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Search for spontaneous hybridization between oilseed rape (*Brassica napus* L.) and wild radish (*Raphanus raphanistrum* L.) in agricultural zones and evaluation of the genetic diversity of the wild species

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Abstract

Thalmann C., Guadagnuolo R. and Felber F. 2001. Search for spontaneous hybridization between oilseed rape (*Brassica napus* L.) and wild radish (*Raphanus raphanistrum* L.) in agricultural zones and evaluation of the genetic diversity of the wild species. Bot. Helv. 111: 107–119.

Possible spontaneous gene flow under agricultural conditions was studied between the crop *Brassica napus* subsp. *napus* (oilseed rape), as pollen donor, and the common weed *Raphanus raphanistrum* (wild radish), as mother plant. Conventional cultivars were used. Around 750 individuals of *R. raphanistrum* were screened by flow cytometry or chromosome counts, and further genetic analysis was performed with random amplified polymorphic DNA (RAPD) on 98 selected plants. No hybrids were detected. The genetic structure of *B. napus* was uniform over the area investigated, which was expected for a cultivated species. In contrast, *R. raphanistrum* was genetically variable within populations, while geographical genetic variation among populations within the regions and even among regions was small. It is interpreted as an indication of gene flow sensu lato over large areas caused by seed dispersal by man.

Key words: Brassica napus, *Raphanus raphanistrum*, gene flow, RAPD, risk assessment, population genetic structure.

Introduction

Oilseed rape (*Brassica napus* L. subsp. *napus*) is an important crop in Switzerland, where 16,500 ha were cultivated in 1990 (Bundesamt für Statistik 1992). The plant is

prone to several diseases and pests and therefore requires intensive treatments with biocides (Geisler 1988). The development and availability of transgenic crops offer new opportunities in disease and pest management, but makes it necessary to investigate aspects of biosafety. Gene flow between crops and wild relatives has the possible consequence of the introgression of the transgene in the crop as herbicides, diseases, stress, or pests tolerance traits as well as altered morphology and/or physiology to wild relatives. The expression of this transgene in the weed may give it competitive advantage and may create ecological risks to the ecosystem (Crawley et al. 1993, Rissler and Mellon 1996, Ammann et al. 1996).

Inter-specific and even inter-generic gene flow from *Brassica napus* to other members of the Brassicaceae have been frequently observed and described as reviewed in Scheffler and Dale (1994) and Metz et al. (1997). Spontaneous hybridization from open pollination occurred with the following species: *B. rapa* L. subsp. *campestris* Clapham (= *B. campestris* L.) (Bing et al. 1991), *B. juncea* (L.) Czern. (Bing et al. 1991), *Hirschfeldia incana* (L.) Lagr.-Foss. (= *B. adpressa* Boiss.) (Lefol et al. 1991), *Sinapis arvensis* L. (Chèvre et al. 1996) and *Raphanus raphanistrum* L. (Chèvre et al. 1992).

Brassica napus (oilseed rape) is an annual to biennial crop and is only known as a cultivated plant, yet it sometimes escapes cultivation. It has the genome constitution AACC (2n = 38) and is an allotetraploid/amphidiploid plant resulting from the hybridization of *B. rapa* (AA, 2n = 20) and *B. oleracea* L. (CC, 2n = 18) (UN. 1934). *B. napus* is regarded as self-compatible and attracts insect pollinators (bees and beetles). As the plants grow densely in fields, wind is also an important pollinator (Hess et al. 1970, Hegi 1986, Geisler 1988, Rufener Al Mazyad 1998).

Raphanus raphanistrum (wild radish) is an annual colonizing plant that grows on fallow land, roadsides, ruderal places and as a weed in agricultural fields. *R. raphanistrum* has 2n = 18 chromosomes and the genome constitution RrRr. It differs from most colonizing species in two ways, it is self-incompatible and insect pollinated (Kercher and Conner 1996) particularly by syrphids and bumblebees (Lee and Snow 1998).

Brassica napus and *R. raphanistrum* co-occur in the same habitats yet the flowering season of *R. raphanistrum* is much longer and comprises the flowering season of *B. napus*, two features that promotes hybridization. Recent research concerning gene flow between the two species has shown a high compatibility when the pollen accepting plant was *B. napus*. Eber et al. (1994) found triploid and amphidiploid hybrids (genome constitution ACRr 2n = 28; AACCRrRr 2n = 56 respectively) between male-sterile *B. napus* and *R. raphanistrum* by open pollination. Baranger (1995a), Baranger et al. (1995b, c), and Chèvre et al. (1997) described one further type of hybrid (ACRrRr 2n = 37), and a broad range of intermediate types. Chèvre et al. (1997) reported four successive generations of backcrosses between hybrids and *R. raphanistrum*. They observed a decrease in the chromosome number with every generation, that positively affected the fitness of the plants. However, they never observed a stable *B. napus* introgression within the genome of *R. raphanistrum* (Chèvre et al. 1998). The first and only successful hybridization in which *R. raphanistrum* acted as female was reported by Darmency et al. (1995, 1998). They found two slowly growing hybrids (ACRr 2n = 28) derived from the same mother plant. It correspond to 0.05% of germinated seeds.

Because more data are needed to assess risks under agricultural and unforced conditions, the main aim of this work was to detect possible spontaneous gene flow between *R. raphanistrum* (seed parent) and *B. napus* under agricultural conditions and with conventional cultivars. Therefore, specific markers for genetic differentiation

of *B. napus* and *R. raphanistrum* were developed. They were also used to assess the genetic structure of the wild species.

Material and methods

Studied sites

Three regions on the Swiss Plateau in the cantons of Vaud, Neuchâtel, Bern, and Fribourg were selected where *B. napus* is cultivated intensively (Table 1). Populations of *R. raphanistrum*, that occur as a common weed in these regions, were observed and mapped over three years (Guadagnuolo and Rufener Al Mazyad unpublished data).

Field work

Field work was performed from May to August 1999 and consisted in the census of populations of *R. raphanistrum* on the border of fields on which *B. napus* was cultivated during the previous year, following the maps of Guadagnuolo and Rufener Al Mazyad (unpublished data). Up to 4–5 fresh leaves per plant were collected. One part was stored at 4°C for quick analysis by flow cytometry and the other was stored at –80°C for subsequent DNA analysis. All sampled plants were tagged and numbered for further identification of hybrids.

Flow cytometry

Individuals were screened for hybrids by flow cytometry (FCM), which measures the DNA content. The difference between the DNA quantity in the nuclei of *R. raphanistrum* (2n = 18) and *B. napus* (2n = 38) was large enough to detect hybrids (2n = 28, 2n = 37, and 2n = 56).

Procedure followed Brown et al. (1991). Leaves (ca. 1 cm^2) were chopped into small pieces with a razor blade in a petri dish in order to release the nuclei into 0.5 ml of ice-cold buffer (sodium metabisulfite (10 mM), $MgCl_2$ (45 mM), 4-morpholinepropane sulfonate (20 mM) pH 7–7.5, 4°C). After treating the nuclei with 20 µl of stain (propidium iodide (1 part) and 10% Triton X-100 (1 part)) for 5 min, the stained nuclei and the buffer were passed through a 30 µm nylon sieve. The analysis was made with a Becton Dickinson (San Jose, CA, USA) FACStrac flow cytometer with an argon laser (wavelength 488 nm). The computer software used was Lysis II Version 1.1 from Becton Dickinson. At the beginning of each session or after every 15 samples, the flow cytometer was calibrated with a plant of known ploidy level, in order to prevent fluctuations in the measures.

DNA extraction and purification

DNA was extracted and purified following Savova-Bianchi (1996). DNA was dissolved and stored in Tris-EDTA (10 mM Tris-HCl, pH 8.0, 1 mM Na_2EDTA) at –20°C. DNA samples were used for polymerase chain reaction (PCR).

Random amplified polymorphic DNA

The protocol for RAPD-PCR (Williams et al. 1990) was based on Guadagnuolo (2000). Amplifications were performed in a final volume of 25 µl with the following concentrations: PCR buffer 1X (Qiagen AG, Basel), $MgCl_2$ 1.5 mM, Q-solution 0.4X (Qiagen AG, Basel), dNTP 0.2 mM, primer 0.2 µM, Taq-pol (Qiagen AG, Basel) 0.03 U/µl and template DNA 1ng/µl. An automatic thermal cycler UNO from Biometra

Table 1. The 18 populations analyzed with RAPD. Sites and coordinates according to the maps from the Bundesamt für Landestopographie, Wabern, Switzerland (scale: 1:25'000).

Population	Site	Coordinates	Pop. size
Region of Vaud			
V1	Montolly	161.925 / 536.050	5
V2	Maupas	161.425 / 535.600	1
V3	Vollangin	159.375 / 538.200	3
V4	La Mèbre	159.300 / 537.500	5
V5	Les Brits	161.075 / 536.100	5
Region of Neuchâtel			
N1	Le Chalet 1	208.650 / 559.375	8
N2	Le Chalet 2	208.550 / 559.475	7
N3	Bois des Aigles 1	208.150 / 556.450	5
N4	Bois des Aigles 2	208.275 / 556.550	11
N5	Bois des Aigles 3	208.350 / 556.550	6
N6	Bois du Millieu	208.250 / 557.125	2
N7	Fin du Chêne	210.400 / 558.075	5
Region of Bern/Freiburg			
B1	Chriegsmatt	200.775 / 582.375	2
B2	Jerisberghof	200.950 / 582.950	12
B3	Unterfeld 1	200.650 / 581.975	1
B4	Unterfeld 2	200.825 / 582.250	1
B5	Oberfeld	200.300 / 582.975	5
B6	Fulmatten	196.925 / 578.150	8

(Biomedizinische Analytik GmbH, Göttingen, D) was used with the following steps: 1 cycle at 94°C for 4 min followed by 38 cycles at 93°C for 1 min, 45°C for 1 min, 72°C for 1 min. The reaction was terminated with a DNA extension at 72°C for 5 min.

Amplified products were analyzed by electrophoreses in 1.6% agarose gels, stained with ethidium bromide and visualized under UV light. Bands of identical size amplified with the same primer were considered to be the same DNA fragment.

RAPD data analysis

The data from the RAPD analysis was used to build up a matrix containing all results of all primers. The bands were represented as presence/absence data (1/0). Genetic similarity between the individuals was expressed by the Jaccard's similarity coefficients (*SJ*):

$$SJ = c / (a + b + c)$$

where c is the number of bands shared by the two individuals, a and b are the number of bands unique to each of the individuals (Van Tongeren 1995).

Principal coordinate (PCO) analysis (Gower 1966) was performed on the genetic distance (dissimilarity matrix (*Di*)):

Table 2. Species and number of plants analyzed by the methods of flow cytometry (FCM), random amplified polymorphic DNA (RAPD) and caryological analysis (chromosome counts) at the different studied regions, as well as the number of hybrids detected.

Region	Vaud	Neuchâtel	Bern/Freiburg	Total
Number of *Raphanus raphanistrum* analyzed by FCM (number of *Brassica napus*)	204 (6)	199 (11)	351 (12)	754 (29)
Number of individuals analyzed by FCM with uncertain amount of DNA	8	21	14	43
Number of *Raphanus raphanistrum* analyzed by RAPD (number of *Brassica napus*)	19 (2)	44 (2)	29 (2)	92 (6)
Number of *Raphanus raphanistrum* analyzed by chromosome counts (number of *Brassica napus*)	0	32 (5)	34	66 (5)
Number of hybrids identified by RAPD and chromosome counts	0	0	0	0

$$Di = 1 - SJ$$

with the Progiciel R 4 (Beta version) software package (Casgrain 1997, http://www.fas.umontreal.ca/biol/casgrain/en/labo/r/v4/). Dissimilarities were computed between all pairs of the 98 entities (including *B. napus*) (Table 2) and arranged in a 98×98 dissimilarity matrix, which, because it is symmetric, can be displayed as a half-matrix. The results of the PCO analysis were visualized with a scatterplot.

The same dissimilarity matrix of the genetic dissimilarity as for PCO analysis and a generated matrix was used to calculate Mantel's test correlation coefficient (Mantel 1967). The generated matrix was built by attributing the values 1 to samples of different species, populations, and regions respectively and 0 to samples of the same species, populations, and regions respectively.

For calculation, the significance levels adjusted for multiple comparisons performed on the same data set, Bonferroni's correction (Rice 1989) was applied. The same software as for the PCO analysis was used.

To explore the potential genetic differentiation of *R. raphanistrum*, an analysis of molecular variance (AMOVA) was calculated (Excoffier et al. 1992). AMOVA was performed with the software package ARLEQUIN 1.1 (Schneider S., Kueffer J. M., Roessli D., Excoffier L. 1997, http://lgb.unige.ch/arlequin).

Caryological analysis

Chromosome counts were carried out on plants with morphology with intermediate characteristics, such as thick or hairless leaves, yellow flowers, or large plant size. Caryological analysis has also been used to confirm the analyses by flow cytometry and RAPD.

Floral buds were fixed in the field with glacial acetic acid (1 part) and (99%) ethanol (3 parts), with iron acetate and acetocarmine and stored for 3 weeks at 4°C. The material was then put in the same implements the material was pre-stained for 60 minutes at room temperature. After heating gently (below boiling point) for two minutes, acetic acid (45%) was added. Afterwards the ovaries, petals, anthers, or young pollen grains were squashed on a slide for observation with a light microscope.

Results

Flow cytometry

A total of 783 plants were analyzed by FCM (Table 2). Of the 754 screened plants of *R. raphanistrum*, 43 individuals had uncertain quantity of DNA and were further analyzed by RAPD.

Random amplified polymorphic DNA

Analysis for differentiation between Brassica napus and Raphanus raphanistrum

The 43 individuals for which FCM revealed uncertain results as well as 49 other plants which figured as a standard for *R. raphanistrum* were selected for RAPD analysis. In addition, six individuals of *B. napus* were analyzed.

Of the 47 screened RAPD primers, five primers were selected to detect hybrids between *B. napus* and *R. raphanistrum* (OPB-6; OPP-8; OPP-15; OPT-1; OPT-7). These primers were selected because they produced at least five specific bands (Fig. 1). Moreover, the number of bands amplified was small enough to ensure that they could be analyzed (up to 13). The five primers yielded a total of 44 bands (5–13 bands/primer, mean 8.8 bands/primer) of which 20 bands turned out to be specific for *R. raphanistrum* and two bands for *B. napus*.

Search for hybrids

The dots on the scatterplot built with RAPD data (Fig. 2) of the PCO analysis form two clearly distinct groups characterized by *B. napus* and *R. raphanistrum*. Individuals with a mixture of both genomes would have been located between the two groups. This assesses that no interspecific hybrid was present in the samples analyzed.

Mantel's test for species distinction with RAPD data was highly significant ($P < 0.0001$) with a high correlation coefficient ($r = 0.58$), which indicates a clear genetic separation of the two species (Table 3).

Analysis of the genetic structure of Raphanus raphanistrum

Mantel's test of the genetic differentiation according to populations was significant at the 5% level although the correlation was low (9.8%), but that according to regions was not significant (Table 3). This is assessed on the scatterplot of the PCO analysis (Fig. 2), on which the individuals of *R. raphanistrum* are quite homogeneously distributed according to the three regions and no grouping was detectable. In addition, no grouping was detectable concerning the investigated populations (data not shown).

When RAPD data set was partitioned within and between populations, most of the genetic variation detected resided within populations (89%), relative to the amount of variation among the three regions (5.4%) and among populations within region (5.7%)

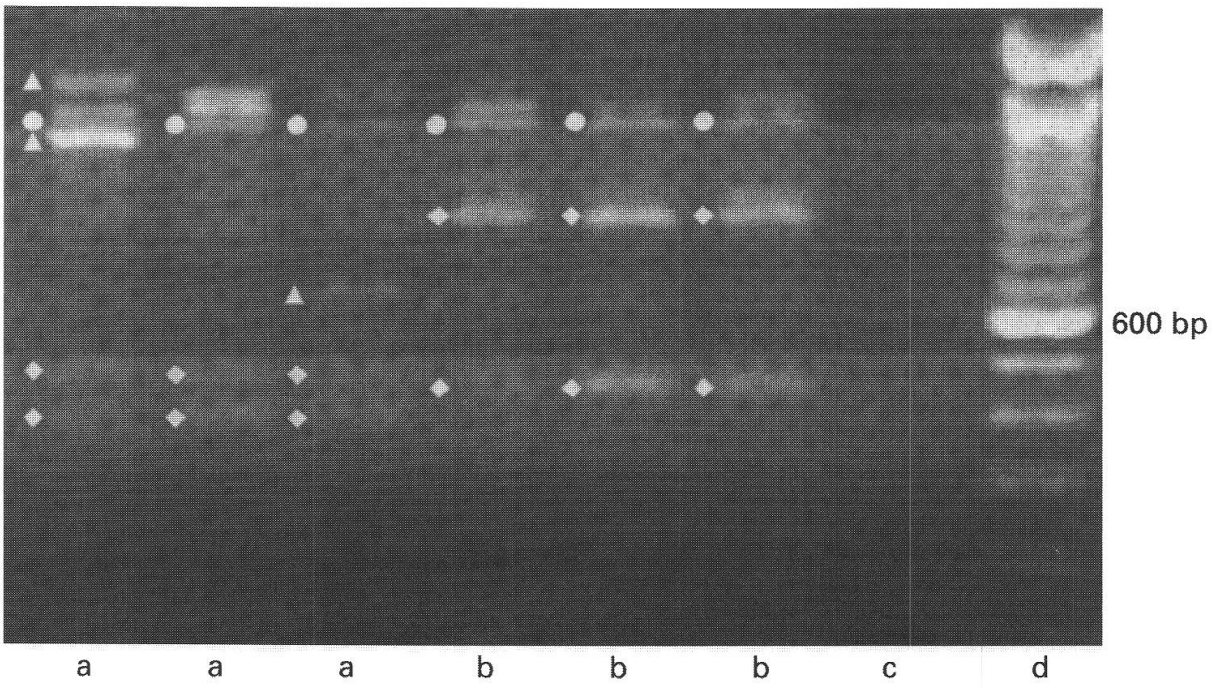

Fig. 1. Agarose gel of RAPD analysis with primer OPP-8 on a) three individuals of *Raphanus raphanistrum* from the site N-14 in Neuchâtel; b) three individuals of *Brassica napus* from Neuchâtel; c) control line with no amplification products; d) 100-bp DNA ladder. The same primer may yield different amplification products: either monomorphic (●), specific (◆) and variable intraspecifically (▲).

(Table 4). AMOVA produced highly significant ($P < 0.00001$) fixation indices. Φ_{ST}, the global differentiation of populations equaled 0.110, Φ_{SC} the differentiation among populations within regions equaled 0.059, and Φ_{CT}, the proportion of differentiation between regions had a value of 0.053.

On the scatterplot of the PCO analysis (Fig. 2) the individuals of *R. raphanistrum* appear homogeneously distributed concerning the three regions and no grouping is detectable. Similarly, no grouping is detectable concerning the different populations (data not shown).

Caryological analysis

A total of 66 individuals of *R. raphanistrum* and 5 of *B. napus* were analyzed by chromosome counts (Table 1). The chromosome numbers corresponded always to the expected number of the species. These findings confirmed the results of RAPD analysis.

Discussion

Analysis for differentiation between Brassica napus and Raphanus raphanistrum

A broad range of species specific markers was found for *R. raphanistrum* and only very few for *B. napus*. It was obvious that the wild species produced more bands and

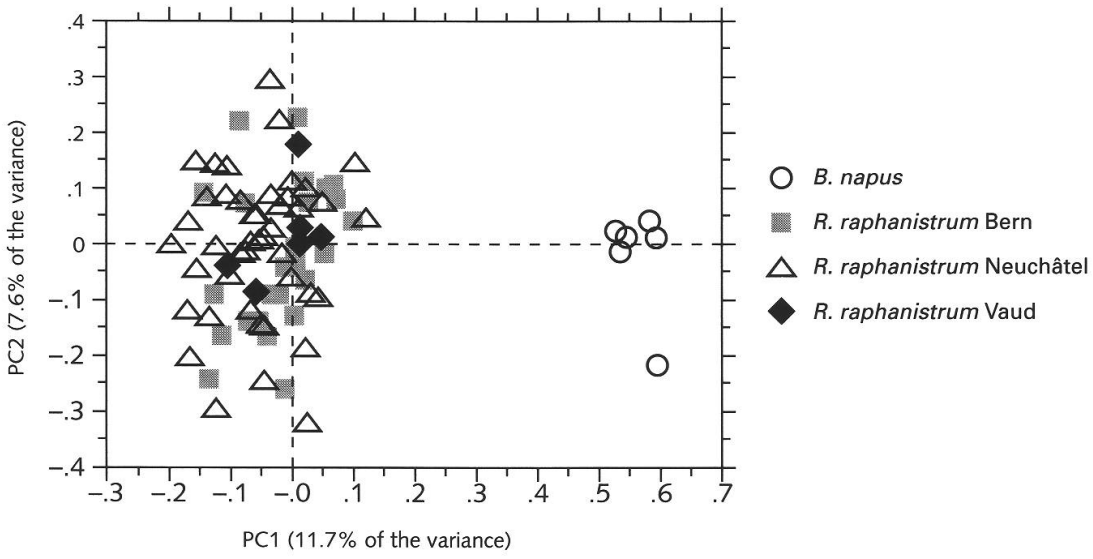

Fig. 2. PCO analysis scatterplot of *Brassica napus* and *Raphanus raphanistrum* which is divided into the three studied regions.

the banding patterns varied much more from one individual to the other compared with *B. napus* which produced less and more uniform patterns. We determined the markers under the assumption that the same bands corresponded to identical DNA fragments. It is conceivable that some *B. napus* specific markers have not been recognized because *R. raphanistrum* produced fragments of the same mobility but from a different locus. However, the scatterplot of the PCO analysis (Fig. 2) and Mantel's test (Table 3) show both a clear genetic separation of *B. napus* and *R. raphanistrum*. Apart from one individual of *B. napus* – outliner on the scatterplot (Fig. 2) – *B. napus* shows a high degree of homogeneity that may indicate a high selection pressure in cultivation (Chan and Sun 1997) and that similar varieties were cultivated over the Swiss Plateau. This particular individual produced the lowest amount of RAPD bands (13 compared with on average 22 of the other 5 *B. napus*), which is probably an artifact.

Search for hybrids

No gene flow between *R. raphanistrum* and *B. napus* was detected in the present study after analyzing 754 individuals of *R. raphanistrum*. Crosschecks with chromosome counts confirmed this result: all individuals had $2n = 18$ chromosomes, the regular number of *R. raphanistrum*. If there is spontaneous gene flow under natural conditions, it must be a rare event. In agriculture, *R. raphanistrum* often grows in lanes along the planted fields of *B. napus*. This arrangement of the plants causes a high pollen pressure from *B. napus* to *R. raphanistrum*. The self-incompatibility of *R. raphanistrum* would even increase the chance of fertilization by pollens of *B. napus*. Even when we did not find evidence for gene flow, the compatibility of the two species has been shown in earlier studies by Darmency et al. (1995, 1998) which grew individual *R. raphanistrum* and transgenic *B. napus* in outdoor cultivation. Therefore, hybridization under agricultural conditions cannot be strictly ruled out.

Table 3. Comparison of the dissimilarity matrix of the real genetic distance with that of the maximum genetic distance. r-statistics were calculated with Mantel's permutation test. 1000 permutations were performed.

Comparison	r	P
Genetic dissimilarity vs. max. dissimilarity between the species *Brassica napus* and *Raphanus raphanistrum*	0.579	**
Genetic dissimilarity vs. max. dissimilarity between the 18 populations of *Raphanus raphanistrum*	0.098	**
Genetic dissimilarity vs. max. dissimilarity between the three regions (Bern, Neuchâtel, Vaud) of the study of *Raphanus raphanistrum*	0.050	n. s.

**: $P < 0.01$; n. s.: non significant

Table 4. Analysis of molecular variance (AMOVA) of the genetic distance of *Raphanus raphanistrum* among regions, among populations within regions and within populations. Phi (ϕ)-statistics is defined by three fixation indices : ϕ_{CT}-proportion of differentiation between regions, ϕ_{SC}-differentiation among populations within a region, ϕ_{ST}-the global differentiation of populations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among regions	2	41.89	$V_a = 0.40$	5.38
Among populations within regions	15	129.53	$V_b = 0.42$	5.67
Within population	74	489.37	$V_c = 6.61$	88.95
Total	91	660.78	$V_t = 7.43$	100

Significance test (1023 permutations)

Analysis of the genetic structure of Raphanus raphanistrum

Results from the RAPD analysis let also draw conclusions from the genetic structure of *R. raphanistrum*. The low significant correlation of the compared matrices in Mantel's test (Table 3) when split by populations and the non-significant when split by regions indicate weak geographic separation of *R. raphanistrum*.

AMOVA (Table 4) corroborates the results of Mantel's test. It detected significantly that the highest amount of genetic variation occurs within populations, which means that the individuals within a population are genetically very variable. This conspicuously high variation within a population can be explained by the self-incompatibility of the species. The Φ_{ST} value of 0.11 (global differentiation of populations) (Table 4) was close to an average of the F_{ST} value of 0.16 for 15 outcrossed annuals, but much lower than average compared to other insect-pollinated (average FST = 0.23) or early-successional (average $F_{ST} = 0.41$) (Loveless and Hamrick 1984). The results of

this study were not very different from studies of self-incompatible weeds that had F_{ST} values ranging from 0.01 to 0.22 (Kercher and Conner 1996). Moreover, genetic variation among populations within the regions and even among regions are small but significant. This is an indication for the low geographic differentiation of *R. raphanistrum* over large areas. This fact is well visible on the scatterplot (Fig. 2) which shows no grouping of the samples into the three regions Bern, Neuchâtel, or Vaud. It can be explained by gene flow over long distances caused by dispersal of either seeds or pollen grains or both. These results are consistent with the study of Kercher and Conner (1996), who concluded that man is responsible of long-distance transport of large numbers of wild radish. Seeds can easily be dispersed by agricultural manipulations such as plowing, harvesting, co-transport with crops and contamination of seeds of crops with weeds. As mentioned above, gene flow by pollen grains can also be an important factor. In pollen dispersal experiments with wild radish (*R. sativus* L.), Ellstrand and Marshall (1985) observed outcrossing of up to 20% from adjacent populations and with populations up to 1000 m away. They concluded that long-range transport of pollen cannot be ruled out. As morphology of the blossoms and the pollinators of *R. raphanistrum* are similar to those of *R. sativus* (Ellstrand and Marshall 1985; Lee and Snow 1998), gene flow over long distances caused by pollen dispersal can also be expected for *R. raphanistrum*.

Genetic variation of *R. raphanistrum* may be influenced by the introgression of genes from *R. sativus*, which is fully compatible to *R. raphanistrum* according to Kercher and Conner (1996). In the present work, wild *R. sativus* was nowhere discovered, however cultivated *R. sativus* subsp. *oleifera* was observed on a place 2 km away from the next population of R. *raphanistrum*. Therefore, gene flow between the two species can probably be neglected this year, while the situation in the years before is not known and therefore gene flow cannot be excluded.

Conclusions

This study demonstrates a strong hybridization barrier between *B. napus* as pollen donor and *R. raphanistrum* even when the latter is exposed to a high amount of pollen of *B. napus*. High hybridization rates under natural conditions can therefore be ruled out and gene flow must be a rare event. Moreover, this study has demonstrated the considerable level of genetic variation within populations of *R. raphanistrum* while genetic differentiation between populations as well as the regions was low. It was interpreted as a consequence of the allogamous breeding system, jointed with either transport of pollen, and/or transport of seeds of *R. raphanistrum* over long distances by humans (Ellstrand and Marshall 1985; Kercher and Conner 1996).

If we refer to the probability of gene dispersal from transgenic crop to the wild flora according to the coding system formulated for Switzerland (Ammann et al. 1996), we have to consider three indices describing the potential of:

a) pollen dispersal and hybridization (here: occasional natural hybridization),
b) diaspores dispersal (here: high seed dispersal),
c) dispersal frequency of the wild relative (here: not frequent but well distributed).

The derived risk category is: low but local effects. This category demands for eventual release experiments first to perform them in containment and afterwards in small

scale releases closely monitored. This statement restrict to transgenes not causing enhanced competitiveness outside agricultural environment. Any other transgenes should be carefully tested in confinements.

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References

Ammann K., Jacot Y. and Rufener Al Mazyad P. 1996. Field release of transgenic crops in Switzerland, an ecological risk assessment of vertical gene flow. In: Schulte E. and Käppeli O. (eds.). Gentechnisch veränderte krankheits- und schädlingsresistente Nutzpflanzen. Eine Option für die Landwirtschaft? Schwerpunktprogramm Biotechnologie SPP des Schweizerischen Nationalfonds, Bern, 101–157.

Baranger A. 1995a. Evaluation en conditions naturelles des risques de flux d'un transgène d'un colza (*Brassica napus* L.) résistant à un herbicide à une espèce adventice (*Raphanus raphanistrum* L.). Thèse de doctorat, Université de Paris-Sud U.F.R. Scientifique d'Orsay no. 3668, 76–90.

Baranger A., Kerlan M. C., Chèvre A. M., Eber F., Vallée P. and Renard M. 1995b. Transgenic oilseed rape. How to assess the risk of outcrossing to wild relatives. In: Gartland K. M. A. and Davey M. R. (eds.). Methods in Molecular Biology, Vol. 44: Agrobacterium Protocols. Humana Press Inc., Totowa, NJ: 393–403.

Baranger A., Chèvre A. M., Eber F. and Renard M. 1995c. Effect of oilseed rape genotype on the spontaneous hybridization rate with a weedy species: an assessment of transgene dispersal. Theor. Appl. Genet. 91: 956–963.

Bing D. J., Downey R. K. and Rakow G. F. W. 1991. Potential of gene transfer among oilseed Brassica and their weedy relatives. Proceedings of the GCIRC VIIIth International Rapeseed Congress, Saskatoon, Canada 4: 1022–1027.

Brown S. C., Bergounioux C., Tallet S. and Marie D. 1991. Flow cytometry of nuclei for ploidy and cell cycle analysis. In: Negrutiu I. and Gharti-Chhetri G. (eds.). A laboratory guide for cellular and molecular plant biology. Birkhäuser, Basel, Boston, Berlin, 327–345.

Bundesamt für Statistik 1992. Eidgenössische Landwirtschafts- und Gartenbauzählung 1990. Statistische Resultate Bd. 1: Landwirtschaftsbetriebe nach Kantonen. Amtliche Statistik der Schweiz, Nr. 355. Bundesamt für Statistik, Bern: 29.

Chan K. F. and Sun M. 1997. Genetic diversity and relationships detected by isozyme and RAPD analysis of crop and wild species of *Amaranthus*. Theor. Appl. Genet. 95: 865–873.

Chèvre A. M., Renard M., Eber F., Vallée P., Deschamps M. and Kerlan M. C. 1992. Study of spontaneous hybridization between male-sterile rapeseed and weeds. Poster abstract, 13th EUCARPIA Congress on Reproductive Biology and Plant Breeding, 6–11 July 1992, 67–68.

Chèvre A. M., Eber F., Baranger A., Kerlan M. C., Barret P., Festoc G., Vallée P. and Renard M. 1996. Interspecific gene flow as a component of risk assessment for transgenic *Brassicas*. Acta Horticulturae 407: 169–179.

Chèvre A. M., Eber F., Baranger A. and Renard M. 1997. Gene flow from transgenic crops. Nature 389: 924.

Chèvre A. M., Eber F., Baranger A., Hureau G., Barret P., Picault H. and Renard M. 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.

Crawley M. J., Hails R. S., Rees M., Kohn D. and Buxton J. 1993. Ecology of transgenic oilseed rape in natural habitats. Nature 363: 620–623.
Darmency H., Fleury A. and Lefol E. 1995. Effect of transgenic release on weed biodiversity: oilseed rape and wild radish. Proc. Brighton Crop Prot. Conf. on Weeds, 433–438.
Darmency H., Lefol E. and Fleury A. 1998. Spontaneous hybridizations between oilseed rape and wild radish. Mol. Ecol. 7: 1467–1473.
Eber F., Chèvre A. M., Baranger A., Vallée P., Tanguy X. and Renard M. 1994. Spontaneous hybridization between a male-sterile oilseed rape and two weeds. Theor. Appl. Genet. 88: 362–368.
Ellstrand N. C. and Marshall D. L. 1985. Interpopulation gene flow by pollen in wild radish, *Raphanus sativus*. Am. Natural. 126: 606–616.
Excoffier L., Smouse P. E. and Quattro J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491.
Geisler G. 1988. Pflanzenbau: Ein Lehrbuch; Biologische Grundlagen und Technik der Pflanzenproduktion. 2. Aufl., Parey, Berlin, Hamburg, 333–345.
Gower J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 325–338.
Guadagnuolo R. 2000. Gene flow in selected crops and related weeds. Risk assessment for the field release of GMOs: case of wheat (*Triticum aestivum* L.) in Northern and Middle Europe. Ph.D. Thesis, University of Neuchâtel, Switzerland.
Hegi G. 1986. Illustrierte Flora von Mitteleuropa. Bd. IV, Teil 1, Verlag Paul Parey, Berlin und Hamburg, 459–464, 499–508.
Hess H. E., Landolt E. und Hirzel R. 1970. Flora der Schweiz und angrenzender Gebiete. Bd. 2. Birkhäuser Verlag, Basel und Stuttgart, 179–187.
Kercher S. and Conner J. K., 1996. Patterns of genetic variability within and among populations of wild radish, *Raphanus raphanistrum* (Brassicaceae). Am. J. Bot. 83: 1416–1421.
Lee T. N. and Snow A. A. 1998. Pollinator preferences and the persistence of crop genes in wild radish populations (*Raphanus raphanistrum*, Brassicaceae). Am. J. Bot. 85: 333–339.
Lefol E., Danielou V., Darmency H., Kerlan M. C., Vallée P., Chèvre A. M., Renard M. and Reboud X. 1991. Escape of engineered genes from rapeseed to wild Brassiceae. Proc. Brighton Crop Prot. Conf. on Weeds, 1049–1056.
Loveless M.D. and Hamrick J.L. 1984. Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Syst. 15: 65–95.
Mantel N. 1967. The detection of disease clustering and a generalized regression approach. Canc. Res. 27: 209–220.
Metz P. L. J., Jacobsen E. and Stiekema W. J. 1997. Aspects of the biosafety of transgenic oilseed rape (*Brassica napus* L.). Acta Bot. Neerl. 46: 51–67.
Rice W. R. 1989. Analyzing tables of statistical tests. Evolution 43: 223–225.
Rissler J. and Mellon M. 1996. The ecological risks of engineered crops. The MIT press, Cambridge, Massachusetts, London, England, 22–70.
Rufener Al Mazyad P. 1998. Gene flow between Swiss crops and related weeds. Morphological character analysis and biogeographical assay for the risk assessment of futur transgenic crops. *Medicago sativa* L. (alfalfa) and *Medicago falcata* L. (sickle medic); *Brassica napus* L. (oilseed rape) and *Brassica rapa* L. ssp. *campestris* Clapham (wild rape). Ph.D. Thesis, University of Neuchâtel, Switzerland.
Savova Bianchi D. 1996. Evaluation of gene flow between crops and related weeds: risk assessment for releasing transgenic barley (*Hordeum vulgare* L.) and alfalfa (*Medicago sativa* L.) in Switzerland. Ph.D. Thesis, University of Neuchâtel, Switzerland.
Scheffler J. A. and Dale P. J. 1994. Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species. Transgenic Res. 3: 263–278.
Van Tongeren O. F. R. 1995. Cluster analysis. In: Ter Braak C. J. F., Van Tongeren O. F. R. and Jongman R. H. Data Analysis in Community and Landscape Ecology. Cambridge University Press, Cambridge, New York, Port Chester, Melbourne, Sydney.

U N. 1934. Genome-analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jap. J. Bot. 7: 389–452.
Williams J. G. K., Kubelik A. R., Livak K. J., Rafalski J. A. and Tingey S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531–6535.

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