. *Pl. Breed.*, **107**, 288–302.
- RAYBOULD, A. F. AND GRAY, A. J. 1993. Genetically modified crops and hybridization with wild relatives: a UK perspective. *J. Appl. Ecol.*, **30**, 199–219.
- RAYBOULD, A. F. AND GRAY, A. J. 1994. Will hybrids of genetically modified crops invade natural communities? *Trends Ecol. Evol.*, **9**, 85–89.
- RÖBBELEN, G. 1966. Beobachtungen bei interspezifischen *Brassica*-Kreuzungen, insbesondere über die Entstehung matromorpher F₁-Pflanzen. *Angew. Bot.*, **39**, 205–221.
- SACKS, E. J., GERHARDT, L. M., GRAHAM, E. B., JACOBS, J., THORRUP, T. A. AND ST CLAIR, D. A. 1997. Variation among 41 genotypes of tomato (*Lycopersicon esculentum* Mill.) for crossability to *L. peruvianum* (L.) Mill. *Ann. Bot.*, **80**, 469–477.
- SAS. 1990. *SAS/STAT User's Guide*, version 6.11. SAS Institute Inc., Cary, NC.
- SCHIEFFLER, J. A. AND DALE, P. J. 1994. Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species. *Transgenic Res.*, **3**, 263–278.
- SNOW, A. A. AND PALMA, P. M. 1997. Commercialization of transgenic plants: potential ecological risks. *BioScience*, **47**, 86–96.
- SONG, K., LU, P., TANG, K. AND OSBORN, T. C. 1995. Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 7719–7723.
- STEBBINS, G. L. 1958. The inviability, weakness, and sterility of interspecific hybrids. *Adv. Genet.*, **9**, 147–215.
- U, N. 1935. Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *Jap. J. Bot.*, **7**, 389–452.