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Long-term monitoring of B-chromosome invasion and neutralization in a population of *Prospero autumnale* (Asparagaceae)

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B chromosomes have been reported in about 15% of eukaryotes, but long-term dynamics of B chromosomes in a single natural population has rarely been analyzed. *Prospero autumnale* plants collected in 1981 and 1983 at Cuesta de La Palma population had shown the presence of B chromosomes. We analyze here seven additional samples collected between 1987 and 2015, and show that B frequency increased significantly during the 1980s and showed minor fluctuations between 2005 and 2015. A mother–offspring analysis of B chromosome transmission, at population level, showed significant drive on the male side ($k_B = 0.65$) and significant drag on the female side ($k_B = 0.33$), with average B transmission rate being very close to the Mendelian rate (0.5). No significant effects of B chromosomes were observed on a number of vigor and fertility-related traits. Within a parasite/host framework, these results suggest that B chromosomes' drive on the male side is the main pathway for B chromosome invasion, whereas B chromosome drag on the female side might be the main manifestation of host genome resistance in this species. *Prospero autumnale* thus illuminates a novel evolutionary pathway for B chromosome neutralization by means of a decrease in B transmission through the nondriving sex.

KEY WORDS: B-chromosomes, drag, drive, invasion, neutralization.

B chromosomes are dispensable supernumerary elements most likely derived from the standard (A) chromosomes or through interspecific hybridization (for review, see Camacho 2005). B chromosomes have been reported in more than 1200 plant species (8% of monocotyledones and 4% of dicotyledones; see Jones and Rees 1982; Camacho 2005; Levin et al. 2005). Their frequency in natural plant populations is associated with mating system (Burt and Trivers 1998), genome size (Trivers et al. 2004; Levin et al. 2005), or number of A chromosomes (Trivers et al. 2004).

As first suggested by Östergren (1945), B chromosomes usually behave as genome parasites, imposing deleterious costs to carriers and promoting their own transmission through a variety of non-Mendelian mechanisms (drive). The main consequence of B-drive mechanisms is an increase in population B frequency, unless severe harmful effects of B-presence on host fitness counteract them. When these effects are slight, however, rapid increase in B frequency can be observed in the course of a few tens of generations, especially when Bs are in the initial invasive stage (Camacho et al. 1997). This invasion dynamic has been reported, for instance, in the grasshopper, *Eyprepocnemis plorans* (Zurita et al. 1998; Riera et al. 2004; Camacho et al. 2015); the fish, *Prochilodus lineatus* (Cavallaro et al. 2000); and the wasp, *Trypoxylon albitarse* (Araújo et al. 2001, 2002). However, B chromosome invasions have never been reported in plants, as B frequency

has rarely been analyzed for long periods in a single natural population.

Population dynamics of B chromosomes in natural populations mainly depend on two main properties, namely inheritance (presence of drive mechanisms) and effects on carrier fitness. A frequent drive mechanism in angiosperms is mitotic nondisjunction during gametophyte formation (Jones et al. 2008). In maize, the nondisjunction takes place during the second mitosis in pollen grain formation (Roman 1947, 1948), in *Aegilops speltoides*, it occurs during first pollen mitosis (Mendelson and Zohary 1972), whereas, in rye, it occurs during the first mitosis in both male and female gametophytes (see Jones et al. 2008).

In most plant species, B chromosomes are parasitic. In rye, B chromosomes show drive through male and female gametophytes, but are harmful to carriers by decreasing fertility (Jiménez et al. 1994), thus being an excellent example of parasitic B chromosomes (Puertas 2002). Likewise, maize B chromosomes are also parasitic by being harmful at high numbers (Staub 1987) and showing several mechanisms of drive (Carlson and Roseman 1992; González-Sánchez et al. 2003). However, in the chive, *Allium schoenoprasum*, up to 20 B chromosomes are found in natural populations. These Bs are highly polymorphic, with more than 12 different types and, most importantly, they show an average transmission rate (0.39) lower than the Mendelian one, but this poor B chromosome transmission is compensated for the higher survival of B-carrying plants between the seed and seedling stages (Plowman and Bougoud, 1994). In fact, this is one of the few well-studied B chromosome polymorphisms pointing to be beneficial for plant fitness.

According to the strength of drive mechanisms, B chromosomes in plants can be classified into three types: (1) highly parasitic, showing more than one mechanism of drive, for example, those in rye; (2) moderately parasitic, showing a single drive mechanism, for example, those in maize and *A. speltoides*; and (3) heterotic, which do not show drive but a beneficial effect to carriers (Puertas 2002). In addition, the coevolution between A and B chromosomes can lead to adaptations for attack and defense in both counterparts (Frank 2000), so that B frequency does not reach stability in natural populations. This is predicted by the near-neutral model of B chromosome evolution (Camacho et al. 1997) by which B chromosome drive is suppressed by A chromosomes, with consequent temporal changes in B frequency depending on whether the population is at the "invasion," "suppression of drive," or "near-neutral" stages. This perspective threw new light on parasitic B chromosomes, such as those in rye and maize, where evidence for A chromosome control of B transmission has been shown (Puertas 2002).

The B chromosome system in *Prospero autumnale* was analyzed in the Iberian Peninsula during 1980s (Ruiz-Rejón et al. 1980a,b; Oliver et al. 1982; Guillen and Ruiz-Rejón 1984; Parker et al. 1991), who found several B-carrying populations with a remarkable effect on the activation of an esterase gene (Ruiz Rejón et al. 1980b; Oliver et al. 1982). However, B chromosome transmission, fitness effects, and long-term frequency changes have never been analyzed. In this article, we analyze temporal variation of B chromosome frequency in an interval of 34 years in a single population, and study B transmission and effects on vigor and fertility-related traits.

Materials and Methods

THE SPECIES

Prospero autumnale (= *Scilla autumnalis*) (L.) Speta (Asparagaceae, Asparagales) is a perennial bulb of circum-Mediterranean distribution than inhabits scrublands and Mediterranean woodlands (De-Yuan 1982; Ebert et al. 1996; Hamouche et al. 2010; Almeida da Silva and Crespi 2013; Almeida da Silva et al. 2014; Jang 2013). Reproduction of this plant is exclusively by seeds (Ainsworth 1980), and we never found vegetatively produced bulbs. *Prospero* (= *Scilla*) *hyacinthoides*, a close relative, showed about 13% of flowers producing seeds through experimental selfer crosses (Shtein et al. 2016). *Prospero autumnale* might show a similar mating system with predominat cross-fertilization.

This plant has been characterized cytogenetically (Battaglia 1957, 1963, 1964a, 1964b; De-Yuan 1982; Ebert et al. 1996; Vaughan et al. 1997; Jang et al. 2013) to show 10 chromosome races over its whole distribution area, presumably including some cryptic species (Vaughan et al. 1997). Recent cytogenetical and molecular analyses have reduced these 10 races to only four diploid and four polyploid cytotypes (Jang et al. 2013). Four of these cytotypes have been found in the Iberian Peninsula: two diploid ($2n = 14$, named AA and B^7B^7) and two tetraploid ($2n = 28$, the allotetraploid AAB^7B^7 and the autotetraploid $B^7B^7B^7B^7$; Parker et al. 1991). The AA cytotype is present only in the Iberian Peninsula and North Africa (Jang et al. 2013), the most western distribution of this species, probably as results of Pleistocene glaciations (Jang et al. 2013). The AA cytotype shows a karyotype consisting of five submetacentric chromosome pairs (no. 1, 2, 3, 5, and 6), one subtelocentric pair (no. 4) and a smaller near-metacentric pair (no. 7), with 7.85 $\pm$ 0.045 pg 1C DNA content and a single nucleolus organizer region (NOR) located adjacent to the centromere on the long arm of chromosome 3.

In addition to the normal karyotype, many natural populations of *P. autumnale* harbor B chromosomes that constitute a highly widespread polymorphism within the Iberian Peninsula (Ruiz-Rejón et al. 1980a,b; Parker et al. 1991). All individuals analyzed here showed the AA diploid cytotype ($2n = 14$), with zero to eight B chromosomes per plant. These

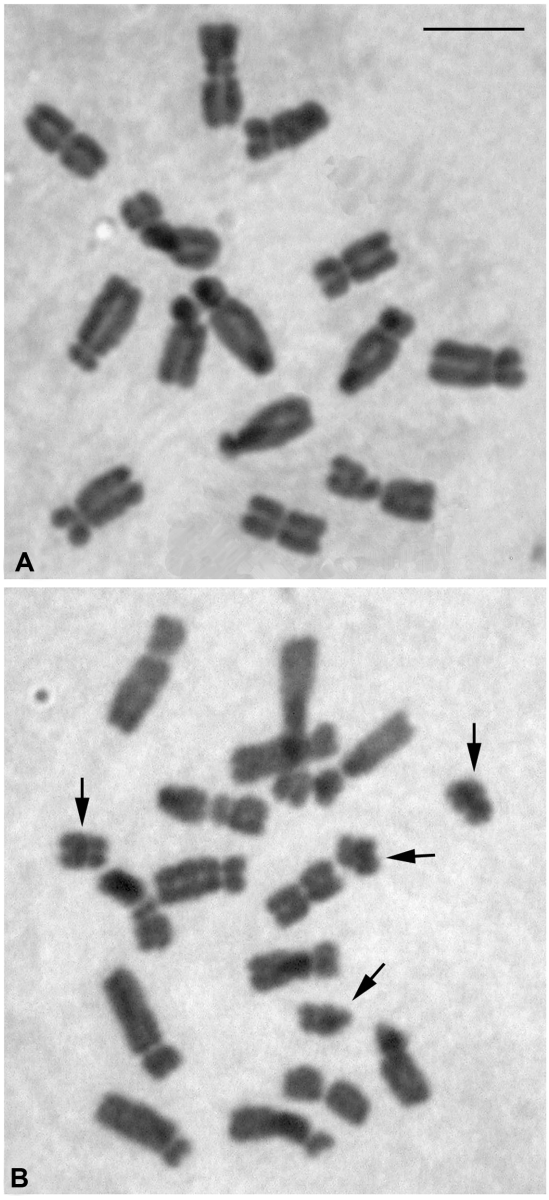

Figure 1. Mitotic metaphase cells from 0B (A) and 4B (B) plants. Note that B chromosomes (arrows) are conspicuously smaller than A chromosomes. Bar in A represents 10 μm.

B chromosomes were metacentric and smaller than the standard (A) chromosomes (Fig. 1), as previously reported by Ruiz Rejón et al. (1980a,b).

PLANT SAMPLING

We collected complete fruit-carrying plants during autumn of 1987, 2005, 2006, 2012, 2013, 2014, and 2015 in Cuesta de La Palma (Loja, Granada, Spain), a population sited at 761 m asl (37°7′6.73″ N; 4°17′0.90″ W) in a Mediterranean scrubland landscape.

DETERMINATION OF B CHROMOSOME NUMBER

Bulbs were submitted to hydroculture until yielding 1-cm long roots, which were cut and immersed into 0.05% colchicine solution for 4 h. The roots were then fixed in 3:1 ethanol–acetic acid solution and stored at 4°C. For chromosome analysis, the roots were immersed into 1N HCl for 1 min, and then in 2% acetic orcein for 15 min. They were then washed in 45% acetic acid and the tip meristem of each root was used to make a squash preparation in a drop of 2% of acetic orcein.

To analyze chromosome number in seeds, they were germinated in Petri dishes on humid filter paper at room temperature at a naturally illuminated area of the laboratory avoiding direct sunlight. When seed roots reached about 5 mm, they were cut and processed as bulb roots.

PARAMETERS MEASURING B CHROMOSOME FREQUENCY

Three parameters of B chromosome frequency were calculated for each population sample. Namely, prevalence (the proportion of B-carrying individuals), mean (the average number of B chromosomes), and load (the mean number of B chromosomes in the B-carrying individuals). The relationship between the three parameters is quite simple, as mean is equal to prevalence times load (Camacho 2005). To get a longer scope of B chromosome evolution in Cuesta de la Palma population, we also included two samples from this same population analyzed in 1981 (Oliver et al. 1982) and 1983 (Guillén and Ruiz-Rejón 1984). In the 1981 sample, the B chromosomes found showed mitotic instability and the authors only scored the presence/absence of B chromosomes, for which reason only B prevalence can be calculated. In the 1983 sample, mitotic instability was observed only in five of the 24 B-carrying plants, and we assigned them a number of B chromosomes coinciding with the median B number observed among the roots analyzed per individual (i.e., four 1B and one 3B individuals), as is usually done for mitotically unstable B chromosomes (see Pardo et al. 1995). No mitotic instability of B chromosomes was observed in the subsequent samples analyzed.

B CHROMOSOME TRANSMISSION AT POPULATION LEVEL

The average transmission of the B chromosome on the male and female sides was analyzed by a mother–offspring analysis of 95 plants collected in 2012. Christiansen and Frydenberg (1973) and Christiansen et al. (1973) showed that analyzing a single offspring from each mother is sufficient to get reliable estimates of average transmission rates at population level. However, several offspring per mother have been analyzed in other cases (e.g., Nur 1977; Camacho et al. 1997). For the present research, we analyzed 347 seeds from the 95 plants collected in 2012, that is, 3.65 seeds per plant on average. The number of B

chromosomes in the seeds from each plant was averaged, and the latter values were averaged per type of plant carrying from zero to eight B chromosomes. This way, the same weight was given to each individual plant for these calculations.

The frequency of B chromosomes found among the offspring of 0B plants, divided by B frequency in the 95 plants, provided an estimate of the average transmission ratio of the B chromosome through the male side at population level. Assuming random mating, we can calculate the B transmission rate through the female side by subtracting the B frequency observed in the progeny of 0B plants to the frequency observed in each class of B-carrying plants (which corresponds to those Bs arrived through the pollen) and dividing by the number of Bs carried by the plant. This can also be done for the whole set of B-carrying plants by using the weighed mean number of B chromosomes in the B-carrying mothers and their offspring.

ANALYSIS OF POLLEN FERTILITY

In 2012, we collected preanthesis inflorescences from 15 plants (eight 0B, two 1B, four 3B, and one 4B). They were fixed in 3:1 ethanol–acetic acid within glass tubes, at 4°C, replacing several times for freshly prepared fixative. To get estimates of pollen fertility, we analyzed eight inflorescences (three 0B, two 1B, two 3B, and one 4B) by means of Alexander's staining (Alexander 1980), which allows differentiating between fertile and infertile pollen grains under the optical microscope.

VIGOR AND FERTILITY-RELATED TRAITS

We measured five vigor traits in the sample of 2005. Namely, the number of scapes per plant, the length of the part of the scape lacking flowers (scape length), inflorescence length, pedicelum length, and bulb diameter, as well as three fertility-related traits, namely the number of fruits, the number of seeds and mean seed weight, and also the seed/fruit ratio. In 2012, we measured the same traits mentioned above plus the number of leaves, the number of flowers, and the fruits/flowers ratio.

STATISTICAL PROCEDURES

Temporal changes in B frequency were analyzed by means of one-way analyses of variance (ANOVA), followed by post hoc Least Significant Difference (LSD) comparisons. Transmission rate of B chromosomes was compared with the Mendelian rate (0.5) by means of the *Z*-test (approximation of a binomial to normal distribution) (López-León et al. 1992; Pardo et al. (1994), using the equation $Z = (k_B - 0.5)/(0.25/N)^{0.5}$, where k_B is the observed transmission rate and N is the number of seeds analyzed. If $Z > 1.96$ or $Z < -1.96$, the null hypothesis is rejected, indicating significant B accumulation and B elimination, respectively. For the analysis of B chromosome phenotypic effects, all vigor and fertility-related traits were transformed to natural logarithms, and comparisons between B-carrying and B-lacking classes were performed by the Student's *t*-test and one-way ANOVA. Pollen viability was compared between B-carrying and B-lacking plants by the Student's *t*-test, and seed survival was compared by a contingency χ^2 test. Type-I errors were minimized by the Bonferroni–Holm method (Holm 1979).

Results

EVOLUTION OF B CHROMOSOME FREQUENCY ACROSS A 34-YEAR PERIOD

We sampled the Cuesta de la Palma population at seven different years between 1987 and 2015. To analyze temporal variation in B frequency for a longer period (1981–2015), the following analyses also include the 1981 sample for prevalence analysis and the 1983 sample for prevalence, mean, and load analyses (Fig. 2; Table S1). We found individuals with zero to eight B chromosomes, and noticed significant changes in B frequency along the 1983–2015 period, which were apparent for prevalence (one-way ANOVA: $F = 7.20$, df $= 8, 601$, $P < 0.000001$, Bonferroni–Holm: Pb < 0.000003) and mean ($F = 3.27$, df $= 7, 541$, $P = 0.0021$, Pb $= 0.0042$), but not for load ($F = 0.85$, df $= 7, 205$, $P = 0.5464$). Post hoc comparisons (Table S2) showed a significant increase in B prevalence and mean frequency between 1987 and 2005 (Fig. 2), whereas B prevalence significantly decreased between 2006 and 2012 and increased between 2012 and 2015. Taken together, these results showed temporal changes in B frequency in Cuesta de la Palma population, with 2.6-fold increase between 1987 and 2005 in prevalence and mean, and 40% decrease in B prevalence between 2006 and 2012.

Remarkably, no changes were found for B-load during the whole period, suggesting that the population reached equilibrium for this parameter. This might result from selection against individuals carrying many B chromosomes. In fact, individuals with more than four B chromosomes were actually rare in most samples (Table S1). This is also evidenced by the lower coefficient of variation (CV) shown by B-load among years (10%) compared to those of prevalence (35%) and mean (34%).

B CHROMOSOME TRANSMISSION AT POPULATION LEVEL

Assuming random mating, the mean number of B chromosomes found among the seeds yielded by B-lacking plants (0.55; Table 1) indicates the average frequency of Bs transmitted through the male side in the natural population. The ratio between this figure and mean B frequency in the 95 plants (0.85; Table 1) yields the average B transmission rate through the male side ($k_B = 0.65$), which was significantly higher than the Mendelian rate (0.5), thus revealing the existence of drive through the male side (Table 1). On the other hand, the B transmission

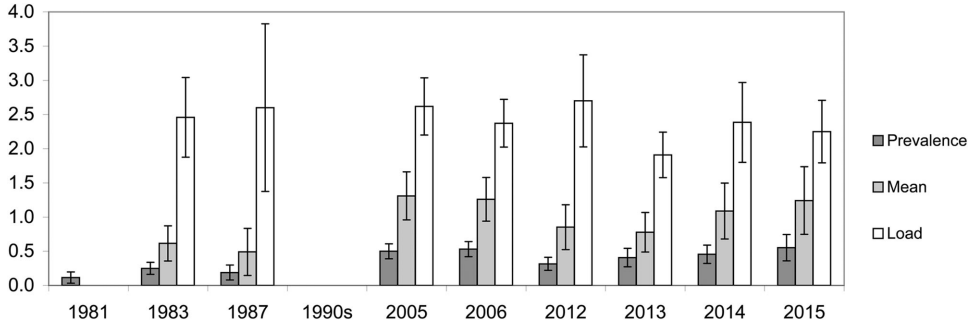

Figure 2. Changes in B chromosome frequency observed in Cuesta de la Palma population between 1983 and 2015, measured as prevalence (the proportion of B-carrying individuals), mean (the mean number of Bs per individual), and load (the mean number of Bs per B-carrying individual). Error bars indicate 95% confidence intervals.

Table 1. Mother–offspring analysis of B chromosome transmission at population level.

Item	Bm	Nm	Ns	Bs	kB	*Z*	Net	Via
B-lacking plants	0	65	236	0.55	0.65	**4.48**	Drive	Male
B-carrying plants	1	9	35	0.86	0.31	**−2.27**	Drag	Female
	2	9	29	1.42	0.43	−0.72		Female
	3	2	6	1.25	0.23	−1.31		Female
	4	7	29	2.20	0.41	−0.94		Female
	5	1	3	2.67	0.42			Female
	7	1	3	0.00	−0.08			Female
	8	1	6	1.50	0.12			Female
Total	0.85	95	347	0.83				
Plants with one to eight Bs	2.7	30	111	1.44	0.33	**−3.62**	Drag	Female
Plants with one to four Bs	2.3	27	99	1.00	0.39	**−2.13**	Drag	Female

Bm = number of B chromosomes in the mother; Nm = number of mothers; Ns = number of seed offspring; Bs = mean number of B chromosomes in the seed offspring; kB = B transmission rate; *Z* = *Z*-test (significant when $1.96 < Z < -1.96$). In B-lacking plants, kB was calculated as the quotient between Bs and the mean number of Bs in the 95 mothers analyzed (0.85), and this represents B transmission rate through the male side. In B-carrying plants, we estimated kB through the female side by subtracting Bs in B-lacking bulbs (0.55) to Bs in the B-carrying mother type, and dividing by the corresponding Bm. In case of joint estimations for B-carrying plants, as a whole, we used weighed means taking into account the number of bulbs into each B-number class. Note that kB indicated significant drive through pollen, but significant drag through the ovum. Significant *Z* values are in bold-type letter, and indicate B drive when positive or B drag when negative.

rate on the female side can be inferred in B-carrying plants by subtracting the 0.55 Bs transmitted through the male side to the mean B frequency observed in each class of B-carrying plants. This revealed a tendency to B chromosome elimination through the female side, which was significant in 1B plants (Table 1). Additional joint analysis of the 30 B-carrying plants showed an average transmission rate ($k_B = 0.33$) being significantly lower than the Mendelian one (Table 1). Due to the presence of only one plant belonging to the 5B, 7B, and 8B classes, we performed this same analysis on plants carrying one to four B chromosomes, which still showed significant drag of B chromosomes on the female side (Table 1). Remarkably, B elimination was higher in plants carrying odd number of B chromosomes.

The mean transmission rate through both sexes ($0.65+0.33)/2 = 0.49$) was, however, very close to the Mendelian one, indicating that B chromosome drive through the male side is counteracted by a similar amount of B chromosome drag through the female side. This explains the high resemblance between the weighted mean number of B chromosomes in plants (0.85) and seed offspring (0.83) (Table 1), and is consistent with the absence of differences in B chromosome prevalence or mean between 2012 and 2013 (Table S2).

NO EFFECTS OF B CHROMOSOMES ON VIGOR AND FERTILITY-RELATED TRAITS

We measured and scored several vigor and fertility-related traits (fruit and seed production) in 2005 and 2012 to compare between years and between B-carrying and B-lacking individuals. A comparison of these plant traits between B-carrying and B-lacking individuals showed absence of significant differences in both years (analyzed separately) (Tables 2 and S3). We also analyzed pollen viability in three B-lacking plants as well as two with 1B, two with

Table 2. Comparison of vigor and fertility-related traits between B-carrying (+B) and B-lacking (0B) plants.

	Mean (0B)	Mean (+B)	*N* (0B)	*N* (+B)	*t*	df	*P*	*Pb*
2005								
Number of scapes	0.02	0.07	40	42	−1.33	80	0.188	
Scape length	5.07	5.10	39	42	−0.42	79	0.673	
Inflorescence length	3.97	3.97	39	42	−0.04	79	0.972	
Pedicelum length	2.44	2.33	38	39	1.53	75	0.130	
Bulb diameter	2.22	2.22	40	42	0.05	80	0.963	
Number of fruits	1.90	1.74	39	42	1.01	79	0.316	
Number of seeds	2.23	2.10	36	36	0.63	70	0.531	
Seeds/fruits	0.25	0.22	36	36	0.20	70	0.839	
Mean seed weight	−6.51	−6.49	34	26	−0.10	58	0.920	
2012								
Number of scapes	0.06	0.12	65	30	−1.05	93	0.297	
Scape length	4.82	4.75	65	30	1.56	93	0.122	
Inflorescence length	4.27	4.19	65	30	1.13	93	0.260	
Pedicelum length	2.44	2.40	65	30	0.87	93	0.386	
Bulb diameter	2.03	1.93	65	30	2.02	93	0.047	0.420
Number of fruits	2.28	2.23	65	30	0.42	93	0.678	
Number of seeds	3.22	3.19	65	30	0.18	93	0.860	
Seeds/fruits	0.94	0.96	65	30	−0.28	93	0.781	
Mean seed weight	−6.53	−6.62	65	30	0.97	93	0.333	

t = Student's *t*-test, df = degrees of freedom, *Pb* = *P*-value corrected by the Bonferroni–Holm method (see full dataset in Table S3).

3B and one with 4B, collected in 2012 (Table S4). The results did not show significant differences associated with B chromosome presence ($t = 0.18$, df $= 6$, $P = 0.86$), with pollen viability being very close to 95% in both B-carrying and B-lacking plants.

Finally, a comparison between the number of seeds with different number of B chromosomes, yielded by plants collected in 2012, and the number of plants collected in 2013 (Table S5), was consistent with absence of B chromosome effects on seed or seedling survival (contingency $\chi^2 = 0.16$, df $= 3$, $P = 0.98$).

Discussion

Temporal changes in B frequency in natural populations have been witnessed in several instances and, in some cases, it was possible to identify the causal factors producing them. For example, in the grasshopper *E. plorans*, the B_{24} variant arose in the Torrox population (Málaga, Spain) as a derivative of the B_2 variant, which is the predominant B variant in all surrounding populations. In only eight years, between 1984 and 1992, B_{24} frequency increased swiftly in this population, leading to the almost complete replacement of B_2. Controlled crosses demonstrated significant drive for B_{24} through females, which suggested that B variant replacement had taken place because of this B_{24} property (Zurita et al. 1998), which was absent in B_2 (López-León et al. 1992). Other examples of dramatic increases in B frequency, typical of an invasion period, were later reported in *E. plorans* from the Mallorca island (Riera et al. 2004) and from the Otivar population in the south of the Iberian Peninsula (Camacho et al. 2015), the Brazilian fish *P. lineatus* (Cavallaro et al. 2000), and the Brazilian wasp *T. albitarse* (Araújo et al. 2001, 2002). However, no similar cases of temporal changes in B frequency have been reported in natural populations of plants.

In all cases, B frequency seemed to reach a maximum for prevalence and load. For instance, in the grasshopper *E. plorans*, the natural population where B chromosomes reached the highest frequency was Torrox (Málaga, Spain) just after B chromosome invasion (Zurita et al. 1998). The invading B_{24} variant showed prevalence values fluctuating among years (0.722 in 1992, 0.827 in 1994, 0.782 in 1998, and 0.826 in 2000; Manrique-Poyato 2010), suggesting that B frequency reached such a limit in 1994 and 2000. Load also fluctuated among these years (1.35, 1.86, 1.34, and 1.63, respectively), also suggesting the existence of a maximum for this parameter. Likewise, B prevalence and B load showed remarkable changes among years in the Cuesta de la Palma population of *P. autumnale*. The lowest value of prevalence was recorded in 1981 (0.11), but it increased to 0.50 in 2005, being this last value close to the upper values observed in subsequent years. This divides the study period into two well-differentiated

stages: the invasion stage during the 1980s, and the near-neutral stage during the 2000s. The rapid invasion of B chromosomes was most likely facilitated by the predominantly cross-fertilizing mating system of *P. autumnale* in consistency with predictions by Burt and Trivers (1998). Comparisons between consecutive samples revealed two significant changes in B prevalence in this population, one between 1987 and 2005 (frequency increase) and the other between 2006 and 2012 (frequency decrease). The frequency increase appeared to be caused by completion of the invasion stage at some point between 1987 and 2005, whereas the frequency decrease between 2006 and 2012 was a characteristic fluctuation of the near-neutral stage. However, no significant differences were observed for B-load during the whole period analyzed, with only slightly fluctuating values from 1.91 in 2013 to 2.70 in 2012. This indicates that B-load cannot increase beyond a threshold, which in *P. autumnale* is clearly higher than in *E. plorans* presumably because the former species shows higher tolerance to B chromosomes, as indicated by the absence of effects on vigor and fertility-related traits reported here, which contrasts with B chromosome effects decreasing female fertility in *E. plorans* (Zurita et al. 1998). As Table S1 shows, *P. autumnale* plants with 5B and 6B were observed in 1983 and 1987, respectively, when prevalence was still low, and this maximum was about the same in the 2005 and 2006 samples, with double values for prevalence.

The male drive mechanism of B chromosomes in *P. autumnale* most likely favors even numbers of B chromosomes, resulting in the frequency of karyomorphs with even number of B chromosomes being higher than the immediately lower odd number (see Table S1). For instance, the frequency of 2B plants was higher than that of 1B ones in 2005, 2006, 2013, 2014, and 2015. This resembles the situation in other plant species showing higher frequency of even than odd B chromosome numbers. An extreme case is rye, where the frequency of plants with odd numbers is very low (see Romera et al. 1991) which is the consequence of B-drive mechanisms involving the directed nondisjunction of B chromosomes to the sperm and egg nuclei during gametophytic postmeiotic mitoses (see Jones and Rees 1982). In *A. speltoides*, plants from Haifa showed a B frequency pattern similarly biased toward even numbers (Cebriá et al. 1995) and B chromosomes show drive on the male side only (Mendelson and Zohary 1972). Therefore, it is conceivable that the drive mechanism in *P. autumnale* involves directed nondisjunction of the B chromosome on the male side.

Our present results shed new light on possible mechanisms explaining the observed changes in B frequency in *P. autumnale* over the 34 years period analyzed here. On the one hand, B chromosomes appear to have little effect on external phenotype and they infringe no harmful effects on fertility-related traits such as pollen fertility, fruit and seed production, or seed survival. Bearing in mind that the two samples analyzed (in 2005 and 2012) most likely belonged to the near-neutral stage, it would be tempting to speculate that selection for more tolerant plants, during the previous invasion stage, would help to explain the absence of B effects in these samples. However, the fact that B-load reached similar values across the whole period monitorized, including the invasion stage, runs against this possibility. On the other hand, our mother–offspring analysis has shown the existence of significant drive through the male side and significant drag on the female side, with average transmission rate (0.49) very close to the Mendelian one (0.5), predicting no change in B chromosome frequency between 2012 and 2013, in consistency with the B-frequency analysis (see Table S2).

Taken together, the former results are consistent with the near-neutral model of B chromosome evolution described for the grasshopper *E. plorans* (Camacho et al. 1997). The absence of net drive and presence of temporal fluctuations in B frequency reflect the near-random walk resulting from drive suppression, with fluctuations mostly due to drift, although some selection against high B numbers cannot be ruled out (Camacho et al. 1997). The main difference with *E. plorans* is that drive suppression in this grasshopper takes place in the driving sex (i.e., the female), whereas B chromosome drive in *P. autumnale* is still operating through the male side ($k_B = 0.65$). Remarkably, B chromosomes showed very low transmission rate through the female side ($k_B = 0.33$), yielding a net B chromosome transmission ($k_B = 0.49$) being highly consistent with B chromosome neutralization ($k_B = 0.50$). Notwithstanding, the clear tendency to B chromosome frequency increase during the 1980s is incompatible with the current k_B values observed in both sexes, as B chromosome initial invasion needs either net B drive (parasitic model) or an increase in host fitness associated to B presence at low numbers (heterotic model) (Camacho et al. 1997), and none of these features were observed in the 2000s. A possible explanation is that, in *P. autumnale*, host genome defense has taken place by decreasing k_B through females instead of by suppressing drive through males. Unfortunately, this hypothesis cannot be tested in Cuesta de La Palma population, because it would have required another mother–offspring analysis in the 1980s. However, it could be done in other populations showing low B chromosome frequency (i.e., with prevalence below 20%), as B invasion could be starting in them, so that temporal changes in male drive and/or female drag could be investigated by mother–offspring analysis in the invasion and neutralization stages. This is an interesting prospect for future research.

The near-neutral model predicts a host's response to B chromosome invasion in form of the increase in frequency of host genes able to suppress B drive, a process leading the system toward a near-neutral random walk. Evidence for genes suppressing B chromosome drive has been reported in plants like rye (Jiménez et al. 1995, 1997) and maize (González-Sánchez et al.

2003), as well as in animals (Shaw and Hewitt 1985; Shaw et al. 1985; Nur and Brett 1985, 1987; Herrera et al. 1996). The case of *P. autumnale* shows a new face for the arms race between parasitic and host chromosomes as B chromosome neutralization appears to have taken place in the nondriving sex, that is, the female side. The fact that transmission rate on the female side was lower in plants with odd number of Bs suggests the possibility that B univalents tend to be lost in polar bodies during female meiosis, so that allelic variants in the host genome promoting this tendency would be selected for during B invasion. Given that drive through the male side was still apparent in 2012, it is conceivable that drive suppression in this species is still evolving, for which reason we observed significant increases and decreases in B frequency at different years, depending on the relative strength of male drive and female drag.

AUTHOR CONTRIBUTIONS

PL, MAGR, and CRR did sampling and cytological analysis; analysis of vigor and fertility-related traits was carried out by PL, FP, MGS, MP, and JPMC; PL, FP, and JPMC analyzed mother–offspring; and PL, FP, MAGR, CRR, MGS, MP, and JPMC wrote the manuscript.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Frequency of B chromosomes observed in Cuesta de la Palma population between 1981 and 2015, measured as B-prevalence (the proportion of B-carrying individuals), B-mean (the mean number of Bs per individual), and B-load (the mean number of Bs per B-carrying individual).

Table S2. Post hoc comparisons after ANOVAs performed on temporal changes for B chromosome prevalence (upper half-matrix) and mean (lower half-matrix) in La Palma population of *Prospero autumnale*.

Table S3. Full dataset of vigor and fertility-related traits measured in *Prospero autumnale* plants from Cuesta de la Palma population.

Table S4. Full dataset for pollen fertility analysis in *Prospero autumnale* plants from Cuesta de la Palma population.

Table S5. Comparison of B frequency between the seeds yielded by plants in 2012 and those analyzed in 2013.