Gene Introgression in Weeds Depends on Initial Gene Location in the Crop: *Brassica napus*–*Raphanus raphanistrum* Model

Katarzyna Adamczyk-Chauvat,^{*} Sabrina Delaunay,[†] Anne Vannier,[†] Caroline François,[†] Gwenaëlle Thomas,[§] Frédérique Eber,[†] Maryse Lodé,[†] Marie Gilet,[†] Virginie Huteau,[†] Jérôme Morice,[†] Sylvie Nègre,[†] Cyril Falentin,[†] Olivier Coriton,[†] Henri Darmency,[‡] Bachar Alrustom,[‡] Eric Jenczewski,[§] Mathieu Rousseau-Gueutin,[†] and Anne-Marie Chèvre^{†,1}

^{*}MaIAGE, INRA, Université Paris-Saclay, 78350 Jouy-en-Josas, France, [†]Institut de Génétique, Environnement et Protection des Plantes, INRA, Agrocampus Ouest, Université Rennes 1, 35653 Le Rheu cedex, France, [‡]Agroécologie, AgroSup Dijon, INRA, University Bourgogne Franche-Comté, 21000, France, and [§]Institut Jean-Pierre Bourgin, INRA, AgroParisTech, CNRS, Université Paris-Saclay, 78026 Versailles Cedex, France

ORCID ID: 0000-0002-1130-1090 (M.R.-G.)

ABSTRACT The effect of gene location within a crop genome on its transfer to a weed genome remains an open question for gene flow assessment. To elucidate this question, we analyzed advanced generations of intergeneric hybrids, derived from an initial pollination of known oilseed rape varieties (*Brassica napus*, AACC, $2n = 38$) by a local population of wild radish (*Raphanus raphanistrum*, RrRr, $2n = 18$). After five generations of recurrent pollination, 307 G5 plants with a chromosome number similar to wild radish were genotyped using 105 *B. napus* specific markers well distributed along the chromosomes. They revealed that 49.8% of G5 plants carried at least one *B. napus* genomic region. According to the frequency of *B. napus* markers (0–28%), four classes were defined: Class 1 (near zero frequency), with 75 markers covering ~70% of oilseed rape genome; Class 2 (low frequency), with 20 markers located on 11 genomic regions; Class 3 (high frequency), with eight markers on three genomic regions; and Class 4 (higher frequency), with two adjacent markers detected on A10. Therefore, some regions of the oilseed rape genome are more prone than others to be introgressed into wild radish. Inheritance and growth of plant progeny revealed that genomic regions of oilseed rape could be stably introduced into wild radish and variably impact the plant fitness (plant height and seed number). Our results pinpoint that novel technologies enabling the targeted insertion of transgenes should select genomic regions that are less likely to be introgressed into the weed genome, thereby reducing gene flow.

KEYWORDS gene flow; intergeneric hybridization; introgression; oilseed rape; wild radish

IN spite of multiple reproductive barriers between species, interspecific hybridization is considered a major evolutionary force in plants due to their ability to produce homoploid (same chromosome number as the parents) or polyploid (whole genome duplication of the F1 hybrid genome) hybrids, or to introgress genomic regions from one species to another.

The latter situation is of particular importance in the evolution of crop-weed complexes (Rieseberg and Willis 2007; Nosil 2008; Ellstrand *et al.* 2013; Gressel 2015). Many studies have demonstrated that closely related plant species are able to hybridize under natural conditions (Jenczewski *et al.* 2003; Andersson and De Vicente 2010; Ellstrand 2014). The rate of interspecific hybridization depends on sympatry and flowering period overlap, the relatedness between species, and their ploidy level. When interspecific or intergeneric hybrids produce fertile progeny after recurrent natural pollinations by the weed, the question remains of the occurrence of crop gene introgression within the genome of wild species.

Genome structure was proposed as one of the main barriers to introgression (Stewart *et al.* 2003). It has been shown that

Copyright © 2017 by the Genetics Society of America

doi: <https://doi.org/10.1534/genetics.117.201715>

Manuscript received March 3, 2017; accepted for publication May 7, 2017; published Early Online May 18, 2017.

Supplemental material is available online at www.genetics.org/lookup/suppl/doi:10.1534/genetics.117.201715/-/DC1.

¹Corresponding author: Institut de Génétique, Environnement et Protection des Plantes, INRA, Agrocampus Ouest, Université Rennes1, BP35327, 35653 Le Rheu cedex, France. E-mail: anne-marie.chevre@inra.fr

particular combinations of parental genomes are selected for the emergence of viable hybrid, offspring generating new species, which can present a transgressive phenotype better adapted to new ecological niches (Rieseberg and Willis 2007; Heredia and Ellstrand 2014). This indicates that some genomic regions may not present the same likelihood of being maintained after an interspecific hybridization event (Hufford *et al.* 2013; Barb *et al.* 2014). Stewart *et al.* (2003) and Ellstrand *et al.* (2013) suggest that chromosomal blocks are the unit of introgression, which can be linked with domestication alleles influencing the stability of the introgressed weed. This situation was demonstrated for gene flow from *Lactuca sativa* to *L. serriola*, with the identification of hotspots or cold regions (Hooftman *et al.* 2011). Such data are missing concerning intergeneric hybrids from species with different ploidy levels, where it depends on the occurrence of intergenomic recombination between the parental genomes. It is thus likely that the genomic regions presenting the highest sequence similarity between two species have the highest probability to recombine in interspecific hybrids (Benavente *et al.* 2008). However, it is still unknown whether the location of a gene within a genome will modify its probability to be introgressed in the genome of a weed belonging to a closely related genus. For this purpose, experiments at the whole genome level are essential, and will aid in deciphering whether some genomic regions are more likely to be introgressed. Results from such studies will determine the best gene location, using new technology to target specific sites (Liu *et al.* 2016), in order to prevent crop gene escape in weeds.

There are several reasons why the Brassiceae tribe (46 genera) (Al-Shehbaz 2012) is a good model system to study the putative impact of initial gene location in a crop, and transfer to a weedy related genome. First, oilseed rape (*Brassica napus*, AACC, $2n = 4x = 38$), an allopolyploid species arising from the hybridization between *B. rapa* (AA, $2n = 2x = 20$) and *B. oleracea* (CC, $2n = 2x = 18$), is one of the main transgenic crops cultivated worldwide (mainly for herbicide tolerance: www.isaaa.org), and has several closely related weedy species belonging to the same tribe (Murat *et al.* 2015). Second, gene flow between oilseed rape and its most frequent weeds has already been assessed (Chèvre *et al.* 2004; Fitzjohn *et al.* 2007; Liu *et al.* 2013). In *B. rapa*, one of the progenitors of *B. napus*, it has been shown that gene introgression can occur in two generations (Mikkelsen *et al.* 1996). It is expected that introgression through homologous recombination occurs more easily when the transgene is located on the A genome (shared between *B. napus* and wild *B. rapa*). On the contrary, when the transgene is located on the C genome of *B. napus*, introgression into *B. rapa* requires homeologous exchanges between the A and C genomes (Stewart *et al.* 2003). However, one of the main factors influencing gene introgression seems to be selection pressure (Warwick *et al.* 2008; Londo *et al.* 2010). For more distant relatives to oilseed rape, such as wild radish (*Raphanus raphanistrum*, RrRr, $2n = 2x = 18$), it has been observed that spontaneous interspecific hybridization with *B. napus* occurs at a low frequency under natural conditions (Darmency *et al.* 1998; Chèvre *et al.* 2000; Rieger

et al. 2001; Warwick *et al.* 2003). Chromosome recombination between the A or C subgenomes of *B. napus* and the wild radish genome was observed in F1 intergeneric hybrids, indicating the possibility of gene exchange in F1 intergeneric hybrids (Kerlan *et al.* 1993; Eber *et al.* 1994). These hybrids produced mainly unreduced gametes from First Division Restitution (FDR)-like at a low frequency under natural conditions, with the same number of chromosomes, but rearranged when compared to the mother plants (Chèvre *et al.* 1998). Using transgenic herbicide resistant lines, we demonstrated that these intergeneric hybrids can generate fertile progeny after open pollination with wild radish under natural conditions. After applying herbicide pressure over five generations, resistant plants presenting a similar morphology to wild radish were obtained, but none had the same number (18) of chromosomes as wild radish (Chèvre *et al.* 1997, 1998, 2007; Gueritane *et al.* 2002). These results suggest that one additional oilseed rape chromosome carrying the transgene is maintained in the resistant plants, albeit without Mendelian inheritance (Al Mouemar and Darmency 2004). In these experiments, we assessed gene introgression only for the genomic region carrying the transgene. An evaluation at the whole genome level of the oilseed rape genomic regions that are more likely to recombine with the wild radish genome is yet to be performed.

Only one additional oilseed rape chromosome was observed in the advanced generations, therefore we wanted to establish if some oilseed loci or chromosomal blocks could be introgressed in the wild radish genome and if there was an effect of the initial position. A precise knowledge of the oilseed rape varieties used to form the initial intergeneric hybrids and the wild radish population recurrently used as pollinators is needed to identify markers specific to the oilseed rape genome. In this paper, we genotyped 307 G5 intergeneric hybrids, with a chromosome number similar to wild radish ($2n = 18$), using oilseed rape specific markers well distributed along the chromosomes. The analyses of these plants, cultivated in field conditions and without selection pressure, revealed that some oilseed rape regions were more or less frequently introgressed to the wild radish genomes. The progeny of some plants carrying different types of introgressions were analyzed, allowing us to demonstrate for the first time that *B. napus* genes can be introgressed in the wild radish genome, but with different impacts on the plant fitness.

Materials and Methods

Plant material and DNA extraction

In order to optimize intergeneric F1 hybrid production, male sterile oilseed rape F1 hybrids were produced by crossing a male sterile European spring type line with Ogu-INRA cytoplasm conferring male sterility (cv. Brutor) with a Canadian spring type line (cv. Westar). These F1 hybrids were cultivated under field conditions in a 1:1 ratio with a local wild radish population. Experimental plots consisted of oilseed rape or wild radish, with plots of each species alternating in three replicates. The F1 male sterile intergeneric hybrids and their four successive generations

were cultivated using the same field design as described in Chèvre *et al.* (2007) (Figure 1). Each generation was representative of the fertility of the plants of the previous generation and the germination rate (Supplemental Material, Figure S1). Out of the 1626 G5 plants, a sample of 307 plants with a chromosome number close to that of wild radish was selected (Figure S2).

For the characterization of potentially introgressed plants, nine G5 plants representative of the different introgression events were selected. Their progenies were obtained via open pollination with wild radish plants (under field conditions), and were analyzed using markers specific to the *B. napus* genome. The wild radish pollinator has to carry the nuclear restorer gene of male sterility in order to produce fertile male plants for further studies. Only two G5 plants with introgressed genomic regions were fertile and produced progeny after crossing with wild radish. The evaluation of male and female transmission rates of oilseed rape genes was performed from seeds harvested from G9 plants with wild radish cytoplasm and introgression, and from seeds harvested from wild radish plants cultivated under the same cages, with two plants per cage (Figure S3).

For fitness assessment, G9 seedlings presenting an A03 introgressed region (see below) were transplanted (INRA Dijon, France) in a 3 × 6 m insect-proof cage. At flowering, 500 g of housefly maggots were deposited weekly into the cage in order to facilitate pollination. Harvest of G10 seeds was carried out plant by plant. Only six plants (out of 19) produced sufficient seeds to enable further experiments. In addition, only one G9 plant had the introgressed A10 markers, and thus was hand-crossed with wild radish in a greenhouse.

G10 seeds were germinated and transplanted every 0.2 m in a 3 × 6 m insect-proof cage with houseflies during the flowering period. The DNA was extracted from each plant and PCR amplifications were performed using the *B. napus* A03 and A10 markers. Developmental traits were assessed, including: date, diameter and color (white, pale yellow, or other) of the first flower, length of the larger rosette leaf, width, and length of the terminal lobe. After harvest, the plant height, the number of main branches, the number and weight of pods, the number of pod segments, and the plant dry weight (after 48 hr at 80°) were measured. Individual data were used in GLM analysis (Systat 10.2), with family (the six G9 original plants) and presence of A03 introgression as factors and interaction. A *t*-test was used to analyze separately the A10 introgression effect in the unique family.

The selection of oilseed rape specific molecular markers was performed by comparing six oilseed rape varieties used to establish different genetic maps (two spring-types: cv. Stellar and Drakkar, three winter-types: cv. Darmor, Darmor-bzh and Samourai, and a Korean type: cv. Yudal), the two varieties used to produce the initial intergeneric hybrids (cv. Brutor and Westar), and the local wild radish population used as pollinator. All of the oilseed rape varieties were provided by the BrACySol Genetic Resource Center (Ploudaniel, France).

Figure 1 Genealogy of the hybrids produced under field conditions.

Cytogenetic studies

Flow cytometry analyses used to assess the chromosome number and meiotic analyses were performed as described in Chèvre *et al.* (2007).

BAC Fluorescent In Situ Hybridization (BAC FISH) was developed as described previously using root tips (Snowdon *et al.* 1997). BAC clone KBrH80A08 (A10, long/C09) and KBrH117M18 (A03, long/C03) (Xiong and Pires 2011) were labeled by random priming with biotin-14-dUTP (Invitrogen, Life Technologies). The ribosomal probe used in this study was pTa 71 (Gerlach and Bedbrook 1979), which contained a 9-kb *EcoRI* fragment of rDNA repeat unit (18S-5.8S-26S genes and spacers) isolated from *Triticum aestivum*. pTa 71 was labeled with Alexa-488 dUTP by random priming. Fluorescence images were captured using a CoolSnap HQ camera (Photometrics, Tucson, AZ) on an Axioplan 2 microscope (Zeiss, Oberkochen, Germany), and analyzed using MetaVue (Universal Imaging, Downingtown, PA).

Molecular analyses

A total of 319 PCR markers, previously genetically mapped on the oilseed rape genome (Delourme *et al.* 2006; Wang *et al.* 2011) and well distributed along all *B. napus* chromosomes, were chosen and used for PCR analysis using the conditions described by Wang *et al.* (2011). The physical location of each selected marker (Table S1) was then determined by blasting each marker sequence against the recently published *B. napus* genome sequence (Chalhoub *et al.* 2014), enabling the comparison of the physical and genetic maps. The homologous relationships between genomic regions were derived from Chalhoub *et al.* (2014).

For some oilseed rape regions (A03, A10, and C09), the *Arabidopsis* orthologous regions were identified. Orthologous genes were identified and primers were designed to specifically amplify the *B. napus* regions. The amplified *B. napus* regions

were sequenced to confirm that they were derived from the initial *B. napus* varieties used to produce the hybrids.

Statistical analyses

Model-based clustering was applied in order to group the molecular markers according to the probability of being observed in G5. The number of plants carrying the selected markers was considered as a sample from a mixture of binomial distributions:

$$f(y) = \sum \pi_k \binom{n}{y} p_k^y (1-p_k)^{n-y}$$

with mixing distribution $\pi_1 \dots \pi_K$ and success probabilities $p_1 \dots p_K$. Here, the parameter π_k is the probability that a marker belongs to a class k and the parameter p_k is the probability that a marker from a class k occurs in G5 generation. The maximum likelihood estimation of model parameters was performed via the EM algorithm (Dempster *et al.* 1977) implemented in the R package *mixtools* (Benaglia *et al.* 2009). Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were applied to determine the number of clusters K . Cluster labels were assigned to the markers according to the Maximum A Posteriori principle.

Data availability

The primers of each marker specific to *B. napus* and absent in *R. raphanistrum* are described in Table S1. The data from the fitness experiment and the presence of introgression in the plants are presented in Table S2 and S3. The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article.

Results

Introgression of oilseed rape markers according to their genomic location

The screening of 319 molecular markers, genetically mapped in oilseed rape (Wang *et al.* 2011), allowed selection of 103 markers specific to oilseed rape. Additionally, two specific PCR markers were designed from certain oilseed rape regions (see below) (Table S1). PCR amplification of these markers was positive in the two oilseed rape varieties used to produce the initial intergeneric oilseed rape-wild radish hybrids but negative (*i.e.*, no amplicon) from the three bulks of 10 wild radish plants of the population used as a pollinator. The retained markers covered 63.4% of the oilseed rape genetic map (Figure 2); most of them (86.5%) were also physically localized on the *B. napus* genome (Figure S4).

The fertility (seed production and germination rate) of the G4 plants was highly variable (Figure S1). As the most fertile G4 plants were expected to contribute more to the following generation, we produced the subsequent G5 progenies by taking into account the G4 fertility (Figure 1). From 60 G4 plants, we analyzed 1626 G5 plants. We selected a subset of 307 G5 plants from 38 different G4 plants that had a chromosome

number (assessed by flow cytometry) close to (± 2 chromosomes) that of wild radish (Figure S2). Among these, 153 plants carried at least one oilseed rape marker, indicating a rate of 49.8% of potential introgression. The frequency of transmission of a given marker from G4 generation was highly variable between markers, ranging from 0 to 28% (Figure 2).

Statistical analyses of specific oilseed rape marker frequency revealed that a mixture model with four components ($K = 4$) best fitted the observed data (Figure 3). Consequently, the oilseed markers were divided into four groups according to their probability of being observed in G5 (Figure 2). The first group (class 1) was characterized by a very low probability ($\hat{p}_1 = 0.003$) of being present among G5 plants, and covered 69.9% of the genome. The expected proportion of markers within this class was $\hat{\pi}_1 = 0.731$. The second group (class 2) had a probability of occurrence $\hat{p}_2 = 0.036$, with an expected proportion of markers of $\hat{\pi}_2 = 0.174$. The markers covered 5.53% of the genome and corresponded to 11 genomic regions on 10 different oilseed rape chromosomes: A01, A03 (three adjacent markers), two regions of A05, one carrying two adjacent markers, A09, A10 (three adjacent markers), C03, C04 (four adjacent markers), C05, C08 (two adjacent markers), and C09. The third group (class 3) had a probability of occurrence $\hat{p}_3 = 0.126$, and an expected proportion of belonging to this class of $\hat{\pi}_3 = 0.076$. These markers were located on A09 (three regions), A10 with two markers flanking a group of markers from class 4 within a unique genomic region (28.7 cM of ~ 4.7 Mb) and C09, consisting of five adjacent markers (48.6 cM of ~ 28.7 Mb). Finally, the fourth group (class 4) contained two markers with the highest probability of occurrence in G5, $\hat{p}_4 = 0.277$, and an expected proportion of markers of $\hat{\pi}_4 = 0.019$. They were located on A10 (6.2 cM of ~ 2.8 Mb) and surrounded by the adjacent markers belonging to class 3.

Concerning the size of the introgressed regions, as the plants had a chromosome number close that of wild radish, three situations were expected. First, a complete *B. napus* chromosome may have been added to the wild radish genome. This may be the case for only three plants that contained all the markers of a linkage group; two plants showed all markers from C01, one of them also harboring all markers from A04, and one plant displayed all markers from A08. These markers belonged to class 1. However, these plants also carried markers from other chromosomes, indicating possible independent introgressions. Second, plants may carry an additional *B. napus* rearranged chromosome due to chromosome exchanges between both species, with a complete or partial oilseed rape chromosome arm or an additional chromosome of oilseed rape partially deleted. In these cases, we could expect that the corresponding centromere to the oilseed rape region detected was maintained. According to the physical location of the markers, only eight genomic regions of oilseed rape contained the centromere (as located by Mason *et al.* 2016), and four genomic regions carried all the markers of an oilseed rape chromosome arm on a total of 549 oilseed rape genomic regions observed in G5 plants (2.5% of the situations observed). Third, we could observe small oilseed rape regions in

Figure 2 Analysis of the G5 plants having a chromosome number similar to wild radish ($2n = 18$). The first circle represents the 19 *B. napus* linkage groups (in cM) and the genetic location of the *B. napus* specific markers, according to Wang *et al.* (2011), is indicated between brackets after the marker name. The second circle indicates the frequency of each marker in the G5 plants; the color corresponds to the four statistical classes to which belongs each marker (red for the hot spot, green for medium spots, brown for low spots, and blue for cold spots). The number of plants carrying one, two, three, four, five, or all adjacent markers per chromosome is indicated in the internal pink circles.

G5 plants. This situation was the most frequent, since 50.6% of introgressions were characterized by the presence of just one oilseed rape marker, and 39.8% by the presence of two or three adjacent markers (Figure 2). For the area on A10 (17 Mb) (Figure S4) with class 4 and 3 markers, the size of oilseed rape regions ranged from 2.8 to 4.9 Mb (max ~27% of the A10 chromosome). The larger region was detected in 13% of plants with the four markers of this genomic region among the

108 plants with markers of this region. This area was observed in 28 different G4 plants out of 38 observed, suggesting that this introgression occurred independently several times. For the other areas belonging to class 3, we assessed the size to be <5 Mb on A9, between 2.7 and 4.9 Mb on A10, and between 3.2 and 28.7 Mb on C09. The last situation was detected in 12% of plants among the 76 plants carrying markers of this region, and the centromere was present in 29 out of 74 G5

Figure 3 Information criteria as the functions of the number of clusters K with $AIC = -2\log(\hat{L}) + 2(2K-1)$ (black line) and $BIC = -2\log(\hat{L}) + (2K-1)\log(N)$ (blue line). $\hat{L}$ denotes the maximized value of the likelihood function for the statistical model, and N is the loci sample size. The model with $K = 4$ groups of markers minimizes both AIC and BIC criteria.

plants. Additionally, 32.6% of markers detected in the G5 plants were located at the extremity of oilseed rape chromosomes, indicating that a proportion of rearrangements involved distal regions of the chromosomes.

Additionally, some oilseed rape homeologous regions (Figure S4) were inserted in the same G5 plants. These situations occurred between markers of class 2 for A03 and C03 (15 plants of 23), A05, and C04 (14 plants of 21). For markers belonging to class 3 on A09 and C09, 22 plants out of 45 carried markers of both chromosomes. Markers most frequently detected on A10 were homeologous to a C09 region with markers of class 3. It is interesting to note that, among the 118 plants carrying markers of A10 and/or C09, 47 plants had markers of both A10 and C09 homeologous regions whereas only seven had markers of C09 and 64 of A10. These plants originated from 28 G4 plants, but those with markers of A10 and C09 were produced from 14 G4 plants. These results indicate that multiple introgressions from the oilseed rape genome can occur, and may be present in previous generations. However, these multiple introgressions were not as frequent, since 77% of the plants presented one to three oilseed rape regions (Figure 4).

Characterization of plants with oilseed rape markers

Nine G5 plants, representative of the different classes, were selected for more precise characterization. The cytoplasm originally used (Ogu-INRA) to produce the hybrids induced a chlorophyll deficiency in G5 plants because of nucleus (mainly radish)–chloroplast (oilseed rape) incompatibility (Gueritain *et al.* 2002); therefore, we selected plants with a restored male fertility, in order to cross these plants on wild radish as female. Only two plants with wild radish cytoplasm and oilseed rape

Figure 4 Number of independent introgressions in the G5 plants carrying oilseed rape markers.

markers produced fertile, vigorous progeny, following the breeding scheme in Figure S3.

The first fertile plant carried markers of three oilseed rape genomic regions: two different genomic regions on A10 (first region of 2.8 Mb from CB10485 to CB10109, and second region with sN8474), and one region on C09 (Bras002, Bras075a, and CB10288, corresponding to an oilseed rape genomic region of 12.4 Mb). The G9 plant selected had 18 chromosomes like wild radish and a regular meiotic behavior with nine bivalents; this suggested that the oilseed rape markers detected were introduced through recombination into wild radish chromosomes. One BAC specific to A10 and C09 of *B. napus* (KBrH080A08) was selected in a genomic region close to sN8474. BAC-FISH allowed detection of four loci carried by chromosomes A10 and C09 in *B. napus* (Figure 5A), but no signal was observed on wild radish chromosomes (Figure 5B). On the G9 introgressed plant, only one signal was detected (Figure 5C). In the G10 progeny of this plant, produced in cages with one plant of wild radish as pollinator, all A10 and C09 markers were transmitted together. The 18.5% transmission rate was observed from the 119 plants obtained from seeds harvested on the introgressed plant, whereas only 7.7% of the 117 plants from seeds harvested from wild radish carried the markers. A BAC FISH control on two plants with introgressed markers indicated that just one signal was always present (Figure 5, D and E). The fitness cost of this introgression was assessed from the progeny derived from one plant. Plants with introgressed markers showed reductions in number of branches, plant weight, pod weight, and article number compared with the respective control (Table 1). According to these results, it is likely that the different oilseed rape genomic regions recombined in previous generations of this plant in order to form a unique block of A10 and C09 markers introgressed in one location on a radish chromosome.

The second fertile plant carried a series of markers distributed along one single ~4 Mb region of *B. napus* A03 chromosome (from CB10388 to Bras029). This plant had 18 wild radish chromosomes and a stable meiosis. We first confirmed that this plant carried an introgressed fragment from *B. napus* by showing that several amplicons specific to A03 produced from this plant had a sequence that was 100% identical to the

Figure 5 (A–E) BAC-FISH/FISH was carried out using BAC KBrH080A08 and 45S rDNA that identify the A10 and C09 chromosomes (red) and all chromosomes carrying the 45S rDNA locus (green), respectively. FISH analyses of somatic metaphase chromosomes of *B. napus* (A), *R. raphanistrum* (B), mother plant with A10 and C09 introgression (C), and two plants of its progeny (D) and (E). Chromosomes were counterstained with DAPI (blue). Bar, 5 µm.

one obtained from the variety we used to produce the initial F1 intergeneric hybrids (*B. napus* cv. Brutor). These sequences were also used to identify the presence of a specific PCR marker in the A03 introgressed region, A38.370, and the absence in the wild radish population used as pollinator. This result was confirmed using three adjacent PCR markers (data not shown). These markers were used to identify a specific BAC clone (KBr117M18), which allowed detection of two pairs of chromosomes in *B. napus* (on A03, characterized by the 45S locus corresponding to the Nucleolus Organizer Region, NOR, and C03, Figure 6A), but no signal was observed on wild radish chromosomes (Figure 6B). Unexpectedly, two signals were detected on one G9 plant, including one on the radish chromosome carrying the NOR (Figure 6C) and the other on a radish chromosome without a rDNA locus, indicating that the A03 oilseed rape region was introgressed twice in two different wild radish chromosomes. In the progeny produced in cages with wild radish as pollinator, we observed,

using molecular markers, a transmission rate of 41.3% from 177 plants produced from seeds harvested on the introgressed plant, and 3.7% from 81 plants produced from seeds harvested on wild radish. Among the 76 plants with introgression, two had the CB10388 and A38.370 markers, two others had the Bras029 marker, and the remainder maintained all the markers. The presence of an introgressed region was verified using BAC FISH for three plants carrying all markers. We observed the expected segregation of the two insertion sites with either one signal on a chromosome carrying the 45S locus (Figure 6D), or one signal on a chromosome without the 45S locus at the heterozygous (Figure 6E) or homozygous stages (Figure 6F). The progeny of six of these plants were studied for growth and reproduction (Table 1). The distribution of the A03 markers was similar among the six families (Table S2, homogeneity $\chi^2 = 5.07$, $P = 0.41$, 5 d.f.), with $47 \pm 6\%$ of the plants possessing the A03 markers. In addition, a few introgressed plants (14) lost some of the markers (Table S2). All of the 258 seedlings analyzed

Table 1 Phenotypic traits measured for every family in the presence or absence of the A3 or A10/C09 introgressions

Family	A03	Plant Number	Lobe Length (cm)	Lobe Width (cm)	Leaf Length (cm)	Flowering (D)	Flower Diameter (cm)	Branches No.	Plant Height (cm)	Plant Weight (g)	Pod No.	Pod dry Weight (g)	Article No.
1	—	15	11.3	8.7	41.2	156	2.31	2.7	105	43.4	171.2	26.0	661
1	+	25	11.9	8.5	44.1	156	2.28	2.1	115	17.1	61.6	8.0	202
2	—	21	11.5	7.5	39.2	153	2.27	2.7	120	30.3	130	16.9	364
2	+	13	11.2	8.2	38.9	153	2.34	1.6	123	25.9	26.9	4.7	91
3	—	18	10.9	7.4	44.2	156	2.26	2.2	119	24.4	97.2	13.4	285
3	+	23	10.7	7.6	42.5	155	2.40	2.6	118	22.7	76.0	10.2	198
4	—	12	11.5	8.8	39.3	158	2.10	1.7	109	30.2	72.2	8.5	263
4	+	15	12.0	7.7	40.6	157	2.18	2.4	121	45.8	73.7	10.7	237
5	—	14	9.7	7.1	34.2	158	1.87	2.1	127	19.2	79.5	10.9	292
5	+	16	9.9	6.8	33.6	160	1.79	1.4	107	11.2	18.3	2.7	67
6	—	22	11.0	7.3	37.2	159	2.29	2.9	114	32.1	67.0	12.1	220
6	+	14	9.7	7.3	36.6	159	2.35	3.9	117	80.4	111.6	15.4	454
A10/C09													
7	—	21	11.5	8.0	48.2	139	2.38	4.6*	131	109.1*	187.8	34.8***	490**
7	+	3	13.7	8.3	44.3	138	2.47	2.0	110	30.9	77.3	7.4	145

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ (Student's t test).

flowered, but 50 (16 with A03 vs. 34 without A03) did not mature to the pod stage. Family had a significant effect for all measurements except plant height and pod number (Table 1 and Table S3). The presence of the A03 introgression reduced, on average, the pod number and weight and the article number. However, an interaction was observed in half of the traits (Table S3). Introgressed plants of family #5 flowered 2 days earlier than their nonintrogressed counterparts, but the difference was not significant after Bonferroni correction (Table 1). Plant height showed different trends in family #4 compared with #5, while there was no difference between introgressed and nonintrogressed plants in the other families. In family #4, there was no difference in plant weight, pod number, pod weight, and number of articles whether or not the A03 introgression was present, while in families #1, #2, #3 and #5 these parameters declined. In family #6, growth and yield parameters improved with the introgression (Table 1). The flower colors were ~1:2 white: pale yellow—never intense yellow as in oilseed rape—and the colors were distributed equally among plants with or without the A03 introgression ($\chi^2 = 1.92$, $P = 0.38$).

Discussion

We demonstrated here that the different genomic regions of oilseed rape were observed with different probabilities in oilseed rape–wild radish intergeneric hybrid plants (G5) with a chromosome number close to wild radish ($2n = 18$) and obtained under field conditions. Four groups of oilseed rape-specific markers were identified from G4 plants, and only 30% of the oilseed rape genome analyzed was detected frequently in the G5 generation. We demonstrated that in the progeny of two G5 plants, introgression can be stably introduced in wild radish chromosomes, but usually with a fitness cost.

Strategy for gene flow assessment at the whole genome level

The initial frequency of intergeneric hybridization between *B. napus* and *R. raphanistrum* is very low, but almost equivalent under field conditions (Darmency *et al.* 1998; Chèvre *et al.* 2000; Rieger *et al.* 2001; Warwick *et al.* 2003). To complete this study, we used male sterile oilseed rape varieties as female parent, and a local wild radish population, in order to facilitate F1 intergeneric hybrids production. The advanced generations were thus generated by pollination with wild radish under field conditions, with the most fertile plants contributing proportionally more to the following generation as under natural conditions. For final assessment, G5 fertile plants were crossed to wild radish (female parent); this strategy avoided the loss of fitness due to cytoplasm–nucleus interactions (Gueritain *et al.* 2002).

Contrary to a strategy based on selection for a specific trait (*i.e.*, herbicide resistance conferred by a transgene located on a specific location on the genome), we never applied selection pressure, and screened for possible introgression at the (oilseed rape) genome scale. The difficulty was to identify specific oilseed markers well distributed in the genome that could be scored reliably. As already suggested by Ellstrand *et al.* (2013), we used SSR as co-dominant markers that are stable over few generations, and that were already genetically mapped in oilseed rape (Delourme *et al.* 2006; Wang *et al.* 2011). We observed a low proportion (32%) of markers amplified in both oilseed rape varieties used but absent in wild radish population. This finding likely reflects the good transferability of the microsatellite markers between these two Brassicaceae species, which diverged only ~8 MYA (Lysak *et al.* 2005; Hedges *et al.* 2015).

Figure 6 (A–F) BAC-FISH/FISH was carried out using BAC KBrH117M18 and 45S rDNA, which identify the A03 and C03 chromosomes (red) and all chromosomes carrying the 45S rDNA locus (green), respectively. FISH analyses of somatic metaphase chromosomes of *B. napus* (A), *R. raphanistrum* (B), mother plant with A03 introgression (C), and three plants of its progeny (D), (E), and (F). Chromosomes were counterstained with DAPI (blue). Bar, 5 μ m.

Frequency of introgression depends on initial gene location

We demonstrated that introgression rates vary with the position of loci in the donor genome, excluding the neutral expectation as already suggested by Ellstrand *et al.* (2013). The counts of plants with oilseed rape markers in the G4 progeny were assumed to follow the mixture of binomial distributions. The components of the mixture correspond to the different probabilities of the occurrence of the markers. A model-based clustering approach allowed assignment of the mixture component to each marker. According to our sampling, a few oilseed rape genomic regions were detected in G5 plants: 73% of specific oilseed rape markers had a probability of being observed close to 0. The remaining 27% were specific to genomic regions carried by 10 different A or C *B. napus* chromosomes. We noted that the whole C02 chromosome as well as a large part of its homeologous A02 (two plants carried one marker of this chromosome) and large genomic regions were never detected in *R. raphanistrum* and may

thus be good candidate locations for the insertion of transgenes in *B. napus* in order to prevent gene flow. In contrast, three genomic regions were detected frequently in the progeny of 74 and 37% of G4 plants, for A10 and C09, respectively. It could be of great interest in the future to insert a transgene conferring herbicide resistance, easy to screen on very large populations in these specific locations through new technologies (Liu *et al.* 2016) in order to validate these expectations.

The size of introgression is variable and linked to genome structure

Stewart *et al.* (2003) proposed that chromosomal blocks are the units of introgression. We observed that, in spite of variations, 90.5% of regions detected did not present more than three adjacent markers. However, the low density of markers did not allow the precise assessment of the physical size of the introgressions. The recent sequencing of *B. napus* (Chalhoub *et al.* 2014), as well as the elucidation of the genome structure of cultivated

radish (*R. sativus*) close to *R. raphanistrum* (Li *et al.* 2011; Shirasawa *et al.* 2011; Jeong *et al.* 2016), will facilitate the development of new specific markers that are physically anchored.

Some genomic regions are more prone to be introgressed in wild radish, and they often shared homeology. For the plants in which we detected the most frequent introgressed regions, A10 and C09, we often observed multiple introgressions of these homeologous oilseed rape regions in the same plant. We can postulate that these rearrangements occurred frequently in previous generations, since they were generally detected from the several independent G4 mother plant (14 different G4 plants of 28 showing A10 and C09 introgressions). Furthermore, we can hypothesize that homeologous exchanges can occur between A10 and C09 chromosomes, and that this block can be introgressed into a radish chromosome, or that the introgression of one of these regions (A10 or C09) favors chromosome exchange, allowing later introgression of the second. This hypothesis is supported by the cosegregation of all A10 and C09 markers in the progeny of the G9 plant with $2n = 18$ and a regular meiotic behavior. These data suggest that homeologous recombination might occur, as previously shown for the introduction of a radish restorer gene into C09 chromosome of oilseed rape varieties (Delourme *et al.* 1998), in which the radish introgression replaced a homeologous oilseed rape region.

For plants carrying A03 markers belonging to class 2, we detected in the G9 plant a regular meiotic behavior but two insertion sites with BAC FISH: one site was on the radish chromosome carrying the NOR and the other on another chromosome. This result can be explained either by open pollination of A03 introgressed hybrids with a plant carrying the A03 introgression on another chromosome, or by two A03 introgressions on two different wild radish chromosomes in the same plant. The two insertion sites showed segregation in its progeny with either one or both introgressions. The transmission rate was much lower than the lowest expected percentage (41–47% instead of 75% when all of the mother plants were heterozygous, and >75% if some of the parents were homozygous), which denotes a strong detrimental segregation bias. We can hypothesize that both introgressions were not the same size since some markers can be lost in the progeny, and recombination between the introgressed and donor genome is expected to be almost null (Delourme *et al.* 1998).

The fitness is affected in the introgressed plants

In spite of their regular meiotic behavior, the complexity of the introgressed regions might explain the skewed segregations and fitness cost observed. For the first plant combining the A10 and C09 markers, we observed a lower transmission rate than expected, and a lower fertility than the control. Similarly, when Delourme *et al.* (1998) introgressed radish regions on chromosome C09 of oilseed rape, they observed that no recombination occurred between the genomic regions, allowing maintenance of the full radish introgression but with a skewed segregation in the progeny. For the A03 introgressions, the presence of two independent introgressions might explain the observation that different derived families did not have the same fitness cost for

different traits. We can hypothesize that the absence of introgression size decrease, combined with a possible linkage with domestication alleles suggested by several authors (Stewart *et al.* 2003; Ellstrand *et al.* 2013), might impose an important barrier preventing the stability of the introgressed weeds.

The integration of information on the effect of initial transgene insertion site in modeling approach could be one way to assess gene flow from a crop to its weeds (Garnier *et al.* 2014). Given the data showing that some oilseed rape genomic regions are more prone to recombine with wild radish, it would be of interest to screen wild radish populations having different histories of cocultivation with oilseed rape, in order to validate our results on a longer evolutionary timescale. Since it is now possible to target the transgene insertion (Liu *et al.* 2016) with CRISPR/Cas9 technology, it becomes possible to insert a transgene in a region with a low frequency of inter-genomic recombination, helping to prevent the escape of transgenes (De Jong and Rong 2013).

Acknowledgments

We acknowledge the Genetic Resource Center [BrACySol, Unité Mixte de Recherche Institut de Génétique, Environnement et Protection des Plantes (UMR IGEPP), Ploudaniel, France) for providing seeds of *B. napus* varieties, Gentyane platform (INRA, Clermont Ferrand, France) for genotyping, the experimental farm (UE La Motte, Le Rheu France) for field experiments, and L. Charlon, P. Rolland, J.P. Constantin, J.M. Lucas and F. Letertre for their technical assistance in greenhouses. This work was funded by ANR OGM ANR-07-POGM-001-01 and by ANR-11-BTBR-0001-GENIUS. We thank Stephen Strelkov (University of Alberta, Canada) and Alina Tollenaere (UMR IGEPP, France and University of Queensland, Australia) for their critical reviews of the manuscript.

Literature Cited

- Al Mouemar, A., and H. Darmency, 2004 Lack of stable inheritance of introgressed transgene from oilseed rape in wild radish. *Environ. Biosafety Res.* 3: 209–214.
- Al-Shehbaz, I. A., 2012 A generic and tribal synopsis of the Brassicaceae (Cruciferae). *Taxon* 61: 931–954.
- Andersson, S., and M. C. De Vicente, 2010 *Gene Flow between Crops and Their Wild Relatives*. The John Hopkins University Press, Baltimore.
- Barb, J. G., J. E. Bowers, S. Renaut, J. I. Rey, S. J. Knapp *et al.*, 2014 Chromosomal evolution and patterns of introgression in *helianthus*. *Genetics* 197: 969–979.
- Benaglia, T., D. Chauveau, D. R. Hunter, and D. S. Young, 2009 mixtools: an R package for analyzing mixture models. *J. Stat. Softw.* 32(6): 1–29.
- Benavente, E., M. Cifuentes, J. C. Dusautoir, and J. David, 2008 The use of cytogenetic tools for studies in the crop-to-wild gene transfer scenario. *Cytogenet. Genome Res.* 120: 384–395.
- Chalhoub, B., F. Denoeud, S. Liu, I. A. Parkin, H. Tang *et al.*, 2014 Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. *Science* 345: 950–953.
- Chèvre, A.-M., F. Eber, A. Baranger, and M. Renard, 1997 Gene flow from transgenic crops. *Nature* 389: 924.

- Chèvre, M. A., F. Eber, A. Baranger, G. Hureau, P. Barret *et al.*, 1998 Characterization of backcross generations obtained under field conditions from oilseed rape-wild radish F1 interspecific hybrids: an assessment of transgene dispersal. *Theor. Appl. Genet.* 97: 90–98.
- Chèvre, M. A., F. Eber, H. Darmency, A. Fleury, H. Picault *et al.*, 2000 Assessment of interspecific hybridization between transgenic oilseed rape and wild radish under normal agronomic conditions. *Theor. Appl. Genet.* 100: 1233–1239.
- Chèvre, A. M., H. Ammitzbøll, B. Breckling, A. Dietz-Pfeilstetter, F. Eber *et al.*, 2004 A review on interspecific gene flow from oilseed rape to wild relatives, pp. 235–251 in *Introgression from Genetically Modified Plants into Wild Relatives*, edited by H. C. M. den Nijs, D. Bartsch, and J. Sweet. CABI Publishing, Cambridge.
- Chèvre, A. M., K. Adamczyk, F. Eber, V. Huteau, O. Coriton *et al.*, 2007 Modelling gene flow between oilseed rape and wild radish. I. Evolution of chromosome structure. *Theor. Appl. Genet.* 114: 209–221.
- Darmency, H., E. Lefol, and A. Fleury, 1998 Spontaneous hybridizations between oilseed rape and wild radish. *Mol. Ecol.* 7: 1467–1473.
- De Jong, T., and J. Rong, 2013 Crop to wild gene flow: does more sophisticated research provide better risk assessment? *Environ. Sci. Policy* 27: 135–140.
- Delourme, R., N. Foisset, R. Horvais, P. Barret, G. Champagne *et al.*, 1998 Characterisation of the radish introgression carrying the Rfo restorer gene for the Ogu-INRA cytoplasmic male sterility in rapeseed (*Brassica napus* L.). *Theor. Appl. Genet.* 97: 129–134.
- Delourme, R., C. Falentin, V. Huteau, V. Clouet, R. Horvais *et al.*, 2006 Genetic control of oil content in oilseed rape (*Brassica napus* L.). *Theor. Appl. Genet.* 113: 1331–1345.
- Dempster, A., N. M. Laird, and D. B. Rubin, 1977 Maximum likelihood from incomplete data via the EM algorithm. *J. R. Stat. Soc. B* 39: 1–38.
- Eber, F., A. M. Chèvre, A. Baranger, P. Vallée, X. Tanguy *et al.*, 1994 Spontaneous hybridization between a male-sterile oilseed rape and two weeds. *Theor. Appl. Genet.* 88: 362–368.
- Ellstrand, N. C., 2014 Is gene flow the most important evolutionary force in plants? *Am. J. Bot.* 101: 737–753.
- Ellstrand, N. C., P. Meirmans, J. Rong, D. Bartsch, A. Ghosh *et al.*, 2013 Introgression of crop alleles into wild or weedy populations. *Annu. Rev. Ecol. Evol. Syst.* 44: 325–345.
- Fitzjohn, R. G., T. T. Armstrong, L. E. Newstrom-Lloyd, A. D. Wilton, and M. Cochrane, 2007 Hybridisation within Brassica and allied genera: evaluation of potential for transgene escape. *Euphytica* 158: 209–230.
- Garnier, A., H. Darmency, Y. Tricault, A. M. Chèvre, and J. Lecomte, 2014 A stochastic cellular model with uncertainty analysis to assess the risk of transgene invasion after crop-wild hybridization: Oilseed rape and wild radish as a case study. *Ecol. Modell.* 276: 85–94.
- Gerlach, W. L., and J. R. Bedbrook, 1979 Cloning and characterization of ribosomal RNA genes from wheat and barley. *Nucleic Acids Res.* 7: 1869–1885.
- Gressel, J., 2015 Dealing with transgene flow of crop protection traits from crops to their relatives. *Pest Manag. Sci.* 71: 658–667.
- Gueritaine, G., M. Sester, F. Eber, A. M. Chevre, and H. Darmency, 2002 Fitness of backcross six of hybrids between transgenic oilseed rape (*Brassica napus*) and wild radish (*Raphanus raphanistrum*). *Mol. Ecol.* 11: 1419–1426.
- Hedges, S. B., J. Marin, M. Suleski, M. Paymer, and S. Kumar, 2015 Tree of life reveals clock-like speciation and diversification. *Mol. Biol. Evol.* 32: 835–845.
- Heredia, S. M., and N. C. Ellstrand, 2014 Novel seed protection in the recently evolved invasive, California wild radish, a hybrid *Raphanus* sp. (*Brassicaceae*). *Am. J. Bot.* 101: 2043–2051.
- Hoofman, D. A., A. J. Flavell, H. Jansen, H. C. Den Nijs, N. H. Syed *et al.*, 2011 Locus-dependent selection in crop-wild hybrids of lettuce under field conditions and its implication for GM crop development. *Evol. Appl.* 4: 648–659.
- Hufford, M. B., P. Lubinsky, T. Pyhäjärvi, M. T. Devengendo, N. C. Ellstrand *et al.*, 2013 The genomic signature of crop-wild introgression in maize. *PLoS Genet.* 9: e1003477.
- Jenczewski, E., J. Ronfort, and A. M. Chèvre, 2003 Crop-to-wild gene flow, introgression and possible fitness effects of transgenes. *Environ. Biosafety Res.* 2: 9–24.
- Jeong, Y. M., N. Kim, B. O. Ahn, M. Oh, W. H. Chung *et al.*, 2016 Elucidating the triplicated ancestral genome structure of radish based on chromosome-level comparison with the Brassica genomes. *Theor. Appl. Genet.* 129: 1357–1372.
- Kerlan, M. C., A. M. Chevre, and F. Eber, 1993 Interspecific hybrids between a transgenic rapeseed (*Brassica napus*) and related species: cytogenetical characterization and detection of the transgene. *Genome* 36: 1099–1106.
- Li, F., Y. Hasegawa, M. Saito, S. Shirasawa, A. Fukushima *et al.*, 2011 Extensive chromosome homoeology among Brassicaceae species were revealed by comparative genetic mapping with high-density EST-based SNP markers in radish (*Raphanus sativus* L.). *DNA Res.* 18: 401–411.
- Liu, D., R. Hu, K. J. Palla, G. A. Tuskan, and X. Yang, 2016 Advances and perspectives on the use of CRISPR/Cas9 systems in plant genomics research. *Curr. Opin. Plant Biol.* 30: 70–77.
- Liu, Y., W. Wei, K. Ma, J. Li, Y. Liang *et al.*, 2013 Consequences of gene flow between oilseed rape (*Brassica napus*) and its relatives. *Plant Sci.* 211: 42–51.
- Londo, J. P., N. S. Bautista, C. L. Sagers, E. H. Lee, and L. S. Watrud, 2010 Glyphosate drift promotes changes in fitness and transgene gene flow in canola (*Brassica napus*) and hybrids. *Ann. Bot. (Lond.)* 106: 957–965.
- Lysak, M. A., M. A. Koch, A. Pecinka, and I. Schubert, 2005 Chromosome triplication found across the tribe Brassicaceae. *Genome Res.* 15: 516–525.
- Mason, A. S., M. Rousseau-Gueutin, J. Morice, P. E. Bayer, N. Besharat *et al.*, 2016 Centromere locations in brassica A and C genomes revealed through half-tetrad analysis. *Genetics* 202: 513–523.
- Mikkelsen, T. R., B. Andersen, and R. Bagger Jorgensen, 1996 The risk of crop transgene spread. *Nature* 380: 31.
- Murat, F., A. Louis, F. Maumus, A. Armero, R. Cooke *et al.*, 2015 Understanding Brassicaceae evolution through ancestral genome reconstruction. *Genome Biol.* 16: 262.
- Nosil, P., 2008 Speciation with gene flow could be common. *Mol. Ecol.* 17: 2103–2106.
- Rieger, A. M., D. T. Potter, C. Preston, and B. S. Powles, 2001 Hybridisation between *Brassica napus* L. and *Raphanus raphanistrum* L. under agronomic field conditions. *Theor. Appl. Genet.* 103: 555–560.
- Rieseberg, L. H., and J. H. Willis, 2007 Plant speciation. *Science* 317: 910–914.
- Shirasawa, K., M. Oyama, H. Hirakawa, S. Sato, S. Tabata *et al.*, 2011 An EST-SSR linkage map of *Raphanus sativus* and comparative genomics of the Brassicaceae. *DNA Res.* 18: 221–232.
- Snowdon, J. R., W. Köhler, W. Friedt, and A. Köhler, 1997 Genomic in situ hybridization in Brassica amphidiploids and interspecific hybrids. *Theor. Appl. Genet.* 95: 1320–1324.
- Stewart, Jr., C. N., M. D. Halfhill, and S. I. Warwick, 2003 Transgene introgression from genetically modified crops to their wild relatives. *Nat. Rev. Genet.* 4: 806–817.

- Wang, J., D. J. Lydiate, I. A. Parkin, C. Falentin, R. Delourme *et al.*, 2011 Integration of linkage maps for the Amphidiploid *Brassica napus* and comparative mapping with *Arabidopsis* and *Brassica rapa*. *BMC Genomics* 12: 101.
- Warwick, S. I., M. J. Simard, A. Légère, H. J. Beckie, L. Braun *et al.*, 2003 Hybridization between transgenic *Brassica napus* L. and its wild relatives: *Brassica rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Erucastrum gallicum* (Willd.). *O.E. Schulz. Theor. Appl. Genet.* 107: 528–539.
- Warwick, S. I., A. Legere, M. J. Simard, and T. James, 2008 Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy *Brassica rapa* population. *Mol. Ecol.* 17: 1387–1395.
- Xiong, Z., and J. C. Pires, 2011 Karyotype and identification of all homoeologous chromosomes of allopolyploid *Brassica napus* and its diploid progenitors. *Genetics* 187: 37–49.

Communicating editor: J. Sekelsky