DOI: 10.1534/genetics.105.042788

A Bayesian Approach for Discriminating Among Alternative Inheritance Hypotheses in Plant Polyploids: The Allotetraploid Origin of Genus Borderea (Dioscoreaceae)

Pilar Catalán,*[,1] José Gabriel Segarra-Moragues,* Marisa Palop-Esteban,[†] Carlos Moreno[‡] and Fernando González-Candelas[†]

*Departamento de Agricultura y Economía Agraria, Escuela Superior Politécnica de Huesca, Universidad de Zaragoza, E-22071 Huesca, Spain, [†]Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Genética Evolutiva, Universitat de València, E-46071 Valencia, Spain and [‡]Departamento de Anatomía, Embriología y Genética, Universidad de Zaragoza, E-50013 Zaragoza, Spain

Manuscript received March 1, 2005
Accepted for publication November 10, 2005

ABSTRACT

Polyploidy is a common phenomenon occurring in a vast number of land plants. Investigations of patterns of inheritance and the origins of plants (*i.e.*, autopolyploidy *vs.* allopolyploidy) usually involve cytogenetic and molecular studies of chromosome pairing, chromosome mapping, and marker segregation analysis through experimental crosses and progeny tests. Such studies are missing for most wild species, for which artificial crosses are difficult, not feasible, or unaffordable. We report here a Bayesian method to discriminate between alternative inheritance patterns in the two extant, tetraploid species of the monocot genus Borderea (Dioscoreaceae), which does not involve progeny array tests. Our approach is based on the screening of a large number of SSR genotypes, which were obtained from successful amplifications of 17 microsatellite regions in individuals of both *B. chouardii* and *B. pyrenaica*. We tested for tetrasomic *vs.* disomic modes of inheritance, using the Bayes factor test. Assignment of genotypes under both alternatives could be unequivocally done for 14 and 11 of the 17 studied microsatellite regions in *B. chouardii* and *B. pyrenaica*, respectively, totaling 9502 analyzed genotypes. The comparison of posterior probabilities for the two competing hypotheses across the surveyed loci clearly favored a disomic inheritance pattern. Linkage tests indicated that none of the studied SSR loci were in linkage disequilibrium, thus representing independent samples of the Borderea genome. These results, along with previous allozyme data, support the allotetraploid origin of this paleoendemic genus and reveal the lowest reported chromosome base number for the family of the yams.

POLYPLOIDY is a common phenomenon in the evolution of angiosperms. High ploidy level taxa account for up to 80% of the total number of species in many families, especially in the monocots (Stebbins 1950, 1971; Grant 1981; Masterson 1994; Otto and Whitton 2000). Although most polyploid plant species are believed to be allopolyploids of hybrid origin (Stebbins 1950, 1971; Wendel 2000), an increasing number of studies have demonstrated the occurrence of autopolyploidy in many groups of higher plants (Soltis and Soltis 1993, 1999, 2000; Galloway *et al.* 2003).

Inheritance patterns are directly influenced by the degree of homology of pairing meiotic chromosome sets, ranging from true diploidized amphidiploids with disomic inheritance to full autopolyploids with polysomic inheritance (Stebbins 1971; Soltis and Soltis 1993, 2000; Wendel 2000). However, intermediate classes of genetic inheritance may involve homeologous chromosome pairs (*i.e.*, segmental allopolyploids) (Stebbins 1947) that do not fit with the expected disomic patterns of the heterologous allopolyploids but that could be mistaken to some extent for a randomized polysomic segregation of homologous autopolyploid chromosomes (Stebbins 1950, 1971). Examples of the latter cases have been described in some plant genomes (Wu *et al.* 1992; De Vicente and Arus 1996; Lerceteau-Köhler *et al.* 2003). Other inheritance types that depart from typical patterns are rare, such as the heterogametic segregation observed in hemisexual polyploid plants (Nybom *et al.* 2004).

Hence, characterizing the mechanisms and patterns of genetic inheritance in polyploids is crucial for evolutionary and population genetic analyses of these organisms. Different models and parameters should be applied to calculate coefficients of genetic variability and structuring of populations, to estimate levels of inbreeding and gene flow, and to infer adaptive changes resulting from intra- or intergenomic interactions (Rieseberg and Doyle 1989; Olson 1997; Ronfort *et al.* 1998; Wendel 2000). Population structure parameters have been modeled for autotetraploid plants on

[1]*Corresponding author:* Departamento de Agricultura y Economía Agraria, Escuela Politécnica Superior de Huesca, Universidad de Zaragoza, C/ Carretera de Cuarte, km 1, E-22071 Huesca, Spain. E-mail: pcatalan@unizar.es

the basis of theoretical and simulated calculations (Moody *et al.* 1993; Ronfort *et al.* 1998). Allopolyploids that behave as perfect amphidiploids could be analyzed as double diploids, using the genetic parameters described for diploid organisms (*e.g.*, Weir and Cockerham 1984). It is noteworthy that inheritance patterns and even the nature of the polyploidy could pass undetected in some instances when molecular markers are not variable enough (Segarra-Moragues and Catalán 2002). In other cases, taxa might have undergone a series of genetically regulated effects, such as gene silencing by mutation and divergent sequence evolution, epigenetic and pleiotropic expressions, and mobile element activation, among others, after polyploidization (Ford and Gottlieb 2002; Liu and Wendel 2002; Mochida *et al.* 2004). Most of these non-Mendelian phenomena affect coding regions, but changes could also involve noncoding repetitive regions, such as microsatellites (Rong *et al.* 2004).

The investigation of segregation patterns of duplicated loci and of the origin of polyploid species usually involves experimental crosses and progeny tests (Stebbins 1971; Grant 1981; Soltis and Soltis 1993, 2000; Wendel 2000; Rong *et al.* 2004). Such studies have been accomplished for a number of cultivated species (Lawton-Rauh 2003) but are lacking for most wild species, for which artificial crosses are often difficult to perform (Otto and Whitton 2000; Nybom *et al.* 2004). On the other hand, potential genetic information that could be traced from putative parental diploids (Stebbins 1950; Wendel 2000) is impractical when the parental species are extinct (Werth and Lellinger 1992). Bayesian procedures have been used to test among alternative inheritance patterns in noncultivated tetraploid species (Olson 1997) although these tests were based on parent–offspring analyses of transmission in two allozyme loci. Inheritance of SSR markers has also been examined in experimental crosses of tetraploid and pentaploid wild roses (Nybom *et al.* 2004).

We report here a Bayesian method to discriminate among alternative inheritance patterns in the two extant, tetraploid species of the monocot genus Borderea (Dioscoreaceae), which does not require progeny array tests. Bayesian methods represent one of the best approaches for selecting among competing evolutionary hypotheses (Huelsenbeck *et al.* 2000, 2001; Beaumont and Rannala 2004; Posada and Buckley 2004) and can be used to evaluate relative support for competing biological alternatives (González-Candelas *et al.* 2003). They are particularly suited for comparing predictions made from different theories that lead to nonnested models (Kass and Raftery 1995). Specifically, we have used Bayes factors, the marginal likelihood assuming the allotetraploid hypothesis H_{allo} over the marginal likelihood assuming the autotetraploid hypothesis H_{auto}, to summarize the support provided by the data for allotetraploidy *vs.* autotetraploidy. Likelihood methods have been applied to test alternative mechanisms of inheritance of microsatellite markers in tetraploid plants through simulation analyses of meiotic behavior of bivalents and multivalents (Wu *et al.* 2001). It is imperative to clarify the genetic inheritance patterns of the tetraploid Borderea taxa to perform further evolutionary and population genetic analyses on them. However, as progeny tests are not readily available within Borderea nor feasible for the highly endangered *Borderea chouardii*, and there are no living diploid relatives of these species, we have conducted a broad survey of 17 microsatellite $(CTT)_n$ regions in the two extant species of Borderea by sampling 851 individuals from the single known population of *B. chouardii* and from 15 populations of *B. pyrenaica*, totaling 14,467 analyzed genotypes of which 9502 were subjected to Bayesian testing.

We have used this large data set to test the inheritance patterns of the highly polymorphic microsatellite markers against the two competing hypotheses for ploidy origin (autopolyploidy *vs.* allopolyploidy) in the genus Borderea, using a Bayesian approach. The correct assignment of alleles and inferred genotypes under each alternative would allow us to derive the inheritance pattern of each Borderea locus on the basis of the relative support received from the data. These results could be of relevance to ascertain the patterns of inheritance and the origins of other polyploid plant taxa.

MATERIALS AND METHODS

Sampling of taxa: Genetic analyses based on microsatellite markers have recently unraveled the tetraploid nature of the Pyrenean endemic plant genus Borderea Miègeville (Segarra-Moragues *et al.* 2003, 2004). The genus Borderea comprises only two extant species [*B. pyrenaica* Miègeville and *B. chouardii* (Gaussen) Heslot] restricted to a narrow geographical area in the Central Pyrenees and Prepyrenees (Gaussen 1965; Segarra-Moragues and Catalán 2002, 2003). The two Borderea species are orophyte taxa that share several biological attributes related to their dioecy, obligate outcrossing, and perennial long life spans of >300 years (García and Antor 1995; García 2003). However, they are differentiated for several morphological traits (Gaussen 1965; Segarra-Moragues and Catalán 2005a) as well as for their ecology and geographical distribution (Segarra-Moragues and Catalán 2005b).

These taxa have long been considered to be diploid and to share a chromosome base number of $x = 12$, deduced from the somatic $2n = 24$ chromosome counts by Heslot (1953) in both species and from the gametic $n = 12$ chromosomes found in pollen mother cells of the more widely distributed *B. pyrenaica* (Heslot 1953; Gaussen 1965). Pilot assays using different sets of primer pairs designed for the amplification of trinucleotide $(CTT)_n$ microsatellite regions in *B. chouardii* (Segarra-Moragues *et al.* 2003) and in *B. pyrenaica* (Segarra-Moragues *et al.* 2004) have revealed polyploid patterns of up to four alleles per individual for most surveyed loci (Segarra-Moragues *et al.* 2003, 2004). Preliminary tests for the cross-amplification of the *B. chouardii* loci in the pilot *B. pyrenaica* population also resulted in similar polyploid allelic patterns for the transferred loci (Segarra-Moragues *et al.* 2004).

TABLE 1

Sampled populations of *Borderea chouardii* and *B. pyrenaica*

Species	Population	Locality	Size	*N*
B. chouardii	Bc01	Spain: Prepyrenees: Sopeira	<2200	47
B. pyrenaica	Bp01	France: NW Pyrenees: La Planette	>5000	60
B. pyrenaica	Bp02	France: NW Pyrenees: Crampettes	>100	60
B. pyrenaica	Bp03	France: NE Pyrenees: Chemin du Cirque	20	20
B. pyrenaica	Bp04	France: NE Pyrenees: Sentier Espugues	>100	60
B. pyrenaica	Bp05	France: NE Pyrenees: Rochers Blancs	>1000	60
B. pyrenaica	Bp06	France: NE Pyrenees: Pailla Nord-Ouest	>100	60
B. pyrenaica	Bp07	France: NE Pyrenees: Pailla Nord-Est	<50	34
B. pyrenaica	Bp08	France: NE Pyrenees: Pailla bas	>1000	60
B. pyrenaica	Bp09	France: NW Pyrenees: Bellevue	1	0
B. pyrenaica	Bp10	France: NE Pyrenees: Hotel de Gavarnie	>100	60
B. pyrenaica	Bp11	France: NE Pyrenees: Hount Blanc	>1000	60
B. pyrenaica	Bp12	France: NE Pyrenees: Pailla NE – Pailla bas	<50	30
B. pyrenaica	Bp13	Spain: SE Pyrenees: Pineta	>1000	60
B. pyrenaica	Bp14	Spain: SW Pyrenees: Ordesa	>500	60
B. pyrenaica	Bp15	Spain: N Prepyrenees: Cotiella, La Vasa Mora	>10000	60
B. pyrenaica	Bp16	Spain: S Prepyrenees: Turbón	>1000	60
Total				851

For each population its code, locality, estimated population size, and number of sampled individuals are given.

B. chouardii individuals were sampled in the single known population of this taxon, at the Prepyrenean locality of Sopeira (Huesca, Spain), totaling 47 samples (Table 1, Bc01). Individuals of *B. pyrenaica* were sampled across the entire geographical range of the species that occupies an area of ~160 km^2 in the Central Pyrenees and Prepyrenees, totaling 804 samples from 15 different localities. Populations Bp01–Bp12 are located on the northern side of the Pyrenees, at Gavarnie (France); Bp13–Bp14 are on the southern side of the Pyrenees, at the Pineta and Ordesa valleys (Spain), respectively; Bp15 is at the Spanish Prepyrenean massif of Cotiella; and Bp16 is at the Spanish Prepyrenean massif of Turbón (Table 1). Sampling included equal sex ratios in most populations.

Transferability of microsatellite loci between *B. chouardii* and *B. pyrenaica*: A total of 17 trinucleotide $(CTT)_n$ microsatellite or simple sequence repeat (SSR) (Tautz 1989) regions were surveyed across all sampled individuals from both species. Ten of these microsatellite regions were previously isolated in *B. chouardii* (Segarra-Moragues *et al.* 2003); the remaining seven SSR regions were isolated from *B. pyrenaica*, as described in Segarra-Moragues *et al.* (2004). In both cases enriched partial genomic libraries were constructed using streptavidin-coated M-280 magnetic beads (Dynal, Great Neck, NY); ligated fragments were transformed into XL10-Gold ultracompetent cells (Stratagene, La Jolla, CA) and positive clones were sequenced for primer design, which was performed with PRIMER3 (Rozen and Skaletsky 1996). Characteristics of primer pair sequences, repeat motifs, sizes of the sequenced regions, annealing temperatures of primer pairs, and GenBank accession numbers of the 17 microsatellite regions used for analysis are given in Segarra-Moragues *et al.* (2003, 2004). PCR reactions for both sets of loci were performed in 20 μl mix containing 3–4 pmol each of the labeled forward and unlabeled reverse primers, 1× *Taq* buffer (Promega, Madison, WI), 2 mm $MgCl_2$, 0.4 mm of each dNTP, 1 unit of *Taq* DNA polymerase (Promega), and ~20 ng DNA. The amplification program consisted of an initial melting step (94°, 4 min) followed by 30 cycles (94°, 1 min; annealing temperature, 1 min; and 72°, 45 sec) and a final extension step (72°, 7 min). PCRs were carried out in a PE GeneAmp 9700 (Applied Biosystems, Foster City, CA). Multiplexed reactions were performed with several groupings of primer pairs, allowing their combination in the same PCR cocktail according to their observed allele size ranges and the fluorescent dye used (Table 2). The products were run on an ABI 310 automated sequencer (Applied Biosystems). Amplified fragment lengths were assigned to allelic classes with the programs GENESCAN and GENOTYPER (Applied Biosystems), using ROX-500 (Applied Biosystems) as the internal lane standard.

Assignment of SSR alleles to potential genotypes: Because of the tetraploidy of the two Borderea species, individual genotypes could not be assigned directly from the scored SSR profiles as for diploids. In this case two main alternative segregation patterns exist, corresponding to autotetraploidy and allotetraploidy.

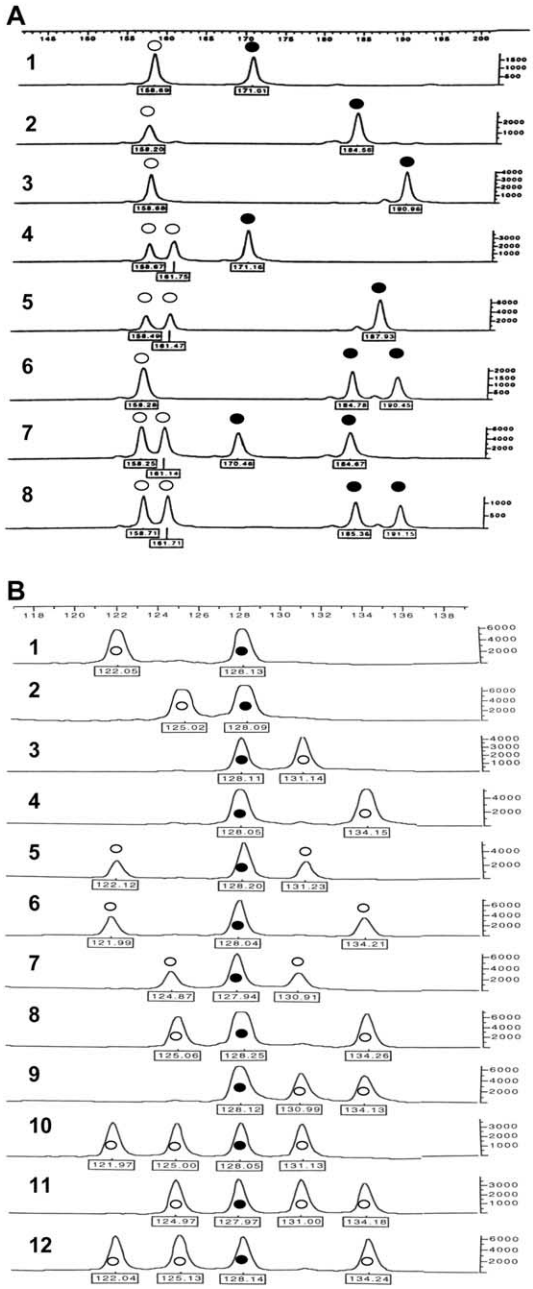
Under the autotetraploid model (polysomic inheritance) individuals with a single amplified product are expected to be homozygous (having four copies of the same allele), if null alleles are absent, and individuals with four amplified products are expected to have a single dose of each of the observed alleles. Individuals with two and three amplified products represent more problematic cases as different genotypic configurations are possible, depending on the alleles being in triple dosage (1:1:1:2/1:2:2:2) or in equal dosages (1:1:2:2) in individuals with two bands, or in double dosage (1:1:2:3/1:2:2:3/1:2:3:3) in individuals with three bands. To designate genotypic configurations we used the recently developed method of microsatellite DNA allele counting-peak ratios (MAC-PR) (Esselink *et al.* 2004) that makes use of the quantitative values for microsatellite allele amplification peak areas. For each locus, all alleles were analyzed in pairwise combinations to determine their dosages in the individual samples by calculating the ratios between the peak areas for all allele pairs that were amplified simultaneously (Figure 1). We considered individuals with the maximum number of alleles (4) as a baseline with all pairwise comparisons of peak ratios

TABLE 2

Amplification products for 17 primer pairs used in Borderea

			Borderea chouardii				*Borderea pyrenaica*			
Primer pair	Motif	Multiplex group	N_A	Allele sizes (bp)	A	D	N_A	Allele sizes (bp)	A	D
Bc1145B	$(CTT)_8$	I	3	a: 91, 103 b: 103	Y	Y	7	85, 88, 94, 97, 103, 106, 109 —	Y	N
Bc1159	$(CTT)_8$	III	4	120, 123, 126, 132	Y	N	3	120, 123, 126	Y	N
Bc1169	$(CTT)_{14}$	III	4	a: 123 b: 142, 145, 152	Y	Y	2	123, 126 single locus	Y	Y
Bc1258	$(CTT)_{12}$	IV	7	a: 159, 162 b: 171, 180, 183, 186, 189	Y	Y	7	a: 156, 162, 165 b: 145, 168, 171, 180	Y	Y
Bc1274	$(CTT)_{12}$	I	5	a: 261, 273 b: 258, 267, 270	Y	Y	24	249, 255, 258, 261, 264, 267, 270, 273, 276, 279, 282, 285, 288, 291, 294, 297, 300, 303, 306, 309, 312, 315, 318, 321 —	N	N
Bc1357	$(CTT)_{10}$	I	6	a: 125 b: 134, 137, 146, 157, 160	Y	Y	7	122, 125, 128, 131, 134, 137, 140 —	Y	N
Bc1422	$(CTT)_7$	IV	5	a: 162 b: 195, 216, 219, 222	Y	Y	18	a: 177, 180, 186 b: 159, 162, 189, 192, 195, 198, 201, 204, 207, 210, 213, 216, 219, 222, 225	Y	Y
Bc1551	$(CTT)_{13}$ ... $(GAA)_5$	I	2	264, 267 Single locus	Y	Y	25	264, 267, 270, 273, 276, 279, 282, 285, 288, 294, 297, 303, 306, 309, 312, 315, 318, 321, 324, 327, 330, 333, 336, 339, 342 —	N	N
Bc1644	$(CTT)_{14}$	III	4	166, 169, 175, 178 Single locus	Y	Y	10	a: 160, 163 b: 169, 172, 175, 178, 181, 184, 187, 190	Y	Y
Bc166	$(CTT)_{13}$	IV	12	182, 185, 188, 191, 194, 197, 203, 206, 215, 218, 221, 224 —	Y	N	4	a: Null, 175 b: 178, 181	Y	Y
Bp126	$(CTT)_8$	II	2	220, 226 Single locus	Y	Y	6	226, 232, 235, 238, 241, 244 Single locus	Y	Y
Bp1286	$(CTT)_8$	II	1	123 Single locus	Y	Y	1	123 Single locus	Y	Y
Bp2214	$(CTT)_7$	I	1	*213* Single locus	Y	Y	2	204, 216 Single locus	Y	Y
Bp2256	$(CTT)_8$	II	2	a: *232* b: 226	Y	Y	3	a: *220* b: *223*, 226	Y	Y
Bp2290	$(CTT)_7$	II	3	a: 130, 133 b: *139*	Y	Y	11	a: 127, 130 b: 143, 149, 152, 155, 158, 161, 164, 167, 170	Y	Y
Bp2292	$(CTT)_7$	I	4	202, 205, 211, 214 —	Y	N	9	199, 202, 205, 208, 211, 214, 217, 220, 223 —	Y	N
Bp2391	$(CTT)_8$	II	2	a: 126 b: *127*	Y	Y	13	a: 123, 126, 129 b: 130, 133, 136, 140, 143, 146, 149, 153, 156, 159	Y	Y

For each taxon and locus we indicate the repeat motif of the sequenced clone, the multiplex group in which each region was amplified, the number of alleles (N_A), the allele sizes in base pairs, and their codification under the assumptions of autotetraploidy (A) and disomic allopoliploidy (D). Y, possible; N, not possible. Underlines indicate shared alleles between both species of Borderea and italics indicate fixed private alleles of each taxon. a and b designate the alleles of each genome under the assumption of allotetraploidy.



	Autotetraploidy	Allotetraploidy	
		a	b
1	159:159:171:171	159:159	171:171
2	159:159:183:183	159:159	183:183
3	159:159:189:189	159:159	189:189
4	159:162:171:171	159:162	171:171
5	159:162:186:186	159:162	186:186
6	159:159:183:189	159:159	183:189
7	159:162:171:183	159:162	171:183
8	159:162:183:189	159:162	183:189

	Autotetraploidy	Allotetraploidy	
		a	b
1	122:122:128:128	122:122	128:128
2	125:125:128:128	125:125	128:128
3	128:128:131:131	131:131	128:128
4	128:128:134:134	134:134	128:128
5	122:128:128:131	122:131	128:128
6	122:128:128:134	122:134	128:128
7	125:128:128:131	125:131	128:128
8	125:128:128:134	125:134	128:128
9	128:128:131:134	131:134	128:128
10	122:125:128:131	???:???	???:???
11	125:128:131:134	???:???	???:???
12	122:125:128:134	???:???	???:????

FIGURE 1.—Sample electropherograms of two microsatellite regions [(A) Bc1258, 8 individuals; (B) Bc1357, 12 individuals], amplifying two genetic dosages (individuals with up to four alleles) in Borderea, and the corresponding genotypes, encoded under autotetraploidy (1 locus) and allotetraploidy (two loci: a and b) assumptions. Genotypes for the autotetraploid hypothesis were inferred following the MAC-PR approach for the allelic dosages in individuals showing two and three peaks (ESSELINK *et al.* 2004). Genotypes for the allotetraploid hypothesis were inferred after the assignment of alleles to each genomic complement (○, alleles of locus a; ●, alleles of locus b), imposing the condition that a given individual cannot present more than two alleles previously assigned to each duplicated locus. Note that in the case of Bc1357 allelic size homoplasy between both loci appears in some tetrallelic individuals (lanes 10–12) that show three alleles previously assigned to locus Bc1357a, precluding thus the encoding of genotypes for the allotetraploid hypothesis. In this case the inferred genotypes for individuals 1–9 may also be inaccurate and therefore this microsatellite region was not considered in the Bayesian analysis.

equaling 1:1 and in such a manner genotypes could be recorded without having parent-offspring information (ESSELINK *et al.* 2004).

Under the allotetraploid model (disomic inheritance) the observed amplification products of an individual may correspond to two different genomic complements, and alleles of each genome should be identified prior to the coding of genotypes. Individuals that show a single band can be explained only by homology or size homoplasy of alleles of the two genomic complements or by the existence of null alleles in one (or both) coexisting genomes, whereas individuals with four bands are interpreted as double heterozygous (for the duplicated loci). Alleles were assigned at random to each genomic complement beginning with individuals that showed two amplified peaks of similar ratio (therefore presumably homozygous for each allele in each complement). This assignment was checked for consistency in individuals with three and four amplified products, imposing the condition that a given individual could not present simultaneously more than two alleles previously assigned to a given complement (Figure 1A). Otherwise, the microsatellite region was considered to present size homoplasy between complements, leading to inaccurate scoring of genotypes (Figure 1B). Those regions that could not be scored confidently for allotetraploidy were not included in the Bayesian analysis of the two competing hypotheses. This was usually due to the impossibility of assigning separate allele complements to each duplicate locus. Once a given individual with three bands was considered to be heterozygous for a given complement, then it was considered to be homozygous for the other, ruling out the possibility of null alleles and size homoplasies. We did this for each individual through a careful examination of both the amplification signal of each allele in the individual electropherograms and the observed patterns of inheritance across the entire sample of individuals for each species (see RESULTS), aiming at reconstructing the corresponding

genotypes in a consistent manner under both hypotheses of tetraploidy.

Bayesian analysis of the nature of Borderea tetraploidy: Likelihood ratios of nested models are regularly used in hypothesis testing (likelihood-ratio tests, LRT) (Edwards 1972). Since we were dealing with two nonnested models, it was not possible to use LRTs to accept/discard one of them. Instead, Bayes factors represent a summary of the evidence provided by the data in favor of each hypothesis, and twice the natural logarithm of this factor, which is on the same scale as the LRT statistic, can be used as a guideline on the strength of the support the data give to one hypothesis with respect to the other (Kass and Raftery 1995; Raftery 1996). In this scale, $2\log_e(B_{\text{allo,auto}}) > 10$ is considered as providing very strong support for the favored hypothesis (Kass and Raftery 1995).

To discriminate between the two possible alternatives for the origin and nature of polyploidy in Borderea, we adopted a Bayesian approach. Hence, following Kass and Raftery (1995), we computed the Bayes factor in favor of allotetraploidy over autotetraploidy for each microsatellite region in each population,

$$B_{\text{allo,auto}} = \frac{p(y \mid H_{\text{allo}})}{p(y \mid H_{\text{auto}})},$$

where the probabilities $p(y \mid H_{\text{allo}})$ and $p(y \mid H_{\text{auto}})$ are the marginal likelihoods of the data (y) at a particular locus in a particular population under each hypothesis. These probabilities were obtained by integrating the joint density of data and the model parameters over the parameter space,

$$p(y \mid H_x) = \int p(y \mid \theta_x, H_x) p(\theta_x, \mid H_x) d\theta_x,$$

where θ_x are the parameters (the allele frequencies at the specified locus) under H_x, $p(y \mid \theta_x, H_x)$ is the likelihood function, and $p(\theta_x, \mid H_x)$ is the prior density.

For both models of inheritance the observed genotype and allele counts at a locus in a population follow a multinomial distribution under the assumption of Hardy-Weinberg equilibrium, while a Dirichlet distribution is proposed as a prior. This prior function is a conjugate prior of multinomial distribution (Johnson and Kotz 1969). Hence, the integral required for the marginal probability is analytically tractable.

Under the autotetraploidy hypothesis, we assume that the alleles segregate in a single locus, thus allowing for all possible allele combinations. The corresponding probability density of the data given the parameters for a specific microsatellite locus and population is defined as

$$p(y \mid \theta_{\text{auto}}, H_{\text{auto}}) = \frac{n!}{\prod_{i=1,n_g} n_i!} \prod_{i=1,n_g} \left(\frac{4!}{\prod_{j=1,n_a} x_{ij}!} \prod_{j=1,n_a} p_j^{x_{ij}} \right)^{n_i},$$

where $\theta_{\text{auto}} = (p_1, \ldots, p_{n_a})'$ is the vector of allele frequencies, n is the sample size, n_g is the number of genotypes in the locus, n_i is the number of individuals with the ith genotype, n_a is the number of alleles in the locus, x_{ij} is the number of times that the jth allele is present in the ith genotype, and p_j is the frequency of the jth allele in the population.

As indicated above, the prior function is a Dirichlet distribution,

$$p(\theta_{\text{auto}} \mid H_{\text{auto}}) = \frac{\Gamma(\sum_{j=1,n_a} \alpha_j)}{\prod_{j=1,n_a} \Gamma(\alpha_j)} \prod_{j=1,n_a} p_j^{\alpha_j - 1},$$

where $\Gamma(\ )$ is the gamma function and $\alpha = (\alpha_1, \ldots, \alpha_{n_a})'$ is the vector of hyperparameters. We had no previous data to estimate the allele frequencies p_j, so we followed Jeffrey's rule to obtain a locally uniform prior, introducing the noninformative prior $\alpha_1 = \cdots = \alpha_{n_a} = \frac{1}{2}$ (Box and Tiao 1973).

The marginal probability of the data under the autotetraploidy hypothesis was finally achieved from the previously defined functions as

$$p(y \mid H_{\text{auto}}) = \frac{n!}{\prod_{i=1,n_g} n_i!} \prod_{i=1,n_g} \left(\frac{4!}{\prod_{j=1,n_a} x_{ij}!} \right)^{n_i} \times \frac{\Gamma(\sum_{j=1,n_a} \alpha_j)}{\prod_{j=1,n_a} \Gamma(\alpha_j)} \frac{\prod_{j=1,n_a} \Gamma(\sum_{i=1,n_g} x_{ij} n_i + \alpha_j)}{\Gamma(4n + \sum_{j=1,n_a} \alpha_j)}.$$

Under the allotetraploidy hypothesis there are some restrictions on the possible genotypes. In this case, the inheritance pattern corresponds to the simultaneous segregation at two loci, one from each parental genome, which may share some alleles, and the likelihood function for a specific microsatellite region and population is defined as

$$p(y \mid \theta_{\text{allo}}, H_{\text{allo}}) = \prod_{h=1,2} \left\{ \frac{n!}{\prod_{i=1,n_{h_g}} n_{h_i}!} \prod_{i=1,n_{h_g}} \left(\frac{2!}{\prod_{j=1,n_{h_a}} x_{h_{ij}}!} \prod_{j=1,n_{h_a}} p_{h_j}^{x_{h_{ij}}} \right)^{n_{h_i}} \right\},$$

where $\theta_{\text{allo}} = (p_{11}, \ldots, p_{1n1a}, p_{21}, \ldots, p_{2n2a})'$ is the vector of allele frequencies of both loci, n_{h_g} is the number of genotypes in the hth locus, n_{h_i} is the number of individuals with the ith genotype in the hth locus, n_{h_a} is the number of alleles in the hth locus, $x_{h_{ij}}$ is the number of times that the jth allele is present in the ith genotype of the hth locus, and p_{h_j} is the population frequency of the jth allele in the hth locus.

Hence, following a development similar to the above one, the marginal probability of the data for this alternative was

$$p(y \mid H_{\text{allo}}) = \prod_{h=1,2} \left\{ \frac{n!}{\prod_{i=1,n_{h_g}} n_{h_i}!} \prod_{i=1,n_{h_g}} \left(\frac{2!}{\prod_{j=1,n_{h_a}} x_{h_{ij}}!} \right)^{n_{h_i}} \times \frac{\Gamma(\sum_{j=1,n_{h_a}} \alpha_{h_j})}{\prod_{j=1,n_{h_a}} \Gamma(\alpha_{h_j})} \times \frac{\prod_{j=1,n_{h_a}} \Gamma(\sum_{i=1,n_{h_g}} x_{h_{ij}} n_{h_i} + \alpha_{h_j})}{\Gamma(2n_h + \sum_{j=1,n_{h_a}} \alpha_{h_j})} \right\},$$

where $\alpha = (\alpha_{11}, \ldots, \alpha_{1n1a}, \alpha_{21}, \ldots, \alpha_{2n2a})'$ is the vector of hyperparameters.

Parametric bootstrap simulation of genotyped SSR loci in Borderea: As stated before, occasionally it was not possible to score confidently the genotypes for some microsatellite regions and populations of *B. chouardii* and *B. pyrenaica* under the allotetraploidy hypothesis. Because of this restraint, these loci had to be excluded from the Bayesian analysis. However, these cases could be explained by the autotetraploidy model and their elimination could represent a bias against this alternative that would lead to an overestimate of the confidence for allotetraploidy. To overcome this potential bias, a parametric bootstrap simulation study (Efron and Tibshirani 1993) was conducted with the genotyped data, intending to test whether the presumed Bayesian support for allotetraploidy was still significant after a simulation that assumes the opposite model (autotetraploidy). For this, the value of the estimated Bayes factor obtained for each microsatellite region and population for which the allotetraploidy model was correctly genotyped was contrasted with an empirical distribution of this statistic. This distribution was obtained from the generation of 100,000 bootstrap replicates that simulated,

under the autotetraploidy model, the corresponding locus, imposing the restriction that all these samples had to be consistent with the allotetraploidy model. Specifically, the data of each replicate were generated from the multinomial distribution $p(y \mid \theta_{auto}, H_{auto})$, with allelic frequencies identical to the observed ones. The simulation analysis was performed with a program written in Fortran language.

Linkage disequilibrium analysis: To check whether the surveyed SSR loci represented an independent set of markers in the Borderea genome, genotypic linkage disequilibrium was tested by Fisher's exact test both for each pair of loci and within each population with GENEPOP v. 3.3 (Raymond and Rousset 1995), using the Markov chain method with 100 batches and 10,000 iterations/batch. Because multiple tests were involved, the sequential Bonferroni-type correction was applied to test for significance (Rice 1989).

RESULTS

Cross-transferability and levels of polymorphism of microsatellite loci in Borderea: All cross-amplifications of microsatellite loci in Borderea were successful. The 10 microsatellite primer pairs designed for *B. chouardii* that rendered 52 alleles in that species (Segarra-Moragues *et al.* 2003) yielded 106 alleles in *B. pyrenaica*. On the other hand, the seven microsatellite primer pairs designed for *B. pyrenaica* that detected 28 alleles in population Bp02 of that taxon (Segarra-Moragues *et al.* 2004) detected 45 alleles across all the studied *B. pyrenaica* populations and also revealed 15 alleles in *B. chouardii*. Levels of SSR polymorphisms varied considerably across loci; however, high values of genetic polymorphism were obtained within this broad survey at both the source species (*B. pyrenaica*) and the target, *B. chouardii* (Table 2). The more widespread *B. pyrenaica* had a total of 152 microsatellite alleles whereas its highly restricted congener *B. chouardii* had only 67 alleles. Thirty-six (19.78%) of the 182 alleles amplified across the 17 microsatellite primer pairs in both species were shared by the two Borderea taxa. Nine and 13 of the 17 analyzed regions detected individuals with up to three or four peaks in *B. chouardii* and *B. pyrenaica*, respectively, thus supporting previous findings of the tetraploid nature of these species (Segarra-Moragues *et al.* 2003, 2004).

Allele sizes fitted the expected values for variation in the number of trinucleotide repeats in all 17 microsatellite regions, suggesting the existence of sequential length mutations of the repeat motifs caused by polymerase slippage (Schlötterer and Tautz 1992). Microsatellite homology was also inferred in Borderea as the two species shared alleles in all but 1 of the 17 studied regions (Table 2). The seven microsatellite primer pairs designed for *B. pyrenaica* detected fewer alleles in the congener *B. chouardii* (15) than in the source species (45), a commonly observed phenomenon in cross-transferability experiments among closely related plant taxa (Palop *et al.* 2000). However, the opposite did not hold true as the 10 microsatellite primer pairs of *B. chouardii* detected more alleles in the target *B. pyrenaica* species (106) than in the source (52). The lower number of SSR alleles detected in *B. chouardii* compared to *B. pyrenaica* could be likely derived from the shorter sampling size and lower effective population size of this endangered species. In general, the number of alleles and their size ranges were similar in the two taxa or, when larger in *B. pyrenaica*, they encompassed most of the allelic variation of *B. chouardii*. Only three microsatellite regions (Bc1159, Bc1169, and Bc166) rendered more alleles in *B. chouardii* than in *B. pyrenaica* (Table 2), although most alleles from the latter taxon are also present in *B. chouardii*. These facts further suggest a strong homology of the common microsatellites in these two species.

The less variable regions included those microsatellites for which the range of allelic variants was small and presented no more than two alleles per individual (Table 2). Locus Bp1286 was monomorphic for allele 123 in all analyzed individuals of both Borderea species. A BLAST search was performed with the sequence of the clone to test whether this locus could correspond to a chloroplast microsatellite region of Borderea. As none of the retrieved sequences with identity values >80% corresponded to plastid DNA it was assumed that the locus could be assigned to an invariable SSR region of the Borderea nuclear genome. Bp2214 was also fixed for allele 213 in *B. chouardii* but presented two alleles (204 and 216) in *B. pyrenaica*. On the other hand, Bp2256 was heterozygous and fixed for private alleles 220 and 223 in *B. pyrenaica*, except for one triallelic individual that also showed allele 226 of *B. chouardii*; this latter taxon showed fixed heterozygosity for alleles 226 and 232 (Table 2).

Other regions that yielded few alleles resulting in mono- or diallelic individuals of *B. chouardii* (Bc1551, Bc1644, Bp126, and Bp2391) detected a broad spectrum of microsatellite allelic variability in *B. pyrenaica* (Table 2), with some individuals presenting up to three or four alleles. Bp2290 was fixed for private allele 139 in *B. chouardii* and Bp2391 showed fixed heterozygosity in this same species. The largest ranges of allele sizes were found in *B. pyrenaica* for regions Bc1422 (18 alleles), Bc1274 (24 alleles), and Bc1551 (25 alleles). Locus Bc166 was more variable in *B. chouardii* than in *B. pyrenaica*, presenting up to 12 alleles in the former taxon but only up to 4 (3 + 1 null) in the latter. The most frequent alleles for this region in *B. pyrenaica* were 175 and 178 and they showed a fixed heterozygous pattern in most studied individuals.

Bayesian analysis of autotetraploidy *vs.* allotetraploidy in Borderea: Distribution patterns of microsatellite alleles varied significantly among the 17 analyzed regions and across individuals of the two Borderea taxa. Assignment of alleles to the potential parental genomes in each alternative case (autotetraploidy with tetrasomic inheritance *vs.* allotetraploidy with disomic inheritance) is a prerequisite for a rigorous statistical testing of the two competing hypotheses. This was achieved

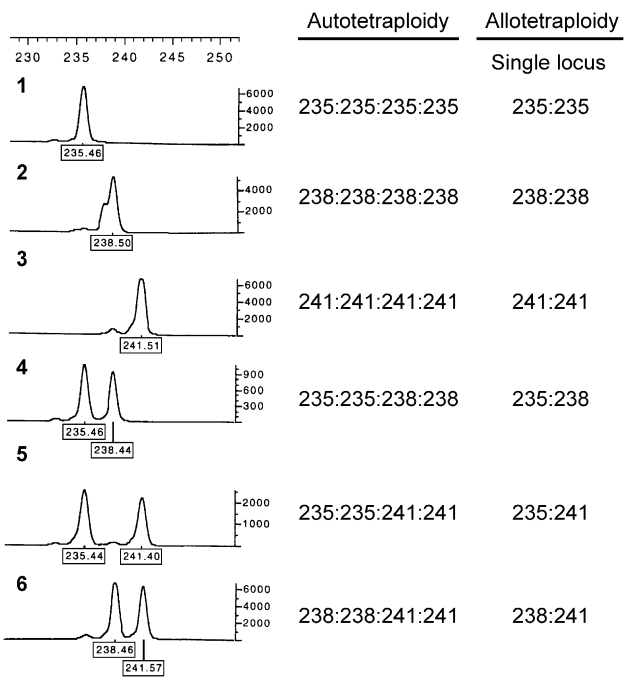


FIGURE 2.—Sample electropherogram of the microsatellite region Bp126 (six individuals) presumably amplifying a single genetic dosage (individuals with up to two alleles) in Borderea and the corresponding genotypes encoded under autotetraploidy and allotetraploidy (1 locus each) assumptions. Note that for the autotetraploid hypothesis all individuals with two bands show similar peak ratios of the amplified products and are therefore encoded as balanced heterozygotes, whereas individuals with a single band are considered homozygous, assuming absence of null alleles. Under the allotetraploid hypothesis all amplified products were assumed to belong to the same locus.

for most of the studied regions on the basis of the comparative analysis of the distribution of alleles and allele sets within individuals of each species and on the basis of the corresponding amplification dosage of those alleles. However, this latter parameter has been interpreted differently when considering autopolyploid and allopolyploid inheritance. For instance, in allopolyploids amplification of alleles from one parental complement is not always as successful as that from the other, as observed in regions Bc1169, Bc1551, Bc1644, Bp126, Bp1286, and Bp2214 (Figure 2), probably due to incomplete identity of the designed primers to the target regions in the heterologous chromosomes (CALLEN *et al.* 1993). Conversely, in autopolyploids, matching of primers is considered equally probable for all homologous chromosomes, thus enabling interpretation of amplification profiles, the allele peak ratios, as a direct consequence of allelic dosage. Assignment of alleles to genotypes was completed for those regions that allowed unequivocal attribution of alleles to both the tetrasomic and the disomic inheritance models. This was performed for those loci that presented <18 alleles and that showed nonoverlapping segregation patterns of alleles in almost all examined individuals.

Fourteen and 11 of the 17 studied microsatellite regions could be coded for via both tetrasomic and disomic inheritance modes in *B. chouardii* and in *B. pyrenaica*, respectively. Nine common microsatellite regions (Bc1169, Bc1258, Bc1644, Bp126, Bp1286, Bp2214, Bp2256, Bp2290, and Bp2391) were coded for both taxa. *B. chouardii* was also coded for regions Bc1145B, Bc1274, Bc1357, Bc1422, and Bc1551 and *B. pyrenaica* for Bc166. Five single-locus cases were present in *B. chouardii* [Bc1551, Bc1644, and Bp126 (polymorphic) and Bp1286 and Bp2214 (monomorphic)] and four in *B. pyrenaica* [Bc1169, Bp126, and Bp2214 (polymorphic) and Bp1286 (monomorphic)]. Fixed diallelic heterozygous patterns were also observed in *B. pyrenaica* for regions Bc166 and Bp2256 and for regions Bp2256 and Bp2391 in *B. chouardii*. Some of the most polymorphic loci could not be satisfactorily coded for the disomic model due to the impossibility of confidently assigning alleles to each parental genome (*i.e.*, Bc1357 in *B. pyrenaica*, Figure 1B) and were not included in the Bayesian analysis.

Marginal probabilities of the data were computed for the two competing hypotheses for each separate species and population across the coded loci. The resulting coefficients and the values of the corresponding Bayes factors are shown in Tables 3 and 4. Monomorphic loci gave values of zero for both the tetrasomic and the disomic models. Bayes factors were always positive for all studied loci except for the monomorphic loci in the only known population of *B. chouardii* and across the 15 analyzed populations of *B. pyrenaica*, always favoring the hypothesis of disomic inheritance over that of tetrasomy. Total Bayes factors give very strong support to the disomic inheritance model over the tetrasomic inheritance model for these microsatellite alleles. In the logarithmic scale reported in Tables 3 and 4, Bayes factors >5 are usually taken as evidence of very strong support in favor of a hypothesis (KASS and RAFTERY 1995; RAFTERY 1996). The corresponding value for the total evidence in *B. chouardii* is 803.38 and that for *B. pyrenaica* is 13,169.28. Hence, these data strongly support the hypothesis that both species exhibit disomic inheritance.

Support for this hypothesis is not provided evenly by the different microsatellite regions. There are two loci in *B. chouardii*, Bp1286 and Bp2214, which do not provide differential support for any hypotheses, as their Bayes factors equal 1.0. The strongest support for the disomic inheritance pattern in this species comes from Bc1258, with a $\log_e$-transformed value of 116.48 for the Bayes factor. Apart from the two equivocal microsatellite regions mentioned above, there is only one *B. chouardii* region that does not provide strong support for the disomic inheritance, namely Bc1145B, with a $\log_e$-Bayes factor of 2.38. The next greater value corresponds to region Bp126 (20.44), which implies a very strong support by itself. The average support across the 14 microsatellite regions compared equals 65.09.

TABLE 3

Bayesian analysis of poliploidy origin in *B. chouardii*

Population/SSR region	Bc1145B		Bc1169		Bc1258		Bc1274		Bc1357		Bc1422		Bc1551	
	D	A	D	A	D	A	D	A	D	A	D	A	D[a]	A
B. chouardii (Bc01)														
$\text{Log}_e\ p(y \mid H_x)$	−6.44	−7.63	−10.28	−5919	−45.47	−103.71	−23.93	−77.90	−21.53	−70.83	−20.90	−69.99	−4.23	−18.64
$2\ \text{Log}_e(B_{\text{allo,auto}})$	2.38		97.82		116.48		107.94		98.60		98.18		28.82	
Boot.	NB		***		***		***		***		***		**	
	Bc1644		Bp126		Bp1286		Bp2214		Bp2256		Bp2290		Bp2391	
	D[a]	A	D[a]	A	D[a]	A	D[a]	A	D	A	D	A	D	A
B. chouardii (Bc01)														
$\text{Log}_e\ p(y \mid H_x)$	−10.24	−34.03	−5.45	−15.67	0	0	0	0	0	−48.87	−4.68	−53.48	0	−48.87
$2\ \text{Log}_e(B_{\text{allo,auto}})$	47.58		20.44		0		0		97.74		97.60		97.74	
Boot.	**		**		NB		NB		**		**		**	
B. chouardii (Bc01)	Total evidence:													
$\text{Log}_e\ p(y \mid H_x)$	−129.22	−530.91												
$2\ \text{Log}_e(B_{\text{allo,auto}})$	803.38													
B. chouardii (Bc01)	Averaged over loci:													
$2\ \text{Log}_e(B_{\text{allo,auto}})$	65.09													

Marginal probability of data $\{\text{Log}_e\ p(y \mid H_x)\}$ obtained for the alternative hypotheses of allotetraploidy (D, single disomic or digenic disomic inheritance) and autotetraploidy (A, tetrasomic inheritance). Bayes factors $\{2\ \text{Log}_e\ B_{\text{allo,auto}} = 2 \times (\text{Log}_e\ p(y \mid H_{\text{allo}}) - \text{Log}_e\ p(y \mid H_{\text{auto}}))\}$ are given for each locus and at the species level. Results based on parametric bootstrap simulation (Boot.) are also given. NB, bootstrap not implemented; $^{**}P < 0.01$; $^{***}P < 0.00001$.

[a] Single-locus microsatellites.

TABLE 4

Bayesian analysis of poliploidy origin in *B. pyrenaica*

Population/ SSR region	Bc1169		Bc1258		Bc1422		Bc1644		Bc166		Bp126		Bp1286		Bp2214		Bp2256		Bp2290		Bp2391		Per Population
	D[a]	A	D	A	D	A	D	A	D	A	D[a]	A	D[a]	A	D[a]	A	D	A	D	A	D	A	
Bp01																							
$\text{Log}_e\ p(y \mid H_x)$	0	0	−9.49	−77.50	−30.86	−103.06	−23.13	−91.03	0	−61.74	−16.02	−88.09	0	0	0	0	0	−61.74	−13.78	−82.29	−37.01	−110.45	
$2\ \text{Log}_e(B_{\text{allo,auto}})$	0		136.02		144.40		135.80		123.48		144.14		0		0		123.48		137.02		146.88		99.20
Boot.	NB		***		***		***		**		**		NB		NB		**		***		***		
Bp02																							
$\text{Log}_e\ p(y \mid H_x)$	0	0	−7.58	−69.26	−32.13	−94.81	−23.92	−94.76	0	−61.74	−10.64	−67.68	0	0	−3.58	−8.59	0	−61.74	−21.74	−90.32	−47.75	−112.50	
$2\ \text{Log}_e(B_{\text{allo,auto}})$	0		123.36		125.36		141.68		123.48		114.08		0		10.02		123.48		137.16		129.50		93.46
Boot.	NB		***		***		***		**		**		NB		**		**		***		***		
Bp03																							
$\text{Log}_e\ p(y \mid H_x)$	0	0	0	−21.96	−9.07	−31.07	−12.16	−37.15	0	−21.96	−6.12	−26.45	0	0	0	0	0	−21.96	−3.33	−25.22	−11.07	−35.64	
$2\ \text{Log}_e(B_{\text{allo,auto}})$	0		43.92		44.00		49.98		43.92		40.66		0		0		43.92		43.78		49.14		32.66
Boot.	NB		**		***		***		**		**		NB		NB		**		**		***		
Bp04																							
$\text{Log}_e\ p(y \mid H_x)$	0	0	0	−61.74	−16.82	−78.69	−13.77	−79.22	0	−61.74	−6.32	−47.75	0	0	0	0	0	−61.74	−8.32	−69.83	−28.52	−106.81	
$2\ \text{Log}_e(B_{\text{allo,auto}})$	0		123.48		123.74		130.90		123.48		82.86		0		0		123.48		123.02		156.58		89.77
Boot.	NB		**		***		***		**		**		NB		NB		**		**		***		
Bp05																							
$\text{Log}_e\ p(y \mid H_x)$	0	0	−4.23	−65.90	−44.63	−107.32	−20.45	−92.61	−8.53	−31.53	−14.70	−93.72	0	0	0	0	0	−61.74	−18.86	−84.27	−30.80	−98.66	
$2\ \text{Log}_e(B_{\text{allo,auto}})$	0		123.43		125.38		144.32		46.00		158.04		0		0		123.48		130.82		135.72		89.74
Boot.	NB		**		***		***		NB		***		NB		NB		**		***		***		
Bp06																							
$\text{Log}_e\ p(y \mid H_x)$	0	0	−7.17	−68.84	−29.82	−92.24	−25.14	−96.16	0	−61.74	−17.44	−91.08	0	0	0	0	0	−61.74	−10.03	−74.10	−32.65	−106.41	
$2\ \text{Log}_e(B_{\text{allo,auto}})$	0		123.34		124.84		142.04		123.48		147.28		0		0		123.48		128.14		147.52		96.37
Boot.	NB		**		***		***		**		***		NB		NB		**		***		***		
Bp07																							
$\text{Log}_e\ p(y \mid H_x)$	0	0	−4.13	−40.02	−18.28	−54.27	−14.86	−51.66	0	−35.96	−14.56	−52.53	0	0	0	0	0	−35.96	−25.10	−63.23	−30.71	−79.86	
$2\ \text{Log}_e(B_{\text{allo,auto}})$	0		71.78		71.98		73.60		71.92		75.94		0		0		71.92		76.26		98.30		55.61
Boot.	NB		**		***		***		**		***		NB		NB		**		***		***		
Bp08																							
$\text{Log}_e\ p(y \mid H_x)$	0	0	−6.78	−68.46	−17.32	−79.10	−33.78	−117.73	0	−61.74	−15.60	−94.63	0	0	0	0	0	−61.74	−12.03	−75.21	−13.67	−78.47	
$2\ \text{Log}_e(B_{\text{allo,auto}})$	0		123.36		123.56		167.90		123.48		158.06		0		0		123.48		126.36		129.60		97.80
Boot.	NB		***		***		***		**		***		NB		NB		**		***		***		
Bp10																							
$\text{Log}_e\ p(y \mid H_x)$	0	0	0	−61.74	−37.83	−100.01	−27.76	−94.48	0	−61.74	−4.23	−17.71	0	0	−6.65	−55.09	0	−61.74	−14.38	−79.09	−21.02	−87.24	
$2\ \text{Log}_e(B_{\text{allo,auto}})$	0		123.48		124.36		133.44		123.48		26.96		0		96.88		123.48		129.42		132.44		92.18
Boot.	NB		**		***		***		**		**		NB		**		**		***		***		

(*continued*)

TABLE 4

(Continued)

Population/ SSR region	Bc1169		Bc1258		Bc1422		Bc1644		Bc166		Bp126		Bp1286		Bp2214		Bp2256		Bp2290		Bp2391		Per Population
	D[a]	A	D	A	D	A	D	A	D	A	D[a]	A	D[a]	A	D[a]	A	D	A	D	A	D	A	
Bp11																							
$\text{Log}_e\ p(y \mid H_x)$	0	0	−3.58	−65.26	−29.04	−94.09	−25.82	−95.49	0	−61.74	−13.46	−66.87	0	0	0	0	0	−61.74	−17.70	−87.81	−28.69	−94.21	
$2\ \text{Log}_e(B_{\text{allo,auto}})$	0		123.36		130.10		139.34		123.48		106.82		0		0		123.48		140.22		131.04		92.53
Boot.	NB		**		***		***		**		**		NB		NB		**		***		***		
Bp12																							
$\text{Log}_e\ p(y \mid H_x)$	0	0	−9.15	−41.05	−13.98	−45.99	−9.53	−41.54	0	−31.97	−13.58	−46.17	0	0	0	0	0	−31.97	−4.85	−36.76	−11.45	−45.61	
$2\ \text{Log}_e(B_{\text{allo,auto}})$	0		63.80		64.02		64.02		63.94		65.18		0		0		63.94		63.82		68.32		47.00
Boot.	NB		***		***		***		**		**		NB		NB		**		**		***		
Bp13																							
$\text{Log}_e\ p(y \mid H_x)$	0	0	−6.63	−68.31	−43.85	−126.07	−20.75	−88.04	−7.50	−28.32	−13.75	−85.36	0	0	0	0	0	−61.74	−26.51	−101.42	−25.96	−98.12	
$2\ \text{Log}_e(B_{\text{allo,auto}})$	0		123.36		164.44		134.58		41.64		143.22		0		0		123.48		149.82		144.32		93.17
Boot.	NB		***		***		***		NB		***		NB		NB		**		***		***		
Bp14																							
$\text{Log}_e\ p(y \mid H_x)$	0	0	−11.04	−72.77	−97.00	−165.39	−34.98	−112.58	−10.27	−35.38	−22.66	−109.37	0	0	0	0	0	−61.74	−43.67	−114.86	−47.97	−127.32	
$2\ \text{Log}_e(B_{\text{allo,auto}})$	0		123.46		136.78		155.20		50.22		173.42		0		0		123.48		142.38		158.70		96.69
Boot.	NB		***		***		***		NB		***		NB		NB		**		***		***		
Bp15																							
$\text{Log}_e\ p(y \mid H_x)$	−11.11	−31.14	−4.36	−66.03	−33.95	−99.41	−26.75	−88.71	−7.78	−7.93	−13.46	−66.60	0	0	0	0	−3.58	−65.26	−26.72	−88.83	−26.94	−101.58	
$2\ \text{Log}_e(B_{\text{allo,auto}})$	40.06		123.34		130.92		123.92		0.30		106.28		0		0		123.36		124.22		149.28		83.79
Boot.	**		**		***		***		NB		**		NB		NB		**		***		***		
Bp16																							
$\text{Log}_e\ p(y \mid H_x)$	0	0	−5.92	−67.60	−65.33	−128.28	−30.58	−89.98	0	−61.74	−5.92	−67.60	0	0	0	0	0	−61.74	−50.95	−120.76	−30.60	−50.76	
$2\ \text{Log}_e(B_{\text{allo,auto}})$	0		123.56		125.90		118.80		123.48		123.36		0		0		123.48		139.62		40.32		83.50
Boot.	NB		**		***		***		**		**		NB		NB		**		***		***		
B. pyrenaica	Total evidence:																						
$\text{Log}_e\ p(y \mid H_x)$	−1913.59	−8498.2																					
$2\ \text{Log}_e(B_{\text{allo,auto}})$	13169.28																						
B. pyrenaica	Per locus:																						Averaged over loci and populations:
$2\ \text{Log}_e(B_{\text{allo,auto}})$	2.67		111.54		117.32		123.70		87.05		111.09		0		7.13		110.76		119.47		121.18		82.90

Marginal probability of data $\{\text{Log}_e\ p(y \mid H_x)\}$ obtained for the alternative hypotheses of allotetraploidy (D, single disomic or digenic disomic inheritance) and autotetraploidy (A, tetrasomic inheritance). Bayes factors $\{2\ \text{Log}_e\ B_{\text{allo,auto}} = 2 \times (\text{Log}_e\ p(y \mid H_{\text{allo}}) - \text{Log}_e\ p(y \mid H_{\text{auto}}))\}$ are given for each locus and at the species level. Results based on parametric bootstrap simulation (Boot.) are also given. NB, bootstrap not implemented; $^{**}P < 0.01$; $^{***}P < 0.00001$.

[a] Single-locus microsatellites.

A similar pattern can be observed in the detailed analysis of *B. pyrenaica*, as only one microsatellite region (Bp1286) gave equal support to both alternatives. One region supported the disomic inheritance mode positively (2.67; Bc1169), although the value of the single population that showed this differential support was >40 (Table 4). The remaining nine regions gave strong support to the allotetraploid hypothesis, although the strength of this support varied from a minimum $\log_e$-transformed Bayes factor of 7.13 (Bp2214) to a maximum of 123.70 (Bc1644). There were two microsatellite regions, Bc166 and Bp2256, in which the disomic pattern was almost perfectly supported by the data in most populations whereas the marginal probability of the alternative was very low. In fact, in all microsatellite-region/population combinations where individual Bayes factors supported the disomic over the tetrasomic inheritance they did so with high support values. The remaining ones were cases where it was not possible to decide between the two alternatives because of the monomorphism of the region in the population.

Parametric bootstrap simulation analysis: Results based on the parametric bootstrap simulation study of each microsatellite region and population are also shown in Tables 3 and 4. Basically, three situations were found: (i) cases where it was not possible to implement the bootstrap procedure (NB) due to the monomorphic nature of the region; (ii) cases where after the generation of 100,000 bootstrap samples none of them was consistent with the allotetraploidy model (which means that the *P*-value for rejecting allotetraploidy is $<10^{-5}$; these cases have been labeled as *** and correspond to >50% of all bootstrapped cases); and (iii) cases where the empirical distribution of the Bayes factor was obtained and the contrast with the estimated Bayes factor from the original sample could be performed. In these latter cases, the high statistical significance observed in all the corresponding tests, which have been identified as **, is remarkable (Tables 3 and 4).

Linkage disequilibrium test: Although some monomorphic loci precluded the testing of all 2801 possible combinations, none of the 1083 tests for linkage disequilibrium performed between pairs of SSR loci within populations of both *B. chouardii* and *B. pyrenaica* was significant at $P < 0.05$ after application of sequential Bonferroni corrections. Although it is not possible that all 17 regions are independent, since there are at most 12 linkage groups in $2n = 24$ species, this result indicates that the set of 17 SSR loci analyzed constitutes a broad sample of the corresponding genomes of the two Borderea taxa.

DISCUSSION

Assessing patterns of inheritance and the origin of polyploidy in plants through Bayesian analysis: The Bayesian approach developed in this study has provided strong support for the disomic inheritance of most microsatellite regions studied and, therefore, for allopolyploid origins of the two Borderea species. The analysis of a broad array of SSR loci has allowed us to conclude that the screening might have covered most of the 12 chromosome pairs of the Borderea genome, which most likely consists of two subgenomes of 6 chromosome pairs each. Consequently, Bayes factor testing is a useful statistical tool for investigating the origin of polyploid taxa when sufficiently large samples and highly variable markers are employed. These methods are relevant and easily transferable for the analysis of patterns of inheritance in other polyploid species for which experimental crosses and progeny tests are precluded.

The use of microsatellite data has been decisive in resolving the allopolyploid origin of the genus Borderea, confirming previous insights based on allozymes. Fixed heterozygous patterns detected for two allozyme loci (IDH and PGI2) in individuals of *B. pyrenaica* and for one locus (PGI2) in those of *B. chouardii*, along with cosegregating allelic patterns observed for the PGI2 locus in pollen grains, pointed to duplicated gene events or to a hybrid origin for these taxa (Segarra-Moragues and Catalán 2002; Segarra-Moragues *et al.* 2004). The combined presence of fixed heterozygous SSR allelic profiles in *B. pyrenaica* individuals (regions Bc166 and Bp2256) and in *B. chouardii* (Bp2256 and Bp2391) and the predominance of independent segregation of different SSR allele sets in both *B. pyrenaica* and *B. chouardii* (Tables 3 and 4) are determinant factors accounting for the overwhelming differences in the degree of support for disomic inheritance over tetrasomic inheritance found through Bayes factors.

The putative allotetraploidy of Borderea is consistent with amphipolyploidy being the most common mechanism for the appearance of polyploidy in angiosperms (Stebbins 1971; Soltis and Soltis 2000; Jenczewski and Alix 2004) and offers new insights into the hybrid origin of this relictual genus and of its sister taxon Tamus L. (Caddick *et al.* 2002). In disagreement with previous reports from Heslot (1953) and from Gaussen (1965) that considered $x = 12$ to be the chromosome base number of the putative diploid Borderea taxa ($2n = 24$) and of the putative tetraploid Tamus ($2n = 48$) (Gaussen 1965), our analysis suggests a lower chromosome base number of $x = 6$ for the allotetraploid Borderea and for a potential allo-octoploid Tamus, which would be the smallest chromosome base number reported for the large and pantropically widespread family of the yams (Heslot 1953; Burkill 1960; Dahlgren *et al.* 1985).

Microsatellite variation and the evolution of the Borderea genome: Our Bayesian analysis of microsatellite variation has provided unequivocal evidence on the allopolyploid nature of the two Borderea species. However, several microsatellite regions have not been

analyzed for either *B. chouardii* (Bc1159, Bc166, and Bp2292) or *B. pyrenaica* (Bc1145B, Bc1159, Bc1274, Bc1357, Bc1551, and Bp2292) due to the impossibility of confidently assigning alleles to each parental genomic complement in the disomic inheritance hypothesis. Some of these microsatellite regions present the highest number of allelic variants for each taxon (Table 2) and thus represent the most polymorphic regions of the whole set. On the basis of the large proportion of disomic inheritance patterns detected for most microsatellite regions in the two Borderea species, it can be assumed that the three- and six-microsatellite regions that could not be confidently tested in *B. chouardii* and in *B. pyrenaica*, respectively, also have an allopolyploid origin. The inability to properly assign alleles to the parental genomes in those noncoded microsatellite regions derives from confounding overlapping ranges of allele sizes (*i.e.*, loci Bc1145B and Bc1357 in *B. pyrenaica*, Figure 1B) and from the risk of assuming the same disomic inheritance in the opposite taxon when allele ranges are large enough therein (*i.e.*, locus Bc166 in *B. chouardii* and locus Bc1551 in *B. pyrenaica*).

However, results obtained from the parametric bootstrap simulation study clearly show that despite the lack of analysis of the above-mentioned microsatellite regions the data are still overwhelmingly supportive of allotetraploidy. This conclusion is based on the following facts: (1) the observed Bayes factor far exceeds the expected Bayes factors under autotetraploidy even after correction for assessment bias (Tables 3 and 4); (2) far more loci would be expected to fail the genotyping procedure under the allotetraploid hypothesis if autotetraploidy was the mode of inheritance; and (3) far more loci would be expected to be in triple dosage, which is a rare event in the original samples, if autotetraploidy was true.

Even if amphidiploidy emerges as the most likely source of origin for most of the studied microsatellite loci, other biological mechanisms that operate in hybrid taxa such as segmental allopolyploidy (Stebbins 1947) should not be ruled out. Although the true nature of plant segmental allopolyploidy has been questioned cytogenetically (Sybenga 1996), segmental allopolyploids have been documented in various lineages of angiosperms on the basis of both genetic and cytogenetic criteria (Stebbins 1971) and on the basis of analyses of codominant and dominant molecular markers with intermediate inherited profiles in natural (Herrero *et al.* 2001) and synthetic (Barone *et al.* 2002) plant species. Segmental allopolyploidy may account for the origin of Borderea species because the ancestral diploid lineages that compose them are genetically similar at some chromosomal complements, as demonstrated by the monomorphic and the overlapped microsatellite regions detected in both taxa. In the absence of parent-offspring information it is not possible to clarify the original nature of the two common microsatellite regions that have not been tested in any of the two Borderea species (Bc1159 and Bp2292) and that might correspond to amphidiploidized loci with confounding overlapping allele-size ranges or to potential segmental allopolyploids.

Clarification of the allopolyploid nature and of the diploid inheritance pattern of Borderea could also help to decipher the identity of the putative ancestors of these hybrid taxa. The stability of the 17 $(CTT)_n$ microsatellite regions across the two Borderea species is reduced when analyzed according to their potential disomic inheritance. Five and four single-locus cases were detected in *B. chouardii* (regions Bc1551, Bc1644, Bp126, Bp1286, and Bp2214) and *B. pyrenaica* (Bc1169, Bp126, Bp1286, and Bp2214), respectively, indicating a single disomic inheritance for those microsatellite regions that do not show priming sites in one of the parental chromosome complements. A similar case was observed for four microsatellite regions in the amphidiploid *Brassica napus*, where only one progenitor's locus was amplified from each primer pair (Lagercrantz *et al.* 1993). Due to the putative ancient hybrid nature of the Borderea species we speculate that the priming sites were present in the chromosomal complement of one diploid ancestral parent but not in the other. This scenario fits well for locus Bp126, which shows to be single disomic and with shared allele 226 in both species, and for loci Bp2214, single disomic in *B. pyrenaica* and monomorphic in *B. chouardii*, and Bc1551, single disomic in *B. chouardii* and uncoded as allopolyploid but sharing alleles 264 and 267 in *B. pyrenaica* (Tables 2–4). However, this possibility is not as clearly supported in other cases, represented by loci Bc1644 and Bc1169, which are single disomic in one species but duplicate disomic in the other, although with shared alleles in presumably the same parental complement in both taxa (Tables 2–4). The most likely explanations for these latter cases would be either the secondary loss of the corresponding SSR priming sites in the alternative chromosomal complements of each species or the result of the hybridization between one common parental species and a different one to produce each Borderea taxa. This latter explanation would fit for Bp2256 as both species share allele 226, although it is rare in *B. pyrenaica*, but have fixed a different alternative allele in the other complement (220 in *B. pyrenaica* and 232 in *B. chouardii*). However, the present data do not allow us to further discern between these alternative evolutionary scenarios (*i.e.*, the allotetraploid Borderea taxa could have had a single hybrid origin followed by a later divergence or could have originated separately, being the only tetraploid remnant species from a larger reticulate complex). Genomic *in situ* hybridization analysis using genomic DNA of one of the species as a probe for the other could be applied to shed some light on this issue, given that the presumed parental taxa of Borderea are now extinct.

We are indebted to Tom Ranker and Pamela Soltis for their critical review of the manuscript and their valuable comments on origins of polyploidy in plants and to Peter Oefner and an anonymous referee for fruitful advice and most encouraging discussion on Bayesian analysis. We thank the technical staff at the Autonomous Community of Aragón (Spain) and at the National Park of the Pyrenees (France) for sampling facilities. This work has been supported by an Aragón Government (DGA) project grant P105/99-AV to P.C. and by a DGA Ph.D. fellowship to J.G.S.-M. M.P.-E. and F.G.-C. were supported by a Ministerio de Educacíon y Ciencia/Comisión Interministerial de Ciencia y Tecnología (P98-1436) grant.

LITERATURE CITED

Barone, A., J. Li, A. Sebastiano, T. Cardi and L. Frusciante, 2002 Evidence for tetrasomic inheritance in a tetraploid *Solanum commersonii* (+) *S. tuberosum* somatic hybrid through the use of molecular markers. Theor. Appl. Genet. **104:** 539–546.

Beaumont, M. A., and B. Rannala, 2004 The Bayesian revolution in genetics. Nat. Rev. Genet. **5:** 251–261.

Box, G. P. E., and G. C., Tiao, 1973 *Bayesian Inference in Statistical Analysis*. Wiley, New York.

Burkill, I. H., 1960 The organography and the evolution of Dioscoreaceae, the family of the yams. Bot. J. Linn. Soc. **56:** 319–412.

Caddick, L. R., P. J. Rudall, P. Wilkin, T. A. J. Hedderson and M. W. Chase, 2002 Phylogenetics of Dioscoreales based on combined analyses of morphological and molecular data. Bot. J. Linn. Soc. **138:** 123–144.

Callen, D. F., A. D. Thompson, Y. Shen, H. A. Phillips, R. I. Richards *et al.*, 1993 Incidence and origin of "null" alleles in the $(AC)_n$ microsatellite markers. Am. J. Hum. Genet. **52:** 922–927.

Dahlgren, R. M. T., H. T. Clifford and P. F. Yeo, 1985 *The Families of the Monocotyledons*. Springer-Verlag. Berlin.

De Vicente, M. C., and P. Arus, 1996 Tetrasomic inheritance of isozymes in sainfoin (*Onobrychis viciaefolia* Scop.). J. Hered. **87:** 54–62.

Edwards, A.W. F., 1972 *Likelihood*. Cambridge University Press, Cambridge, UK.

Efron, B., and R. J. Tibshirani, 1993 *An Introduction to the Bootstrap*. Chapman & Hall, New York.

Esselink, G. D., H. Nybom and B. Vosman, 2004 Assignment of allelic configuration in polyploids using the MAC-PR (microsatellite DNA allele counting-peak ratios) method. Theor. Appl. Genet. **109:** 402–408.

Ford, V. S., and L. D. Gottlieb, 2002 Single mutations silence PgiC2 genes in two very recent allotetraploid species of *Clarkia*. Evolution **56:** 699–707.

Galloway, L. F., J. R. Etterson and J. L. Hamrick, 2003 Outcrossing rate and inbreeding depression in the herbaceous autotetraploid *Campanula americana*. Heredity **90:** 308–315.

García, M. B., 2003 Demographic viability of a relict population of the critically endangered plant *Borderea chouardii*. Conserv. Biol. **17:** 1672–1680.

García, M. B., and R. J. Antor, 1995 Age and size structure in populations of a long-lived dioecious geophyte: *Borderea pyrenaica* (Dioscoreaceae). Int. J. Plant Sci. **156:** 236–243.

Gaussen, H., 1965 Révision de *Dioscorea* (*Borderea*) Pyrénéens. Bull. Soc. Hist. Nat. Toulouse **100:** 392–398.

González-Candelas, F., M. A. Bracho and A. Moya, 2003 Molecular epidemiology and forensic genetics: application to a hepatitis C virus transmission event at a haemodialysis unit. J. Infect. Dis. **187:** 352–358.

Grant, V., 1981 *Plant Speciation*, Ed. 2. Columbia University Press, New York.

Herrero, A., S. Pajarón and C. Prada, 2001 Isozyme variation and genetic relationships among taxa in the *Asplenium obovatum* group (Aspleniaceae, Pteridophyta). Am. J. Bot. **88:** 2040–2050.

Heslot, H., 1953 Le nombre chromosomique des Dioscoréacées pyrénéennes et leur rattachement au genre *Borderea* Miégeville. Compt. Rend. Acad. Sci. Paris **237:** 433–434.

Huelsenbeck, J. P., B. Rannala and J. P. Masly, 2000 Accommodating phylogenetic uncertainty in evolutionary studies. Science **288:** 2349–2350.

Huelsenbeck, J. P., F. Ronquist, R. Nielsen and J. P. Bollback, 2001 Bayesian inference of phylogeny and its impact on evolutionary biology. Science **294:** 2310–2314.

Jenczewski, E., and K. Alix, 2004 From diploids to allopolyploids: the emergence of efficient pairing control genes in plants. Crit. Rev. Plant Sci. **23:** 21–45.

Johnson, N. L., and S. Kotz, 1969 *Discrete Distributions*. Wiley, New York.

Kass, R. E., and A. E. Raftery, 1995 Bayes factors. J. Am. Stat. Assoc. **90:** 773–795.

Lagercrantz, U., H. Ellegren and L. Andersson, 1993 The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Res. **21:** 1111–1115.

Lawton-Rauh, A., 2003 Evolutionary dynamics of duplicated genes in plants. Mol. Phylogenet. Evol. **29:** 396–409.

Lerceteau-Köhler, E., G. Guérin, F. Laigret and B. Denoyes-Rothan, 2003 Characterization of mixed disomic and polysomic inheritance in the octoploid strawberry (*Fragaria* × *ananassa*) using AFLP mapping. Theor. Appl. Genet. **107:** 619–628.

Liu, B., and J. F. Wendel, 2002 Non-Mendelian phenomena in allopolyploid genome evolution. Curr. Genomics **3:** 489–506.

Masterson, J., 1994 Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science **264:** 421–423.

Mochida, K., Y. Yamazaki and Y. Ogihara, 2004 Discrimination of homoeologous gene expression in hexaploid wheat by SNP analysis of contigs grouped from a large number of expressed sequence tags. Mol. Genet. Genomics **270:** 371–377.

Moody, M. E., L. D. Mueller and D. E. Soltis, 1993 Genetic variation and random drift in autotetraploid populations. Genetics **134:** 649–657.

Nybom, H., G. D. Esselink, G. Werlemark and B. Vosman, 2004 Microsatellite DNA marker inheritance indicates preferential pairing between two highly homologous genomes in polyploid and hemisexual dog-roses, *Rosa* sect. *Caninae* DC. Heredity **92:** 139–150.

Olson, M. S., 1997 Bayesian procedures for discriminating among hypotheses with discrete distributions: inheritance in the tetraploid *Astilbe biternata*. Genetics **147:** 1933–1942.

Otto, S. P., and J. Whitton, 2000 Polyploid incidence and evolution. Annu. Rev. Genet. **34:** 401–437.

Palop, M., C. Palacios and F. González-Candelas, 2000 Development and across-species transferability of microsatellite markers in the genus *Limonium* (Plumbaginaceae). Conserv. Genet. **1:** 177–179.

Posada, D., and T. R. Buckley, 2004 Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst. Biol. **53:** 793–808.

Raftery, A. E., 1996 Approximate Bayes factors and accounting for model uncertainty in generalised linear models. Biometrika **83:** 251–266.

Raymond, M., and F. Rousset, 1995 GENEPOP. Version 3.3: population genetics software for exact test and ecumenism. J. Hered. **86:** 248–249.

Rice, W. R., 1989 Analysing tables of statistical tests. Evolution **43:** 223–225.

Rieseberg, L. H., and M. F. Doyle, 1989 Tetrasomic segregation in the naturally ocurring autotetraploid *Allium nevii* (Alliaceae). Hereditas **111:** 31.

Rong, J., C. Abbey, J. E. Bowers, C. L. Brubaker, C. Chang *et al.*, 2004 A 3347-locus genetic recombination map of sequence-tagged sites reveals features of genome organization, transmission and evolution of cotton (*Gossypium*). Genetics **166:** 389–417.

Ronfort, J. L., E. Jenczewski, T. Bataillon and F. Rousset, 1998 Analysis of population structure in autotetraploid species. Genetics **150:** 921–930.

Rozen, S., and H. J. Skaletsky, 1996 *Primer 3*. Whitehead Institute for Biomedical Research, Cambridge, MA (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

Schlötterer, C., and D. Tautz, 1992 Slippage synthesis of simple sequence DNA. Nucleic Acids Res. **20:** 211–215.

Segarra-Moragues, J. G., and P. Catalán, 2002 Low allozyme variability in the critically endangered *Borderea chouardii* and in its congener *Borderea pyrenaica* (Dioscoreaceae), two paleoendemic relicts from the central Pyrenees. Int. J. Plant Sci. **163:** 159–166.

Segarra-Moragues, J. G., and P. Catalán, 2003 Life history variation between species of the relictual genus *Borderea* (Dioscoreaceae): phylogeography, genetic diversity, and population genetic structure assessed by RAPD markers. Biol. J. Linn. Soc. **80:** 483–498.

Segarra-Moragues, J. G., and P. Catalán, 2005a *Borderea* Miégeville, pp. 11–14 in *Flora Iberica*, Vol. 21, edited by C. Aedo and A. Herrero. Real Jardín Botánico, Departamento de Publicaciones del CSIC, Madrid.

Segarra-Moragues, J. G., and P. Catalán, 2005b Species boundaries and population divergence in the Pyrenean endemic relict genus *Borderea* (Dioscoreaceae) as revealed by microsatellite (SSR) and other hypervariable markers. Aliso **22:** 541–555.

Segarra-Moragues, J. G., M. Palop-Esteban, F. González-Candelas and P. Catalán, 2003 Characterization of ten trinucleotide microsatellite loci in the critically endangered Pyrenean yam *Borderea chouardii* (Dioscoreaceae). Mol. Ecol. Notes **3:** 265–267.

Segarra-Moragues, J. G., M. Palop-Esteban, F. González-Candelas and P. Catalán, 2004 Characterization of seven $(CTT)_n$ microsatellite loci in the Pyrenean endemic *Borderea pyrenaica* (Dioscoreaceae). Remarks on ploidy level and hybrid origin assessed through allozymes and microsatellite analyses. J. Hered. **95:** 177–183.

Soltis, D. E., and P. S. Soltis, 1993 Molecular data and the dynamic nature of polyploidy. Crit. Rev. Plant Sci. **12:** 243–273.

Soltis, D. E., and P. S. Soltis, 1999 Polyploidy: recurrent formation and genome evolution. Trends Ecol. Evol. **14:** 348–352.

Soltis, D. E., and P. S. Soltis, 2000 The role of genetic and genomic attributes in the success of polyploids. Proc. Natl. Acad. Sci. USA **97:** 7051–7057.

Stebbins, G. L., 1947 Types of polyploids: their classification and significance. Adv. Genet. **1:** 403–429.

Stebbins, G. L., 1950 *Variation and Evolution in Plants.* Columbia University Press, New York.

Stebbins, G. L., 1971 *Chromosomal Evolution in Higher Plants.* Arnold, London.

Sybenga, J., 1996 Chromosome pairing affinity and quadrivalent formation in polyploids: Do segmental allopolyploids exist? Genome **39:** 1176–1184.

Tautz, D., 1989 Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res. **17:** 6463–6471.

Weir, B. S., and C. C. Cockerham, 1984 Estimating F-statistics for the analysis of population structure. Evolution **38:** 1358–1370.

Wendel, J. F., 2000 Genome evolution in polyploids. Plant Mol. Biol. **42:** 225–249.

Werth, C. R., and D. B. Lellinger, 1992 Genomically preserved plants and their nomenclature. Taxon **41:** 513–521.

Wu, K. K., W. Burnquist, M. E. Sorrells, T. L. Tew, P. H. Moore *et al.*, 1992 The detection and estimation of linkage in polyploids using single-dose restriction fragments. Theor. Appl. Genet. **83:** 294–300.

Wu, R., M. Gallo-Meagher, R. C. Littell and Z-B. Zeng, 2001 A general polyploid model for analysing gene segregation in outcrossing tetraploid species. Genetics **159:** 869–882.

Communicating editor: P. J. Oefner