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Destiny of a transgene escape from *Brassica napus* into *Brassica rapa*

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Abstract Transgenic *Brassica napus* can be easily crossed with wild *Brassica rapa*. The spread of the transgene to wild species has aroused the general concern about its effect on ecological and agricultural systems. This paper was designated, by means of population genetics, to study the fate of a transgene escape from *B. napus* to *B. rapa*. Three models were proposed to survey the change in gene frequency during successive backcross processes by considering selection pressures against aneuploids, against herbicide-susceptible individuals, and by considering A-C intergenomic recombination and the effect of genetic drift. The transmission rate of an A-chromosome gene through an individual to the next generation was 50%, irrespective of the chromosome number; while that of a C-chromosome transgene varied from 8.7% to 39.9%, depending on the chromosome number of the individual used in the backcross. Without spraying herbicide, the frequency of an A-chromosome gene was 50% in the BC_1 generation, and decreased by 50% with the advance of each backcross generation; that of a C-chromosome gene was around 39.9% in BC_1, 7.7% in BC_2, 1.2% in BC_3 and 0.1% in the BC_4 generation. Under the selection pressure against herbicide-susceptible individuals, the frequency of a transgene reached a stable value of about 5.5% within six generations of successive backcrossings. The effect of genetic drift and intergenomic exchange on gene transmission rate was discussed. It is suggested that the transgene integrated on a C-chromosome (or better on a cytoplasm genome) is safer than that on an A-chromosome. The transgenic cultivars should be cultivated rotationally by year(s) with other non-transgenic varieties in order to reduce the transfer of the transgene to wild *B. rapa* species.

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Introduction

Oilseed rape (*Brassica napus*) is one of the most-important oil-producing crops in the world. Infestation of the weedy species in rapeseed fields causes heavy loss of the seed yield and reduction of the extracted oil and meal (Rose and Bell 1982; McMullan et al. 1994). The requirements for more-economic and successful weed control in a rapeseed field and the advance of transformation technology have brought the transgenic *B. napus* to be tolerant to the herbicide glyphosate and glufosinate ammonium (Oelck et al. 1995). However, such transgenic varieties have raised general concern about their possible adverse impact on the agricultural and ecological systems (Raybould and Gray 1993; Scheffler and Dale 1994). Such a transgene(s) might further escape into weedy relatives of the crop species, such as weedy *Brassica rapa*, by cross-pollination in fields where the crop and weedy species exist in close proximity, resulting in the formation of weedy forms resistant to the same herbicides. Studies have revealed that *B. napus* and *B. rapa* hybridize readily (Jørgensen and Anderson 1994; Bing et al. 1996) and that the interspecific hybrids can backcross with *B. rapa* under field conditions (Jørgensen et al. 1996). According to genetic studies, using isozymes, random amplified polymorphic DNA (RAPD), markers and transgenes, the introgression of genes from *B. napus* into its diploid wild relative *B. rapa* is considered to be inevitable (Jørgensen and Anderson 1994; Mikkelsen et al. 1996). In evaluating the risk of a transgene escape from *B. napus* to *B. rapa*, most of the studies have been based on the data collected from F_1 and BC_1 generations, or based on very limited samples of a population. Very few studies have been performed to survey the fate of a transgene through more than three generations after the interspecific crossing event. As we show in this paper, it is very difficult to estimate the gene frequency or gene

transmission rate in BC_2 and advanced generations by means of molecular investigations like RAPD markers, due to the complexity of backcross generations in their chromosome number.

In order to assess the fate of a transgene escape from *B. napus* into wild *B. rapa*, an approach from the viewpoint of population genetics is the most plausible. The key point to make such an approach is how to estimate the fitness of the individuals with a specific chromosome number in the interspecific progenies.

In a previous paper (Lu and Kato 2001), we defined two parameters, that is fertilization fitness and relative fitness, to describe the competitive ability of gametes, and demonstrated that the fitness of gametes in interspecific progeny between *B. napus* and *B. rapa* was predominantly determined by the chromosome number and modified to a minor extent by genes, such as those for pollen fertility and seed fertility, which influence the intensity of competition among fertilized ovules in a pod. The frequency distribution of the chromosome numbers in the meiotic MII stage of the sesquidiploids was in good agreement with the binomial distribution. Gametes with different chromosome numbers from n = 10 to n = 19 showed no significant difference in competitive ability before fertilization but they differed conspicuously in survival rate after fertilization. By using relative fitness, we predicted the frequencies of the chromosome numbers in the progeny of interspecific hybridization between *B. napus* and *B. rapa* and showed good agreement with the published results from actual observation. In this study, we use the relative fitness of gametes estimated in the previous paper to analyze the fate of a transgene integrated in the A- and in the C-genome, respectively, based on the principle of population genetics.

Materials and methods

The transmission process of a transgene from *B. napus* (2n = 38, AACC) to *B. rapa* (2n = 20, AA) was monitored in this study. The target population was initiated as a hybrid (2n = 29, AAC) between a *B. rapa* and a transgenic herbicide-resistant *B. napus* line. The hybrid population was backcrossed repeatedly onto *B. rapa* to produce BC_1 to BC_6 generations. The frequencies of the chromosome numbers and the transgene were computed for all the resultant backcross progenies and generations. Without selection pressure, the chromosomes and the genes were supposed to act in a Mendelian manner and be described by a binomial distribution. Under selection pressures, the frequency of chromosome numbers and the gene were treated through the principle of population genetics. Several factors influencing the gene frequency were considered in the present study: (1) selection pressure due to variation of the chromosome number; (2) selection pressure due to application of the herbicide; (3) genetic drift due to small sample size; and (4) intergenomic recombination. It is assumed through the entire paper that no mutation and no migration are involved in the backcross process. Since the main fitness effect in the introgression process of an unselected gene from *B. napus* into *B. rapa* is the number of C-chromosomes (Lu and Kato 2001), and the present studies are based on population means instead of individual data, the effects of genomic structure and of different C-chromosomes are assumed to be negligible.

Three models were used in the present study.

Model I

Model I provides a theoretical situation, in which no selection pressure is involved. All the gametes and individuals in the backcross process have same possibility to survive and to produce offspring through a normal sexual process. In this case, the frequency distribution of chromosome numbers in the progeny from the cross between an individual with 2n = y (2n = 20 to 29) and *B. rapa* follows the binomial distribution. The frequency of the chromosome number 2n = x in the backcross progeny from the cross between an individual with 2n = y (2n = 20 to 29) and *B. rapa* was computed by using the formula:

$$T_{x/y} = {}_rC_m(1/2)^r \times 100,$$

in which $T_{x/y}$ is the binomial frequency of the progeny with the chromosome number 2n = x derived from the individuals with 2n = y; r = the number of the C-chromosomes harbored by the individual used for the backcross parent, and m = number of C-chromosomes harbored by its backcross progeny with a chromosome number 2n = x. For example, $T_{22/27} = {}_7C_2\ (1/2)^7 \times 100 = (7 \times 6)/(2 \times 1) \times (1/2)^7 \times 100 = 16.4$; that is to say, 16.4% of the progeny will be 2n = 22. In the F_1 generation, all the individuals are supposed to be 2n = 29. Therefore, $T_{i/BC_1} = T_{i/29}$ (T_{i/BC_1} in Table 1).

In other backcross generations (BC_n), the individual with the chromosome number 2n = x is derived from individuals with various chromosome numbers from 2n = x to 29 of the BC_{n-1} generation. The frequency of the individuals with the chromosome number 2n = x in the BC_n generation (T_{x/BC_n} in Table 1) was assigned as T_{x/BC_n}:

$$T_{x/BCn} = \sum_{i=x}^{29} T_{i/BCn-1} T_{x/i},$$

in which $T_{i/BC_{n-1}}$ is the frequency of the individuals with the chromosome number 2n = i (i = x to 29) in the last generation(BC_{n-1}); $T_{x/i}$ is the binomial frequency of the individuals with the chromosome number 2n = x derived from the individuals with 2n = i (i = x to 29).

Model II

Model II simulates an actual situation, in which selection pressure is involved against aneuploid gametes in the backcross process. The survival rate of gametes with a specific chromosome number is determined by the relative fitness (Lu and Kato 2001). The relative fitness of gametes with a specific chromosome number was estimated in the previous paper by using the sesquidiploid AAC-N, which was a hybrid between natural *B. napus* cv Lisandra (2n = 38, AACC) and *B. rapa* cv Osaka-Shirona (2n = 20, AA) (Lu and Kato 2001). Since no significant difference was found in the relative fitness between male and female gametes (Lu and Kato 2001), the mean relative fitness of the male and female gametes was used in this paper in order to reduce random error. The relative fitness (rFi) of the backcross progeny used in the present study is 100% for the chromosome number 2n = 20, 9.5% for 2n = 21, 1.8% for 2n = 22, 1.0% for 2n = 23, 0.5% for 2n = 24 and 2n = 25, 0.6% for 2n = 26, 1.3% for 2n = 27, 4.2% for 2n = 28 and 33.5% for 2n = 29, corresponding to the gamete chromosome numbers from n = 10 to n = 19 in the previous paper (Lu and Kato 2001).

The frequency distribution of chromosome numbers in progeny of the cross between an individual with 2n = y and *B. rapa* was computed according to the principle of population genetics (Hartl and Clark 1997). The frequency of the progeny with 2n = x derived from the individuals with 2n = y was assigned as $Q_{x/y}$:

$$Q_{x/y} = T_{x/y} rF_x / \sum_{i=20}^{29} T_{i/y} rF_i,$$

in which $T_{x/y}$ is the binomial frequency of the progeny with the chromosome number 2n = x derived from the individuals with 2n = y; rF_x is the relative fitness of the individuals with the chromo-

some number 2n = x; $T_{i/y}$ is the binomial frequency of the progeny with the chromosome number 2n = i (i = 20 to 29) derived from the individuals with 2n = y; rF_i is the relative fitness of individuals with the chromosome number 2n = i (i = 20 to 29). In the BC_1 generation which is derived from F_1 individuals (2n = 29), the frequency (Q_{x/BC_1}) of individuals with 2n = x was computed by $Q_{x/BC_1} = Q_{x/29}$ (see Table 1).

The frequency distribution of chromosome numbers in other backcross generations from BC_2 to BC_n (Q_{x/BC_n} in Table 1) was computed by using the formula:

$$Q_{x/BC_n} = \sum_{i=x}^{29} Q_{x/BC_{n-1}} Q_{x/i},$$

in which Q_{x/BC_n} is the frequency of the chromosome number 2n = x in the BC_n generation, $Q_{x/BC_{n-1}}$ is the frequency of the individuals with the chromosome number 2n = i (i = x to 29) in the last generation (BC_{n-1}); $Q_{x/i}$ is the frequency of the individuals with the chromosome number 2n = x derived from the individuals with 2n = i (i = x to 29).

When a transgenic individual with the chromosome number 2n = y is crossed with *B. rapa*, the transmission rate of the transgene is dependent upon its location: on an A-chromosome or on a C-chromosome (Table 4).

An A-chromosome transgene acts as a general Mendelian gene. An A-chromosome transgene is always transmitted to half of the progeny. The frequency ($A_{x/y}$) of an A-chromosome transgene in the individuals with 2n = x derived from the transgenic individuals with 2n = y (Table 4) was computed by using:

$$A_{x/y} = Q_{x/y}/2.$$

In the BC_n generation, the frequency of an A transgene in the individuals with 2n = x (Table 2) was calculated by using

$$A_{x/BC_n} = Q_{x/BC_n}/2.$$

A C-chromosome gene is transmitted with one of the C-chromosomes. In an unselected backcross progeny (BC_1–BC_n), no individual with 2n = 20 should be transgenic without intergenomic recombination and all the individuals with 2n = 29 should be transgenic. The more C-chromosomes (x – 20) does the progeny harbour, the more probable it is transgenic. In the BC_n generation, the frequency of a transgene in individuals with 2n = x (Table 2) was computed by using:

$$C_{x/BC_n} = Q_{x/BC_n}(x-20)/9,$$

and the probability ($C_{x/y}$) of the transgene in the progeny with 2n = x derived from transgenic individuals with 2n = y can be computed by using the formula (Table 4):

$$C_{x/y} = Q_{x/y}(x-20)/(y-20).$$

The effect of sampling size on the observed gene frequency in the BC_1 population (Table 5) was assessed by using the formula:

$$\sigma_p = [p(1-p)/N]^{1/2},$$

in which σ_p is the standard deviation of gene frequency, or the square root of variance of binomial distribution, *N*, is the sampling size (individual numbers) and *p* is the expected gene frequency. A 95% confidence interval of the gene frequency was estimated by $L_1 = p - 1.96\sigma_p$ and $L_2 = p + 1.96\sigma_p$.

Model III

Model III was designated to simulate the situation where herbicide is applied to kill all the non-transgenic plants and selection pressure was against aneuploids (Table 3). In this model, the relative fitness of the non-transgenic plants is given zero. For the transgenic plants, the relative fitness is the same as in Model II.

When intergenomic recombination is involved (see the column of frequency[b] in Tables 2 and 3), the frequency of a C-chromosome gene was calculated by using the formula:

$$Pr/c = rPa + (1-r)Pc,$$

and the frequency of an A-chromosome gene was calculated by using the formula:

$$Pr/a = rPc + (1-r)Pa,$$

where r is the A-C intergenomic recombination rate of the transgene; Pa and Pc are the frequency of the transgene on the A-genome and on the C-genome, respectively, when intergenomic recombination is not involved.

Results

Interspecific hybrids (F_1) between *B. rapa* (2n = 20, AA) and *B. napus* (2n = 38, AACC) proved to be sesquidiploids (AAC, 2n = 29), and the BC_1 progeny derived from the cross between the sesquidiploids and *B. rapa* had varying numbers of C-chromosomes (AA + 0–9 C) (Lu and Kato 2001). The frequency distribution of the chromosome number changes with the successive backcrossing process. The frequency of a transgene changes with the chromosome number and depends on its location either on an A-chromosome or on a C-chromosome.

Frequency distribution of chromosome numbers and a transgene in various backcross generations

Table 1 shows the frequency distribution of chromosome numbers in various backcross generations from BC_1 to BC_4. A significant difference was found between Model I and Model II in the frequency distribution and in the mean of chromosome numbers. With the advance of backcrossing, the frequency distribution of chromosome numbers changes toward 2n = 20. The mean chromosome number for a generation decreased more rapidly under selection pressure against aneuploids (Model II) than without the selection pressure (Model I).

Table 2 shows the frequency distribution of a transgene in various backcross generations based on Model II.

For an A-chromosome transgene (A-transgene), the gene frequency is 50% in BC_1, 25% in BC_2, 12.5% in BC_3 and 6.3% in BC_4, being reduced by a half with the advance of each backcross generation. The transgene is distributed among individuals with different chromosome numbers. In the BC_1 generation, the A-transgene was distributed quite evenly among the individuals with chromosome numbers from 2n = 20 to 29. About 70% of the transgene in BC_2, 93% in BC_3 and 99% in the BC_4 generation appeared in individuals with 2n = 20. For a C-chromosome transgene (C-transgene), the gene frequency is 39.9% in BC_1, 7.7% in BC_2, 1.2% in BC_3 and 0.1% in the BC_4 generation. No C-transgene is present on individuals with the chromosome number 2n = 20 if no intergenomic exchange occurs. In the BC_1 generation, the C-transgene was present at a similar frequency of

Table 1 Frequency distribution of chromosome numbers in various backcross generations

Type of frequency[a]	Generation	Chromosome number										Generation mean (2n)
		20	21	22	23	24	25	26	27	28	29	
$T_{x/BCn}$	BC_1	0.2	1.8	7.0	16.4	24.6	24.6	16.4	7.0	1.8	0.2	24.5
	BC_2	7.5	22.5	30.0	23.3	11.7	3.9	0.9	0.1	0.0	0.0	22.2
	BC_3	30.1	38.6	22.1	7.4	1.6	0.2	0.0	0.0	0.0	0.0	21.1
	BC_4	60.3	29.3	9.0	1.4	0.1	0.0	0.0	0.0	0.0	0.0	20.5
$Q_{x/BCn}$	BC_1	16.1	13.9	10.4	13.6	9.8	9.1	8.3	7.5	6.1	5.4	23.6
	BC_2	69.4	14.9	5.7	4.4	2.0	1.4	0.9	0.6	0.4	0.3	20.7
	BC_3	93.3	4.7	1.0	0.6	0.2	0.1	0.1	0.0	0.0	0.0	20.1
	BC_4	99.1	0.8	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0

[a] $T_{x/BCn}$: frequency based on Model I; $Q_{x/BCn}$: frequency based Model II

Table 2 Frequency distributions of a transgene in various backcross generations, based on Model II

Gene location and (type of frequency)	Generation	Chromosome number (2n)										Transgene frequency[a]	Transgene frequency[b]
		20	21	22	23	24	25	26	27	28	29		
A-genome (A_{x/BC_n})	BC_1	8.1	6.9	5.2	6.8	4.9	4.6	4.2	3.7	3.0	2.7	50.0	49.0
	BC_2	17.4	3.7	1.4	1.1	0.5	0.3	0.2	0.2	0.1	0.1	25.0	23.3
	BC_3	11.7	0.6	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	12.5	11.4
	BC_4	6.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.3	5.6
C-genome (C_{x/BC_n})	BC_1	0.0	1.5	2.3	4.5	4.4	5.1	5.5	5.8	5.4	5.4	39.9	40.9
	BC_2	0.0	1.7	1.3	1.5	0.9	0.8	0.6	0.5	0.3	0.3	7.7	9.5
	BC_3	0.0	0.5	0.2	0.2	0.1	0.1	0.0	0.0	0.0	0.0	1.2	2.3
	BC_4	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.7

[a] No intergenomic recombination with the transgene is involved
[b] When the intergenomic recombination rate of the transgene is 10%

Table 3 Frequency distribution of a C-chromosome transgene under the selection pressure against herbicide-susceptible individuals and against aneuploids (Model III)

Generation	Chromosome number										Transgene frequency[a]	Transgene frequency[b]
	20	21	22	23	24	25	26	27	28	29		
BC_1	0.0	3.9	5.8	11.4	10.9	12.7	13.9	14.5	13.5	13.5	39.9	40.9
BC_2	0.0	16.0	14.5	18.8	12.5	11.2	9.0	7.5	5.7	4.9	14.9	18.4
BC_3	0.0	26.2	17.4	18.4	10.6	8.7	6.5	5.2	3.8	3.2	8.4	12.5
BC_4	0.0	31.5	17.8	17.4	9.6	7.7	5.6	4.5	3.3	2.7	6.4	10.7
BC_5	0.0	33.6	17.7	16.9	9.2	7.3	5.4	4.2	3.1	2.5	5.7	10.1
BC_6	0.0	34.3	17.6	16.7	9.1	7.2	5.3	4.2	3.0	2.5	5.5	9.9

[a] No intergenomic recombination with the transgene is involved
[b] When the intergenomic recombination rate of the transgene is 10%

about 5% in individuals with chromosome numbers from 2n = 23 to 2n = 29. About 21% (1.7/7.7) of the transgene in BC_2, 44% (0.5/1.2) in BC_3 and 69% (0.9/0.13) in the BC_4 generation appeared in individuals with 2n = 21. If A-C intergenomic recombination is involved, the transgene frequency in a population may increase a little in each generation (Table 2).

Table 3 shows the frequency distribution of a C-chromosome transgene in various backcross generations under the condition of Model III, in which the selection pressures against aneuploids and against herbicide-susceptible genotypes were involved. Results showed that the frequency of a transgene became stabilized at about 5.5%, and the frequency distribution of a transgene also became constant within six generations of backcrossing onto *B. rapa*. Compared with the result based on Model II where no herbicide is applied, more aneuploid individuals were maintained in the population due to the application of the herbicide. The consequence of A-C intergenomic recombination is more conspicuous in Model III than in Model II (Table 3 vs Table 2).

Table 4 Transmission rate of a gene through individuals with various chromosome numbers under selection pressure against aneuploids (based on Model II)

Gene location and (type of frequency)	Parent (2n)	Backcross progeny (2n)										Transmision rate
		20	21	22	23	24	25	26	27	28	29	
A-genome ($A_{x/y}$)	29	8.1	6.9	5.2	6.8	4.9	4.6	4.2	3.7	3.0	2.7	50.0
	28	13.0	10.0	6.8	7.8	4.6	3.7	2.3	1.4	0.6		50.0
	27	18.1	12.1	7.1	6.8	3.2	1.9	0.8	0.3			50.0
	26	23.0	13.2	6.4	4.9	1.8	0.7	0.2				50.0
	25	27.8	13.3	5.2	3.0	0.7	0.2					50.0
	24	32.5	12.4	3.6	1.4	0.2						50.0
	23	37.0	10.6	2.1	0.4							50.0
	22	41.4	7.9	0.8								50.0
	21	45.6	4.4									50.0
	20	50.0										50.0
C-genome ($C_{x/y}$)	29	0.0	1.5	2.3	4.5	4.4	5.1	5.5	5.8	5.4	5.4	39.9
	28	0.0	2.5	3.4	5.9	4.6	4.6	3.4	2.4	1.1		27.7
	27	0.0	3.5	4.0	5.8	3.7	2.7	1.4	0.5			21.5
	26	0.0	4.4	4.3	4.9	2.3	1.2	0.3				17.4
	25	0.0	5.3	4.1	3.5	1.1	0.3					14.4
	24	0.0	6.2	3.6	2.1	0.3						12.2
	23	0.0	7.1	2.7	0.8							10.6
	22	0.0	7.9	1.5								9.4
	21	0.0	8.7									8.7

Table 5 Sampling size and the 95% confidence interval of the gene frequency in the BC_1 generation of transgenic *B. napus* onto *B. rapa*

[a] The expected frequency *p* is 50% for an A-chromosome gene and 39.9% for a C-chromosome gene

Gene location	Confidence interval[a]	Sampling size (individuals)									
		20	40	60	80	100	120	140	160	180	200
A genome	L_1	28.1	34.5	37.3	39.0	40.2	41.1	41.7	42.3	42.7	43.1
	L_2	71.9	65.5	62.7	61.0	59.8	58.9	58.3	57.7	57.3	56.9
C genome	L_1	18.4	24.7	27.5	29.2	30.3	31.1	31.8	32.3	32.8	33.1
	L_2	61.6	55.2	52.4	50.7	49.6	48.8	48.1	47.6	47.1	46.8

Transmission rate of a transgene through individuals with various chromosome numbers under the condition of Model II

Table 4 shows the transmission rate of a transgene through individuals with various chromosome numbers under the condition of Model II. For an A-chromosome gene, the transmission rate was 50%, irrespective of the chromosome number. For a C-chromosome gene, the transmission rate varied according to the chromosome number of the individuals used in the backcross. The more chromosomes the parental plant harbors, the higher the transmission rate of the transgene was to the next generation. The transmission rate of a C-chromosome gene was the highest (39.9%) when the transgenic individuals had the chromosome number 2n = 29. Individuals with 2n = 21 to 2n = 23 passed their gene to the next generation at a rate of about 10%.

Effect of sampling size on the observed gene frequency in the BC_1 generation

The expected frequency of an A-chromosome gene is 50% and that of a C-chromosome gene is 39.9% in the BC_1 generation. A small sample may lead to a significant deviation in the observed frequency from the expected one (Table 5). With a confidence probability of 95%, and a sample of 100 individuals, the confidence interval of the frequency was 40.2–59.8% for an A-chromosome gene and 30.3–49.6% for a C-chromosome gene. Even with a sample of 200 individuals, partial overlapping occurs between the interval of an A-chromosome gene and that of a C-chromosome gene (Table 5), indicating that it is not sound to distinguish whether a gene is on an A- or a C-chromosome based on the observed frequency of a small sample.

Discussion

In order to evaluate whether a transgene is likely to introgress into wild species, one can follow the transmission of genetic markers in controlled crosses or field experiments with the species in question (Jørgensen and Anderson 1994; Mikkelsen et al. 1996). This method is valid for the BC_1 generation but is not efficient for the BC_2 and advanced backcross generations. This is because the BC_1 population consists of individuals with chromosome numbers ranging from 2n = 20 to 2n = 29 (Lu and Kato 2001) and the transmission rate varies with the chromosme number of the BC_1 individuals used in the backcross (Table 4). Usually only a few BC_1 individuals have been used to produce a BC_2 generation in the

investigation of gene frequency by using gene markers. This may lead to genetic drift due to a small sampling size. McGrath and Quiros (1990) noticed that transmission of a transgene to the BC_2 generation was much more variable than to the BC_1 generation. A small sample may lead to significant variation in the observed gene frequency not only for the BC_2 and advanced generations but also for the BC_1 generation (Table 5). This is why it is difficult to estimate the actual gene frequency of a transgene in BC_3 and advanced generations by using gene markers in controlled crosses. In this connection, an approach based on population genetics is the most plausible.

The present studies showed that without selection pressure, genetic drift, mutation and immigration being involved, the frequency of a transgene is 50% in the BC_1 generation and is reduced by 50% with the advance of each backcross generation wherever the gene is located on an A-chromosome or on a C-chromosome. Under selection pressure against aneuploids, the frequency of an A-chromosome gene did not change and the transmission rate of an A-chromosome transgene was 50%, irrespective of the chromosome number. Just like a general Mendelian gene, a transgene present on an A-chromosome may be transferred into *B. rapa* within two generations. However, the transmission rate of a C-chromosome gene through an individual was reduced and varied with the chromosome number from about 9% to about 40%. The transgene frequency in the BC_1 generation was about 40% and reduced rapidly to about 1% within three generations (Table 2). When herbicide is used, the frequency of a transgene became stabilized at about 6% in a population within about four or five generations (Table 3). McGrath and Quiros (1990) investigated the transmission rates of C-chromosomes in the same type of backcross, using diagnostic C-chromosome markers (RFLPs and isozymes). They found that a mean C-chromosome transmission rate to the BC_1 generation was 32.1% (27 BC_1 individuals analyzed), with transmission of a single chromosome ranging between 23.1 and 42.3%. In a comparative study with two transgenic herbicide-tolerant varieties of oilseed rape, Metz et al. (1997) investigated the frequency of a transgene in the BC_1–BC_4 generations under application of herbicide. They found that the frequency of the C-chromosome transgenic plants in the BC_1 generation was 26% and 36%, and that of the A-chromosome transgenic plants was 46% and 54%. The frequency of the herbicide-resistant individuals was 5% in BC_2, 11% in BC_3 and 9% in BC_4 (Metz et al. 1997). Based on their results, they concluded that a C-chromosome gene is transmitted in a relatively lower frequency and diminishes more rapidly in subsequent backcross generations than an A-chromosome gene, in good agreement with our conclusion. Regarding the frequency of a transgene in the BC_2 and BC_4 generations, Metz et al. (1997) concluded that the transmission of a C-chromosome gene is stabilized at about 10% due to inter-genomic recombination between the A- and C-genomes, chromosome substitutions, or disomic chromosome additions. Tomiuk et al. (2000) argued that the result observed by Metz et al. (1997) can also be explained by selection against the transgenic A-chromosomes of *B. napus* during the backcross process. According to the present study, the frequency of 10% in advanced backcross generations observed by Metz et al. (1997) may be derived from two possibilities: one is due to the crosses between transgenic individuals with 2n = 21 to 2n = 23 and *B. rapa* (Table 4), because Metz et al. (1997) produced BC_2, BC_3 and BC_4 populations by using only 1–4 herbicide-resistant individual plants, and the transmission rate of a gene through individuals with 2n = 21–23 ranges from 8.7–10.6% (Table 4). Another possibility may be due to the balance between two selection pressures against aneuploids and against herbicide-susceptible individuals (Model III), and due to A-C intergenomic recombination (Table 3). The result observed by Metz et al. (1997) in BC_2, BC_3 and BC_4 can not be explained by selection against the transgenic A-chromosomes of *B. napus* during the backcross process (Tomiuk et al. 2000), because the fitness of an individual in the backcross progeny is principally determined by the chromosome number rather than by a gene(s) (Lu and Kato 2001).

Most of the authors explained the reduced transmission rate of a C-chromosome in the BC_1 generation by conjecture at the loss of C-chromosome(s) during meiosis (McGrath and Quiros 1990; Metz et al. 1997; Tomiuk et al. 2000) and pollen certation (Maykay 1977) in the sesquidiploids. We demonstrated, however, that it was selection pressure against aneuploids during the embryo developmental stage that resulted in reduced transmission of the C-chromosomes (Lu and Kato 2001).

The results indicated that integration of a transgene on a chromosome of the C-genome (or better on cytoplasm genomes) is safer than integration on an A-chromosome, when ecological risk is considered. For a C-chromosome transgene, the frequency changes from 39.9% in BC_1, through 7.7% in BC_2, 1.2% in BC_3 to 0.1% in the BC_4 generation, while for an A-chromosome transgene, the frequency is 50% in BC_1, 25% in BC_2, 12.3% in BC_3 and 6.1 in BC_4. Without repeated immigration of the transgene from transgenic *B. napus* to wild *B. rapa* and without favorable selection for the transgene, such as the application of herbicides, the transgene will diminish from the population within several generations. If a measure is taken to cultivate the herbicide-resistant varieties rotationally by year(s) with other type of varieties, the risk of transmission of the transgene to *B. rapa* may be largely reduced.

In a natural population, the transmission rate of a C-chromosome gene may increase due to exchange between the A and C genomes (Tables 2 and 3). Röbbelen (1960) found in a sesquidiploid population, which was derived from a re-synthesized *B. napus*, that about 50% of the cells contained two trivalents. Namai (1976) reported that the number of trivalent chromosomes in meiosis of the sesquidiploid (2n = 29, AAC) ranged from 0.13 to 0.72 per cell in each plant. Quiros et al. (1987)

observed intergenomic recombination for some of the markers in the progeny between a *B. rapa* and a re-synthesized *B. napus* line at frequencies between 6% and 20%. A gene closer to the centromere may show a lower recombination rate. In the assessment of ecological risk, most of the researchers have emphasized interspecific crossability and intergenomic recombination, but they have neglected the most important fact that almost all the intergenomic recombinants are aneuploids, because they harbour C-chromosomes, and individuals with a few C-chromosomes are much lower in fitness than *B. rapa*. Tables 2 and 3 demonstrate that even if intergenomic recombination occurs, the gene frequency in the interspecific progeny would not change too much within several generations. Intergenomic exchange does not mean the immediate introgression of the gene into *B. rapa*. In the long run, however, if a rapeseed variety with a herbicide-resistant gene, whether present on an A-chromosome or on a C-chromosome, is cultivated in areas where herbicide is constantly used, the introgression of the transgene from *B. napus* to wild *B. rapa* might be inevitable due to intergenomic recombination and gene accumulation.

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