B chromosomes and Robertsonian fusions of *Dichroplus pratensis* (Acrididae): intraspecific support for the centromeric drive theory

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Abstract. We tested the centromeric drive theory of karyotypic evolution in the grasshopper *Dichroplus pratensis*, which is simultaneously polymorphic for eight Robertsonian fusions and two classes of B chromosomes. A logistic regression analysis performed on 53 natural populations from Argentina revealed that B chromosomes are more probably found in populations with a higher proportion of acrocentric chromosomes, as the theory predicts. Furthermore, frequencies of B-carrying individuals are significantly negatively correlated with the mean frequency of different Robertsonian fusions per individual. No significant correlations between presence/absence or frequency of Bs, and latitude or altitude of the sampled populations, were found. We thus provide the first intraspecific evidence supporting the centromeric drive theory in relation to the establishment of B chromosomes in natural populations.

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Two different types of B chromosomes have been described in the South American grasshopper *Dichroplus pratensis* (Melanoplinae, Acrididae) (Bidau, 1986, 1987). One of these Bs carries an active NOR and exhibits assortment-distortion with respect to the X in male meiosis (Bidau, 1986; Bidau et al., 2004). The other is mitotically unstable and its numbers vary from 0 to 5 between and within testis follicles in B male carriers (Bidau, 1987; Martí, 2002). Nevertheless, and despite the enormous geographical distribution of the species and the large number of natural populations sampled to date (Bidau and Martí, 2002, 2004), these B chromosomes are rare within the

species. Their frequencies are very low in marginal populations hundreds of kilometers apart, while they are virtually absent in intermediate populations except in hybrid zones (Bidau and Martí, 2002).

D. pratensis is also a classical example of Robertsonian (Rb) variation in nature. The species, which is widespread in Argentina, Uruguay and Southern Brazil, is polymorphic and polytypic for a complex system of eight centric fusions that involve the six large autosomal pairs of the $2n = 19\,\mathrm{O}^{\!\!\!\nearrow}/20\,\mathrm{Q}$ standard all-acrocentric karyotype (Bidau et al., 1991). These translocations have profound effects on chiasma frequency and localisation (Bidau, 1990, 1993; Bidau and Martí, 1995) and it was recently found that the distribution of the polymorphisms shows a central-marginal variation (Bidau and Martí, 2002, 2004). Thus, *D. pratensis* offers a unique opportunity to test intraspecifically, the predictions of the centromeric drive theory of karyotypic evolution (Pardo-Manuel de Villena and Sapienza, 2001a, b) and its extension to B chromosomes as Palestis et al. (2004) have done interspecifically for mammals. Under this theory, it is hypothesized that B chromosomes should be favored in populations in which there is a bias in chromosomal segregation during female meiosis, resulting in acrocentric chromosomes driving toward the functional (oocyte) pole of the spindle that, in this case, is the more efficient one in capturing centromeres. B chromosomes add new centromeres to the cell thus, in populations where the female spin-

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Table 1. Characteristics of the *Dichroplus pratensis* populations polymorphic for B chromosomes. Rb fusions indicates the different arm combinations found in metacentrics of each population; Type indicates if populations are ecologically marginal or central, or if they belong to a hybrid zone; F is the mean frequency of different Rb fusions per individual; B frequency (%) represents the number of individuals harbouring Bs irrespective of sex or B chromosome type

Population	Latitude (S)	Altitude (masl)	Rb fusions	Type	F	B frequency (%)
Volcán	23º55'	2474	None	Marginal	0.00	3.0
Estación Mazán	28º44'	646	2.5	Marginal	1.00	11.0
Carrizal	28º54'	522	2.5	Marginal	1.00	25.0
La Granja	33º30'	125	1.6, 3.4, 2.5	Central	1.72	5.5
Manantiales	33º33'	134	1.6, 3.4, 2.5	Central	1.61	7.1
Tandil	37º13'	171	1.6, 3.4	Central	2.00	6.7
Balcarce	37º49'	97	1.6, 3.4	Central	1.80	10.0
Villa Ventana	38º04'	161	1.6, 3.4, 1.2, 5.6	Hybrid zone	3.00	2.0
Sierra de la Ventana	38º06'	250	1.6, 3.4, 1.2, 5.6	Hybrid zone	2.33	2.2
Cerro Ceferino	38º06'	456	1.2, 3.4, 5.6	Hybrid zone	3.00	5.6
El Atravesado	38º08'	240	1.6, 3.4, 1.2, 5.6	Hybrid zone	2.69	11.0
Saldungaray	38º12'	242	1.6, 3.4, 1.2, 5.6	Hybrid zone	1.73	5.0
Km784	40º04'	43	1.6, 3.4, 5.6	Hybrid zone	1.20	5.0
Istmo Ameghino	42º27'	55	1.4, 5.6	Marginal	0.25	15.8
El Doradillo	42º48'	20	1.4, 5.6	Marginal	0.15	20.0
Puerto Madryn	42º49'	18	1.4, 5.6	Marginal	0.26	20.0
Lago Musters	45º30'	261	None	Marginal	0.00	9.1
Diadema Argentina	45º47'	326	None	Marginal	0.00	12.1
Rada Tilly	45º57'	0	None	Marginal	0.00	14.3

dle polarity is reversed (i.e. the functional pole is the one that captures less centromeres), a high frequency of biarmed (meta- and submetacentric) chromosomes is expected, but also, a low frequency of B chromosomes. The former hypothesis was tested in 53 natural populations of *D. pratensis* spanning the whole geographic range of the species.

Materials and methods

For the analysis of B chromosome distribution in relation to metacentric vs acrocentric karyotypes within the species, we used our own published and unpublished data on B chromosome frequencies and Robertsonian (Rb) fusions frequencies (Bidau, 1986, 1987, 1990; Bidau and Martí, 1995, 2002, 2004; Martí, 2002). A total of 67 natural populations were scored but, for statistical analyses, only the 53 where at least 20 individuals had been karyotyped, were considered. Of these, 19 populations were polymorphic for one or both types of B chromosomes (Table 1). Frequency of Bs per population was calculated as the number of B carriers (regardless of sex and B chromosome type) within the total population sample. Since most populations are polymorphic for up to 4 Rb fusions, and the number of metacentrics in the karyotype varies between 0 and 6, we used the mean frequency of different Rb fusions per individual (F) as a measure of the degree of metacentricity/acrocentricity of the karyotype. A value of F = 0 implies an all acrocentric fixed karyotype; a value of F = 3 may occur in a population where three fusions have become fixed (thus, all individuals carry 6 biarmed chromosomes), or in a polymorphic one where, if the frequency of each fusion is very high, individuals may carry from 3 to 6 biarmed autosomes (Bidau and Martí, 1995). If the frequencies of the fusions are intermediate or low, the F value will be lower than 3 since individuals with 2, 1 or 0 fusions occur. The analyses were performed using logistic and linear regressions. To control for environmental factors that could presumably affect B frequencies in relation to Rb fusion frequencies, we also included latitude and altitude of the sampled populations as independent variables.

Results and discussion

The B chromosomes of *D. pratensis* occur at low frequencies in 19 of 67 (29%) populations of the species' geographical range (Bidau, 1986; Bidau and Martí, 2002; Martí, 2002). The maximum observed frequency of individuals carrying B chro-

mosomes was 25% in the marginal Andean population of Carrizal. However, the distribution of Bs seems to be non-random (Fig. 1) (Bidau and Martí, 2002). Considering the 53 populations where at least 20 individuals were karyotyped, a logistic regression of mean number of different fusions per individual per population (F) vs presence/absence of Bs indicated that populations with higher proportions of acrocentric chromosomes are more likely to harbour B chromosomes (r = −0.322; P = 0.01646). Mean F values for the non-B and B populations were 1.90 and 1.25 respectively (Student's t test; P = 0.01539). No statistically significant associations were found between presence/absence of Bs and either latitude (r = 0.133; P = 0.33771) or altitude (r = 0.100; P = 0.47186). Furthermore, when only the 19 populations harbouring Bs were considered, a significant negative correlation between frequency of B chromosomes and F was obtained in a multiple regression analysis using F, latitude and altitude as independent variables (r = −0.522; P = 0.02184) (Fig. 2). No significant correlations between Bs frequency and latitude (r = 0.199; P = 0.41407), and altitude (r = −0.244; P = 0.31409) were observed.

A classic problem in evolutionary biology concerns the origin of karyotypic differences between species (White, 1978; King, 1993). Although chromosomal rearrangements such as Rb fusions or fissions usually accompany (and in some cases might cause) speciation, the mechanisms of fixation of the rearrangements in natural populations are controversial (White, 1978; King, 1993; Pardo-Manuel de Villena and Sapienza, 2001b). Recently, Pardo-Manuel de Villena and Sapienza (2001a, b) developed the theory of centromeric drive, that explains chromosomal evolution as a result of drive in the polarized meiosis of female mammals and other organisms. According to this theory, the functional spindle pole (the one that will give origin to the mature oocyte) may be the more or the less efficient in capturing centromeres. In each case, acrocentric or biarmed chromosomes will be favored respectively, and this fact can explain trends in karyotypic evolution that are not assessed by other models (Pardo-Manuel de Villena and Sapienza, 2001b).
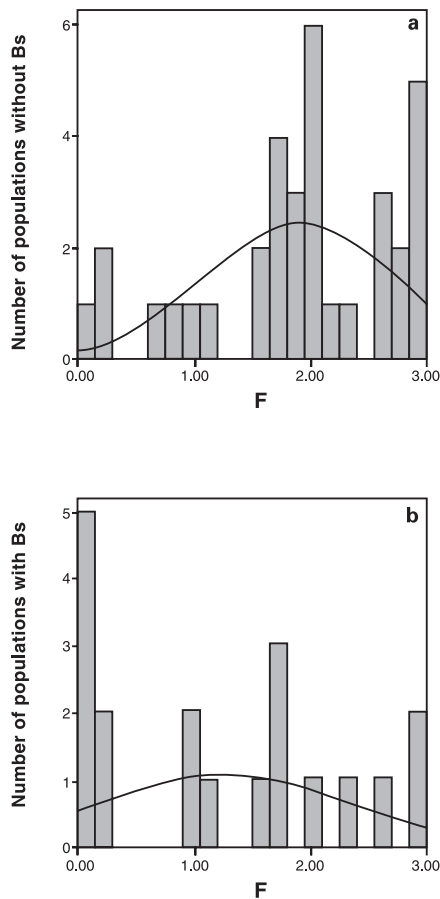
Fig. 1. Histograms of the number of *Dichroplus pratensis* populations with different F (mean number of different fusions per individual) values. (**a**) Populations without B chromosomes. (**b**) Populations with B chromosomes.

Fig. 2. Linear regression of frequency of B chromosomes (%) vs F (mean number of different fusions per individual) in 19 populations of *Dichroplus pratensis*. The regression equation is, Bfreq = 14.02 – 3.20*F.

Palestis et al. (2004) tested this theory using mammalian B chromosomes, under the hypothesis that, since Bs are usually mantained by drive (Hewitt, 1979; Jones and Rees, 1982; Bell and Burt, 1990; Jones, 1991, 1995; Camacho et al., 2000) and they provide the cell with an extra centromere, a bias towards more centromeres in the functional pole during female meiosis should favor the establishment of B chromosomes, while a bias towards fewer centromeres should have the opposite effect. Indeed, Palestis et al. (2004) found, in a sample of 1116 mammals, that species with a higher proportion of acrocentrics in their karyotype tend to bear B chromosomes more probably than species with fewer acrocentrics.

The centromeric drive theory may be tested in organisms other than mammals, since the polarity of female meiosis, that can sponsor centromeric drive, is common to most eukaryotes including insects. For example, grasshoppers have typically asymmetric oocyte spindles and B chromosomes are known to drive during female meiosis in some species (Hewitt, 1979; Cano and Santos, 1989; Santos et al., 1993). Thus, *D. pratensis*, being polymorphic for Bs and Rb fusions is an exceptional
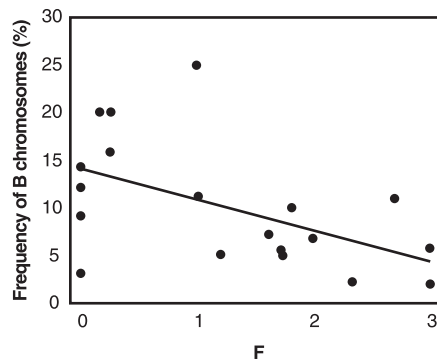
model to test the centromeric drive theory as applied to B chromosomes. As already noted (Bidau and Martí, 2002, 2004) B chromosomes in this species are more frequent in ecologically and geographically marginal populations which also have the lowest frequencies of Rb polymorphisms. In extreme marginal areas, populations have the all-acrocentric standard karyotype of 2n = 19♂/20♀ (Bidau and Martí, 2002). The central-marginal distribution of Rb fusions has been interpreted by us in terms of natural selection favoring all-acrocentric karyotypes in marginal areas and Rb polymorphisms in central parts of the range, because of the effects of Rb translocations on recombination (Bidau and Martí, 2002). However, the origin of the tendency of *D. pratensis* to generate many different Rb fusions which may or may not become established in different populations, has not been determined. In the case of *D. pratensis*, the results of logistic regression analysis of the mean number of different Rb fusions per individual in a population vs presence/absence of B chromosomes indicated that the centromeric drive theory as demonstrated for mammalian B chromosomes, also applies intraspecifically since Bs are more probably found (and with a higher frequency) in populations with basically acrocentric karyotypes. Furthermore, the centromeric drive theory offers a plausible explanation for the origin of differences in Rb fusion frequencies between marginal and central habitats. Central populations might have accumulated Rb fusions because of a change in polarity of the female meiotic spindle poles producing a bias towards less centromeres (thus, towards biarmed chromosomes) captured by the functional pole. Then, natural selection could have acted to mantain the resulting polymorphisms if they protected coadapted supergenes (Bidau and Martí, 2002). But, as a consequence of the change in polarity of the female spindle, Bs found more difficulty to prosper in central populations.

Our results are clearly consistent with the predictions of the centromeric drive theory, although it must be kept in mind that drive has not yet been demonstrated in *D. pratensis*. Furthermore, it is possible that only the mitotically stable B shows drive through females because of its tendency to co-segregate with the X during male meiosis (Bidau, 1986; Bidau et al.,

2004) thus landing preferentially in females where it would show drive. The unstable B probably drives through males (Bidau, 1987). In that case, only the first B should show an association with Rb fusion frequency. However, data are at present insufficient to analyse both Bs separately.

A further point of interest is that, of those populations harbouring Bs but having otherwise high Fs, six belong to two hybrid zones between chromosomal races (Table 1). Of these, five (Villa Ventana, Sierra de la Ventana, Cerro Ceferino, El Atravesado and Saldungaray) are included in the Sierra de la Ventana hybrid zone which resulted from the reproductive interaction of a Southern race, centered at the locality of Monte Hermoso and polymorphic for Rb fusions 1.2, 3.4 and 5.6, and a Northern race from central Buenos Aires province, bearing Rb fusions 1.6 and 3.4 (Chiappero et al., 2004). None of the parental races shows B chromosome polymorphisms despite extensive sampling (Martí, 2002). Thus, it is possible that the existence of B chromosomes within the hybrid zone resulted from the initial contact between the races when one or both still had B chromosomes which were in the process of disappearance because of drive towards fewer centromeres (and more metacentrics). The persistence of Bs in the hybrid zone would be thus a consequence of the supernumeraries being incorporated to new, hybrid genomes in which drive at female meiosis was not yet fully established.

Acknowledgements

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