

Hybridisation within *Brassica* and allied genera: evaluation of potential for transgene escape

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Abstract Determining the potential for hybridisation between transgenic crops and their relatives is a major component of risk assessment. Recent assessments of the extent of reproductive compatibility between crops and their relatives draw heavily on existing data from experimental crosses to infer the likelihood of hybridisation and introgression. Since the literature in this area continues to grow at a rapid pace, it is essential that such analyses can be easily updated. We used a database approach to assemble data on reproductive compatibility for eight crop species in *Brassica*, *Raphanus* and *Sinapis*, using data from hand pollination, spontaneous (unassisted) pollination and trials using in vitro techniques (e.g. embryo rescue), incorporating 326 studies and 216

species combinations. We found many reports for major crop species (*B. juncea*, *B. napus*, *B. oleracea* and *B. rapa*), but fewer for minor crops (*B. carinata*, *B. nigra*, *Raphanus sativus* and *Sinapis alba*). Many species combinations remain untested, and we highlight these information gaps. While reproductively incompatible species can be discounted as targets for transgene escape, compatible species must be evaluated further in the particular context where transgenic crops are grown. Because the data is retained in a database in a relatively unmodified form, multiple views of the data can be generated; this review represents one possible view of this data. Our approach also allows new data to be easily incorporated into future reanalyses and can be extended to other crop groups, and as such is a useful method of assembling, analysing and sharing data for risk assessment.

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Introduction

Global concern about negative environmental consequences resulting from the release of transgenic crops has motivated significant research aimed at minimising the degree of uncertainty surrounding the potential for transgene escape via hybridisation (Ellstrand 2003). While reports of spontaneous

hybridisation (resulting from cross pollination under natural conditions) provide the strongest positive evidence that transgenes are able to escape via hybridisation, they are not alone adequate for determining the likelihood of hybridisation (Armstrong et al. 2005). Spontaneous hybridisation is ephemeral and difficult to detect due to pre-pollination barriers (Grant 1994; Arnold 1997). Importantly, a lack of evidence of spontaneous hybridisation does not indicate that particular pairs of species will not cross in the field. Reports of experimental hybridisation (manual hand pollination) between crops and their relatives are a useful source of information, as they allow measurement of the degree of reproductive compatibility between species and the identification of incompatible species combinations. This allows conservative assessment of species that should be considered in their local context for their potential as targets for transgene escape.

In this review we focus specifically on the potential for transgenes to escape in the globally significant crops in the genus *Brassica* and allied genera. These species form a complex of partially reproductively compatible species, presenting many potential opportunities for transgene escape via hybridisation. This group has been at the forefront of development of transgenic crops, with extensive field trials of transgenic cultivars of *Brassica napus* and *B. rapa* (OECD 1999). Fewer trials have also been carried out with transgenic cultivars of *B. carinata*, *B. juncea*, *B. nigra*, *B. oleracea*, *Raphanus sativus* and *Sinapis alba* (OECD 1999). Transgenic *B. napus* and *B. rapa* have been approved for environmental release in several countries (AGBIOS 2005), and transgenic *B. napus* accounts for 19% of the 23 million hectares of canola grown worldwide (James 2004).

Detailed summaries of hybridisation data in *Brassica* and allied genera have previously been compiled, originally as a source of information for breeders (e.g. Davey 1959; Prakash and Hinata 1980; Warwick et al. 2000). More recently, reports have focused on hybridisation with *B. napus* from a transgene escape perspective (e.g. Scheffler and Dale 1994; Bourdôt et al. 1999; Rieger et al. 1999; Salibury 2002; Chèvre et al. 2004). However, since forms of *B. rapa* are currently deregulated (AGBIOS 2005) and transgenic forms of the other crop species exist, it is necessary that these species are also considered. As

the quantity of hybridisation information continues to grow, it is pertinent that any new attempt to synthesise these data is readily updateable. To increase future utility of the data, the particular method of display should not govern the method of data storage.

In this paper, we update and expand upon the findings of previous reviews of hybridisation in *Brassica* and allied genera. We include all crop species in Brassicaceae, tribe Brassiceae, (*B. carinata*, *B. juncea*, *B. napus*, *B. nigra*, *B. oleracea*, *B. rapa*, *Raphanus sativus* and *Sinapis alba*), and incorporate data from papers published since the last major reviews. We synthesise data from experimental and spontaneous crosses using a database approach in which the storage and presentation of information are independent, allowing multiple views of the same data to be generated while retaining data in a format as close to the original as possible. This database approach allows us to assemble a body of information that may be conveniently updated and reused in future. Our aim here is not to present all the information we have collected; rather, we seek to highlight the general trends and relevant information likely to be of interest to regulators and breeders, while introducing a novel system of representing the results of large numbers of crossing trials.

Methods

Study group

The genus *Brassica* contains several important crop species, used for a variety of purposes. *Brassica napus* and *B. rapa* are the most important for their use as oilseed crops (canola, rapeseed; Ellstrand 2003). *Brassica* and the related genus *Raphanus* include vegetables for human consumption: swede (*B. napus*), turnip and Chinese cabbage (*B. rapa*), and cauliflower, cabbage, etc. (*B. oleracea*), Indian mustard (*B. juncea*), and radish (*R. sativus*). Considerable variability exists within each species, and many similar forms exist across different species (Prakash and Hinata 1980; Stewart 2002). Three *Brassica* species and one species in the related genus *Sinapis* are ‘mustards’; *B. carinata* (Ethiopian mustard), *B. juncea* (Indian mustard), *B. nigra* (black mustard) and *Sinapis alba* (white mustard). Cultivation of *B. carinata* as an oilseed and vegetable crop is largely

restricted to Ethiopia and India (Hemingway 1995; Stewart 2002). Forms of *B. napus*, *B. oleracea*, *B. rapa* and *R. sativus* are also grown as fodder crops. Three of the crop *Brassica* species are diploids (*B. nigra*, *B. oleracea* and *B. rapa*), while the other three are amphidiploids; the result of hybridisation and subsequent chromosome doubling. *Brassica napus* is an amphidiploid of *B. oleracea* and *B. rapa*, *B. juncea* of *B. nigra* and *B. rapa*, and *B. carinata* of *B. nigra* and *B. oleracea*.

Literature survey

We used the CABI abstracting database and reviews of hybridisation to find articles reporting attempted hybridisation involving any of the crops in *Brassica*, *Raphanus* and *Sinapis*. The reviews included Prakash and Hinata (1980), Scheffler and Dale (1994), Bourdôt et al. (1999), Rieger et al. (1999), Warwick et al. (2000) and Salibury (2002). We considered only studies reporting crosses involving the F₁, F₂ and BC₁ generations and excluded studies dealing solely with later generations. Studies involving spontaneous crosses between species (i.e. non-assisted crosses under field conditions) were included, but treated separately. We also included studies using ‘in vitro’ methods (embryo rescue, etc.), but have also treated these separately since success using these techniques does not necessarily indicate that a pair of species will cross under natural conditions. However, failure to cross with such assistance may give some indication as to which species will not cross. We excluded studies describing somatic hybridisation. We included articles only in English and covered up to June 2005.

Databasing

As part of a wider project examining gene flow and environmental risks of transgenic crops, we developed a database, the New Zealand Plant BioSafety Database, designed to hold diverse information for risk assessment, including hybridisation data. This is an attribute database in which data are associated with pairs of taxa. The database was implemented using the Microsoft .Net framework and SQL server. Analysis and graphing routines were written in R (R Development Core Team 2005). To increase future reusability of the data, particular attention was paid to recording information in a

form as closely resembling that of the original source as possible. We applied this principle to both the taxonomic names used and to the hybridisation data. We recorded a range of information on production of fruit, seeds and hybrids, as well as data on the fertility of the hybrids (e.g. chromosome associations, pollen stainability). However, we only present information on hybrid production here.

Studies reporting hybridisation do so at a variety of taxonomic ranks (e.g. species, subspecies, variety, or cultivar). Because cross-compatibility may vary with the particular genotype used and with the polarity of the cross (i.e. which species was the maternal parent; Arnold 1997), we recorded the exact names given (including cultivars), and the cross polarity, using the Landcare Research Plant Names Database (Allan Herbarium 2000). Taxonomic names were converted into currently accepted names at analysis following the nomenclature of Zhou et al. (2001) and Warwick et al. (2000). This approach keeps taxonomic translation explicit, and would allow reanalysis of data at alternative taxonomic ranks in the future. We recorded where the polarity of a cross was unclear, and these data have largely been excluded from our analysis.

Analysis

Within a study, many crosses may be performed, using different cultivars, subspecies or experimental treatments. While previous studies summarising hybridisation information have collapsed these different attempts to a single row per species, we have retained the individual attempts. We define a ‘trial’ to mean any distinguishable crossing attempt; for example, different experimental conditions, years or genotypes within the same study would constitute different trials. Retaining individual trials provides useful information about the variability of success of a cross, while still allowing the data to be collapsed in future.

In the literature, information on production of hybrids may be reported quantitatively (i.e. rate of hybrid production) or as a binary variable (i.e. success or failure of hybrid production). To maximise the information available for analysis, we here express hybrid production in both forms. Importantly, while all quantitative information can be displayed as

binary data (any item greater than zero treated as success), binary data cannot be converted to quantitative data. This affects the amount of data available, and was one motivation for retaining the data as close to its original form as possible. The only quantitative data presented in this paper is hybrid production from experimental crossing trials, expressed as hybrids produced per flower pollinated. Where data were given as ranges, we used the midpoint of that range. Where only a subset of seeds produced were planted (common in trials that involved species with high levels of reproductive compatibility), we took this into account by recalculating to estimate the number of hybrids that would have been produced per pollination.

Spontaneous hybridisation experiments, in which pollination between experimentally planted species is allowed to occur without assistance, adds both additional biological reality and complexity to characterising hybridisation under natural conditions. Between studies, many experimental designs are used, with varying plant ratios, densities and placement patterns. Because of the difficulty of comparing across different experiments with divergent methods, we have summarised spontaneous hybridisation to show only where a study successfully produced or failed to produce hybrids. The only ‘experimental design’ factors we have included here are (1) where the female parent in the cross was male sterile, as absence of competition from conspecific pollen will greatly increase the likelihood of hybrid formation, (2) whether hybridisation was observed on naturally occurring populations, or in an experimental system, (3) and if (in an experimental system) plants were caged.

When reporting backcrosses and F_2 production, most papers referred to seed production, rather than plant production. For analysis, we created a derived variable, in which a backcross or F_2 cross was considered successful if plants were produced or (in the absence of information on plant production) if seeds were produced. If seeds or plants were not produced, the backcross was considered unsuccessful. This may overestimate the success of these later generations, but enables better coverage. As noted above, this analysis could be rerun in future with different criteria for production of backcrosses or F_2 generations. We included backcrossing to both crop and weed species.

Results

We databased 326 articles covering hybridisation in *Brassica* and allied genera. Results are presented below to show production of F_1 hybrids, and then BC_1 and F_2 formation, reflecting the steps that must occur for transfer of transgenes from a crop into a related species. Within the F_1 section we cover data from experimental hybridisation studies first, then spontaneous hybridisation, and finally hybridisation involving ‘in vitro’ techniques.

F_1 production

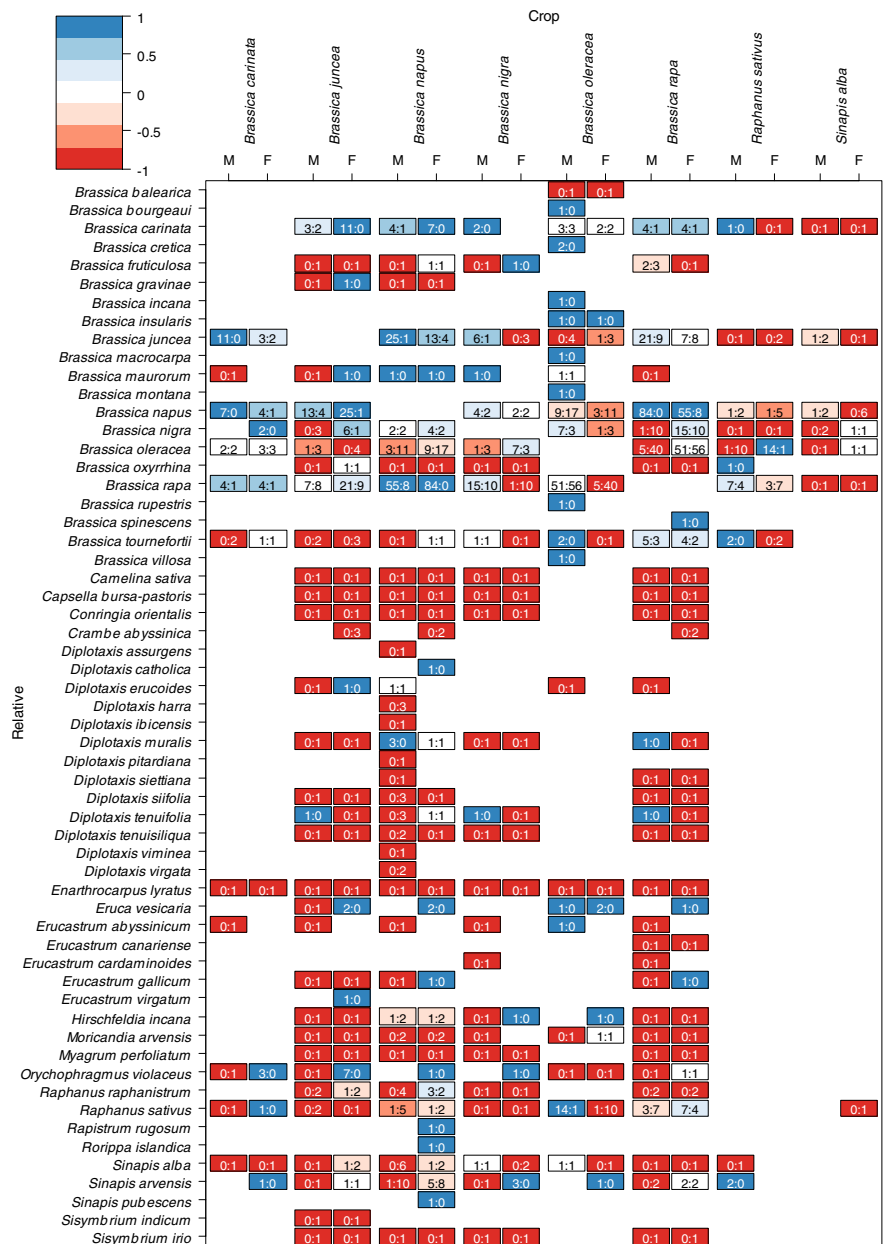
Production of hybrids within *Brassica* and allied genera has been attempted between many combinations of species (Fig. 1). The major crop species were well covered, with attempted crosses reported for 43 species with *B. napus*, 35 for *B. rapa*, 34 for *B. juncea*, 27 for *B. nigra* and 26 for *B. oleracea* (Table 1). However, *B. carinata*, *Raphanus sativus* and *Sinapis alba* have far fewer reported combinations (Table 1). Most reported combinations were unsuccessful and most crosses have only been reported once (Fig. 1). Where crosses were successful, rates of hybrid production were typically very low; the median number of hybrids per pollination for combinations with at least one report of successful hybridisation was only 0.007 hybrids per pollination (Fig. 2).

Brassica napus (canola, rapeseed)

Of the 43 species with reports of attempted hybridisation involving *B. napus*, 23 had at least one reported case of successful hybridisation (Fig. 1, Table 1). We found considerable variability in rates of hybrid production within most combinations (Fig. 3), both within and between studies.

Crosses between *B. napus* and *B. rapa* (a progenitor of *B. napus*) were very successful with a median rate of 2.29 hybrids/pollination when *B. napus* was the female parent in the cross (Fig. 2), but lower (0.44 hybrids/pollination) when *B. napus* was the male parent in the cross. In both directions, rates of production ranged widely across trials (Fig. 3). In contrast, crosses between *B. napus* and *B. oleracea* (*B. napus*’ other progenitor) were very difficult, especially where *B. napus* was the male parent in the

Fig. 1 Reported successes and failures of hybrid production between crops (along x-axis) and relatives in *Brassica* and allied genera (along y-axis). Crops are divided into cases where the crop was the female parent ('F') or male parent ('M') in the cross. Numbers in the cells indicate how many successful and unsuccessful trials have been reported (successes:failures). Cells are shaded to indicate the proportion of trials that were successful. Articles used are listed in the supplementary material



cross (Figs. 1, 3). Hybrids between *B. napus* and *B. oleracea* have been successfully produced using sexual hybridisation (U 1935; Mizushima 1950; Honma and Summers 1976; Chiang et al. 1977; Wahiduzzaman 1987), but success was found generally only in some combinations of genotypes used, or in one direction within a study, and most of the successful combinations in Chiang et al. (1977) involved tetraploid *B. oleracea* (*B. oleracea* is

normally diploid). Several studies have failed to produce hybrids between *B. napus* and *B. oleracea* without the use of in vitro techniques (Calder 1937; Takeshita et al. 1980; Ayotte et al. 1987; Kerlan et al. 1992).

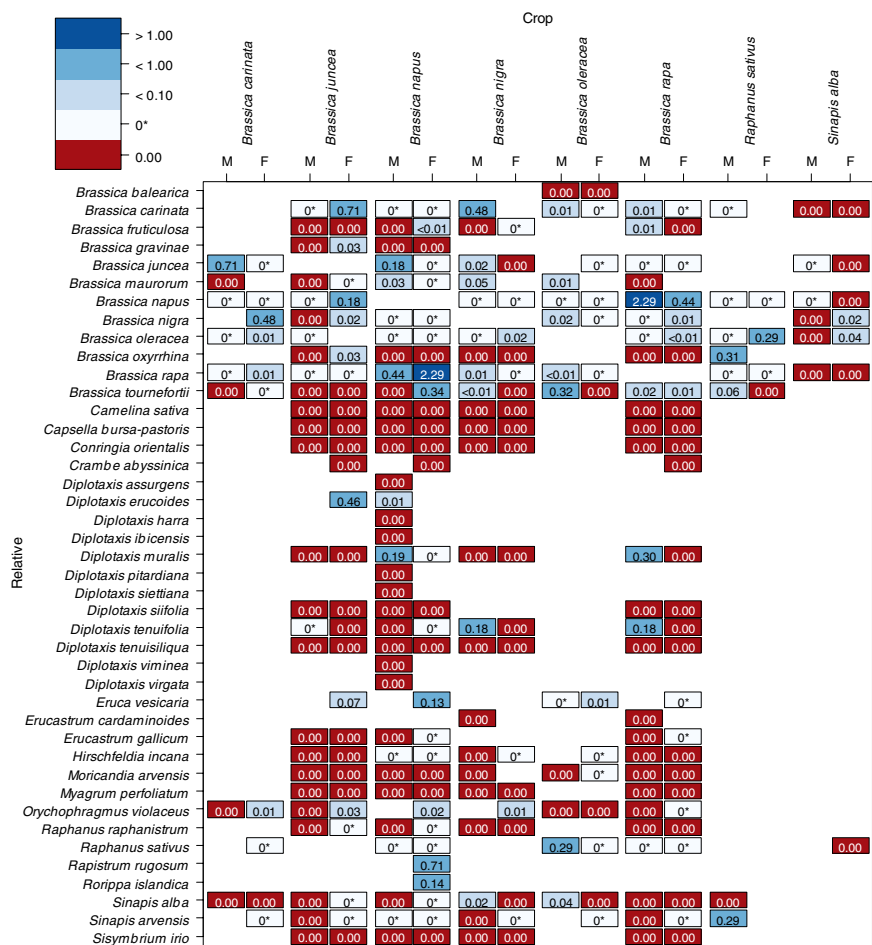
Many studies reported successful hybridisation between *B. napus* and *B. juncea* (Fig. 1), and Choudhary and Joshi (1999) reported a relatively high rate of hybrid production (0.18 hybrids/pollina-

Table 1 Summary of crossing results for crop species

| Crop species | F ₁ | | | | F ₂ | | | | BC ₁ | | | |
|--------------------------|----------------|----|----|----|----------------|---|---|----|-----------------|---|---|----|
| | A | S | N | T | A | S | N | T | A | S | N | T |
| <i>Brassica carinata</i> | 2 | 7 | 4 | 13 | 2 | 4 | 0 | 6 | 4 | 0 | 2 | 6 |
| <i>Brassica juncea</i> | 1 | 15 | 18 | 34 | 5 | 6 | 4 | 15 | 5 | 0 | 5 | 10 |
| <i>Brassica napus</i> | 7 | 16 | 20 | 43 | 10 | 8 | 3 | 21 | 13 | 5 | 5 | 23 |
| <i>Brassica nigra</i> | 3 | 10 | 14 | 27 | 3 | 3 | 4 | 10 | 3 | 1 | 6 | 10 |
| <i>Brassica oleracea</i> | 12 | 10 | 4 | 26 | 8 | 5 | 4 | 17 | 2 | 4 | 2 | 8 |
| <i>Brassica rapa</i> | 2 | 13 | 20 | 35 | 5 | 7 | 5 | 17 | 6 | 5 | 9 | 20 |
| <i>Raphanus sativus</i> | 4 | 5 | 3 | 12 | 1 | 1 | 3 | 5 | 3 | 1 | 0 | 4 |
| <i>Sinapis alba</i> | 0 | 4 | 3 | 7 | 0 | 1 | 1 | 2 | 1 | 0 | 1 | 2 |

For the F₁, F₂ and BC₁ generations, the numbers of species are listed where all reports of hybridisation were successful (A), some reports were successful (S) and where no reports were successful (N). ‘T’ is the total number of species with which hybridisation was attempted (A+S+N). Results presented are the combination of crosses with the crop as both maternal and paternal parent

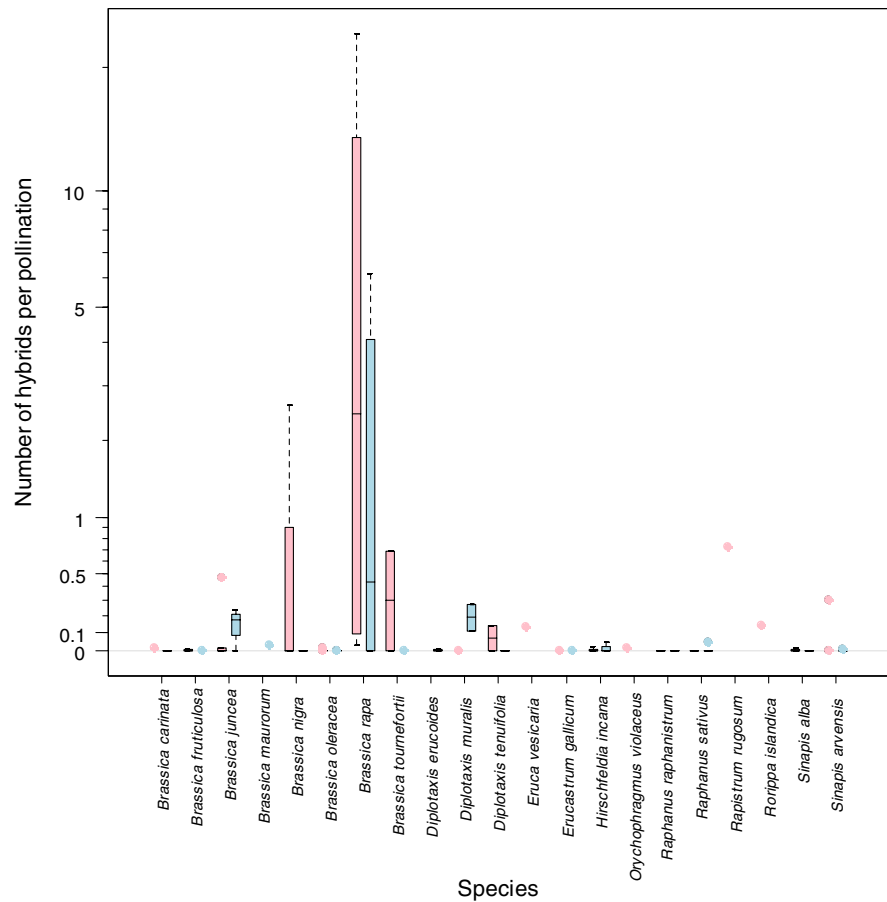
Fig. 2 Median rate of hybrid production (hybrids per pollination) for crosses between crops and wild species in *Brassica* and allied genera. Where a cell contains 0*, the median rate of hybrid production was zero, but at least one case of hybrid production was reported for the species combination. See Fig. 1 for further details



tion with *B. napus* as the female parent). Other studies reported high rates of seed production for this cross, but did not report quantitative rates of hybrid

production (Roy 1980; Wahiduzzaman 1987; Sharma and Singh 1992; Frello et al. 1995). Heyn (1977) reported a very high rate of hybrid formation for the

Fig. 3 Variation in rates of hybrid production (hybrids per pollination) in crosses involving *Brassica napus*. Within each species, pink boxes (on left) indicate crosses where *B. napus* was the maternal parent, blue boxes (on right) indicate crosses where *B. napus* was the paternal parent. Note that the y-axis is log transformed. Species with no quantitative reports of successful hybrid production were excluded from the plot (see Fig. 2)



cross *B. napus* × *B. nigra* (2.61 hybrids/pollination, although this was from only 18 pollinations). While we found no other quantitative rates of hybrid production in this cross, successful hybridisation was reported elsewhere (Mizushima 1950; Struss et al. 1991; Zhu and Struss 1991), although failed hybridisation has also been reported (Diederichsen and Sacristan 1988; Kerlan et al. 1992). Within *Brassica*, successful hybridisation has also been reported between *B. napus* and the following species: *B. carinata* (U 1935; Roy 1980; Wahiduzzaman 1987; Fernandez-Escobar et al. 1988; Chen and Heneen 1992; Rashid et al. 1994; Getinet et al. 1997), *B. fruticulosa* (Heyn 1977), *B. maurorum* (Bijral et al. 1995; Chrungu et al. 1999), and *B. tournefortii* (Heyn 1977). We found only unsuccessful reports of hybridisation between *B. napus* and both *B. gravinae* (Nanda Kumar et al. 1988b) and *B. oxyrrhina* (Salisbury 1991).

Many intergeneric crosses have been attempted with *B. napus*, and the majority of these were unsuccessful (Fig. 1). Successful hybridisation with *B. napus* has been reported for *Diplotaxis catholica* (Bijral and Sharma 1998), *D. erucoides* (Ringdahl et al. 1987), *D. muralis* (Fan et al. 1985; Ringdahl et al. 1987; Salisbury 1991; Bijral and Sharma 1996b), *D. tenuifolia* (Heyn 1977), *Eruca vesicaria* (Heyn 1977; Bijral and Sharma 1996a), *Erucastrum gallicum* (Lefol et al. 1997), *Hirschfeldia incana* (Lefol et al. 1996b), *Orychophragmus violaceus* (Cheng and Séguin-Swartz 2000), *Raphanus raphanistrum* (Kamala 1983; Lefol et al. 1997; Chèvre et al. 1998a), *Raphanus sativus* (Paulmann and Röbbelen 1988; Huang et al. 2002), *Rapistrum rugosum* (Heyn 1977), *Rorippa islandica* (Bijral and Sharma 1995), *Sinapis alba* (Heyn 1977), *S. arvensis* (Mizushima 1950; Inomata 1988; Moyes et al. 1999, 2002), and *S. pubescens* (Inomata 1994).

Brassica rapa (rapeseed, turnip, Chinese cabbage)

Brassica rapa is the next most comprehensively studied crop, with 35 combinations tested. As with *B. napus*, relatively few studies report success of hybrid production quantitatively (Fig. 2). Aside from the cross between *B. rapa* and *B. napus* (see above), most effort has focused on crosses between *B. rapa* and *B. oleracea* or *B. juncea* (Fig. 1). Crosses with *B. oleracea* had very low rates of success (Fig. 2), but were more successful where *B. rapa* was the female parent in the cross (Fig. 1). Crosses with *B. juncea* (of which *B. rapa* is a progenitor) were often successful, especially where *B. rapa* was the male parent (Fig. 1). Other crosses within *Brassica* were generally successful, with reported successes between *B. rapa* and the following species: *B. carinata* (Howard 1942; Mizushima 1950; Struss et al. 1991; Meng et al. 1998; Choudhary et al. 2000a; Rahman 2001), *B. fruticulosa* (Nanda Kumar et al. 1988a, b), *B. nigra* (Ramanujam and Srinivasachar 1943; Mizushima 1950; Olsson 1960; Prakash 1973a, b; Bing et al. 1996a), *B. spinescens* (Inomata 2000), and *B. tournefortii* (Sikka 1940; Prakash and Narain 1971; Narain and Prakash 1972; Choudhary and Joshi 2001). Hybridisation failed in all reported attempts only for crosses between *B. rapa* and *B. maurorum* (Chrungu et al. 1999) and *B. oxyrrhina* (Salisbury 1991).

While intergeneric hybridisation involving *B. rapa* was attempted with many species (Fig. 1), it was only successful with *Diplotaxis muralis* (Salisbury 1991), *D. tenuifolia* (Salisbury 1991), *Eruca vesicaria* (Mizushima 1950), *Erucastrum gallicum* (Lefol et al. 1997), *Orychophragmus violaceus* (Li and Heneen 1999), *Raphanus sativus* (Morris 1936; Richharia 1937; Mizushima 1950; Ellerström 1978; Namai 1980; Luo et al. 2000; Matsuzawa et al. 2000) and *Sinapis arvensis* (Mizushima 1950). Hybridisation failed for 18 other intergeneric combinations (Fig. 1).

Brassica juncea (Indian mustard)

Attempts at hybridisation with *B. juncea* have been reported for 34 species, though with typically fewer attempts per combination than *B. rapa* (Fig. 1). Successful hybridisation was reported between *B. juncea* and both its progenitors, *B. rapa* (see above), and

B. nigra (Fig. 1). However, successful hybridisation with *B. nigra* has only been reported where *B. juncea* was the female parent in the cross (Morinaga 1931; Ramanujam and Srinivasachar 1943; Mizushima 1950; Olsson 1960; Anand et al. 1985); crosses where *B. juncea* was male have failed (Ramanujam and Srinivasachar 1943; Olsson 1960; Ghosh Dastidar and Varma 1999). Crosses between *B. juncea* and *B. carinata* were successful (Fig. 1; U 1935; Rahman 1976, 1978; Anand et al. 1985; Katiyar and Gupta 1987; Getinet et al. 1994, 1997; Subudhi and Raut 1994b; Katiyar and Chamola 1995), with a relatively high rate of hybrid formation where *B. juncea* was the female in the cross (0.71 hybrids/pollination; U 1935; Fig. 2). Only one study reported successful hybridisation between *B. juncea* and *B. oleracea* (Fig. 1, Struss et al. 1991). Crosses were also possible between *B. juncea* and *B. gravinae* (Nanda Kumar et al. 1988b), *B. maurorum* (Bijral et al. 1995) and *B. oxyrrhina* (Bijral and Sharma 1999b). Crosses failed with *B. fruticulosa* (Salisbury 1991) and *B. tournefortii* (Salisbury 1991; Goyal et al. 1997; Ghosh Dastidar and Varma 1999).

Of intergeneric crosses, successful hybridisation has been reported between *B. juncea* and *Diplotaxis eruroides* (Inomata 1998), *D. tenuifolia* (Salisbury 1991), *Eruca vesicaria* (Bijral and Sharma 1999a; Goswami and Devi 2002), *Erucastrum virgatum* (Inomata 2001), *Orychophragmus violaceus* (Li et al. 1998), *Raphanus raphanistrum* (Kamala 1983), *Sinapis alba* (Bijral et al. 1991) and *S. arvensis* (Mizushima 1950). Hybridisation in the above crosses was often possible only in one direction (usually with *B. juncea* as female), and was often not successful across all studies (e.g. the cross *B. juncea* × *B. oxyrrhina* failed in Salisbury 1991, but succeeded in Bijral and Sharma 1999b). No successful hybridisation was reported for 16 other attempted intergeneric combinations (Fig. 1).

Brassica oleracea (cabbage, kale)

Aside from extensive trials with *B. napus* and *B. rapa* (see above), the number of trials involving *B. oleracea* was usually low (Fig. 1). Hybrids have been reported with *B. carinata*, of which *B. oleracea* is a progenitor (Morinaga 1933; U 1935; Mizushima 1950; Barcikowska et al. 1983; Rahman 2001), although rates of hybrid production in this cross were low

(Fig. 2). Ljungberg et al. (1993) reported high rates of hybrid production in the cross *B. tournefortii* × *B. oleracea* (0.63 hybrids/pollination), while Narain and Prakash (1972) reported a much lower rate (0.003 hybrids/pollination). Successful hybridisation has been reported between *B. oleracea* and several members of the *B. oleracea* cytodeme (Warwick et al. 2000): *B. bourgeauii*, *B. cretica*, *B. incana*, *B. insularis*, *B. macrocarpa*, *B. montana*, *B. rupestris*, and *B. villosa* (von Bothmer et al. 1995). Outside of the cytodeme and the crop species discussed above (*B. juncea*, *B. napus* and *B. rapa*), successful hybridisation between *B. oleracea* and other species within *Brassica* has only been reported for *B. maurorum* (Chrungu et al. 1999). Crosses with *B. balearica* were unsuccessful (Snogerup and Persson 1983) and *B. nigra* (Frandsen 1947; Mizushima 1950; Pearson 1972; Prakash et al. 1984; Sarla et al. 1987; Sarla and Raut 1988, 1991).

In comparison with *B. napus*, *B. rapa* and *B. juncea*, relatively few attempts of intergeneric hybridisation involving *B. oleracea* have been reported (Fig. 1), accounting for the relatively few failed reports of hybridisation for *B. oleracea* (Table 1). Successful intergeneric hybridisation has been reported for crosses between *B. oleracea* and the following species: *Eruca vesicaria* (U et al. 1937; Eenink 1974), *Erucastrum abyssinicum* (Sarmah and Sarla 1998), *Hirschfeldia incana* (Quiros et al. 1988), *Moricandia arvensis* (Apel et al. 1984), *Raphanus sativus* (Karpechenko 1924; Richharia 1937; McNaughton 1973; Ellerström and Zagorcheva 1977; Opena and Lo 1978; Ellerström 1978; McCollum 1979; Sarashima and Matsuzawa 1979; Namai 1980), *Sinapis alba* (U et al. 1937) and *S. arvensis* (Mizushima 1950). Evidence for unilateral incompatibility was observed in the cross between *B. oleracea* and *Raphanus sativus*, with only one report of success where *B. oleracea* was the female parent (Opena and Lo 1978), but 13 reports of success where *B. oleracea* was the male parent (Fig. 1).

Brassica nigra (black mustard)

Hybridisation has been reported between *B. nigra* and 27 species, although most combinations were represented by a single trial (Fig. 1). Aside from combinations discussed above (with *B. juncea*, *B. napus*,

B. oleracea and *B. rapa*) hybrids have been successfully produced between *B. nigra* and *B. carinata* (of which *B. nigra* is a progenitor, Fig. 1), with high rates of hybrid formation (U 1935; Figs. 1, 2, also Mizushima 1950), although we found no reports of this cross with *B. nigra* as the female parent (Fig. 1). Within *Brassica*, hybrids have also been reported between *B. nigra* and *B. fruticulosa* (Salisbury 1991), *B. maurorum* (Chrungu et al. 1999) and *B. tournefortii* (Narain and Prakash 1972). Successful intergeneric crosses were reported between *B. nigra* and the following: *Diplotaxis tenuifolia* (Salisbury 1991), *Hirschfeldia incana* (Salisbury 1991), *Orychophragmus violaceus* (Li and Heneen 1999), *Sinapis alba* (Choudhary and Joshi 2000) and *S. arvensis* (Mizushima 1950; Banga and Labana 1991; Salisbury 1991). Attempts with 14 other species failed (Fig. 1).

Brassica carinata (Ethiopian mustard)

Brassica carinata is the least studied of all *Brassica* crops, with reported hybridisation attempts for only 13 species combinations. Aside from combinations discussed above (with *B. juncea*, *B. napus*, *B. nigra*, *B. oleracea*, *B. rapa*), successful hybridisation with *B. carinata* was reported for *B. tournefortii* (Joshi and Choudhary 1999), *Orychophragmus violaceus* (Li et al. 1998), *Raphanus sativus* (Richharia 1937) and *Sinapis arvensis* (Mizushima 1950). Attempts with four other species failed (Fig. 1): *Brassica maurorum* (Chrungu et al. 1999), *Enarthrocarpus lyratus* (Gundimeda et al. 1992), *Erucastrum abyssinicum* (Rao et al. 1996) and *Sinapis alba* (Sridevi and Sarla 1996).

Raphanus sativus (radish) and *Sinapis alba* (white mustard)

Very few combinations involving *Sinapis alba* or *Raphanus sativus* have been reported, and most of these involve the *Brassica* crops discussed above (Fig. 1). Of non-crop species, successful hybridisation has been reported between *R. sativus* and *B. oxyrrhina* (Bang et al. 1997), *B. tournefortii* (Choudhary et al. 2000b) and *S. arvensis* (Mizushima 1950; Bang et al. 1996), both with *R. sativus* as male. Successful hybridisation involving *R. sativus* has also been reported with *Eruca vesicaria* (Dayal 1987) and

with *Raphanus raphanistrum* (Eber et al. 1994), but the polarity of these crosses was not clear. Hybridisation between *R. sativus* and *S. alba* was unsuccessful (Bang et al. 1996). We found no reported cases of attempted hybridisation between *S. alba* and any non-crop species (Fig. 1).

Spontaneous hybridisation

Brassica napus

Compared with experimental hybridisation, far fewer studies have examined the occurrence of spontaneous hybridisation between *B. napus* and related species (Table 2). Most attempts involved *B. rapa*, with which hybrids were readily obtained through spontaneous hybridisation, as well as observed in naturally occurring populations. Chèvre et al. (1998b) reported spontaneous hybridisation between *B. napus* and *B. oleracea*, but provided few details about this experiment. Successful spontaneous hybridisation involving *B. napus* was also reported with *B. juncea*, *Hirschfeldia incana*, *Raphanus raphanistrum*, and *Sinapis arvensis*, while only unsuccessful spontaneous hybridisation was reported with *B. nigra*, *Erucastrum gallicum*, *R. sativus* and *S. alba* (Table 2). Note that spontaneous hybridisation between *B. napus* and these species was possible even though in some cases rates of experimental hybridisation were very low (Figs. 1, 2).

Male-sterile *B. napus* was commonly used in spontaneous hybridisation experiments (Table 2). Spontaneous hybrids between *B. napus* (female) and *Hirschfeldia incana* have only been reported using male-sterile *B. napus*, but the reciprocal cross has produced hybrids with male-fertile *H. incana* as the maternal parent. We found no reported attempts in this cross with male-fertile *B. napus* as female. Crosses between *S. arvensis* and *B. napus* have only been successful when using male-sterile *B. napus* as female (Chèvre et al. 1996; Lefol et al. 1996a), failing where male-fertile *B. napus* was used (Bing et al. 1996b), or where *S. arvensis* was the female parent (Bing et al. 1996b; A. Brown et al. 1996; Lefol et al. 1996a; Moyes et al. 2002). Recently, however, a study has reported a hybrid between these species found after a field trial of male-fertile *B. napus* (Daniels et al. 2005), but it is not clear if *B. napus* or *S. arvensis* was its female parent.

Other crop species

We found few reports of spontaneous hybridisation not involving *B. napus*. Bing et al. (1996b) reported unsuccessful spontaneous hybridisation in the combinations *B. juncea* × *B. rapa*, *B. nigra* × *B. juncea*, *B. nigra* × *B. rapa* and *S. arvensis* × *B. juncea*, while Chèvre et al. (1998b) reported successful spontaneous hybridisation in the combinations *R. sativus* × *R. raphanistrum* and *R. sativus* × *S. arvensis* (using male-sterile *R. sativus*), and *B. oleracea* × *B. nigra*.

In vitro hybridisation

Plant breeders have increasingly used techniques such as ovule culture and embryo rescue to increase the likelihood and frequency of hybrid production in very wide crosses, and this has increased the pool of species available for hybridisation with crop species (Sharma et al. 1996). Ignoring cross polarity, 100 distinct species combinations were attempted using in vitro techniques, of which 93 were successful in at least one case. Of the 93 successful combinations, 81 were also attempted using just experimental hybridisation; 34 of these were not possible without the use of in vitro techniques, while 47 were successful with experimental hybridisation alone. While success using in vitro techniques does not indicate that a pair of species will cross under natural conditions, failure to cross even with such assistance may give some indication about which species will not cross. We found seven cases where all attempts at hybridisation using in vitro techniques failed (Fig. 4). However, of these cases, four were possible using experimental hybridisation (*B. napus* × *D. tenuifolia*, *B. napus* × *Sinapis pubescens*, *S. alba* × *B. nigra* and *B. oleracea* × *S. arvensis*). This indicates that failure to produce hybrids through in vitro techniques does not always provide proof of incompatibility. Of the 93 combinations that successfully produced hybrids using in vitro techniques, we found no data for experimental crossing for 12 combinations (Fig. 5); for these combinations it is not possible to infer whether they are able to cross under natural conditions.

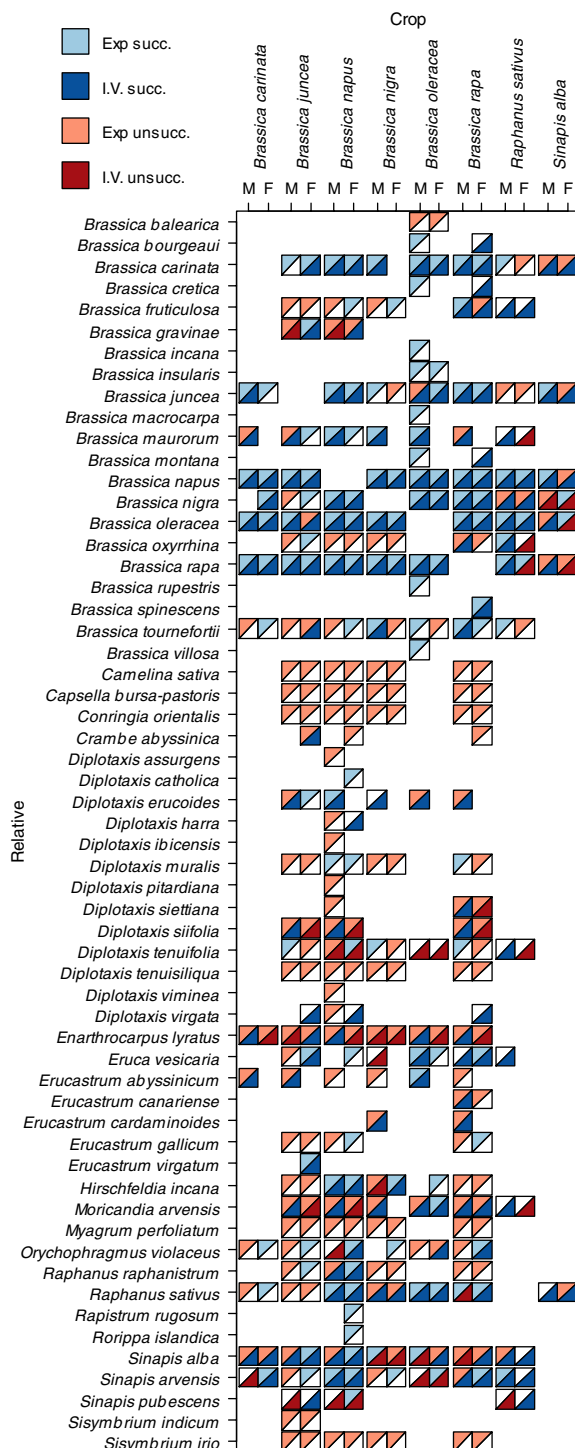
BC₁ and F₂ production

Backcross and F₂ data were less frequently reported than F₁ data, and for most species combinations only

Table 2 Results of spontaneous and natural hybridisation experiments involving *Brassica napus* (hybridisation without manual pollination)

| Polarity | Relative | Successful | References |
|------------------------|------------------------------|------------|---|
| <i>B. napus</i> female | | | |
| | <i>Brassica juncea</i> | True | Bing et al. (1996b) and Jørgensen et al. (1998) |
| | <i>Brassica nigra</i> | False | Bing et al. (1996b) |
| | <i>Brassica oleracea</i> | False | Wilkinson et al. (2000) ^b |
| | <i>Brassica rapa</i> | True | Palmer (1962), Jørgensen and Andersen (1994), Bing et al. (1996b), Mikkelsen et al. (1996), Jørgensen et al. (1998), and Wilkinson et al. (2000) ^b |
| | | False | Calder (1937) |
| | <i>Hirschfeldia incana</i> | True | Lefol et al. (1991) ^a , Eber et al. (1994) ^a , Chèvre et al. (1996) ^a , Lefol et al. (1996b) ^a |
| | <i>Raphanus raphanistrum</i> | True | Eber et al. (1994) ^a , Baranger et al. (1995) ^a , Chèvre et al. (1996) ^a , Darmency et al. (1998) ^{a,c} , Chèvre et al. (2000), and Rieger et al. (2001) |
| | | False | Darmency et al. (1998) ^a |
| | <i>Sinapis arvensis</i> | True | Chèvre et al. (1996) ^a and Lefol et al. (1996a) ^{a,c} |
| | | False | Bing et al. (1996b) |
| <i>B. napus</i> male | | | |
| | <i>Brassica juncea</i> | True | Frello et al. (1995), Bing et al. (1996b), and Jørgensen et al. (1998) |
| | <i>Brassica nigra</i> | False | Leckie et al. (1993) ^c , Bing et al. (1996b), A. Brown et al. (1996), and Daniels et al. (2005) |
| | <i>Brassica oleracea</i> | True | Chèvre et al. (1998b) |
| | <i>Brassica rapa</i> | True | Calder (1937), Palmer (1962), Mackay (1973) ^d , Leckie et al. (1993) ^c , Jørgensen and Andersen (1994), Jørgensen and Andersen (1994) ^b , Landbo et al. (1996) ^b , Bing et al. (1996b), A. Brown et al. (1996), Mikkelsen et al. (1996), Jørgensen et al. (1998), Scott and Wilkinson (1998) ^b , Jenkins et al. (2001), Halfhill et al. (2002), Pertl et al. (2002), Warwick et al. (2003), Warwick et al. (2003) ^b , Halfhill et al. (2004), and Daniels et al. (2005) |
| | <i>Erucastrum gallicum</i> | False | Warwick et al. (2003) ^b |
| | <i>Hirschfeldia incana</i> | True | Lefol et al. (1996b) and Darmency and Fleury (2000) |
| | | False | Lefol et al. (1996b) ^c |
| | <i>Raphanus raphanistrum</i> | True | Darmency et al. (1998), Chèvre et al. (2000), Rieger et al. 2001, and Warwick et al. (2003) |
| | | False | Darmency et al. (1998) ^c , Thalmann et al. (2001) ^b , Warwick et al. (2003) ^b , Halfhill et al. (2004), and Daniels et al. (2005) |
| | <i>Raphanus sativus</i> | False | Daniels et al. (2005) |
| | <i>Sinapis alba</i> | False | Daniels et al. (2005) |
| | <i>Sinapis arvensis</i> | True | Leckie et al. (1993) ^c |
| | | False | Lefol et al. (1996a), Bing et al. (1996b), A. Brown et al. (1996), Moyes et al. (2002), Warwick et al. (2003) ^b , and Daniels et al. (2005) |
| Polarity unknown | | | |
| | <i>Brassica rapa</i> | True | Hansen et al. (2001) ^b , and Wilkinson et al. (2003a) ^b |
| | <i>Sinapis arvensis</i> | True | Daniels et al. (2005) |

^a Male-sterile *B. napus* used as the female parent^b Naturally occurring hybridisation (see text)^c Plants caged^d Individual pairs of plants caged



a single trial was reported (Figs. 5, 6). These data are most relevant where F_1 production was possible, since only these combinations may lead to the escape of transgenes. Of the 95 species combinations in

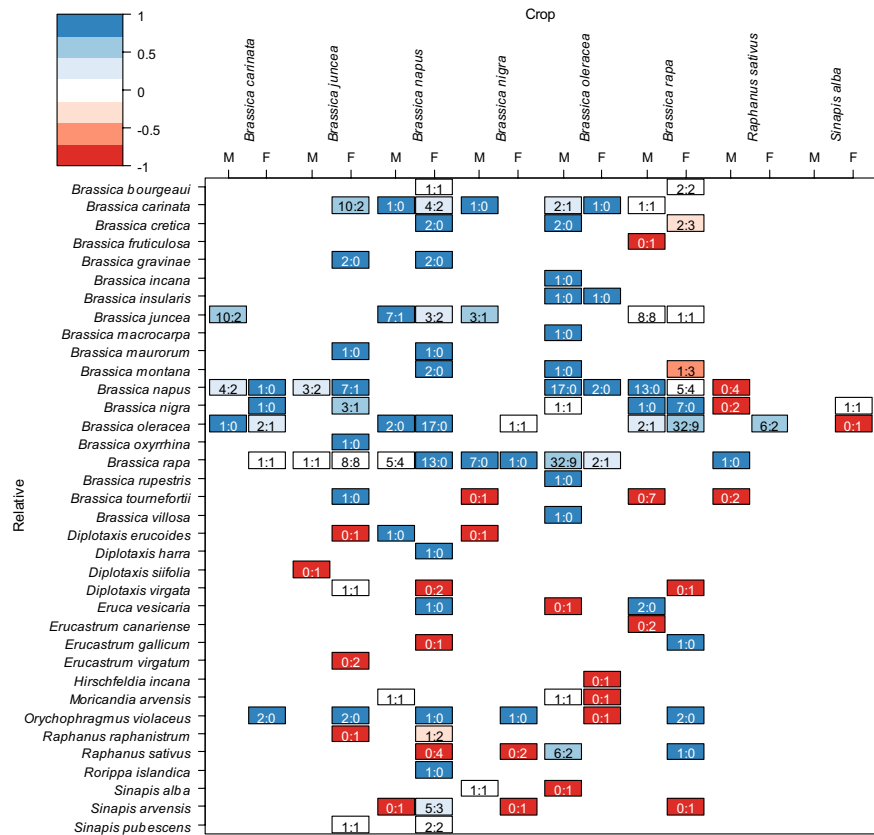
Fig. 4 Result of crosses using in vitro (e.g. embryo rescue) techniques, compared to experimental hybridisation alone for crops in *Brassica* and allied genera. The top-left (light-shaded) triangle displays results from experimental hybridisation, and the bottom-right (dark-shaded) triangle displays results from in vitro hybridisation. Where any success was reported in hybridisation, triangles are coloured blue, where all trials were unsuccessful triangles are coloured red, and where no data were available, triangles are not coloured

which successful F_1 production was reported (independent of cross polarity), we found reports of attempted backcrosses for 45 (47%), F_2 production for 58 (61%) and either of these for 73 (77%). We found additional reports of attempted backcross and F_2 production for species combinations with no reports of successful experimental hybridisation.

A hybrid may backcross to its maternal or paternal parent. Since the success of the F_1 cross varies depending on the polarity (i.e. which species is female), similar variation might be expected for backcrosses (i.e. which of its parents a hybrid is crossed with). This is important because most breeding programmes use the crop species as the recurrent parent in backcrosses, but for assessment of the likelihood of transgene escape from transgenic crops, backcrossing to the wild species is required for introgression of the transgene into the wild species. However, we identified just one case where backcrossing was reported as possible to one parent but not the other; in the cross *R. sativus* \times *B. oleracea*, Ellerström and Zagorcheva (1977) produced seeds backcrossing to *B. oleracea*, while backcrossing to either parent was unsuccessful when attempted by Karpechenko (1924). We found 8 combinations where backcrossing to both parents failed in all cases, 18 combinations where backcrossing to both parents was successful at least once, and 40 combinations where only one parent was tested. For the few combinations where quantitative data for seed production was presented, large differences were observed in the rate of backcross production between these different backcross combinations (e.g. Lefol et al. 1997).

Backcross success varied depending on whether the hybrid was the maternal or paternal parent in the cross, and this might be expected given the generally low male fertility of F_1 hybrids. For example, failure to produce backcrosses using the F_1 hybrid as pollen parent but success using the F_1 hybrid as the female parent has been observed for *B. nigra* \times *Raphanus sativus* (Matsuzawa and Sarashima 1986) and

Fig. 5 Reported backcross (BC₁) production for hybrids between species in *Brassica* and allied genera. See Fig. 1 for further details



B. napus × *Raphanus raphanistrum* (Lefol et al. 1997). In addition, the polarity of the initial cross may affect backcrossing success; e.g. in the cross between *Eruca vesicaria* and *B. rapa*, backcrosses succeeded where *E. vesicaria* was the female in the initial cross (Agnihotri et al. 1990), but failed where *B. rapa* was the initial female (Roberts et al. 1999). However, in most cases where backcrossing was successful using one polarity of the initial cross, it was possible in the alternate direction (Fig. 5).

Brassica napus

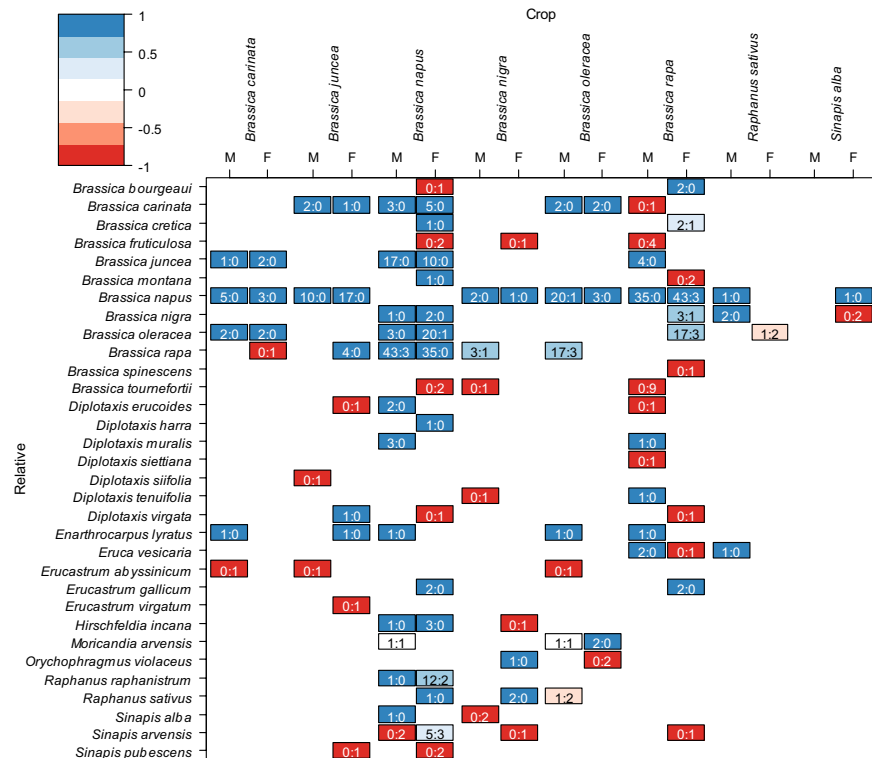
Successful backcrosses and F₂ production from hybrids involving *B. napus* have been reported for 18 species (Table 1, Figs. 5, 6). These include its progenitors (*B. rapa* and *B. oleracea*), as well as the other crop *Brassica* species (*B. nigra*, *B. carinata*, *B. juncea*), although we found no reports of attempted F₂ production between *B. napus* and *B. nigra* (Fig. 6). Both backcrossing and F₂ production were possible from many intergeneric crosses (Fig. 6). Of the

combinations where F₁ production was successful, we found no reports of backcross attempts with *B. maurorum*, *Diplotaxis catholica*, *D. tenuifolia*, *Eruca vesicaria*, *Orychophragmus violaceus*, *Rapistrum rugosum* or *Rorippa islandica*, and no reports of attempted F₂ production with *B. fruticulosa*, *B. nigra*, *B. tournefortii*, *D. catholica*, *D. muralis*, *D. tenuifolia*, *Hirschfeldia incana*, *Rapistrum rugosum* or *Sinapis alba*. Backcrossing failed in all attempts with *B. fruticulosa*, *B. tournefortii*, *D. virgata* and *S. pubescens*, and F₂ production failed with *D. virgata*, *Erucastrum gallicum* and *Raphanus sativus* (see supplementary material).

Brassica rapa

Successful backcrosses have been reported between *B. rapa* and 11 species, and F₂ production with 12 (Table 1, Figs. 5, 6). Of the species that formed F₁ hybrids with *B. rapa*, we found no reports of backcross attempts for *Orychophragmus violaceus* or *Raphanus sativus* (Fig. 5), and no reports of

Fig. 6 Reported F₂ production for hybrids between species in *Brassica* and allied genera. See Fig. 1 for further details



attempted F₂ production for *B. spinescens*, *D. muralis* or *D. tenuifolia* (Fig. 6). Backcrossing and F₂ production were successful with most attempted species in *Brassica*, and several species of other genera (Fig. 5).

Brassica juncea

We found relatively few reports of backcrossing involving *B. juncea* (Fig. 5). Of species that formed F₁ hybrids with *B. juncea*, we found no reports of backcross attempts with *B. gravinae*, *B. maurorum*, *B. nigra*, *B. oleracea*, *B. oxyrrhina*, *Diplotaxis tenuifolia*, *Eruca vesicaria*, *Orychophragmus violaceus*, *Raphanus raphanistrum*, *Sinapis alba* or *S. arvensis*, and no reports of attempted F₂ production with *B. oleracea*, *D. tenuifolia*, *E. vesicaria*, *S. alba* or *S. arvensis*.

Brassica oleracea

Similar to *B. juncea*, we found many more reports of attempted F₂ production involving *B. oleracea* than BC₁ production (Table 1), although most of the F₂ attempts involved species from the *B. oleracea*

cytodeme (Fig. 6; von Bothmer et al. 1995). Of species that formed F₁ hybrids with *B. oleracea*, we found no reports of backcross attempts with *B. bourgeauii*, *B. cretica*, *B. incana*, *B. insularis*, *B. juncea*, *B. macrocarpa*, *B. maurorum*, *B. montana*, *B. nigra*, *B. rupestris*, *B. tournefortii*, *B. villosa*, *Eruca vesicaria*, *Hirschfeldia incana* or *Sinapis arvensis*, and no reports of attempted F₂ production with *B. bourgeauii*, *B. juncea*, *B. maurorum*, *B. tournefortii*, *Erucastrum abyssinicum* or *Sinapis arvensis*.

Other crops

We found few reports of backcross or F₂ production for the other crop species (*B. carinata*, *B. nigra*, *Raphanus sativus* and *Sinapis alba*: Table 1). For the *Brassica* crops, BC₁ and F₂ production was generally successful for crosses within *Brassica*.

Discussion

As noted by Scheffler and Dale (1994), it is not possible to make simple statements about the

reproductive compatibility and incompatibility of *Brassica* crops. Reproductive compatibility relationships are complicated within this group, with partial reproductive barriers between many pairs of species. However, by bringing the available data together, it is possible to examine the extent and limits of the evidence for or against reproductive compatibility as a component of risk assessment. New hybridisation data are continually being reported for this group, and therefore no review is ever complete. We have brought together new results and combinations published since previous reviews (Scheffler and Dale 1994; Warwick et al. 2000). Here, we discuss issues surrounding the interpretation of our data, the approach we have used, and how this approach may be useful for risk assessment for transgenic crops.

Information gaps

By identifying species combinations that are known to be reproductively compatible and also those that are incompatible, research, risk assessment and containment strategies can be designed that minimise both cost and potential negative consequences. Despite decades of research on hybridisation in *Brassica* and related genera, the level of reproductive compatibility between many species combinations remains untested (Figs. 1, 2, 4). It is very likely that many of the ‘untested combinations’ have been attempted but failed, and were therefore not reported due to a tendency not to publish negative results. Highlighting such gaps is an important component of risk assessment. While decisions need to be made with the best available evidence (Snow et al. 2005), species combinations for which there is no available information must still be taken into account when performing risk assessments.

Because experimental hybridisation studies are designed to optimise the likelihood of successful hybridisation (e.g. through use of bud pollination), they may create a bias towards positive reports of hybridisation between species that may be unlikely to spontaneously cross. Most reports of experimental hybridisation derive from the breeding literature rather than the risk-assessment literature, and so are primarily concerned with introgressing useful characters from wild species to crop species. For risk assessors to have the right information, more research needs to be directed towards assessing how crop

genes may introgress into wild populations, and over the last 15 years a substantial literature has built up examining this, particularly for *B. napus*.

Where cross-compatibility information is lacking, inferences can be made about the likelihood of hybridisation between species lacking experimental hybridisation data by incorporating other sources of information. Using information on hybridisation by means of *in vitro* methods (e.g. embryo rescue), we were able to identify an additional three species combinations that are likely to be reproductively incompatible. However, some combinations that could not hybridise using *in vitro* methods have been reported to hybridise using experimental hybridisation alone, so caution must be exercised when interpreting these data.

Interpretation issues

Successful experimental hybridisation does not necessarily imply that a pair of species will hybridise under natural conditions; simply that they are reproductively compatible to some extent. Clearly, the ease with which a crop and its wild relatives can be hybridised through manual cross-pollination reveals little about the potential influence of pre-pollination and other ecological barriers (reviewed in detail elsewhere; e.g. Grant 1994; Arnold 1997). Within species, different genotypes may differ in their propensity to cross with another species; for this reason, experimental hybridisation studies for plant breeding often incorporate diverse genotypes. This means that even if one genotype may cross with a wild relative, others within the same species may not. Potential for hybridisation may also vary across different geographic regions; for example, *R. sativus* (radish) and *R. raphanistrum* (jointed charlock) do not hybridise in parts of Europe (e.g. the Netherlands), but hybridise extensively in California (Ellstrand 2003). However, data on the degree of intrinsic reproductive compatibility between species is useful precisely because it is independent of the ecological context in which the species occur. If two species are known to be reproductively incompatible, then spontaneous hybridisation between them cannot happen anywhere. Conversely, if species can be freely hybridised in the glasshouse, research should be directed at determining the extent to which pre-pollination barriers control rates of hybridisation in

the field. Importantly, spontaneous hybridisation has been observed in several combinations of *Brassica* (Table 2), even where rates of hybridisation were low in experimental crosses. This illustrates that species combinations where experimental hybridisation is possible should be treated differently to those for which hybridisation with a wild relative is known to be impossible, even where rates of hybrid formation are low.

Problems of interpretation also result from the large variation in sampling effort among studies we surveyed. For example, the number of pollinations attempted varied from just a single flower (e.g. Snogerup and Persson (1983) in *B. balearica* × *B. oleracea*, which failed to produce a hybrid), to tens of thousands of flowers (e.g. Raut and Kaul (1982) made 65,206 pollinations in the cross *B. rapa* × *B. oleracea* to produce just two hybrids). Furthermore, some studies did not report the number of attempted pollinations, making meaningful comparisons among studies difficult. Lastly, most combinations were represented by only a single trial (Fig. 1), and therefore caution should be exercised if concluding that a pair of species cannot hybridise based on this, especially given large variability in hybrid formation rates between trials (Fig. 3).

To incorporate a transgene in the genome of a wild species, at least one successful backcrossing event between the hybrid and wild species must occur. A hybrid may backcross to either of its progenitor parents, and be either the maternal or paternal parent in such a cross. Clearly, transfer of the transgene can only happen where backcrossing is with the wild species. Since polarity can affect the outcome of an initial F_1 cross, the particular backcross combination performed may also influence success. Despite this concern, where backcrossing was reported as being possible in one combination, it was generally possible in the other, including across different polarities of the initial cross. However, the different rates of backcross production with different combinations (e.g. Lefol et al. 1997) may have important consequences on the likelihood and rate of backcrossing (and therefore introgression) under natural conditions.

Reuse of data

Our approach to collecting and displaying information on hybridisation represents an advance for risk

assessment of transgenic crops. By storing data in a database, we have separated content from presentation, allowing multiple views of the data to be presented. Since it is not practical to present all collected information in this paper, we plan to deliver a more comprehensive set of information in a web application. Hybridisation information naturally lends itself to a system of linked graphs, allowing transition from a broad overview (e.g. Fig. 1) through more detailed summaries (e.g. Fig. 3) to the actual data itself (not shown). A prototype application is available at <http://biosafety.landcareresearch.co.nz>, which will be developed if it proves useful for the risk assessment and/or plant breeding community. As more data on hybridisation in *Brassica* are published, we will add these to our database, meaning future analysis will be much more rapid, and that work collecting studies here and in previous reviews need not be repeated as the body of literature increases. Our approach is easily applicable to other crop groups, and we have applied it to *Allium* crops (in prep.), and several other minor crop groups.

Conclusions

As the number of countries growing transgenic crops increases, along with the area planted in these crops, the potential for gene flow via hybridisation will need to be considered for an increasing number of sympatric crop–wild species combinations. Data on reproductive compatibility is applicable across geographic regions where a particular combination of crop and relative occur. In contrast, because spontaneous hybridisation is influenced by pre-pollination barriers it is more context dependent and would be expected to vary from one geographic region to another. In general, collecting data on the level of reproductive compatibility is more straightforward than collecting data on spontaneous rates of hybridisation, and as such reproductive compatibility data represent an early ‘tier’ of risk assessment (Wilkinson et al. 2003b; Raybould and Wilkinson 2005). The more complicated assessment of rates of spontaneous hybridisation forms a later tier of assessment and needs to be done only for reproductively compatible species. Our approach makes the level of information for each species combination clear and therefore allows identification of reproductively incompatible

species that need not be subjected to later tiers of analysis, as well as identification of species combinations where spontaneous hybridisation is particularly likely. However, occurrence of spontaneous hybridisation between species that are only marginally compatible highlights the importance of considering all sympatric compatible species. Future research is required to determine how rates of experimental hybridisation translate into rates of spontaneous or natural hybridisation in different regions, and identification of specific factors that control this. This would reduce the need for repeating experiments that have been carried out with *B. napus* for other crops where rates of experimental hybridisation are well known.

While we have concentrated entirely on the possibility of interspecific hybridisation, intraspecific gene flow is a more likely route for the escape of transgenes from genetically modified crops. Intraspecific gene flow may occur between conspecific crops, or between a crop and a conspecific wild form (Ellstrand 2003; Newstrom et al. 2003). When a transgenic crop is grown in close proximity to these potential targets, gene flow will typically be governed by pre-pollination barriers alone. However, intraspecific gene flow can be predicted more easily than interspecific gene flow; a priori, we would expect few reproductive barriers to exist between forms of the same species. In contrast, between species there exist a wide range of reproductive compatibility levels, making it more difficult to determine which species combinations cannot hybridise. In practice, regulators may have to determine for which wild relatives the probability of hybridisation is acceptably low, and for which the potential for hybridisation should be investigated under local conditions.

Management options will also affect the likelihood of hybridisation under natural conditions. Progress is being made on developing ‘biological confinement’ techniques that seek to reduce the likelihood of gene flow from transgenic crops, for example sterility (male and/or female), or genetic modification of maternally inherited chloroplast, rather than biparentally inherited, nuclear DNA (NRC 2004). Since such strategies are in their infancy, and will include monitoring components, knowledge of potential target species for transgene escape via hybridisation is still critical.

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