

Fitness of backcross and F₂ hybrids between weedy *Brassica rapa* and oilseed rape (*B. napus*)

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With the cultivation of genetically modified crops, transgenes may spread by introgression from crops into weedy and wild populations of related species. The likelihood of this depends in part on the fitness of first and later generation hybrids. We here present results on the fitness of F₂ and backcross hybrids between oilseed rape (*Brassica napus*) and weedy *B. rapa*. Two populations of *B. rapa*, two varieties of *B. napus*, and their F₁ hybrids were used for controlled crosses, and seed development, survival in the field, pollen viability, pod- and seed-set were estimated for the offspring. Offspring from F₂ and backcrosses had a reduced fitness relative to their parents for most of the fitness components and for a combined estimate of fitness, with F₂ offspring suffering the lowest fitness. Despite their lower fitness on average, some of the hybrids were as fit as the parents. Significant fitness differences were detected between backcross and F₂ offspring from different *B. rapa* populations, *B. napus* varieties, and parental plants. Our results suggest that introgression of transgenes from oilseed rape to *B. rapa* will be slowed down, but not hindered, by the low fitness of second generation hybrids.

Keywords: backcrosses, Brassicaceae, controlled crosses, F₂, interspecific hybrids, introgression.

Introduction

The role and significance of hybridization and introgression in evolution has been debated for many years (discussed by Rieseberg & Wendel, 1993; Arnold, 1997). A variant of this debate has recently become of public interest, because of the concern that transgenes inserted into crops may spread to related weedy or wild species via spontaneous hybridization and introgression (e.g. Ellstrand & Hoffman, 1990; Raybould & Gray, 1993, 1994; Snow & Palma, 1997). The phenotype and thus the ecological profile of the recipients may be altered by the transgenes, which, in the worst possible case, might create new aggressive lineages spreading over large areas, perhaps replacing other species.

To assess whether such a scenario is at all likely, a first step is to study whether crops can indeed hybridize with related species in their surroundings, and whether the hybrids are able to survive and reproduce, and thereby pass genes on to subsequent generations. Spontaneous introgression of crop

genes into related species has been described or suggested (discussed by, e.g. Harlan, 1982; Rieseberg & Wendel, 1993; Raybould & Gray, 1994), but in general very little is known about this. The fitness of some crop–wild hybrids has recently been shown to be relatively high (e.g. Klinger & Ellstrand, 1994; Arriola & Ellstrand, 1997; Hauser *et al.*, 1998; Snow *et al.*, 1998), suggesting that introgression may take place. Only F₁ hybrids were studied, though, and because some theoretical models predict fitness to be depressed preferentially in the second hybrid generation (e.g. Stebbins, 1958; Lynch, 1991), we do not really know whether there is still a fitness barrier to introgression in these species.

Oilseed rape (*Brassica napus*) has already been genetically engineered for traits such as herbicide and disease resistance, and transgenic varieties are now being marketed in the EU and elsewhere. Several related species occur as weeds, some of which may be able to exchange genes with the crop (e.g. Scheffler & Dale, 1994; Bing *et al.*, 1996). Among these, *B. rapa* (= *B. campestris*) has been shown to hybridize and backcross spontaneously with *B. napus*, both in experiments and in the field

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(Jørgensen & Andersen, 1994; Landbo *et al.*, 1996; Mikkelsen, 1996; Mikkelsen *et al.*, 1996). The fitness of F₁ hybrids can be high relative to *B. rapa* (Hauser *et al.*, 1998), and it thus seems that introgression between the two species may be frequent, and that transgenes contained in oilseed rape may soon be found in weedy *B. rapa* populations. However, the fitness of backcross and F₂ plants may be more depressed than in the F₁, partly because many such plants will be aneuploid.

To evaluate whether second generation hybrids are indeed less fit, we crossed F₁ plants with each other as well as with the parental species, and evaluated seed development, survival and reproduction of the offspring under field conditions. Because parents originated from two weedy *B. rapa* populations, two varieties of *B. napus*, and their F₁ hybrids (Hauser *et al.*, 1998), we were able to examine genotypic variation between populations and varieties in the fitness of their backcross and F₂ descendants.

Materials and methods

Plant material

Brassica rapa (abbreviated Br; diploid, 2n = 20) grows in Denmark in oilseed rape and fallow fields, along roadsides, and in other disturbed areas. It is self-incompatible, with pollinations performed by insects. Oilseed rape, *B. napus* (abbreviated Bn; amphidiploid, 2n = 38) is commonly grown in Denmark, mostly as a winter crop. *Brassica napus* has an intermediate selfing rate (Becker *et al.*, 1992),

with the flowers being pollinated by insects and wind.

In an earlier experiment, reciprocal crosses were performed among three weedy *B. rapa* populations and three *B. napus* varieties (for a description, see Hauser *et al.*, 1998). For the present experiment, parents were taken from two of the populations (Br25 and Br54) and varieties (Topas and Westar), and from their four hybrid (Br(female) × Bn(male)) combinations (Fig. 1).

As parental plants we used 16 from each *B. rapa* population (two from each of eight full-sib families from the first generation of crossings), 22 from each F₁ population (two from each of 11 full-sib F₁ seed families), and 16 from each *B. napus* variety (from the same seed lot as used in the first generation of crossings; not family structured). *Brassica rapa* and F₁ plants were checked by morphology, isozymes and chromosome counts to confirm their species/hybrid status (for methods, see Jørgensen & Andersen, 1994). Parental plants were separated into two groups to serve as maternal recipients and paternal donors, and placed in separate growth chambers to avoid unintended pollinations. The climate in the chambers was ≈16 h light/18°C, and 8 h darkness/15°C.

Pollinations

The crosses performed between populations, varieties, and their F₁ hybrids are illustrated in Fig. 1. At the individual level, maternal plants were pollinated with only a subset of the donors from a given population/variety/F₁ group.

		<i>B. rapa</i>		<i>B. napus</i>		F ₁ hybrids			
		Br25	Br54	Topas	Westar	Br25 × Topas	Br25 × Westar	Br54 × Topas	Br54 × Westar
<i>B. rapa</i>	Br25	X				X	X		
	Br54		X					X	X
			<i>Bn × Bn</i>		<i>Bn × F₁</i>				
	Topas			X		X		X	
<i>B. napus</i>	Westar				X		X		X
			<i>F₁ × Br</i>		<i>F₁ × Bn</i>		<i>F₁ × F₁</i>		
F ₁ hybrids	Br25xTopas	X		X		X			
	Br25xWestar	X			X		X		
	Br54xTopas		X	X				X	
	Br54xWestar		X		X				X

Fig. 1 Crossing design. On the left is shown the maternal *Brassica rapa*, *B. napus* and F₁ populations, and on top the paternal populations. Combinations of maternal and paternal populations involved in this study are indicated by X. Abbreviations for the types of crossings are shown in italic.

Each *B. rapa* and *B. napus* maternal plant received pollen from three donors from its own population or variety (Br × Br or Bn × Bn crosses, respectively), and from four donors from each of the two F₁ populations derived from that population/variety (backcrosses: Br × F₁ or Bn × F₁, respectively) (Fig. 1). Each F₁ maternal plant received pollen from four donors from its own F₁ population (F₁ × F₁ crosses) and from the *B. rapa* population or *B. napus* variety from which the F₁ population was derived (backcrosses: F₁ × Br and F₁ × Bn, respectively). Donors pollinated one flower per maternal plant; 11 flowers were thus pollinated on *B. rapa* and *B. napus* maternal plants, and 12 on F₁ plants; 880 flowers in total.

Pollen donors were randomly assigned to maternal plants (except that pollinations were never performed within parental *B. rapa* and F₁ sib-families) and used for an equal number of pollinations for a given population/variety/F₁ combination. Flowers were emasculated prior to anthesis, and pollinated by rubbing a couple of anthers on the stigma surface. Surplus flowers on maternal plants were removed.

The proportion of flowers developing into pods was estimated, and for each pod seeds were sorted into fully developed, germinated within pods, and aborted (less than full size and shrunk), and counted. The fully developed seeds were then used in a field trial.

Field trial

From each of the 644 developed pods, six seeds were sown into three sphagnum pots (5 × 5 cm) filled with standard soil (two seeds per pod; if fewer seeds were available, these were distributed among pots). A total of 2057 seeds was sown in 1346 pots (Br × Br: 112; Br × F₁: 191; F₁ × Br: 202; F₁ × F₁: 137; F₁ × Bn: 240; Bn × F₁: 326; Bn × Bn: 138). The pots were transplanted to the field in a randomized design of three blocks, each containing one of the pots per pod. Plant density and field management was as in the F₁ generation (Hauser *et al.*, 1998).

Emerged plants were counted at day 25 after sowing, and thinned randomly to one per pot. Plants were counted again at harvest. Pollen viability was estimated on one backcross offspring from eight of the Br, Bn and F₁ maternal plants, and on one F₂ offspring from eight of the F₁ maternal plants (methods in Jørgensen & Andersen, 1994); 400 pollen grains were counted per plant. Pollen viabilities of pure *B. rapa* and *B. napus* offspring were not estimated in the field, but on the parental plants.

Plants were harvested when pods were ripe, and the number of pods per plant counted (if they appeared to contain at least one developed seed). Eight pods per plant were selected at random 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 × F₁, F₁ × Br, F₁ × F₁, F₁ × Bn, Bn × F₁ and Bn × Bn) were tested by a one-way ANOVA (SAS, 1990).

The following fitness components of the offspring were analysed in detail: the proportion of fully developed seeds within pods, the proportion of plants alive at day 25 that survived to harvest, the number of pods per offspring plant, the number of seeds per pod, and the proportion of viable pollen. These components (except pollen fertility) were further combined by multiplying the proportion of fully developed seeds within a pod (i.e. zygote survival) by the survival and fertility components of each offspring derived from that pod. Germination and initial survival (until day 25) were excluded from the combined estimate, because seed dormancy of *B. rapa* and its backcrosses is confounded with their initial survival (Landbo & Jørgensen, 1997).

The fitness components and their combination were analysed by two models.

(a) A test for whether *B. rapa* populations and *B. napus* varieties gave rise to F₂ and backcross plants that differed in fitness. The Br × F₁, F₁ × Br, F₁ × F₁, F₁ × Bn and Bn × F₁ crossing types were analysed separately, using the following model:

$$Y_{ijk} = \mu + brpop_i + bnpop_j + (brpop \times bnpop)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} are the individual fitness observations of the offspring, μ the overall mean, $brpop_i$ the effect of the i th *B. rapa* population ($i = 1, 2$), $bnpop_j$ the effect of the j th *B. napus* variety ($j = 1, 2$), and ε_{ijk} the variation of individual observations around means.

(b) A test for whether parental *B. rapa* and F₁ families, and parental *B. napus* plants, gave rise to backcross and F₂ offspring that differed in fitness. Offspring from *B. rapa*, F₁ or *B. napus* parents were analysed separately by models including effects of parental families (or plants), nested within parental populations, and effects of crossing type and interactions.

For all the analyses, data on counts and the multiplicative combination of components were log-transformed, and data on proportions trans-

Table 1 Means of the characters scored on maternal plants and offspring from 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 the 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 × F ₁	F ₁ × Br	F ₁ × F ₁	F ₁ × Bn	Bn × F ₁	Bn × Bn
Proportion of pollinated flowers developing into pods on maternal plants	0.89 ^a (0.05, 44)	0.70 ^{bc} (0.04, 120)	0.65 ^{bc} (0.04, 176)	0.60 ^c (0.04, 176)	0.80 ^{ab} (0.03, 176)	0.89 ^a (0.03, 128)	0.96 ^a (0.03, 48)
Number of seeds per pod on maternal plants	14.03 ^b (1.08, 39)	4.54 ^d (0.40, 84)	2.23 ^e (0.17, 115)	1.40 ^e (0.11, 106)	2.04 ^c (0.14, 140)	9.59 ^c (0.57, 114)	22.78 ^a (1.14, 46)
Proportion of fully developed seeds*	0.932 ^a (0.016, 39)	0.578 ^d (0.031, 84)	0.749 ^{bc} (0.031, 115)	0.582 ^d (0.038, 106)	0.554 ^d (0.029, 140)	0.774 ^c (0.021, 114)	0.913 ^{ab} (0.021, 46)
Survival from day 25 to harvest*	1.000 ^a (0.000, 103)	0.978 ^{ab} (0.011, 180)	0.928 ^b (0.019, 181)	0.980 ^a (0.014, 102)	0.985 ^a (0.009, 197)	0.990 ^a (0.006, 308)	0.977 ^{ab} (0.013, 132)
Number of pods per offspring plant*	304 ^a (27, 91)	260 ^b (24, 164)	190 ^{bc} (18, 152)	205 ^c (33, 88)	436 ^a (27, 175)	418 ^a (20, 284)	321 ^a (20, 120)
Number of seeds per pod on offspring plants*	14.3 ^b (0.7, 89)	5.0 ^e (0.3, 145)	5.3 ^e (0.3, 136)	4.6 ^e (0.5, 74)	7.8 ^d (0.4, 171)	11.3 ^c (0.4, 279)	23.1 ^a (0.6, 118)
Pollen viability	0.910 ^b (0.017, 31)	0.433 ^d (0.051, 27)	0.378 ^d (0.038, 29)	0.478 ^d (0.037, 25)	0.496 ^d (0.049, 24)	0.706 ^c (0.034, 31)	0.983 ^a (0.004, 32)
Combined fitness components	4320 ^a (456, 91)	901 ^c (117, 173)	884 ^c (114, 177)	663 ^d (147, 111)	1725 ^b (153, 197)	3704 ^a (227, 287)	7137 ^a (537, 123)

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

formed to the arcsine of their square roots, after analyses of the normality and variance of the residuals. Type III sums of squares of Proc GLM, SAS Statistical Software for Windows (SAS, 1990) were used for tests. For means comparisons we used Tukey's test (SAS, 1990).

Results

All the characters that were measured on maternal plants and their offspring differed significantly among the Br × Br, Br × F₁, F₁ × Br, F₁ × F₁, F₁ × Bn, Bn × F₁ and Bn × Bn crossing types (Table 1). In general, fewer pods and seeds developed on the maternal plants from F₂ and backcrosses than from pure *B. rapa* and *B. napus* crosses, and the backcross and F₂ offspring were less viable, produced fewer pods and seeds, and had lower proportions of viable pollen. Among the F₂ and backcrosses, Bn(female) × F₁(male) resulted in the highest maternal pod- and seed-set, and in offspring with the highest survival and reproduction, whereas F₂ crosses resulted in the lowest (Table 1).

When the single fitness components of the offspring were combined into a multiplicative estimate, *B. napus* and *B. rapa* were the fittest, together

with Bn × F₁ offspring, all other backcross classes were significantly less fit, and F₂ plants the least fit (Table 1).

Parental *B. rapa* populations and *B. napus* varieties gave rise to backcross offspring that differed significantly in survival, number of seeds per pod, and pollen viability (details in Table 2). The combined estimate of fitness for Br × F₁ and F₁ × F₁ offspring was significantly and positively influenced by population Br25, and the fitness estimate of F₁ × F₁ by variety Topas. Interactions between populations and varieties affected some fitness components (Table 2), and especially F₁ × F₁ offspring of Br54 and Westar had a very low fitness.

Parental *B. rapa* and F₁ families and parental *B. napus* plants also gave rise to backcross and F₂ offspring that differed significantly in fitness. (*Brassica rapa* families: proportional seed development (MS = 0.394, d.f. = 14, *P* < 0.05), pod-set (MS = 8.5, d.f. = 14, *P* < 0.01), and combined estimate (MS = 18, d.f. = 14, *P* < 0.01); F₁ families: pod-set (MS = 5, d.f. = 40, *P* < 0.001), seed-set (MS = 0.6, d.f. = 40, *P* < 0.05), and combined estimate (MS = 10, d.f. = 40, *P* < 0.05); *B. napus* parental plants: seed-set (MS = 0.70, d.f. = 28, *P* < 0.05) and combined estimate (MS = 9, d.f. = 28, *P* < 0.01).

Table 2 Effects of *Brassica rapa* populations and *B. napus* varieties on the fitness components and their combination for F₂ and backcross offspring. Estimated effects (with SE) are shown for population Br25 (*brpop*), variety Topas (*bnpop*), and their combination (*brpop* × *bnpop*); all other effects are set to zero (PROC GLM; SAS, 1990). Significances of effects, as estimated from the ANOVA, are indicated by asterisks

		Br × F ₁	F ₁ × Br	F ₁ × F ₁	F ₁ × Bn	Bn × F ₁
Proportion of fully developed seeds	<i>brpop</i>	−0.01 (0.12)	−0.06 (0.14)	−0.07 (0.18)	−0.05 (0.13)	0.14 (0.09)
	<i>bnpop</i>	−0.03 (0.12)	0.08 (0.15)	0.11 (0.19)	−0.03 (0.13)	0.06 (0.09)
	<i>brpop</i> × <i>bnpop</i>	−0.16 (0.17)	−0.04 (0.20)	0.09 (0.24)	0.17 (0.18)	−0.17 (0.13)
Survival from day 25 to harvest	<i>brpop</i>	0.06 (0.03)	0.06 (0.06)	−0.03 (0.06)	0.00 (0.03)	0.01 (0.02)
	<i>bnpop</i>	0.04 (0.03)	0.10 (0.06)*	0.00 (0.06)	−0.04 (0.03)	0.01 (0.02)
	<i>brpop</i> × <i>bnpop</i>	−0.06 (0.04)	−0.03 (0.08)	0.00 (0.07)	0.02 (0.04)	−0.04 (0.02)
Number of pods per offspring plant	<i>brpop</i>	−0.3 (0.5)	0.0 (0.5)	1.2 (1.1)	0.2 (0.3)	0.1 (0.2)
	<i>bnpop</i>	−1.0 (0.5)	−0.6 (0.5)	1.3 (1.1)	0.5 (0.3)	0.3 (0.2)
	<i>brpop</i> × <i>bnpop</i>	1.5 (0.6)*	0.7 (0.6)	−1.1 (1.2)	−0.3 (0.4)	−0.1 (0.3)
Number of seeds per pod on offspring plants	<i>brpop</i>	0.19 (0.13)*	0.21 (0.16)	0.84 (0.34)	−0.11 (0.15)	−0.22 (0.11)
	<i>bnpop</i>	0.03 (0.14)	−0.14 (0.17)	0.96 (0.36)*	−0.01 (0.16)	−0.06 (0.12)
	<i>brpop</i> × <i>bnpop</i>	−0.01 (0.18)	−0.07 (0.21)	−1.01 (0.40)*	0.05 (0.21)	0.34 (0.16)*
Pollen viability	<i>brpop</i>	0.11 (0.16)	−0.22 (0.12)	−0.10 (0.13)	−0.02 (0.19)	−0.05 (0.10)
	<i>bnpop</i>	−0.13 (0.16)	−0.25 (0.12)	−0.08 (0.13)	−0.08 (0.18)	0.17 (0.10)*
	<i>brpop</i> × <i>bnpop</i>	0.24 (0.23)	0.46 (0.16)**	0.24 (0.17)	0.24 (0.23)	0.04 (0.14)
Combined fitness components	<i>brpop</i>	0.5 (0.6)*	0.9 (0.7)	2.5 (1.1)*	0.6 (0.6)	0.1 (0.3)
	<i>bnpop</i>	−0.6 (0.6)	0.2 (0.7)	2.6 (1.2)*	0.5 (0.6)	0.3 (0.3)
	<i>brpop</i> × <i>bnpop</i>	0.9 (0.8)	−0.0 (0.9)	−2.0 (1.4)	−0.1 (0.8)	−0.2 (0.5)

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

Discussion

Reciprocal differences

The fitness of backcross offspring was higher for many of the components when *B. rapa* or *B. napus* served as maternal plants, than when F₁ plants were mothers (Table 1). In the backcrosses to *B. napus*, F₁(female) × Bn(male) and Bn × F₁ plants carry different cytoplasmic genomes, the former from *B. rapa* and the latter from *B. napus*, where that of *B. napus* may have a positive effect on vigour in the field. Alternatively, environmental influences of the maternal plants may create these reciprocal differences, e.g. seeds developed on F₁ hybrids are somewhat smaller than those developed on *B. napus* (pers. obs.). In the backcrosses to *B. rapa*, both the Br × F₁ and F₁ × Br offspring carry *B. rapa* cytoplasm, and hence their fitness differences can only be attributed to environmental maternal effects.

Only for one fitness component did we find the opposite reciprocal difference, that lower proportions of seeds developed upon backcrosses to *B. rapa* mothers than upon backcrosses to F₁ mothers. This may suggest that the maternal influence of F₁ plants is more beneficial than that of *B. rapa* plants,

possibly as hybrids are vegetatively more vigorous. Alternatively, backcross seeds may be under larger competitive pressure on *B. rapa*, where they compete for resources with conspecific seeds in other pods. On the F₁ maternal plants, all seeds are F₂ or backcrosses, where the latter may actually be at an advantage.

Fitness components and their combination

The lower fitness that we estimated for the backcross and F₂ offspring (except Bn × F₁) may be contrasted to the fitness of F₁ hybrids that was estimated for the first crossing generation (Hauser *et al.*, 1998). For the same fitness components, populations, and varieties, we found F₁ hybrids to be intermediate to their parents. Obviously, comparisons of fitness between experiments may be prone to errors even for the same components. The combined fitness estimate for Br × Br offspring was thus much lower in the first generation than in the second (917 and 4320, respectively), whereas that of Bn × Bn was about the same (7396 and 7137) (discussed in Hauser *et al.*, 1998). Despite this variation, our results do suggest that both the relative and absolute

fitnesses of F_2 and backcross plants were lower in the second than in the first hybrid generation.

This pattern across generations may be expected from chromosomal and genetic differences between the two species. F_1 hybrids (genomic composition AAC) carry complete but haploid sets of A chromosomes from *B. rapa* (AA), and A and C chromosomes from *B. napus* (AACC), which enable them to function relatively well, at least vegetatively. Heterosis, especially between loci on the two A genomes, may contribute to a high fitness. Backcross and F_2 plants are, in contrast, often aneuploid with unbalanced C chromosomes (backcrosses: AA+0–9 C; F_2 : AA+0–18 C). This may seriously reduce fitness, as indicated by significant negative correlations between the number of chromosomes (≥ 20) and pollen fertility of $F_1 \times$ Br backcross plants (Mikkelsen, 1996). Translocations between the A and C chromosomes of *B. napus* may also reduce fitness, because some backcross and F_2 plants will inherit only incomplete sets of genes from the original *B. napus* A-genome (J. Tomiuk, unpubl. results). Finally, heterosis is reduced in backcross and F_2 plants, as are positive epistatic interactions of the parents (Lynch, 1991).

Estimated and realized fitness

By combining the single fitness components into a multiplicative estimate, we hope to come closer to an estimate of the lifetime fitness realized by F_2 and backcross plants in the field. Realized fitness is notoriously difficult to estimate, partly because experimental procedures and conditions greatly influence the results.

We have some indications that even the low fitness of backcross and F_2 offspring is overestimated in our experiment. If some backcross and F_2 zygotes abort so early that they are not detectable, the proportional development of seeds that we calculated and used as an estimate of zygote survival, would overestimate the real survival. In fact, the total numbers of counted seeds (aborted, germinated and mature) in pods on *B. rapa* and *B. napus* were lower for backcrosses than for conspecific crosses, suggesting that some backcross zygotes do abort early. Such early abortion has not been detected in our previous experiments on F_1 hybrids (Hauser *et al.*, 1997, 1998).

The fitness of hybrids may also be overestimated by not including pollen viability. All backcross and F_2 plants produced significantly lower proportions of viable pollen (Table 1), which may contribute to a lower male fitness. As discussed for the F_1 plants

(Hauser *et al.*, 1998), this may be compensated to some extent by producing many flowers. Backcross plants were in general larger and produced more flowers than *B. rapa* (pers. obs.), but because we did not count flowers but only pods, we are not able to evaluate the contribution of low pollen viability to fitness.

Neither early abortion nor reduced male fitness would change the above conclusion, though, that both the relative and absolute fitnesses of backcross and F_2 hybrids are depressed relative to the parental species and F_1 hybrids.

Recently, Arnold & Hodges (1995) and Arnold (1997) have questioned the generalization that hybrids are less fit than their parents, and reviewed species-pairs where hybrids were as fit or more fit than their parents. Our results illustrate how complex this discussion really is. Judging from the F_1 generation (Hauser *et al.*, 1998), we argued that hybrids between *B. rapa* and *B. napus* were very fit, and that introgression from *B. napus* into *B. rapa* seems to be very probable. The present results show that there still is a fitness barrier between the two species, that is preferentially expressed in the second hybrid generation. Had we studied only the first generation, we would simply have missed this effect.

Fitness variation

From theoretical considerations, the fitness of hybrids is expected to be highly variable in the F_2 and backcross generations, because of segregation and recombination. This complicates a discussion of hybrid fitness, as some backcross and F_2 plants may be reasonably fit, even when fitness is on average severely depressed. As illustrated in Fig. 2, some of the backcross and F_2 plants in our study scored as high for the combination of fitness components as the 10% or 20% best *B. rapa* and *B. napus* plants. Similarly, Mikkelsen *et al.* (in Mikkelsen, 1996) found that the pollen fertility of $F_1 \times$ Br plants was highly variable (29–97%), and that some plants, selected as morphologically like *B. rapa* and with the same number (20) of chromosomes, had pollen fertilities $> 90\%$.

Some of the variation in fitness was found to be genotypically determined in our study, so that parental populations and varieties gave rise to backcross and/or F_2 offspring that differed in fitness. This was especially interesting, as it turned out that the ranking of the populations and varieties (Br25 $>$ Br54 and Topas $>$ Westar) was the same as in the F_1 experiment (although differences were nonsignificant there; Hauser *et al.*, 1998). In that

experiment, Br54 × Westar hybrids also had the lowest fitness. Other studies of agriculturally relevant hybrids have found genotypic differences in the F₁ generation that carry over to the backcross/F₂ generation (e.g. beans: Mejia-Jimenez *et al.*, 1994; *Brassica*: Roy, 1980).

Implications

The relatively low average fitness of F₂ and backcross plants could suggest that introgression of genes from oilseed rape to weedy *B. rapa* would be somewhat restricted, despite the reported ease of hybridization and high fitness of F₁ hybrids. Nevertheless, many of the F₂ and backcross plants did survive and reproduce, and some of these were as fit as weedy *B. rapa*. If such plants carried a transgene from oilseed rape, it would probably be transferred to plants of the next generation.

The genotypic variation for fitness of F₂ and backcross hybrids suggests that the likelihood of hybridization and introgression may vary between

populations and varieties, stressing that risk assessment of transgenic plants should preferentially cover a range of crop varieties and recipient wild populations.

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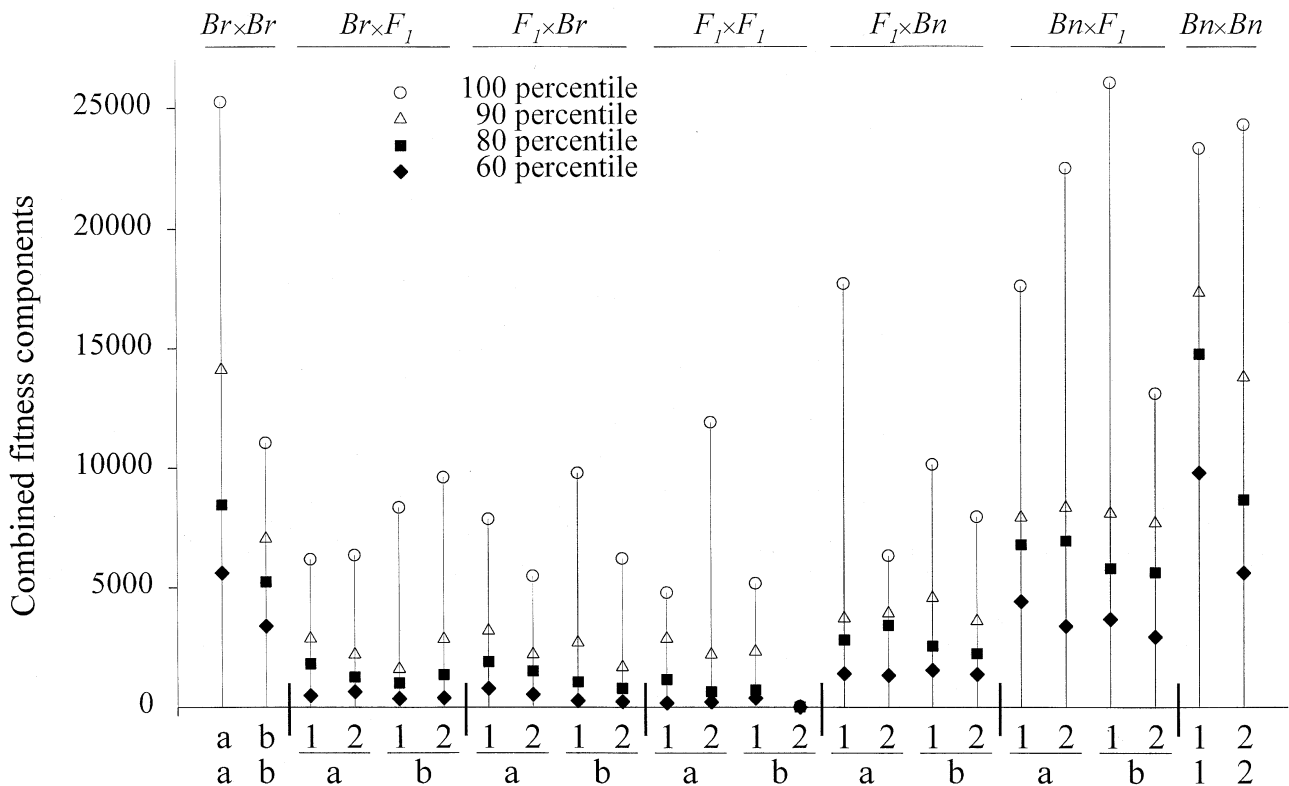


Fig. 2 Combined estimate of fitness (upper percentiles) for the different backcross and F₂ combinations of the *Brassica rapa* populations (a: Br25, b: Br54) and *B. napus* varieties (1: Topas, 2: Westar). The crossing type of the offspring is indicated above the columns, and the populations and varieties from which the offspring are derived below the abscissa.

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