PrBn, a Major Gene Controlling Homeologous Pairing in Oilseed Rape (*Brassica napus*) Haploids

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ABSTRACT

Precise control of chromosome pairing is vital for conferring meiotic, and hence reproductive, stability in sexually reproducing polyploids. Apart from the *Ph1* locus of wheat that suppresses homeologous pairing, little is known about the activity of genes that contribute to the cytological diploidization of allopolyploids. In oilseed rape (*Brassica napus*) haploids, the amount of chromosome pairing at metaphase I (MI) of meiosis varies depending on the varieties the haploids originate from. In this study, we combined a segregation analysis with a maximum-likelihood approach to demonstrate that this variation is genetically based and controlled mainly by a gene with a major effect. A total of 244 haploids were produced from F_1 hybrids between a high- and a low-pairing variety (at the haploid stage) and their meiotic behavior at MI was characterized. Likelihood-ratio statistics were used to demonstrate that the distribution of the number of univalents among these haploids was consistent with the segregation of a diallelic major gene, presumably in a background of polygenic variation. Our observations suggest that this gene, named *PrBn*, is different from *Ph1* and could thus provide complementary information on the meiotic stabilization of chromosome pairing in allopolyploid species.

POLYPLOIDY has played a major role in the evolution of higher plants. Recent estimates suggest that up to 70% of all angiosperms have experienced one or more episodes of polyploidization during the course of their evolution (Masterson 1994) or domestication (Hilu 1993; van Raamsdonk 1995). Nonetheless, most if not all polyploids behave as diploids at meiosis, indicating that precise control of chromosome pairing is a prerequisite and confers evolutionary advantages in polyploid species. In allopolyploids containing homeologous chromosomes with sufficient homology to be able to pair at meiosis, cytological diploidization requires homeologous pairing to be suppressed. This process can be achieved by two complementary systems: (i) differentiation of homeologous chromosomes due to either structural changes or gene mutations, which leads to differential affinity and preferential pairing of homologs, and (ii) a genetic control that distinguishes between the differentiated sets of chromosomes and precludes pairing between homeologues. Although the presence of genetic systems regulating pairing has been suspected in a wide range of polyploids [*e.g.*, cotton (Kimber 1961), oat (Gauthier and McGinnis 1968), and fescues (Jauhar 1975)], evidence to date has been circumstantial. It is only in wheat that the presence of pairing regulators has been indisputably demonstrated with the characterization of the *Ph1* locus (Riley and Chapman 1958; Sears and Okamoto 1958) that suppresses homeologous pairing and contributes to the karyotypic stability of wheat (Sánchez-Morán *et al.* 2001). Several other weaker loci have also been shown to either restrict or promote homeologous pairing (Riley and Law 1965). The question remains whether similar pairing regulators are widespread among polyploid species and have therefore a general evolutionary significance. Differences in meiotic pathways among closely related species (Shaw and Moore 1998; Cuñado and Santos 1999) and in strategies for bivalent formation in different allopolyploids (Jenkins and Rees 1991) indicate that the genetic mechanisms characterized in wheat are not the same as in other polyploid species. It is therefore necessary to explore new and complementary models to further understanding of polyploid meiotic diploidization. This issue has practical applications. A large number of successful alien introgressions have been achieved in wheat through homeologous recombination (Friebe *et al.* 1996) and, notably, by suppressing the control exerted by *Ph1* (*e.g.*, Riley *et al.* 1968; Luo *et al.* 1996 and references therein; Benavente *et al.* 2001). It may be anticipated that a better understanding of the genetic systems regulating homeologous pairing in other polyploid species could help in promoting and engineering introgressions.

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Oilseed rape (*Brassica napus*) is an allopolyploid species (AACC; $2n = 38$) that originated from hybridization between *B. oleracea* (CC; $2n = 18$) and *B. rapa* (AA; $2n = 20$). This species exhibits a clear bivalent-pairing regime and a disomic inheritance, which demonstrate that homologs pair at meiosis at the expense of homeologous pairing. The basis of such a diploid-like meiotic behavior is hypothetical. Different authors have proposed that homeologous pairing is genetically regulated in oilseed rape (Attia and Röbbelen 1986a; Sharpe *et al.* 1995) and its close relatives (Prakash 1974; Hardberg 1976; Eber *et al.* 1994). Renard and Dosba (1980) and Attia and Röbbelen (1986a) observed that the amount of chromosome pairing in haploid plants (AC; $n = 19$) originating from different oilseed rape varieties was variable and identified high- and low-pairing varieties (at the haploid stage). Chiasmata were formed between paired chromosomes and resulted in both rod- and ring-shaped bivalents, but also in multivalents.

The objectives of this study are to determine if a large part of the variation observed for the amount of chromosome pairing in oilseed rape haploids is genetically based and to establish the genetic basis of this variation. To do so, we analyzed the meiotic behavior of haploids produced from a high- and a low-pairing line and developed proper statistical analyses to account for the different sources of variation (genetic and environmental determinants). We studied the segregation of the meiotic behavior in a population of haploids produced from F_1 hybrids between the high- and low-pairing lines and used likelihood-ratio (LR) statistics to test for alternative modes of inheritance and interpret genetic distribution in terms of both major and minor gene effects.

MATERIALS AND METHODS

Plant materials: The genealogy and structure of the data sets are detailed in Figure 1. All haploids were isolated using microspore cultures as described by Polsoni *et al.* (1988). A total of 13 and 27 haploids were isolated from a spring Korean line (*Yudal*) and a French dwarf winter line (*Darmor-bzh*), which are known to vary in their meiotic behavior at the haploid stage (Figure 1). All the diploid lines used to produce these parental haploids (*Darmor-bzh* F_3 and F_4 progenies and *Yudal* F_9 and F_{13} progenies) were obtained by single-seed descent (SSD). These haploids comprise the parental data set. The parental genotypes differed in their response to produce haploids so that twice as many haploids were scored for *Darmor-bzh* as for *Yudal*. A total of 244 haploids were isolated from F_1 hybrids between *Darmor-bzh* and *Yudal* and comprise the offspring data set.

In an initial phase, three and seven haploids were produced from a few diploid plants from *Yudal* F_9 and *Darmor-bzh* F_3 progenies, respectively. A total of 45 haploids were isolated from F_1 hybrids obtained by crossing a single plant of the *Darmor-bzh* F_3 progeny to a single plant of the *Yudal* F_9 progeny (Figure 1). These parental and offspring haploids were grown together in the greenhouse and floral buds were sampled on almost the same date (three dates within 15 days); these haploids comprise the first set of observations (series 1). In a second phase, 10 and 20 haploids were isolated from *Yudal* F_{13} and *Darmor-bzh* F_4 progenies. A total of 199 haploids were isolated from a few F_1 hybrids obtained by crossing a single *Darmor-bzh* F_4 plant by a single *Yudal* F_{13} plant; three microspore cultures were needed to isolate all the haploids. Accordingly three series of haploids were successively grown in the greenhouse and analyzed separately (series 2–4 of observations). Only one set was grown simultaneously with parental haploids (series 3). For series 2 and 4, floral buds were sampled on three to four dates within 15 days. For series 3, floral buds were sampled on three dates within 1 month. For series 1 and 3, some haploids were observed at each date (or at least more than once) and showed the same meiotic behavior (data not shown). Sixteen other haploids were observed in 2 consecutive years to test for the repeatability of the amount of pairing. These haploids were chosen within series 2–4 to encompass the whole range of meiotic behaviors [$3.3 <$ no. of univalents < 10]. These haploids were conserved as cuttings.

Meiotic observations: Floral buds were fixed in Carnoy's solution (ethanol-chloroform-acetic acid, 6:3:1) for 24 hr and stored in 50% ethanol. Observations on the pollen mother cells (PMCs) were performed at the metaphase I (MI) stage from anthers squashed and stained in a drop of 1% acetocarmine solution. On average, 20 PMCs (minimum, 14; maximum, 149) were examined for each haploid, regardless of their origin.

Statistical analysis: Statistical analyses were performed mainly on the number of univalents. This variable was chosen because it can be reliably scored and because it measures the whole extent of pairing in a synthetic way, reflecting by subtraction the number of chromosomes associated as both bivalents and multivalents. Parental data were first analyzed on their own, to determine to what extent variation in the amount of pairing among *Darmor-bzh* and *Yudal* haploids was genotypically determined. On the basis of this preliminary analysis, the offspring and parental data were then analyzed simultaneously, so that parental and offspring distributions could be compared within a single model. In the models below, we denote by $Y_{g,lij}$ the number of univalents in the PMC j of haploid i observed in the series l from population g, where g refers to haploids produced from *Darmor-bzh* ($g = D$), *Yudal* ($g = Y$), or *Darmor-bzh* × *Yudal* F_1 hybrids ($g = H$).

Analysis of parental data: The model employed for each parental genotype was

$$Y_{g,lij} = \mu_g + \gamma_l + b_{g,li} + \varepsilon_{g,lij}, \quad (1)$$

where μ_g is the mean for population g (g is either genotype D or genotype Y), γ_l is the effect of series l ($l = 1$ or 3), $b_{g,li}$ is a random haploid plant effect, and $\varepsilon_{g,lij}$ is a residual error term. The $b_{g,li}$ and $\varepsilon_{g,lij}$ random effects were assumed to follow independent normal and centered distributions, with variances denoted by τ_g^2 for haploid effects and by σ_g^2 for residual errors. The parameter estimates in model (1) and their asymptotic standard errors were calculated by residual maximum likelihood (REML) with the PROC MIXED procedure of SAS (SAS Institute 1999). Note that for variance parameter estimates, the standard errors are known to be unreliable and should not be used to construct confidence intervals. The hypotheses on the absence of series effects ($\gamma_1 = \gamma_3 = 0$ *vs.* $\gamma_1 \neq \gamma_3$) and of haploid effects ($\tau_g^2 = 0$ *vs.* $\tau_g^2 > 0$) were tested by an analysis of variance performed with the PROC GLM procedure of SAS. The RANDOM statement of this procedure was used because the haploid random factor was nested within the series factor. On two occasions in results, we propose to quantify and compare the contributions to the variability between haploids that are due to the different factors of the models. For factors with random effects, contributions are

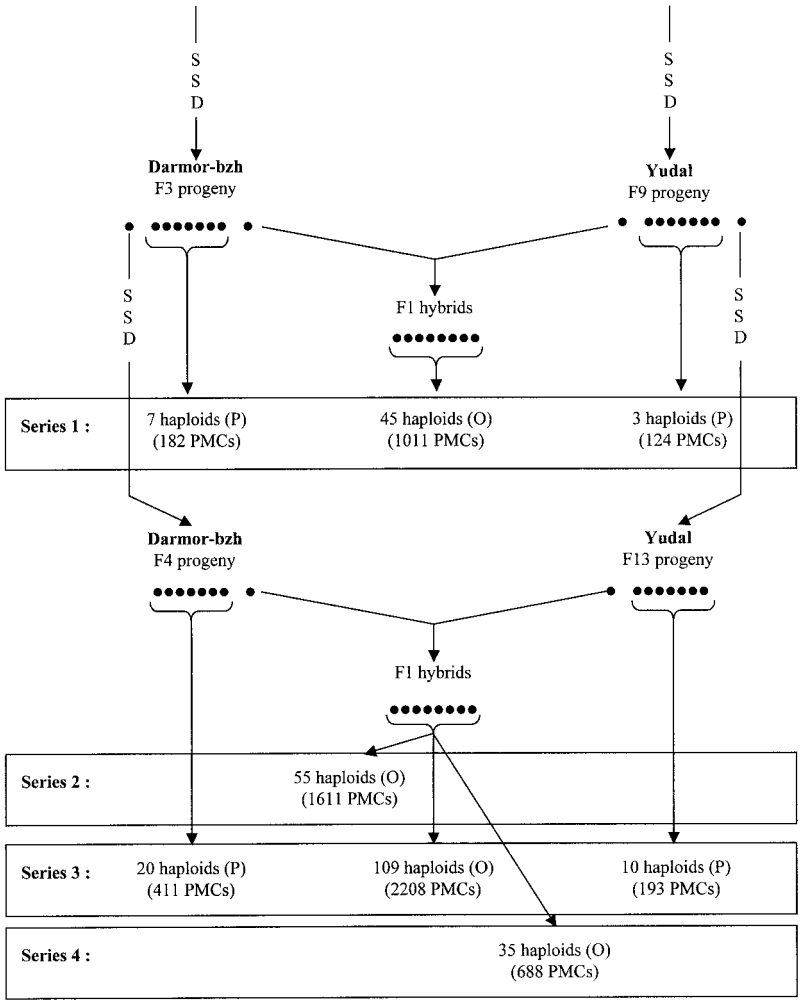

FIGURE 1.—Genealogy and structure of the parental (P) and offspring (O) subpopulations employed for segregation analyses. The total number of pollen mother cells (PMCs) observed is indicated for each series in each subpopulation. All the diploid lines used to produce the parental haploids were obtained by single-seed descent (SSD).

given by the estimated variance parameters. For factors with fixed effects, contributions are calculated as the average squared estimates of the factor effects.

Simultaneous analysis of parental and offspring data: We considered a model with both a segregating diallelic major gene and a completely additive polygenic background. This model allowed the presence of two subpopulations in the offspring data set, one with a behavior similar to *Darmor-bzh* haploids (denoted HD) and the other one with a behavior similar to *Yudal* haploids (denoted HY). Our general model was

$$Y_{D,lij} = \mu_D + \gamma_l + b_{D,li} + \varepsilon_{D,lij}$$

$$Y_{Y,lij} = \mu_Y + \gamma_l + b_{Y,li} + \varepsilon_{Y,lij}$$

$$Y_{H,lij} = \begin{cases} \mu_{\mathrm{HD}} + \gamma_l + b_{\mathrm{HD},li} + \varepsilon_{H,lij} & \text{with probability } p \\ \mu_{\mathrm{HY}} + \gamma_l + b_{\mathrm{HY},li} + \varepsilon_{H,lij} & \text{with probability } 1-p, \end{cases} \quad (2)$$

where μ_D, μ_Y, γ_l, $b_{D,li}$, $b_{Y,li}$, $\varepsilon_{D,lij}$, and $\varepsilon_{Y,lij}$ are as defined for the parental data; μ_{HD} and μ_{HY} are the means for the two subpopulations HD and HY; $b_{\mathrm{HD},li}$ and $b_{\mathrm{HY},li}$ are the random haploid plant effects for the two subpopulations HD and HY; $\varepsilon_{H,lij}$ is a residual error term; and p and $1 - p$ are the transmission probabilities of the *Darmor-bzh* and *Yudal* major-locus alleles, respectively. The $b_{g,li}$ and $\varepsilon_{g,li}$ random effects were assumed to follow independent normal and centered distributions. Haploid variances τ_g^2 (with $g = D$, Y, HD, or HY) were assumed to depend on the genotype, whereas the residual variance σ^2 was assumed to be the same for all PMCs. Model (2) involves factors with fixed (genotype and series) and random effects (plants) and is therefore a mixed model. In the offspring data, it includes the mixture of two distributions with different means and variances (EVERITT and HAND 1981).

The vector of parameters is $\theta = (p, \gamma_1, \gamma_2, \gamma_3, \gamma_4, \mu_D, \mu_Y, \mu_{\mathrm{HD}}, \mu_{\mathrm{HY}}, \tau_D^2, \tau_Y^2, \tau_{\mathrm{HD}}^2, \tau_{\mathrm{HY}}^2, \sigma^2)$, with the constraints $\gamma_1 + \gamma_2 + \gamma_3 + \gamma_4 = 0$ to avoid overparameterization and $\mu_{\mathrm{HY}} - \mu_{\mathrm{HD}} > 0$ to ensure that the parameters can be identified. The parameter estimates and their asymptotic standard errors were calculated by Gaussian likelihood maximization. For each g, l, i ($g = D$, Y, or H), $\mathbf{Y}_{g,li}$ denotes the vector of observations on all PMCs from plant i of genotype g in series l. In the model, the $\mathbf{Y}_{g,li}$'s are mutually independent and so the total log-likelihood is equal to the sum of the log-likelihoods for each vector $\mathbf{Y}_{g,li}$. $\phi(\mathbf{Y};\mathbf{m},\Sigma)$ denotes the density of a Gaussian vector with expectation $\mathbf{m}$ and covariance matrix Σ, calculated in $\mathbf{Y}$. For $g = D$ or Y, the likelihood for $\mathbf{Y}_{g,li}$ is $\phi(\mathbf{Y}_{g,li};(\gamma_1 + \mu_g)\mathbf{1}, \tau_g^2\mathbf{J} + \sigma^2\mathbf{I})$, where $\mathbf{1}$ denotes the vector of ones of appropriate length, $\mathbf{I}$ is the identity matrix, and $\mathbf{J}$ is the matrix of ones. For $g = H$, the likelihood for $\mathbf{Y}_{g,li}$ is $p\phi(\mathbf{Y}_{g,li};(\gamma_l + \mu_{\mathrm{HD}})\mathbf{1}, \tau_{\mathrm{HD}}^2\mathbf{J} + \sigma^2\mathbf{I}) + (1 - p)\phi(\mathbf{Y}_{g,li};(\gamma_l + \mu_{\mathrm{HY}})\mathbf{1}, \tau_{\mathrm{HY}}^2\mathbf{J} + \sigma^2\mathbf{I})$, which we denote by $f(\mathbf{Y}_{g,li};\theta)$.

The maximization of the likelihood coming from a mixture model is usually carried out using an expectation-maximization (EM) algorithm (DEMPSTER *et al.* 1977). In this study, the numerical procedure was improved by using a generalization of this algorithm, namely an expectation-conditional-maximization (ECM) algorithm (MENG and RUBIN 1993). In-

deed, the ECM algorithm allowed us to maximize the log-likelihood more efficiently, by separating maximization with respect to the variance parameters and maximization with respect to the other model parameters. The algorithm was programmed using the MATLAB software (MathWorks 2000).

The testing procedure was based on the LR test statistic. This procedure tests the null hypothesis that the vector of parameters satisfies a set of q linear constraints against the alternative that at least one of these constraints is not satisfied. LR equals twice the difference between the maximum log-likelihoods under the alternative and null hypotheses. The null hypothesis is rejected at level 5% when the test statistic is greater than the 95% quantile of a χ^2 distribution with q d.f. This test proved to be approximately of level 5% when the numbers of haploids and PMCs per haploid are large (Graybill 1976).

Finally, we used the estimated model parameters to predict the major-locus genotype of all haploids in the offspring data set. According to Bayes' theorem, the probability that a haploid from the offspring data set carries the *Darmor-bzh* allele, conditionally to its vector $\mathbf{Y}_{H,li}$ of observed values, is

$$P(D/\mathbf{Y}_{H,li}) = \frac{p\phi(\mathbf{Y}_{H,li};(\gamma_l + \mu_{HD})\mathbf{1}, \tau^2_{HD}\mathbf{J} + \sigma^2\mathbf{I})}{f(\mathbf{Y}_{g,li};\theta)}$$

and the probability that it carries the *Yudal* allele is $P(Y/\mathbf{Y}_{H,li}) = 1 - P(D/\mathbf{Y}_{H,li})$ (Everitt and Hand 1981).

For estimating the repeatability of our observations, we computed the correlation between repeated measures (mean number of univalents) on the 16 haploids from the offspring data set that have been observed in 2 consecutive years (Falconer and Mackay 1996).

RESULTS

Analysis of the variation among *Darmor-bzh* and *Yudal* haploids: Typical pairing patterns at MI for *Darmor-bzh* and *Yudal* haploids are illustrated in Figure 2. Figure 3A presents the mean numbers of univalents for each plant in the parental data set. Averaged meiotic behaviors for *Darmor-bzh* and *Yudal* haploids estimated by REML and corrected for the series and haploid effects (Table 1) demonstrate that pairing patterns in *Darmor-bzh* and *Yudal* haploids are clear cut. Haploids produced from *Darmor-bzh* showed far more pairing than those originating from *Yudal*; 80% of the PMCs observed in the *Darmor-bzh* haploids had less than six univalents whereas 95% of the PMCs scored in the *Yudal* haploids had more than eight univalents. On average, only 36.8% of the chromosomes paired in the *Yudal* haploids while >75% of the chromosomes were associated in the *Darmor-bzh* haploids. Similar differences were observed with the number of multivalents: 41 trivalents (III) and 60 quadrivalents (IV) were scored in a total of 593 PMCs from 27 *Darmor-bzh* haploids, whereas 19 III and only 2 IV were scored over the 317 PMCs analyzed from the 13 *Yudal* haploids. Regardless of the genotype, bivalents and multivalents were held by chiasmata.

Using the number of univalents as a variable, the estimated values (plus or minus their standard errors) for the parameters of model (1) were $\mu_D = 4.82$ (± 0.08), $\gamma_1 = -\gamma_3 = 0.98$ (± 0.08), $\tau^2_D = 0.02$ (± 0.04), $\sigma^2 = 2.52$ (± 0.15) for *Darmor-bzh* haploids and $\mu_Y = 12.03$ (± 0.44), $\gamma_3 = -\gamma_1 = 0.26$ (± 0.33), $\tau^2_Y = 0.86$ (± 0.44), $\sigma^2 = 3.78$ (± 0.31) for *Yudal* haploids, respectively. The analyses of variance (Table 2), performed separately on each parental line, showed that significant differences existed between the two series of haploids (series 1 and 3) produced from *Darmor-bzh* ($P = 1.8e\text{-}11$), which differed on average by the association of two chromosomes as a bivalent. By contrast, no differences were observed between the two series of haploids produced from *Yudal* whereas haploids within each series were significantly different from one another ($P = 2.2e\text{-}09$). This result is surprising because the diploid *Yudal* plants used for microspore culture were from the same F_9 or F_{13} progenies and were therefore genetically almost homogeneous. By contrast, no differences were detected among the haploids of the same generation produced from *Darmor-bzh*.

According to parameter estimates, 93% of the observed variability for the number of univalents could be

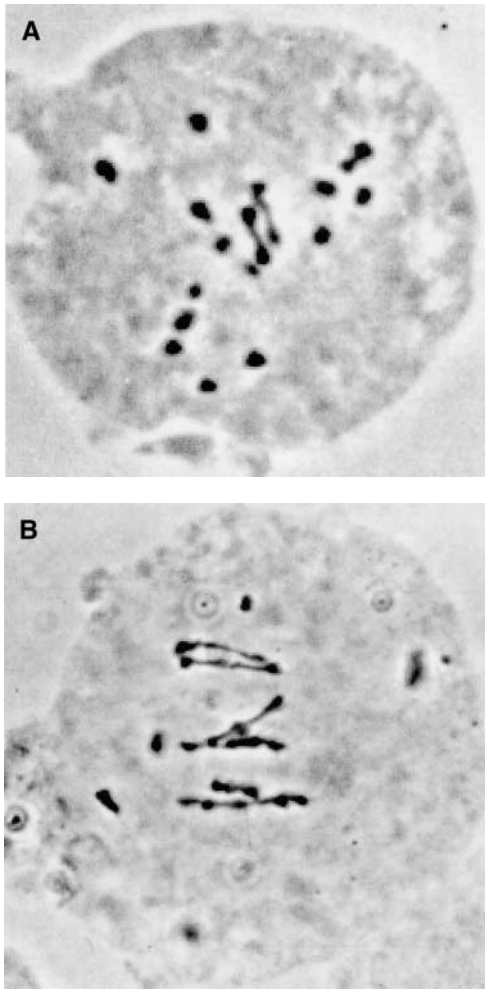

Figure 2.—First metaphase of meiosis in 1% acetocarmine-stained squashes of pollen mother cells of two oilseed rape haploids produced from the parental lines: (A) low pairing in a *Yudal* haploid, with two bivalents and 15 univalents; (B) high pairing in a *Darmor-bzh* haploid, with eight bivalents and only 3 univalents.

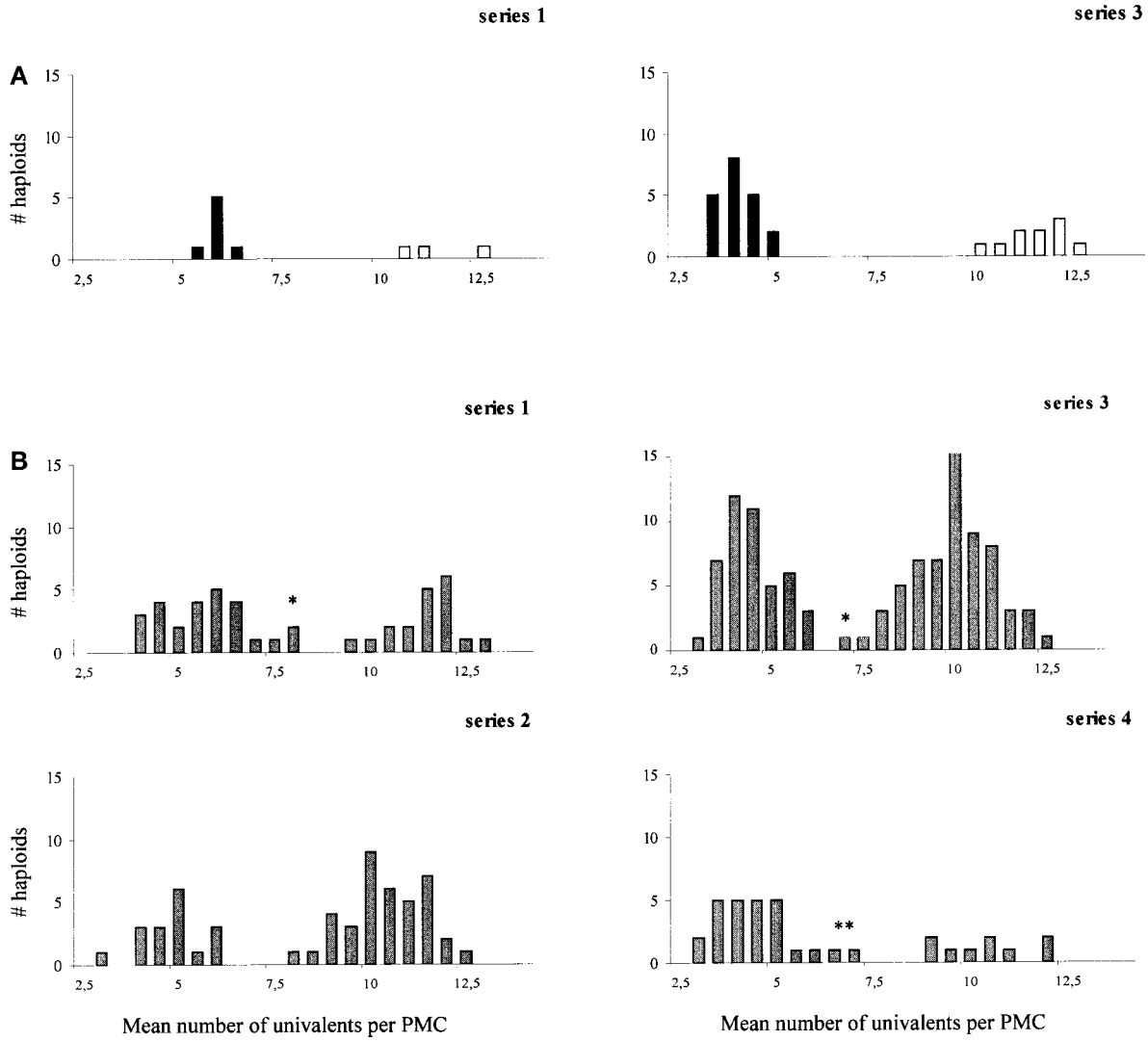

FIGURE 3.—Mean numbers of univalents per PMC for each haploid produced from either (A) the parental lines or (B) the hybrids between *Darmor-bzh* and *Yudal*. Haploids produced from *Darmor-bzh* are represented by solid histograms and those produced from *Yudal* by open histograms. Asterisk (*) points to haploids with intermediate behavior.

attributed to differences between the parental genotypes, 4% to differences between series (calculated by averaging over the two parental lines), and 3% to differences between haploids within a series.

Analysis of the whole data set, including the segregating population of haploids: Figure 3B presents the mean numbers of univalents for each plant in the offspring data set. These values proved to be very repeatable and reliable using Falconer's method; a very high correlation ($r_F = 0.96$) was observed among repeated measures on the 16 haploids that had been chosen in the offspring data set to encompass the whole range of meiotic behaviors. The maximum absolute differences in the mean number of univalents between the 2 years of observation for a plant were 1.7 and then 1.05.

The distribution of the mean number of univalents in the offspring data set was clearly bimodal with a mixture of two distinct distributions (Figure 4). Ac-

TABLE 1

Averaged meiotic behavior in haploids produced from the two parental lines

	No. of cells	I	II	III	IV
Darmor-bzh	597	4.82 ± 0.08	6.77 ± 0.05	0.07 ± 0.01	0.11 ± 0.01
Yudal	317	12.03 ± 0.33	3.37 ± 0.136	0.07 ± 0.02	0.007 ± 0.004

I, II, III, and IV are the mean numbers of univalents, bivalents, trivalents, and quadrivalents per PMC, respectively. These values ±standard errors have been estimated by REML.

TABLE 2

Analyses of variance on the numbers of univalents observed among haploids produced from *Darmor-bzh* and *Yudal* parental lines, respectively

	Darmor-bzh haploids			*Yudal* haploids		
Factor	d.f.	MS	*F* value	d.f.	MS	*F* value
Series	1	462.18	150.34***	1	17.54	0.57 NS
Haploid	25	3.0	1.2 ns	11	23.7	6.3***
Residual	566	2.5		304	3.8	
Error for series	22.63[a]	3.07		10.07[a]	31.03	

NS, not significant; MS, mean square. ***$P < 10^{-3}$.
[a] Approximated degrees of freedom.

cording to model (2), the parameter estimates (plus or minus their standard errors) were $p = 0.46$ (± 0.03), $\mu_D = 4.50$ (± 0.36), $\mu_Y = 12.06$ (± 0.38), $\mu_{HD} = 4.67$ (± 0.35), $\mu_{HY} = 10.27$ (± 0.60), $\gamma_1 = 1.08$ (± 0.36), $\gamma_2 = -0.05$ (± 0.27), $\gamma_3 = -0.56$ (± 0.35), $\gamma_4 = -0.48$ (± 0.62), $\tau^2_D = 0.005$ (± 0.07), $\tau^2_Y = 0.97$ (± 1.05), $\tau^2_{HD} = 0.70$ (± 0.21), $\tau^2_{HY} = 0.91$ (± 1.09), and $\sigma^2 = 3.11$ (± 0.17). Adjusted and observed distributions of the mean number of univalents for each haploid of the offspring data set were in close agreement (Figure 4).

We initially tested whether the distribution of the number of univalents was consistent with the Mendelian segregation of a major gene. The full model that treated transmission probability as an unknown parameter was compared, using a likelihood-ratio test, with the restricted model that fixed $p = 0.5$. As the full model did not provide a better fit than the restricted one ($P = 0.25$), the hypothesis $p = 0.5$ was accepted at the 5% level (Table 3). Therefore the distribution of the mean number of univalents in the offspring data set supports the presence of a major gene with two alleles.

To analyze whether this major gene was the only genetic source of variation, we tested whether the distribution of the number of univalents in the offspring data set was consistent with the mixture of the two parental distributions within a given series: this would be expected if the amount of pairing was completely controlled by the major gene. We compared the full model that treated μ_{HD}, μ_{HY}, τ^2_{HD}, and τ^2_{HY} as free parameters with restricted models that fixed $\mu_D = \mu_{HD}$, $\mu_Y = \mu_{HY}$, $\tau^2_{HD} = \tau^2_D$, or $\tau^2_{HY} = \tau^2_Y$. Note that, as a consequence of using a mixture model, each test was performed on the whole data set and took account of the uncertainty on the subpopulations of offspring haploids. Results, presented in Table 3, clearly showed that the distribution of the number of univalents in the offspring data set was not a mixture of the two parental distributions. First, the mean number of univalents was significantly lower in the HY subpopulation than in the parental *Yudal* haploids. By contrast there were no differences between the *Darmor-bzh* mean and that of the HD subpopulation ($P = 0.17$), although μ_{HD} was slightly higher than μ_D. Second, although τ^2_{HY} was not significantly different from τ^2_Y, the variance for the number of univalents was

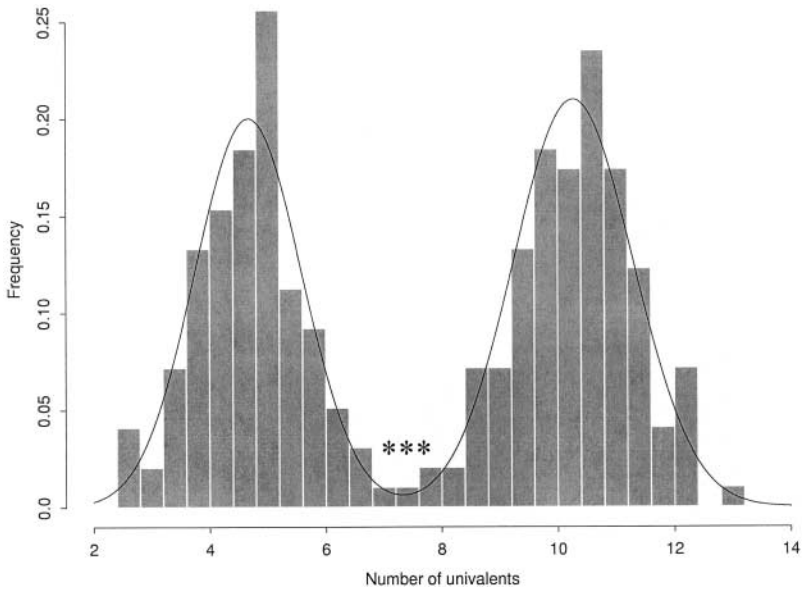

FIGURE 4.—Comparison of the observed (histogram) and estimated (solid curve) frequency distributions of the mean number of univalents in the offspring haploids. The observed distribution was obtained by pooling observations (mean number of univalents, adjusted for the estimated series effects) from all the series of the offspring data set. Asterisks (*) point to haploids with intermediate behavior.

TABLE 3

Likelihood-ratio (LR) tests for different hypotheses assuming a Mendelian single-locus model for the inheritance of the meiotic behavior in oilseed rape haploids

Hypothesis	Parameter constraints	Likelihood-ratio statistic	d.f.	*P* value
Mendelian segregation	$P = 0.5$	1.3	1	0.25
Adjustment of means	$\mu_D = \mu_{HD}$	1.895	1	0.17
	$\mu_Y = \mu_{HY}$	17.23	1	$<10^{-3}$
Adjustment of variance	$\tau_D = \tau_{HD}$	19.13	1	$<10^{-3}$
	$\tau_Y = \tau_{HY}$	0.017	1	0.90
	$\tau_Y = \tau_{HY} = \tau_{HD}$	1.11	2	0.57

significantly higher in the HD subpopulation than in the parental *D* subpopulation; actually, τ^2_Y, τ^2_{HY}, and τ^2_{HD} were not different from one another (Table 3).

According to the parameter estimates of model (2), 86% of the observed variability for the number of univalents in the offspring data set was due to differences between the HD and HY subpopulations, 5% to differences between series, and 9% to differences between haploids within a series and a subpopulation. Interestingly, several haploids in the offspring data set exhibited an intermediate pairing behavior (Figures 3B and 4). Prediction of the major-locus genotype of each haploid in the offspring data set showed that three of them had probabilities $P(D/\mathbf{Y}_{H,li})$ and $P(Y/\mathbf{Y}_{H,li})$ <0.9. These three plants had an averaged meiotic behavior of 6.77 univalents (I) + 5.97 bivalents (II) + 0.025 III + 0.05 IV; 90% of their PMCs had 5 I + 7 II, 7 I + 6 II, or 9 I + 5 II. These patterns were quite different from those observed in the other haploids of the offspring data set since 82% of the PMCs had more than nine univalents in the HY subpopulation or less than five univalents in the HD subpopulation. The amount of pairing in two of these intermediate haploids was measured twice and values were similar in both measurements (data not shown).

DISCUSSION

Several authors have observed a variation in the extent of pairing among oilseed rape haploids (Renard and Dosba 1980; Attia and Röbbelen 1986a). In this study, we demonstrated that this variation is genetically based and controlled mainly by the major gene *PrBn* (*Pairing regulator in B. napus*).

Investigations on the meiotic behavior of poly-haploids have been made on a wide range of allopolyploid species, usually at MI or later stages (Kimber and Riley 1963; Magoon and Khanna 1963). These studies have usually demonstrated a low level of bivalent formation and/or various types of secondary associations of univalents (*i.e.*, not held by chiasmata). In this study, we observed a high level of pairing in the oilseed rape haploids isolated from *Darmor-bzh*, with up to 75% of the chromosomes being associated at MI. Even in the low-pairing haploids isolated from *Yudal*, a minimum of two to three bivalents were systematically observed. All these associations were held by chiasmata and probably resulted from both auto- and allosyndesis within and between the A and C genomes of oilseed rape. Autosyndesis, the pairing between two chromosomes originating from the same genome, has been reported within *B. oleracea* (Armstrong and Keller 1982) and *B. rapa* (Armstrong and Keller 1981) as a result of intragenomic duplications (Prakash and Hinata 1980; Schmidt *et al.* 2001). As only one or two autosyndetic pairs are possible within the C and A genomes (Mizushima 1950, 1972; Armstrong and Keller 1981, 1982), additional associations should be considered as allosyndetic (*i.e.*, involving chromosomes from different genomes). This assertion is consistent with the close proximity of A/C homeologous genomes of oilseed rape and with their high affinity for pairing, which is indirectly supported by the high amount of pairing reported in *B. rapa* × *B. oleracea* interspecific hybrids (Attia and Röbbelen 1986b); for example, Inomata (1980) observed that 81% of the PMCs in such a hybrid contained 1 I + 9 II, 8 II + 1 III, 8 II + 3 I, or 2 I + 7 II + 1 III and that the frequency of quadrivalents was ~10%.

Interestingly, high-pairing haploids of oilseed rape exhibit a meiotic behavior that is almost similar to that of raw *B. rapa* × *B. oleracea* interspecific hybrids (direct comparisons are ongoing). This suggests that these haploids express the largest extent of pairing affinities between the A and C genomes. By contrast, low-pairing haploids show a severe restriction in pairing potentialities. Such restriction has been used to infer the presence of pairing regulators (Kimber 1961; Riley and Law 1965) or interpreted as the consequence of an overdifferentiation of homeologous chromosomes since the origin of the polyploid state. This last proposal seems unlikely in oilseed rape haploids for several reasons. First, meiosis in *Darmor-bzh* × *Yudal* F_1 diploid hybrids

is regular (19 II; data not shown), suggesting that these genotypes do not differ by extensive chromosomal rearrangements. In addition, high levels of chromosome pairing in A × AC and AC × C hybrids indicated that the A/C genomes in oilseed rape have remained essentially unaltered with respect to the A/C genomes of their progenitors (Olsson and Hagberg 1955; Attia *et al.* 1987; see also Parkin *et al.* 1995). Finally our study provided direct evidence that the differences between the high- and low-pairing haploids are genetically based.

Our study combined a segregation analysis with a maximum-likelihood approach to test for different modes of inheritance of the pattern of chromosome pairing in oilseed rape haploids. A similar approach has been recently advocated by Wu *et al.* (2001) to combine quantitative genetic and population genetic principles. Our approach assumed normality of the underlying distributions, which appeared consistent with the observed mean numbers of univalents and simplified the form of the likelihood functions. Our statistical treatment provides a powerful and flexible framework to investigate the different sources of variation, test for parameter adjustment in the different parental (*D*, *Y*) and offspring (HD, HY) subpopulations independently, and interpret genetic data in terms of both major and minor gene segregation.

Segregation analysis combined with LR tests clearly demonstrates that pairing patterns in oilseed rape haploids are inherited in a Mendelian fashion and supports the presence of a single major gene. However, the distribution of the number of univalents in the offspring data set was not consistent with the mixture of the two parental distributions; an obvious asymmetry in the evolution of mean and variance parameters in the HD and HY subpopulations (*i.e.*, $\mu_D = \mu_{HD}$ and $\tau^2_{HD} > \tau^2_D$ while $\mu_Y > \mu_{HY}$ and $\tau^2_{HY} = \tau^2_Y$) was detected. This pattern may have resulted from the segregation of additional weaker genes with nonadditive effects that are confounded with the major gene activity or the range of chromosome pairing affinities, environmental variation affecting HD and HY haploids in a different way, or both. These interpretations are tentative although they are supported by additional observations. On the one hand, the meiotic behavior of *Yudal* haploids, which were taken and observed at the same date, was related to their position in the greenhouse (data not shown); this indicates that a large part of the unexpected variation observed between these haploids (Table 2) was due to environmental heterogeneity. By contrast, no variation was detected among *Darmor-bzh* haploids (Table 2), suggesting that pairing in high-pairing haploids was less susceptible to environmental variation than pairing in low-pairing haploids. On the other hand, strong differences between the *Y* and HY subpopulations (Table 3), the presence of intermediate haploids with a repeatable behavior, and the increased variance in the HD subpopulations (while pairing patterns in *Darmor-bzh* haploids did not vary) are consistent with the presence of a polygenic background.

Our results suggest that control of chromosome pairing in oilseed rape haploids is roughly similar to that in wheat in that major genes are involved in both cases. However, it is likely that *PrBn* is different from *Ph1*. First, polymorphism observed among oilseed rape haploids is natural whereas there is hardly any natural polymorphism for *Ph1* (see Ozkan and Feldman 2001); the only known wheat lines defective for *Ph1* have been induced through irradiation (Sears 1977; Giorgi 1978; Roberts *et al.* 1999). Second, *Ph1* prevents homeologous pairing at both the haploid and diploid stage. By contrast, all *B. napus* accessions, regardless of the frequency of chromosome pairing in their dihaploid forms, display regular bivalent associations and disomic inheritance. This indicates that, if the presence of *Ph1* is essential for chromosome stability and fertility in wheat (Sánchez-Morán *et al.* 2001), *PrBn* is not required. Alternatively, *PrBn* could contribute to the regularity of chromosome pairing in all diploid forms of *B. napus*, but the allele present in genotypes with a high-pairing behavior at the haploid stage could be ineffective at the hemizygous stage or at least less efficient than that in the diploid state. Such haplo-ineffective regulating systems have been described in hexaploid fescues (Jauhar 1975) and different Aegilops species (Cuñado and Santos 1999).

This last hypothesis is tentative and clearly deserves further examination. Ongoing genetic mapping and subsequent cloning of *PrBn*, comparative analysis of chromosome pairing at prophase I in high- and low-pairing haploids, and direct studies of the amount of recombination in oilseed rape diploids and haploids should further our understanding of the genetic regulation of chromosome pairing in this species. Then, combined with the extensive and continuous characterization of *Ph1* (Roberts *et al.* 1999), the Brassica model could provide new insights into the nature of the meiotic stabilization of allopolyploid species.

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