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Male fitness of oilseed rape (*Brassica napus*), weedy *B. rapa* and their F_1 hybrids when pollinating *B. rapa* seeds

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The likelihood that two species hybridise and backcross may depend strongly on environmental conditions, and possibly on competitive interactions between parents and hybrids. We studied the paternity of seeds produced by weedy *Brassica rapa* growing in mixtures with oilseed rape (*B. napus*) and their F_1 hybrids at different frequencies and densities. Paternity was determined by the presence of a transgene, morphology, and AFLP markers. In addition, observations of flower and pollen production, and published data on pollen fertilisation success, zygote survival, and seed germination, allowed us to estimate an expected paternity. The frequency and density of *B. napus*, *B. rapa*, and F_1 plants had a strong influence on flower, pollen, and seed production, and on the paternity of *B. rapa* seeds. Hybridisation and backcrossing mostly occurred at low densities and at high frequencies of *B. napus* and F_1, respectively. F_1 and backcross offspring were produced mainly by a few *B. rapa* mother plants. The observed hybridisation and backcrossing frequencies were much lower than expected from our compilation of fitness components. Our results show that the male fitness of *B. rapa*, *B. napus*, and F_1 hybrids is strongly influenced by their local frequencies, and that male fitness of F_1 hybrids, when pollinating *B. rapa* seeds, is low even when their female fitness (seed set) is high.
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Introduction

Over the last decade there has been much focus on hybridisation between domesticated plants and their wild relatives, partly due to the potential risks of transgenes escaping from genetically modified crops into wild populations of related plants (eg, Ellstrand and Hoffmann, 1990; Snow and Palma, 1997). In a recent review, Ellstrand *et al* (1999) concluded that most domesticated plant taxa are cross-compatible with wild relatives somewhere in the world; escape of transgenes to some wild relatives is thus highly likely. That reproductive isolation between crops and wild relatives is incomplete may not be surprising in the light of the rather recent origin of most domesticated plants; strong biological barriers may not have had sufficient time to evolve. Questions that remain to be understood are how frequently hybridisation and introgression take place, and how these processes are modified by various ecological conditions.

Several different barriers, both pre- and postzygotic, have the potential to reduce gene flow between species, eg, temporal divergence, gametic and zygotic incompatibility, hybrid inviability and sterility (Levin, 1978). If the barriers are incomplete, first and later generation hybrids may be formed and function as a bridge for gene transfer. Different alleles may flow from one species into another at different rates, depending on rates of dispersal, the strength of selection, breeding system, and linkage to selected loci (Barton and Hewitt, 1985; Christiansen *et al*, 1995). Thus, the dynamics of an introgression process depends on a number of factors; two of the more important ones are the fitness of hybrids and the selective value of the genes (and among them, transgenes). To complicate this, fitness of hybrids is often strongly dependent on the environment (Arnold, 1997). Hybrids may function best under certain ecological conditions that are not necessarily like those preferred by the parents, or they may be more or less tolerant to stress, herbivory, diseases etc (Arnold, 1997; Fritz *et al*, 1999). Competitive interactions between parents and their hybrids may also affect hybridisation frequencies and fitness of hybrids: eg, several studies have shown that heterospecific pollen is less successful in fertilising ovules when competing against conspecific pollen (Carney *et al*, 1996; Hauser *et al*, 1997).

Cultivated oilseed rape (*Brassica napus*) and weedy *B. rapa* are able to hybridise and backcross spontaneously in fields and in experiments (Jørgensen and Andersen, 1994; Jørgensen *et al*, 1996; Mikkelsen *et al*, 1996), even though the frequency of hybrid seeds varies a lot between experiments (Jørgensen and Andersen, 1994), fields (Landbo *et al*, 1996) and geographical regions (Wilkinson

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et al, 2000). Surprisingly, F_1 plants sometimes set many more seeds than *B. rapa* (Hauser *et al*, 1998b) but much fewer in other cases (Mikkelsen, 1996; Jørgensen *et al*, 1996). We argued (Hauser *et al*, 1998b) that different frequencies of parents and hybrids, and thereby different compositions of the pollen cloud, might be one explanation for the discrepant results. If F_1 and backcross zygotes are preferentially aborting within *B. rapa* pods, whereas backcross zygotes are preferentially surviving within F_1 pods, then the seed set of *B. rapa*, *B. napus*, and F_1 hybrids should vary with their relative frequencies in the population. Both the male and female fitness of hybrids (ie, paternity through pollen donation and seed set, respectively) should thus be frequency dependent.

To study these effects in more detail, we conducted two experiments, one focussing on female fitness (Hauser *et al*, unpublished), and the present focussing on the male fitness of *B. napus*, *B. rapa*, and F_1 hybrids. The three plant types were grown in mixtures of different proportions and densities, and during the course of the experiment we counted the number of open flowers, pollen and seed production. After harvest, the paternity of seeds developed on *B. rapa* was determined, and the observed paternity compared with the paternity expected from observed fitness components in this and other studies. With these data, we could compare the magnitude of female and male fitness, and determine whether fitness is indeed frequency dependent.

Materials and methods

Plant material

Oilseed rape, *B. napus* (abbreviated *Bn*; $2n = 38$, chromosomal composition AACC) is commonly grown in Denmark, and is subject to worldwide intensive breeding including genetic modification. The crop is self-compatible with an intermediate selfing rate (Becker *et al*, 1992), and is insect- and wind-pollinated. *B. rapa* (abbreviated *Br*; $2n = 20$, AA) is a common weed in Denmark, occurring in oilseed rape fields, along roadsides, and in other disturbed habitats. The species is self-incompatible and pollinated by insects and to some extent by wind.

Brassica napus plants used for the experiment originated from the non-transgenic spring cultivar 'Drakkar', *B. rapa* from two populations on Zealand, Denmark (Br25 and Br45 in Landbo *et al*, 1996 and Hauser *et al*. 1998a, b; all plants had a pollen fertility >90% and were checked morphologically to avoid inclusion of possible hybrids), and F_1 hybrids from controlled crosses between a homozygous transgenic glufosinate tolerant Drakkar (♀) and *B. rapa* (Br25). Hybrids were thus hemizygotic for the bar-gene giving tolerance to the herbicide glufosinate. The parents and production of their hybrids are described in Snow *et al* (1999).

In order to break seed dormancy, *B. rapa* and F_1 seeds were germinated on filter paper wetted by 0.2% KNO_3 and exposed to temperature cycles of 16 h at 20°C and 8 h at 30°C in darkness (Landbo and Jørgensen, 1997). After germination they were transferred to trays in the greenhouse. *B. napus* seeds were sown directly in trays. All plants were transferred to the field when approximately three to five leaves had developed.

Experimental design

Brassica napus, *B. rapa*, and F_1 plants were grown in mixtures of six different proportions (including pure plots of *B. rapa*), with two densities for each proportion (Table 1). Within plots (combinations of one mixture and one density) the plant types were randomised. The 12 plots were placed within a barley field at Risø National Laboratory, with a minimum distance of 10 m between plots.

The number of open flowers per plant was counted once a week on 10 plants from each plant type in each plot, except in proportion 35:1:0 (*Bn*:*Br*:F_1) and 0:1:35 where flowers on all *B. rapa* plants were counted.

Pollen production was estimated four times during the flowering season by counting the number of grains within 21 anthers from each plant type and density (three flowers from seven randomly selected plants). The anthers were stored in a staining solution containing 70% ethanol, detergent and 0.5% methylene blue (Dafni, 1992). The viability of the pollen was estimated by the proportion of round and deeply stained pollen.

The number of fully developed seeds (round and black) produced per plant was counted on nine to 12 individuals per plant type in each plot, except on *B. rapa* in proportions 35:1:0 and 0:1:35 where all plants were counted. The number of seeds per plant was estimated from the total weight of seeds divided by the weight of 100 randomly selected seeds.

To determine the paternity (*Bn*, *Br*, or F_1) of the seeds harvested on *B. rapa*, samples of seeds from each plant were germinated as described above. Paternity was determined by a combination of tolerance to glufosinate combined with PCR for the transgenic construct, by morphology, and by *B. napus*-specific AFLP markers.

Among the backcross offspring, sired by hemizygous transgenic F_1 parents, half are expected to carry the bar gene and thus be tolerant to glyphosinate (references in Snow *et al*, 1999). To evaluate tolerance, 5 × 5 mm filter papers wetted with a 0.2% glufosinate solution (Basta) were placed on a leaf of each plant (when ~5 leaves were present). Resistance (no leaf damage) was assessed after 4–5 days. All tolerant plants were additionally tested by PCR analysis with primers specific to the bar-gene promotor. Only plants with both tolerance and a positive PCR test were counted as transgenic. The total number of offspring sired by F_1 was then calculated as the double of the number of bar-positive plants; the expected 50% non-transgenic backcross offspring were taken from the group of offspring plants determined to be backcrosses based on morphology.

Plants that were sensitive to glufosinate were divided into three groups based on morphology: pure *B. rapa* are readily distinguished from F_1 plants, that are more robust, blue-green, usually with fewer hairs, and an elongated raceme like *B. napus*. A third group of plants was somewhat aberrant in morphology, and considered to be backcrosses. To confirm the paternity of the three groups, the presence of *B. napus*-specific AFLP markers was determined in subsets of the plants: none of the plants determined to be *B. rapa* carried *B. napus* markers, whereas plants determined to be F_1 carried all 10 markers, as expected. One of the plants considered to be backcrosses carried two of the 10 markers and the rest of the plants no markers; these plants could therefore either be backcrosses carrying no C chromosomes or pure *B. rapa* plants with an atypical morphology.

Table 1 Number of plants, total number of open flowers counted per plant over the season, average number of pollen grains per flower, and average pollen viability of *Brassica napus* (*Bn*), *B. rapa* (*Br*), and F_1 hybrids in the different mixtures and densities. Symbols are explained in the Methods

Mix proportion Bn:Br:F$_1$	*Plant type*	*High density (100 m*$^{-2}$*)*				*Low density (16 m*$^{-2}$*)*			
		No. of plants P_{ij1}	*No. of open flowers per plant* Q_{ij1}	*No. of pollen per flower* ($\times 10^3$) $R_{i\cdot1}$	*Pollen viability* $s_{i\cdot1}$	*No. of plants* P_{ij2}	*No. of open flowers per plant* Q_{ij2}	*No. of pollen per flower* ($\times 10^3$) $R_{i\cdot2}$	*Pollen viability* $s_{i\cdot2}$
3:1:1	*Bn*	120	31	111.1	0.95	120	73	114.7	0.95
	Br	40	23	95.4	0.91	40	75	107.7	0.92
	F_1	40	92	105.2	0.33	40	456	106.5	0.33
1:3:1	*Bn*	40	26	111.1	0.95	40	37	114.7	0.95
	Br	120	21	95.4	0.91	120	63	107.7	0.92
	F_1	40	129	105.2	0.33	40	142	106.5	0.33
1:1:3	*Bn*	40	12	111.1	0.95	40	59	114.7	0.95
	Br	40	13	95.4	0.91	40	100	107.7	0.92
	F_1	120	81	105.2	0.33	120	223	106.5	0.33
35:1:0	*Bn*	175	24	111.1	0.95	175	88	114.7	0.95
	Br	5	25	95.4	0.91	5	93	107.7	0.92
	F_1	0	–	–	–	0	–	–	–
0:1:35	*Bn*	0	–	–	–	0	–	–	–
	Br	5	16	95.4	0.91	5	65	107.7	0.92
	F_1	175	33	105.2	0.33	175	124	106.5	0.33

Markers specific to *B. napus* used in this experiment were found after screening plants in the experiment (50 *B. rapa*, 50 F_1 and 30 *B. napus)* and six *B. oleraceae* plants (CC genome) with the two primer combinations E-AG/M-ACG (yielding seven markers) and E-CAG/M-CT (three markers) (for methods: see Shim and Jørgensen, 2000).

Data analysis

Flower production per plant and numbers of pollen per flower was analysed by ANOVA (SAS, 1998), with a model including effect of plant type, density, week, and their interactions. The same model was used for analysis of pollen viability by logistic regression (Proc Genmod; SAS, 1998). The effects of mixture and density on the numbers of offspring sired by *B. napus* and F_1 were analysed by chi-square and G-tests of contingency tables; none of these tests are robust, though, due to the many low counts and zero observations (for the G-tests, all counts were incremented by one to avoid empty cells).

The observed data on flower production, phenology, pollen production and viability were combined with published data (references below) on pollen fertilisation success, zygote survival, and seed germination to estimate an expected relative paternity of *B. napus*, *B. rapa*, and F_1 fathers among seeds harvested on *B. rapa*.

The expected relative paternity of *B. rapa* seeds harvested was calculated as:

$$e_{ijk} = v_{i\cdot\cdot} \cdot u_{i\cdot\cdot} \cdot t_{i\cdot\cdot} \cdot \frac{P_{ijk}}{P_{\cdot jk}} \cdot \sum_{t=1}^{t=7} \frac{s_{i\cdot k}(t) \cdot R_{i\cdot k}(t) \cdot Q_{ijk}(t)}{s_{\cdot\cdot k}(t) \cdot R_{\cdot\cdot k}(t) \cdot Q_{\cdot jk}(t)} \cdot \frac{Q_{2jk}(t)}{Q_{2jk}},$$

$$i = 1, 2, 3;\ j = 1, ., 6;\ k = 1, 2,$$

where P_{ijk} is the number of plants of plant type i (i = 1: *Bn*; i = 2: *Br*; i = 3: F_1) in mixture j at density k, $Q_{ijk}(t)$ the number of open flowers per plant at time t, $R_{i\cdot k}(t)$ is the number of pollen produced per flower at time t (not determined for each mixture, only for densities), $s_{i\cdot k}(t)$ the proportional viability of pollen at time t (not determined for each mixture, only for densities), $t_{i\cdot\cdot}$ the fertilisation success of pollen landed on a *B. rapa* stigma, $u_{i\cdot\cdot}$ the survival of zygotes to mature seeds, and $v_{i\cdot\cdot}$ the proportional germination of seeds. The denominator of the left-hand fraction of the summation symbolises the total production of viable pollen among the three plant types at time t (in the given plot), and Q_{2jk} the sum of all open *B. rapa* flowers over the seven countings (in the given plot).

Pollen fertilisation success within *B. rapa* styles, and relative to competing *B. rapa* pollen, was taken from Hauser *et al* (1997). Only the success of *B. rapa* and *B. napus* pollen was determined in that study, though, and we therefore conservatively set the relative success of F_1 pollen to be equal to that of *B. napus* ($t_{2\cdot\cdot} = 1$; $t_{1\cdot\cdot} = t_{3\cdot\cdot} = 0.72$), since no better estimates are available.

Survival of *BrxBr* and F_1 zygotes within *B. rapa* pods was found in Hauser *et al* (1997); the survival values were determined for pods containing mixtures of both types of zygotes. No data were available for the survival of *Br*(♀)×F_1 zygotes in pods of mixed parentage, only data from single donor crosses in Hauser *et al* (1998a, b). To account for the effect of competition and preferential abortion, we calculated a 'competition factor' by scaling the survival of F_1 zygotes (relative to *B. rapa*) within mixed pods (0.48; Hauser *et al*, 1997) to their relative survival in single-donor pods (0.74; Hauser *et al*, 1998a; 'competition factor' = 0.65). The survival of backcross zygotes in mixed pods was then estimated by multiplying their survival in pure pods ((relative to *B. rapa*) = 0.62; Hauser *et al*, 1998b) with the competition factor: $u_{3\cdot\cdot} = 0.62 \times 0.65 = 0.40$.

Germination of seeds harvested on *B. rapa* mothers was found in Landbo and Jørgensen (1997): $v_{1\cdot\cdot} = 0.91$, $v_{2\cdot\cdot} = 0.46$, $v_{3\cdot\cdot} = 0.47$ (their Table 3 and 4: treatment 1, and comments in text); these estimates come from seeds that had not been treated to break dormancy. In the present experiment, we treated seeds as described above, but ger-

mination proportions were still low and similar to the proportions that could be expected from untreated seeds (Landbo and Jørgensen, 1997). We therefore assume that our treatment for some reason did not work.

The expected paternity, as calculated by combining the different male fitness components, was compared by a chi-square test to the observed paternity among seeds from *B. rapa* plants.

Results

Flower phenology and production

Brassica rapa started flowering ca. 1 week earlier than *B. napus* and F_1, and peaked about 1 to 2 weeks earlier (except in pure plots at low density). Still, their flowering periods overlapped extensively (Figure 1). F_1 plants produced many more open flowers than their parents, especially when growing at low density (Figure 1, Table 1); *B. napus* and *B. rapa* produced approximately even numbers of flowers. In general, plants at low density produced more flowers and flowered later than plants at high density; the composition of the mixtures did not affect flowering.

Brassica rapa growing in pure plots flowered at about the same time as those in mixtures (except for a surge of late flowering at low density; Figure 1), but produced more flowers (Figure 1, Table 1).

Pollen production and viability

The number of pollen grains produced per flower (Table 1) was slightly, but non-significantly, higher in *B. napus* and F_1 than in *B. rapa* (Table 1). Pollen production declined somewhat over time at high density (*Bn*: from 124.6 to 91.4; *Br*: 114.9–76.7; F_1: 116.9–71.8; all numbers $\times 10^3$), but increased at low (*Bn*: 111.3–116.3; *Br*: 98.3–113.0; F_1: 105.5–110.1; all numbers $\times 10^3$) .

Pollen viability was much lower in F_1 hybrids than in the two parental species (Table 1). In *B. rapa* and F_1, the viability declined slightly over the season (*Br*: from 0.93 to 0.89; F_1: 0.37–0.27).

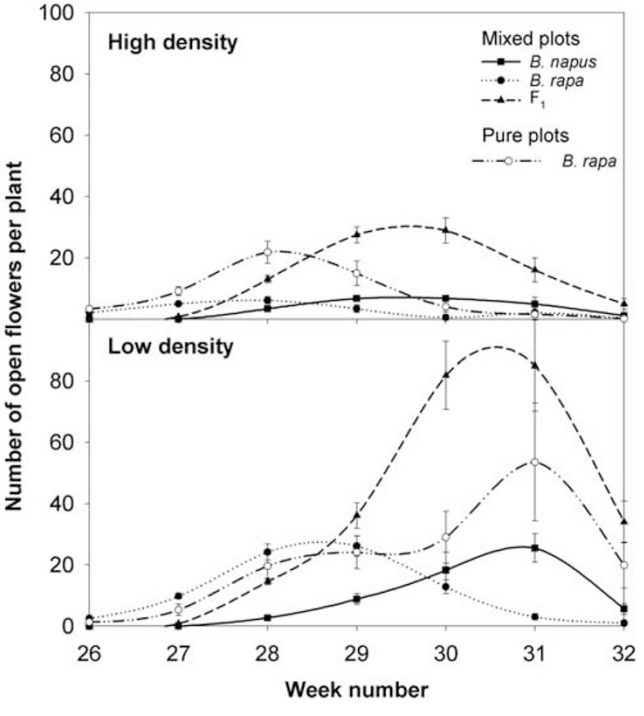

Figure 1 Flowering phenology shown as the average number of open flowers per plant (with standard error shown as bars) over time.

Seed production and germination

The seed weight varied strongly between the plant types, with *B. rapa* weighing the least (0.11×10^{-2} g), and F_1 and *B. napus* the most (0.21 and 0.24×10^{-2} g, respectively). F_1 hybrids produced more seeds per plant than the parents in most of the plots, and *B. napus* more than *B. rapa*. Seed set of F_1 hybrids did not differ systematically between mixtures, whereas *B. rapa* and *B. napus* produced more seeds in plots with high frequencies of themselves. Seed set was significantly higher at low density (Table 2), and in the pure plots *B. rapa* produced more seeds than in the mixtures (Table 2).

Relatively low proportions of the seeds harvested on *B. rapa* germinated, despite the dormancy breaking treatment (Table 3). In two plots, germination was below 10%, which limited the number of seedlings available for paternity analysis.

Paternity

Brassica napus and F_1 almost only sired offspring on *B. rapa* in the mixtures with high proportions of themselves (Table 3, Figure 2; this is significant by G-test ($P < 0.001$) for both species; these statistics are biased, however, due to the many low counts and zero observations). In addition, *B. napus* almost only sired offspring at low density (Table 3, Figure 2; G-test: $P < 0.001$).

A few of the *B. rapa* plants produced almost all the heterospecific offspring while others produced none at all (Table 3). A few F_1 offspring were scored in the 0:1:35 plots, where none should be expected; these are most likely backcross plants with F_1 morphology and all C-chromosomes present, but could also result from pollen dispersal from *B. napus* in other plots (these offspring were omitted from further analysis).

The expected paternity of *B. rapa* seeds, based on combined fitness components, is presented in Figure 2. The expected number of F_1 and backcross offspring was significantly higher than that observed (chi-square analysis: $P < 0.001$) in all plots but one (35:1:0 at low density).

Discussion

Our results clearly show that the frequency and density of *B. napus*, *B. rapa*, and F_1 plants in mixtures had a strong influence on their flower, pollen, and seed production, and on the paternity of *B. rapa* seeds. *B. napus* and F_1 hybrids almost only fathered offspring at high frequencies of themselves and *B. napus* almost only at low density. This indicates that their male fitness among seeds produced by *B. rapa* is rather low. Paternity of seeds set by *B. napus* and F_1 was not studied here, but we can speculate that only few of those would be sired by pollen from F_1 fathers, either, due to low pollen viability and strong competition from *B. rapa* and *B. napus* pollen and sired zygotes. Male fitness of F_1 hybrids is therefore probably low overall. On the contrary, the seed set of F_1 hybrids was very high (as also found by Hauser *et al*, 1998b and unpublished), and generally higher than

Table 2 Average number of seeds of *Brassica napus* and *B. rapra* produced per plant in the different mixtures and densities (with standard error and sample size in parenthesis)

Mix proportion Bn:Br:F$_1$	*High density*			*Low density*		
	B. napus	*B. rapa*	F_1	*B. napus*	*B. rapa*	F_1
3:1:1	666 (58; 10)	202 (14; 11)	1195 (111; 10)	5389 (287; 11)	1119 (112; 11)	6608 (520; 10)
1:3:1	839 (52; 11)	390 (27; 10)	1386 (80; 9)	1262 (92; 10)	1532 (152; 10)	973 (95; 10)
1:1:3	509 (54; 10)	126 (13; 9)	1009 (135; 10)	2581 (186; 10)	1212 (99; 9)	5751 (267; 10)
35:1:0	1397 (58; 10)	175 (25; 5)	– –	7138 (159; 10)	1010 (321; 4)	– –
0:1:35	– –	247 (71; 3)	2722 (133; 10)	– –	779 (62; 5)	426 (28; 10)
0:1:0	– –	1556 (184; 10)	– –	– –	4046 (409; 12)	– –

Table 3 Number of *Brassica rapa* plants from which seeds were harvested, average germination percentage of seeds (range in parenthesis), number of offspring seedlings assigned to different paternities, and the number of *B. rapa* maternal plants from which these seedlings originated

Mix proportion Bm:Br:F$_1$	*High density*					*Low density*				
	No. of mother plants	*Germination percentage*	*Paternity*	*No. of seedlings*	*From no. of plants*	*No. of mother plants*	*Germination percentage*	*Paternity*	*No. of seedlings*	*From no. of plants*
3:1:1	11	56 (24–80)	*Bn*	1	1	11	45 (12–77)	*Bn*	10	1
			Br	244				*Br*	258	
			F_1	0				F_1	4	2
1:3:1	10	57 (7–85)	*Bn*	0		10	63 (36–87)	*Bn*	0	
			Br	246				*Br*	250	
			F_1	0				F_1	0	
1:1:3	9	55 (7–90)	*Bn*	0		9	5 (0–21)	*Bn*	0	
			Br	185				*Br*	68	
			F_1	0				F_1	0	
35:1:0	5	33 (19–48)	*Bn*	1	1	4	7 (0–25)	*Bn*	19	1
			Br	78				*Br*	18	
			F_1	0				F_1	0	
0:1:35	3	49 (0–100)	*Bn*	1	1	5	38 (11–88)	*Bn*	11	2
			Br	266				*Br*	269	
			F_1	14	2			F_1	18	4

both parents, indicating that female fitness of F_1 at the same time is very high.

In all but one of the plots, much fewer F_1 and backcross offspring were found than should be expected based on the combined fitness components (Figure 2). This shows that selection against matings and/or offspring from the *B. napus* and F_1 fathers is even stronger than suggested by our current estimates of male fitness components. A detailed inspection of the components gives some insight into which life stages may be responsible for their low male fitness.

Male fitness components

F_1 plants set many more flowers than either of the parents, and is thus favoured at this life stage. *B. napus* was observed to set as many flowers as *B. rapa*, which is probably unrealistic since *B. napus* usually produces many more flowers than *B. rapa*. This could be due to the counting once a week, where only flowers open at that day, and not the turnover is assayed. The developmental time from flower opening to stigma receptivity is about the same in the three plant types (TP Hauser, personal observations), but *B. rapa* flowers probably stay open for a longer time, as they are self-incompatible (in contrast to *B. napus*) and have to wait for full fertilisation by pollen from other plants.

Brassica napus, *B. rapa* and F_1 hybrids did not differ much in pollen production, as seen in Figure 2. However, F_1 pollen was much less viable than *B. rapa* and *B. napus* pollen, as has been found in several other studies (eg,

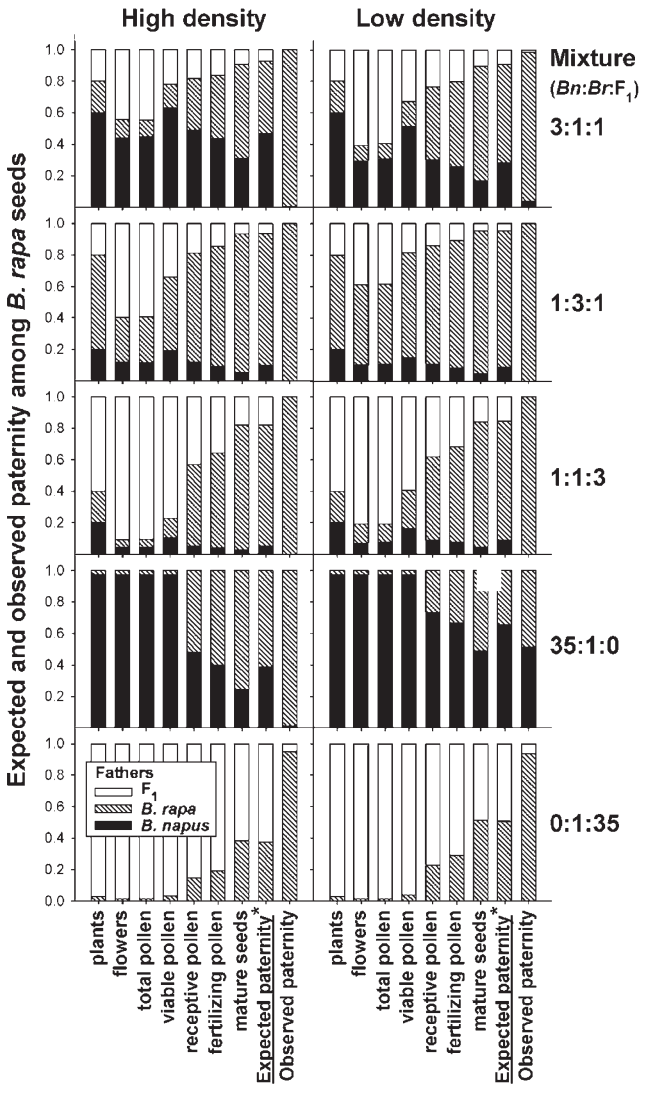

Figure 2 Paternity proportions (*Brassica napus*, *B. rapa*, and F_1) among *B. rapa* seeds, shown for each combination of densities and mixtures. The first eight columns show the paternity expected from the cumulation of successive male fitness components (relative proportions of plants, flower production, total and viable pollen production, pollen synchroneous with receptive *B. rapa* styles, pollen fertilization success, seed survival and germination), and the last column the observed proportions. (*Expected paternity includes, in addition to previous components, the seed germination rate.)

Jørgensen and Andersen, 1994; Jørgensen *et al*, 1996). Matings by F_1 fathers are thus selected against at this life stage.

Brassica napus and F_1 plants are not flowering precisely at the same time as *B. rapa*, which reduces their paternity potential strongly (see 'receptive pollen' in Figure 2). No published data are available on the fertilisation success of pollen from F_1 hybrids, when competing against *B. rapa* and *B. napus* pollen within *B. rapa* styles. To be conservative, we therefore assumed it to be equal to *B. napus*. In reality, F_1 pollen are probably weaker competitors than *B. napus*, due to their aneuploid genome constitution (A + 0–9C) (see for example, results by Lu and Kato, 2001), and the expected paternity of F_1 is probably overestimated for this fitness component.

The survival of backcross zygotes in competition with F_1 and *B. rapa* zygotes has not been studied either. We therefore used a conservative estimate based on the survival of backcross zygotes within pods of single parentage (from Hauser *et al*, 1998b), but scaled down by a 'competition factor' (see Methods). We here assume that competition with *B. rapa* zygotes has the same relative impact on F_1 and backcross zygotes. However, the competitive ability of backcross zygotes is probably lower than that of F_1 zygotes, partly due to their aneuploid genome composition (see also results by Lu and Kato, 2001), and the expected paternity of F_1 is therefore likely overestimated by this fitness component.

Only relatively low proportions of offspring seed germinated, much lower than should be expected for seeds that are treated to break dormancy (Landbo and Jørgensen, 1997). Probably the non-germinating seeds are pure *B. rapa* (or backcross seeds) that for some reason are not affected by our treatment. If this is so, our (observed) paternity analysis is biased with too many *B. napus* (and perhaps also non-dormant backcross) offspring, relative to what the total seed pool actually contains (see similar discussion by Landbo *et al*, 1996). However, when comparing the observed and expected paternity, we have corrected for this by including estimates of seed germination from untreated seeds in the calculation of expected paternity.

Our estimates for pollen fertilisation success, zygote survival, and seed germination come from previously published experiments, which could bias our estimates of paternity if the conditions and material in those experiments were different. However, all the previous studies used plants from one or both of the *B. rapa* populations used in the present study, and from the same cultivar Drakkar, minimising at least the genetic differences between experiments.

Expected and observed paternity

Considering the fitness components together, we see that flower production most likely is underestimated for *B. napus* fathers, whereas pollen fertilisation success and zygote survival probably is overestimated for F_1 fathers. If flower production of *B. napus* was in reality larger than our estimates suggest, selection against *B. napus* pollen or F_1 offspring must have been even stronger at later life stages, if we are to account for the low proportions of observed F_1 offspring. Since we have no indices that the pollen production or flowering time data are biased, this suggests that *B. napus* pollen and/or F_1 zygotes are doing worse than extected after pollen deposition. Similarly, the most reasonable explanation for the deficit of observed backcross offspring (relative the expected) also is a stronger selection against F_1 pollen and/or backcross zygotes than assumed.

We know from results by Hauser *et al* (1997) that selection against *B. napus* pollen and F_1 zygotes within *B. rapa* styles and pods is much stronger when competing against *B. rapa* pollen and zygotes, respectively. Our new results indicate that such frequency-dependent processes also play a major role in determining the paternity, and thus male fitness, in the field: despite producing a very large numbers of viable pollen, *B. napus* and F_1 fathers are prevented from producing offspring on *B. rapa* by competition from *B. rapa*'s own pollen and zygotes, or by selective abortion from the *B. rapa* mother.

Conclusions

This study clearly demonstrates that frequencies of parents and hybrids may greatly affect male fitness in plant mixtures. This has important implications for the understanding of hybrid fitness (eg, Arnold and Hodges, 1995; Rieseberg, 1995; Arnold, 1997) and the dynamics of hybrid zones (see discussion in Arnold, 1997): hybrid fitness not only depends on the environmental conditions in which they live, but also on the frequencies of parental plants and other hybrids with which they interact. Further, our study suggests that even if F_1 hybrids have a high female fitness (seed set), they may simultaneously suffer from a very low male fitness.

Our results also have implications for the discussions on transgene escape. Hybridisation was most pronounced at low plant density, which suggests that introgression of transgenes into *B. rapa* is most likely at set-aside land, ruderal sites, and fields where Brassicas are weedy in other crops. Among these localities, the most likely is probably fields with inefficient weed management (Hansen *et al*, 2001). Our results also suggest that local frequencies of *B. rapa*, *B. napus*, and their hybrids are important in determining the transfer of transgenes to *B. rapa*. Finally, the large difference in male and female fitness strongly imply that the likelihood of gene transfer is also dependent on the possible introgression routes: who are fathers and who are mothers.

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