

Prevalence of B chromosomes in Orthoptera is associated with shape and number of A chromosomes

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Abstract We analyze the prevalence of B chromosomes in 1,601 species of orthopteran insects where chromosome number and shape are known. B chromosomes have been reported in 191 of these species. Bs are not uniformly distributed among orthopteran superfamilies, with evident hotspots in the Pyrgomorphoidea (32.3% of species carrying Bs), Grylloidea (14.9%), Acridoidea (14.6%) and Tetrigoidea (14.3%). As expected under the theory of centromeric drive, we found a correlation between B chromosome presence and A chromosome shape—Bs are more frequent in karyotypes with more acrocentric A chromosomes. We also found that Bs are less common in species with high chromosome numbers and appear to be most common at the modal chromosome number ($2n = 24$). Study effort, measured for each genus, was not associated with B prevalence, A chromosome shape or A chromosome number. Our results thus provide support for centromeric drive as an important and prevalent force in the karyotypic evolution of Orthoptera, just as it appears to be in mammals. We suggest that centromeric drive may provide a mechanistic explanation for White's principle of karyotypic orthoselection.

Keywords B chromosome · Centromeric drive · Chromosome number · Chromosome shape · Insects · Orthoptera

Introduction

B chromosomes are additional dispensable chromosomes which constitute one of the most widespread numerical polymorphisms in many eukaryote species (reviewed in Camacho 2005; Burt and Trivers 2006). Many of them show a selfish behaviour during transmission, thus gaining a crucial advantage for invasion of new populations. This property, named drive or accumulation, is also essential for B chromosome maintenance in natural populations. In addition, many B chromosomes are associated with a fitness decrease in the individuals carrying them, thus behaving as true parasites. These two properties, drive and harmfulness, justify their consideration as genome parasites (Östergren 1945). Although most B chromosomes fit this parasitic model, where B frequency in natural populations may reach a stable equilibrium between drive and deleterious effects, there are also cases where Bs are frequent but lack apparent drive, some even showing a benefit for those individuals carrying them. This alternative model, the heterotic one, was first proposed by Darlington (1958) and was supported by White (1973), who claimed that Bs might prosper in the absence of drive, and their frequency might also reach equilibrium if they are beneficial at low number but harmful at high numbers. In addition, parasitic chromosomes may lose drive because of selection for resistance genes in the A chromosomes, thus becoming near-neutral elements. In this last case, B chromosome frequency does not necessarily reach equilibrium but is constantly changing, with Bs passing through parasitic,

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drive-suppressing and near-neutral stages (Camacho et al. 1997). In this kind of B, the near-neutral stage is much longer than the other two and, during this time, it is even possible for the appearance of a B-derived variant to recover drive and become parasitic again (Zurita et al. 1998). It is thus possible that many B chromosome systems are long-lasting, as is also suggested by the 2 million year age calculated for maize B chromosomes (Lamb et al. 2007) and the 750,000 years for Bs in *Locusta migratoria* (Teruel et al. 2010). Some B chromosome systems may even survive speciation events (Camacho 2005).

Previous analyses of B chromosome prevalence across species have shown that they are unequally spread over taxonomical groups, being particularly frequent in monocots (especially Commelinales and Liliales) among plants, and orthopteran insects among animals (see Jones and Rees 1982; Camacho 2005; Levin et al. 2005). In looking for reasons to explain the distribution of B chromosomes across species, previous research has investigated the possible association between B chromosome presence and factors such as breeding system, genome size, and A chromosome shape, number and ploidy (reviewed in Palestis et al. 2004b). In summary, these authors showed that, in plants, (1) outbreeding favors harmful Bs whereas beneficial Bs do best under inbreeding (Burt and Trivers 1998), (2) presence of B chromosomes is positively correlated with genome size (Trivers et al. 2004; Levin et al. 2005), and (3) B chromosomes are more likely in species with few A chromosomes (Trivers et al. 2004). In addition, Palestis et al. (2004a) showed that, in mammals, B chromosomes are more common in species with acrocentric chromosomes, in agreement with expectations from the theory of centromeric drive put forward by Pardo-Manuel de Villena and Sapienza (2001a, b).

The theory of centromeric drive suggests the existence of a bias during female meiosis in the ability for capturing centromeres by the oocyte and polar body poles, a bias that frequently reverses in mammals so that most species either have mainly acrocentric or mainly metacentric chromosomes (Pardo-Manuel de Villena and Sapienza 2001a, b). In species with karyotypes predominantly composed of biarmed chromosomes, we can infer that Robertsonian translocations have found an easy pathway to fixation, and this could have taken place by a greater ability of the polar body to capture centromeres, thus capturing the two acrocentrics in a trivalent of a heterozygous female, whereas the metacentric would tend to go to the oocyte. In a species where the greater ability to capture centromeres is shown by the oocyte, the predominant A chromosome shape would be uni-armed and, since B chromosomes represent extra centromeres, they would also tend to go to the oocyte thus finding an easy way to drive and increase in frequency (Palestis et al. 2004a).

In this report, we investigate whether chromosome shape and number are associated with B chromosome presence in the order Orthoptera, the insects with the highest number and frequency of Bs. The last comprehensive review of B chromosome occurrence in Orthoptera was performed by Hewitt (1979), who reported B presence in 130 species. These last 31 years have been very intensive in orthopteran cytogenetic research, and now this figure is 210. Here we provide evidence that, in Orthoptera, chromosome shape and number are associated with B chromosome presence, in agreement with trends shown previously in other organisms.

Methods

We built a spreadsheet database including cytogenetical and taxonomical information on orthopteran species where chromosome number and shape has been analysed. For this purpose, we first reviewed the 3,206 entries in a Reference Manager database we had previously built with all reprints collected for our 35 years of orthopteran chromosome research. In addition, we reviewed the main cytogenetics books including information on orthopteran chromosomes, such as Makino (1951), White (1973), Hewitt (1979) and Jones and Rees (1982). The taxonomical fields in our spreadsheet database (suborder, superfamily, family, subfamily, tribe, genus and species) were established according to the Orthoptera Species File Online database (<http://orthoptera.speciesfile.org>). The cytogenetic fields were Bs (presence or absence), chromosome number in females ($2n$), number of chromosome arms in females (FN), proportion of one-armed chromosomes, and B chromosome shape (number of arms in the B chromosome). Information about genome size in orthopteran species was obtained from the Animal Genome Size database (Gregory 2010: <http://www.genomesize.com>).

The major limitation to determining the factors that make a species more likely to have B chromosomes is that Bs, by definition, are not present in all individuals in a species. Bs are therefore more likely to be observed in well-studied species (Palestis et al. 2004b; Trivers et al. 2004). For the Orthoptera it would be preferable to have a species-level index of study effort (e.g. number of published cytogenetic papers for each species in the dataset), but creating such an index for 1,601 species would be extremely labor-intensive and probably incomplete (Palestis et al. 2004b; Trivers et al. 2004). To create a substitute index of study effort, we calculated the proportion of species in a genus with known karyotypes. For this purpose, we compared the number of species of a given genus in our spreadsheet database with the total number of species taxonomically described for that genus, as found in

the Orthoptera Species File Online database (<http://orthoptera.speciesfile.org>). Species without published karyotypes are excluded from all other analyses to avoid creating a downward bias in estimates of B prevalence across taxa (Palestis et al. 2004b). We also tested for relationships between study effort and the predictor variables to ensure no biases toward specific variables were present (Palestis et al. 2004b; Trivers et al. 2004). Unfortunately, within-genus study effort does not necessarily correlate with within-species study effort, so we also tested for an effect of study effort at the genus level.

We tested whether A chromosome shape and number affect the likelihood that a species is reported to have Bs, controlled for study effort, using logistic regression. Independent variables were the proportion of chromosome arms that are on acrocentrics, $2n$ chromosome number, and the study effort index. The dependent variable was presence or absence of Bs. The proportion of arms is used rather than the proportion of chromosomes, because one metacentric has the same number of arms and could be formed by the same number of evolutionary events as two acrocentrics (Palestis et al. 2004a). We also tested for a relationship between genome size and chromosome number (see Trivers et al. 2004) using simple regression. Because data on genome size was available for only a small number of species ($n = 39$ for genome size, $n = 1601$ for the other variables), we performed a separate logistic regression on 1-C genome size alone rather than including genome size with the other variables. If more than one C-value was given for a species in Gregory (2010), then we used the mean. Nearly all of the C-values were calculated using Feulgen densitometry, and there appeared to be very little variation among methods for measuring genome size.

The relationship between A chromosome shape and B prevalence was tested further using independent taxonomic contrasts (Felsenstein 1988; Burt 1989), following the methodology of Burt and Trivers (1998). To do so we created a discrete variable for A chromosome shape: more than half versus less than half of chromosome arms on acrocentrics; species with exactly 50% of arms on acrocentrics were excluded (Palestis et al. 2004a). We then created for each independent taxonomic group a 2×2 table giving the number of species in each category of chromosome shape (more than half, less than half) and B presence (present, absent). The likelihood of a positive correlation between B presence and chromosome shape occurring by chance ($P+$) was calculated using <http://statpages.org/ctab2x2.html>. The expected number of positive correlations across species is given by the sum of $P+$ values across taxonomic groups (Burt and Trivers 1998).

Statistical analyses were performed using SPSS 12.0. Unless otherwise noted, all tests are two-tailed at an alpha level of 0.05.

Results

Our first data collection included 210 orthopteran species where B chromosome presence has been reported. However, A chromosome shape was reported in only 191 of these species, so that our subsequent analyses were limited to the database collection that included chromosome shape. Overall, 191 of 1,601 species (11.9%) with known karyotypes are reported to have B chromosomes. However, there is much variation among taxonomic groups within the Orthoptera in the prevalence of Bs. Table 1 shows variation at the superfamily level in B prevalence and karyotype, and also includes data on large families (number of species with known karyotypes ≥ 50). A contingency χ^2 test shows significant differences for B prevalence among superfamilies ($\chi^2 = 41.42$, $df = 8$, $P < 0.001$). With the notable exception of Grylloidea, the superfamilies with a relatively high proportion of species harboring Bs tend to have nearly all of their chromosome arms on acrocentric chromosomes (Table 1). No patterns related to chromosome number or study effort (proportion of species in a group with known karyotypes) are apparent at the superfamily level (Table 1; Fig. 1). The data presented have already been partly corrected for study effort by excluding species lacking karyotypic information.

We performed a logistic regression at the species level including the proportion of A chromosome arms on acrocentrics, diploid chromosome number, and an index of study effort (see methods) as independent variables, and presence or absence of Bs as the dependent variable. Bs are significantly positively associated with acrocentric karyotypes and negatively associated with chromosome number (Table 2). Study effort was not a significant predictor of the reported presence of Bs. Study effort is also not associated with either of the other two variables, so there does not appear to be a bias among genera toward studying species with acrocentric chromosomes or with fewer chromosomes (linear regressions, $r^2 = 0.003$ for chromosome shape and 0.001 for chromosome number). In addition to an overall logistic regression across the entire dataset, we also performed separate logistic regressions for each of three large superfamilies: Among Acridoidea, significant effects of chromosome shape and number remain ($P = 0.016$ and 0.004, respectively) with no effect of study effort ($P = 0.976$); among Grylloidea none of the variables are significant predictors; among the Tettigoniodea the only significant predictor was study effort ($P = 0.013$). However, this significant study effort effect is opposite the predicted direction and appears to result from three poorly studied genera with reports of Bs.

Although statistically significant, the relationships with acrocentric As and chromosome number are very weak (in the overall logistic regression $r^2 = 0.012$), in part because

Table 1 Variation in prevalence of Bs and karyotypes across superfamilies and large families

Superfamily	Family	N	Prop. B	Proportion arms on acro.		Modal 2n	Prop. all species ^a
				Mean (SD)	Mode		
Pyrgomorphoidea		31	0.323	0.95 (0.18)	1	20	0.248
Grylloidea		121	0.149	0.48 (0.34)	0	20	0.117
	Gryllidae	114	0.14	0.48 (0.33)	0	20	0.12
Acridoidea		834	0.146	0.90 (0.19)	1	24	0.249
	Acrididae	700	0.171	0.89 (0.20)	1	24	0.241
	Romaleidae	50	0.04	0.98 (0.05)	1	24	0.439
Tetragoidea		28	0.143	1.00 (0.00)	1	14	0.093
Tettigonioidea		434	0.081	0.90 (0.16)	1	32	0.17
	Tettigoniidae	434	Same as for superfamily				
Rhaphidophoroidea		30	0.033	0.77 (0.22)	1	46	0.17
Eumastacoidea		91	0.011	0.78 (0.27)	1	18	0.231
Tridactyloidea		11	0	0.11 (0.19)	0	14	0.204
Stenopelmatoidea		18	0	0.48 (0.29)	0.71	12	0.237

Only species with known karyotypes are included. Superfamilies with fewer than five karyotyped species are excluded

^a Indicates the proportion of named species that have been karyotyped as a rough index of study effort across major taxa. The proportions here are likely to be overestimates, because the totals are based on the total number of species for each genus in our dataset (from <http://orthoptera.speciesfile.org>) and genera with no karyotyped species would not be represented

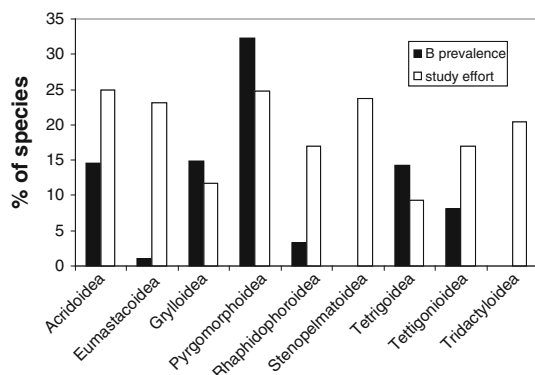


Fig. 1 Variation among superfamilies in B prevalence (% karyotyped species with reports of Bs) and study effort (% species that have been karyotyped), following Table 1

Table 2 Results of logistic regression analysis across species with presence or absence of Bs as the dependent variable ($r^2 = 0.012$)

Item	B (SE)	Probability	Odds ratio
Proportion arms on acrocentrics	0.78 (0.37)	0.036	2.18
Chromosome number	-0.035 (0.016)	0.027	0.97
Study effort	-0.41 (0.26)	0.111	0.66

there is very little karyotypic variation in the Orthoptera—most have acrocentric karyotypes (see Table 1; Fig. 2). On average, species with Bs have 91% (SD = 17%) acrocentric chromosomes, with 87% ($\pm 22\%$) arms on acrocentrics, while those without reports of Bs have 89% ($\pm 21\%$)

acrocentric chromosomes and 85% ($\pm 25\%$) arms on acrocentrics. Mean (\pm SD) chromosome number is 23.66 (± 4.47) for species with Bs and 24.36 (± 5.95) for species lacking Bs. Examination of variation in B prevalence across karyotypes reveals the following patterns: (1) B chromosomes are rare in species with fewer than 20% of A chromosome arms on acrocentrics (Fig. 2). (2) Although B frequency is numerically highest among species with all of their A chromosome arms on acrocentric chromosomes, B prevalence varies little once the percentage of arms on acrocentrics is above 20% (Fig. 2). (3) Bs are rare in species with more than 32 A chromosomes (Fig. 3). (4) Rather than increasing at low chromosome numbers, B prevalence peaks at $2n = 24$, the modal chromosome number (Fig. 3).

We tested the relationship between B prevalence and acrocentric As further in an analysis of independent taxonomic contrasts across 27 taxa (28 minus one tie). In 19 groups there was a positive correlation between having mostly acrocentric A chromosomes (defined as $>50\%$ arms on acrocentrics) and the likelihood of harboring Bs, and the relationship was negative in only 8 groups (Table 3). The expected number of positive correlations by chance is 15.75 (SE = 2.22), and the comparison approaches statistical significance ($Z = 1.464$, one-tailed $P = 0.072$).

Because our index of study effort is at the genus level and we are comparing karyotypes of species with and without Bs, we also tested for a relationship between study effort and the proportion of species with Bs across genera (excluding genera containing fewer than five species with

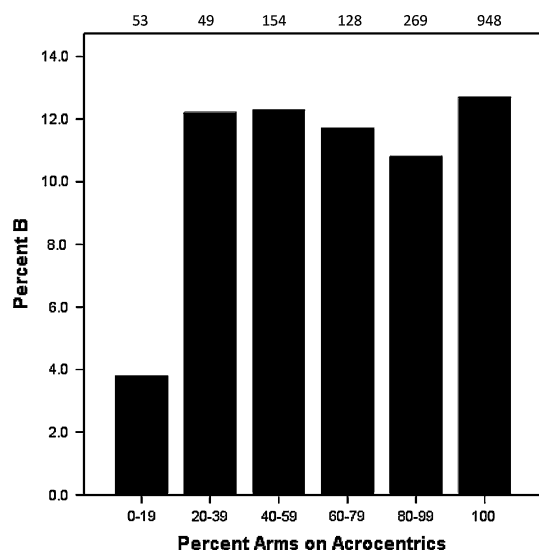


Fig. 2 The percentage of species with B chromosomes across karyotypes differing in the percentage of A chromosome arms on acrocentrics. Numbers across the *top* indicate sample size (number of species)

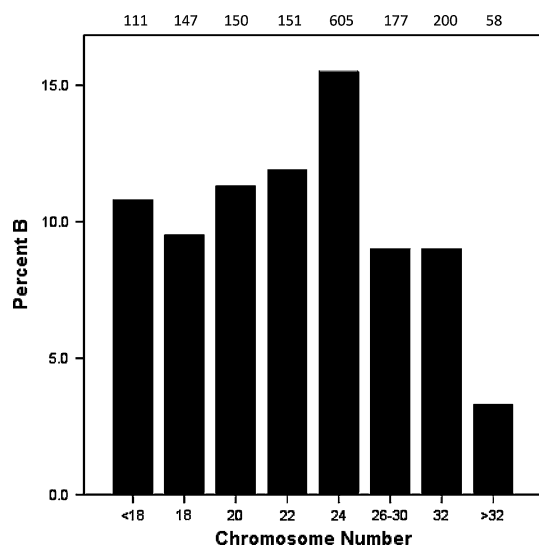


Fig. 3 The percentage of species with B chromosomes across karyotypes differing in the number of A chromosomes. Numbers across the *top* indicate sample size (number of species)

known karyotypes). There is no significant relationship (in a simple regression, $F_{1,89} = 0.441$, $P = 0.508$, $r^2 = 0.005$; also not significant if tested in multiple regression including karyotype variables).

As is true for A chromosomes, most B chromosomes in Orthoptera are acrocentric. Despite little variability across species in B shape, there appears to be a relationship between the shape of As and the shape of Bs (Table 4). Comparing only species with exclusively acrocentric Bs to those with exclusively metacentric or submetacentric Bs this relationship borders on statistical significance (Mann–Whitney

U -test, $Z = 1.825$, $P = 0.068$). The comparison is significant if iso-B chromosomes are included with metacentrics and submetacentrics ($Z = 2.288$, $P = 0.022$).

There are too few species present with genome size data to have included genome size as an additional variable in the logistic regression analysis described above. There appears to be a weak trend for Bs to be more prevalent in species with larger genomes. The mean 1-C genome size for species with Bs ($n = 13$) is 9.80 ± 3.72 pg, and the mean for species lacking Bs ($n = 26$) is 8.54 ± 3.36 pg. Analyzing genome size alone in a logistic regression reveals no significant relationship ($P = 0.29$, $r^2 = 0.042$). There is a highly significant negative relationship between chromosome number and genome size ($F_{1,37} = 12.692$, $P = 0.001$, $r^2 = 0.255$), although there are few species present with genome size data and high numbers of chromosomes. There is no relationship between study effort and genome size ($F_{1,37} = 0.888$, $P = 0.352$, $r^2 = 0.023$).

Discussion

The long B chromosome life is compatible with their conspicuous presence in about 15% of eukaryotes, including fungi, plants, and animals (see Jones 1995; Camacho 2005; Burt and Trivers 2006). B chromosomes may even be more widespread among eukaryotes if they were not almost obligately vertically transmitted parasites, i.e. that can only prosper through sexual reproduction, at the microevolutionary level, and common descent (after speciation events) at macroevolutionary level. With some horizontal transmission we would expect an even greater proportion of species harbouring B chromosomes. Interspecific hybridization seems to give rise to B chromosomes in *Nasonia vitripennis* (McAllister and Werren 1997; Perfecti and Werren 2001), and Bs could also pass among species during these hybridization events. If this kind of phenomenon were frequent, the prevalence of B chromosomes would likely be higher.

Every analysis of B chromosome presence across species leads necessarily to underestimates, because the fact of not finding Bs in a population does not grant that they are not present in any other population of that same species, and thus the observed 15% may grow as more species and populations are analysed cytogenetically. As an example, White (1973) reported the presence of B chromosomes in 53 species of orthopteran insects. Later on, Jones and Rees (1982) included 92 orthopteran species carrying Bs in their atlas of B chromosomes, and Hewitt (1979) reported 130 species in his compilation. Finally, our present database includes 210 species. This means that the proportion of orthopteran species known to harbor B chromosomes (0.7% of the about 30,000 known species) has grown

Table 3 Independent taxonomic contrasts

Taxon	B-class	<50%	>50%	Sign	P+
Genera					
<i>Abracris</i>	+B	1	0	–	0.67
	–B	0	2		
<i>Acrida</i>	+B	0	3	+	0.75
	–B	1	8		
<i>Circotettix</i>	+B	1	4	+	0.04
	–B	4	0		
<i>Dichroplus</i>	+B	0	2	+	0.89
	–B	1	15		
<i>Gampsocleis</i>	+B	0	2	+	0.48
	–B	2	3		
<i>Gryllotalpa</i>	+B	1	1	0	N/A
	–B	2	2		
<i>Gryllus</i>	+B	3	0	–	0.49
	–B	14	4		
<i>Loxoblemmus</i>	+B	1	1	+	0.29
	–B	5	0		
<i>Oxya</i>	+B	0	3	+	0.83
	–B	1	14		
<i>Pyrgomorpha</i>	+B	0	1	+	0.6
	–B	2	2		
<i>Scapsipedus</i>	+B	0	1	+	0.67
	–B	1	1		
<i>Scotussa</i>	+B	1	3	–	0.43
	–B	0	3		
<i>Trimerotropis</i>	+B	1	7	–	0.63
	–B	1	30		
Tribes					
Abracrini	+B	0	1	+	0.94
	–B	1	14		
	(less <i>Abracris</i>)				
Copiphorini	+B	1	0	–	0.25
	–B	5	2		
Dichroplini	+B	0	1	+	0.96
	–B	1	20		
Gryllini	+B	1	0	–	0.57
	–B	2	4		
	(less <i>Gryllus</i> , <i>Loxoblemmus</i> , <i>Scapsipedus</i>)				
Keyacidini	+B	0	1	+	0.33
	–B	2	0		
Melanoplina	+B	0	10	+	0.62
	–B	1	15		
Pteronemobiini	+B	0	1	+	0.5
	–B	16	15		
Subfamilies					
Gomphocerinae	+B	0	8	+	0.78
	–B	3	88		

Table 3 continued

Taxon	B-class	<50%	>50%	Sign	P+
Gryllinae	+B	1	1	–	0.54
	–B	1	5		
	(less Gryllini)				
Luzarinae	+B	0	1	+	0.4
	–B	3	1		
Phaneropterinae	+B	0	19	+	0.87
	–B	1	124		
Families					
Gryllidae	+B	1	4	+	0.53
	–B	7	15		
	(less Gryllinae, Luzarinae, Pteronemobiini)				
Rhaphidophoridae	+B	1	0	–	0.82
	–B	4	23		
Tettigoniidae	+B	0	13	+	0.82
	–B	4	253		
	(less Phaneropterinae, Copiphorini, <i>Gampsocleis</i>)				
Suborder					
Caelifera	+B	0	77	+	0.05
	–B	23	526		
	(less Gomphocerinae, Dicroplini, Melanoplina, Abracrini, Keyacidini, <i>Acrida</i> , <i>Circotettix</i> , <i>Trimerotropis</i> , <i>Oxya</i> , <i>Pyrgomorpha</i>)				
	Sum of P+ = 15.7				

For each taxon the number of species with and without Bs is given, divided among species with less than and greater than 50% of their autosome arms on acrocentric chromosomes. The sign indicates whether there is a positive or negative correlation between the proportion of arms on acrocentrics and B status. The probability of observing a positive correlation under the null hypothesis of independence is indicated by P+

fourfold in little more than 30 years. This frequency is expected to continue growing as more species are analysed cytogenetically in the coming years. In this study we include only orthopteran species with known karyotypes and find that approximately 12% of them have reports of Bs.

Our data on B chromosome distribution across orthopteran species reveal sharp differences in B prevalence among different superfamilies and families. For instance, B chromosomes are most frequent in the superfamily Pyrgomorphae, where more than 30% of the 31 species analyzed cytogenetically (which represents 25% of species taxonomically described) have been reported to carry B chromosomes (see Table 1). By contrast, no species carrying B chromosomes has been found in the superfamilies Tridactyloidea and Stenopelmatoidea, even though 20 and 23% of species, respectively, have been analyzed

Table 4 Relationship between shape of Bs and shape of As

Shape of Bs	Proportion acrocentric As		N
	Mean	SD	
Acrocentric	0.95	0.14	115
Mixed (Acro + others)	0.9	0.15	16
Metacentric/Submeta.	0.86	0.24	22
Isochromosome	0.87	0.17	13

cytologically. Therefore, with almost equal study effort, B chromosome prevalence is much higher in the Pyrgomorphaeidea. As illustrated in Fig. 1, B prevalence varies among superfamilies much more than does study effort. Several superfamilies (Grylloidea, Acridoidea and Tetrigoidea) show intermediate B frequency, i.e. about 14% of species, whereas only 8% of B-carrying species were found in the Tettigonioidea, 3% in the Raphidophoroidea and 1% in the Eumastacoidea (Table 1; Fig. 1).

This variation among taxa suggests the existence of hotspots for B chromosome presence, i.e. certain evolutionary lineages where Bs are more likely to occur (Levin et al. 2005). In Orthoptera these hotspots are represented, at the superfamily level, by the Pyrgomorphaeidea, Acridoidea, Grylloidea and Tetrigoidea. At lower levels the situation seems to be similar. For instance, within the superfamily Acridoidea, the family Acrididae shows 17.1% of species carrying B chromosomes whereas only 4% of species belonging to the family Romaleidae have been shown to carry them (see Table 1). The Acrididae thus seem to be another hotspot for B chromosome presence. The high prevalence of acrocentric A chromosomes (see below) does not explain this difference since it is even higher in the Romaleidae.

In looking for possible causes of this unequal distribution of B chromosomes, we performed logistic regression analyses which showed significant association of B prevalence with both A chromosome shape and number, with no effect of study effort (see Table 2). This relationship was positive with the proportion of arms in acrocentric chromosomes, and negative with chromosome number. When the same analysis was performed within well represented superfamilies, the results were similar for Acridoidea but not for Grylloidea or Tettigonioidea. Overall, B prevalence is very low among species with mostly biarmed As and with high chromosome numbers, but does not increase steadily as acrocentricity increases or as chromosome number decreases (see Figs. 2, 3).

Although the relationship between B presence and A chromosome shape is weak in Orthoptera, it is in complete agreement with previous findings in mammals, where B chromosomes were shown to be more frequent in species with karyotypes composed of a higher proportion of

acrocentric chromosomes, as predicted by the theory of centromeric drive (Palestis et al. 2004a). The association we have found in Orthoptera is weaker than that found in mammals, presumably because orthopteran karyotypes are more biased toward a higher proportion of acrocentric chromosomes (90%) than mammals (44%). We can speculate that the unequal centromere number rule, which determines when two centromeres are superior to one, and vice versa, in reaching the egg spindle pole during segregation (Pardo-Manuel de Villena and Sapienza 2001a) and thus constitutes the basis of the segregation distortion on which B chromosomes could take advantage (Palestis et al. 2004a), has been reversed fewer times in the evolution of orthopteran than mammalian karyotypes. Therefore most orthopteran species would still conserve a higher ability of the oocyte to capture centromeres. However, centromeric drive would not explain why B chromosomes are much more frequent in Acrididae than Romaleidae.

In any case, it is logical to find a weak relationship between B presence and A chromosome shape, since there are a number of factors obscuring it. For instance, centromeric shifts, including pericentric inversions, notably change A chromosome shape but not the number of centromeres, thus producing background noise (Pardo-Manuel de Villena and Sapienza 2001a). Polymorphisms for centric shifts are very frequent in Orthoptera, and cases where related species of Orthoptera differ by pericentric inversions have also been reported (White 1973; Hewitt 1979). In addition, other chromosome changes, such as tandem fusions, produce the contrary effect, i.e. they decrease chromosome number but do not change chromosome shape. Overall rates of chromosomal rearrangement may also be independently associated with B frequency (Trifonov et al. 2002). Another difficulty is that B chromosome presence is not a characteristic of species but populations, so that many of the species considered as non-B carriers might actually have been misclassified. Taking together all this background noise and relatively low variation in chromosome shape, it is remarkable to find a significant signal of B chromosome presence with A chromosome shape.

In support of this association, B chromosomes in Orthoptera frequently show meiotic drive in females and the mechanism of drive is related to a large asymmetry in the meiotic spindles (Hewitt 1976). Female meiotic drive has been demonstrated in the grasshoppers *Melanoplus femur-rubrum* (Lucov and Nur 1973; Nur 1977), *Myrmeleotettix maculatus* (Hewitt 1973, 1976), *Heteracris litoralis* (Cano and Santos 1989), *Omocestus burri* (Santos et al. 1993), and *Eyprepocnemis plorans* (Zurita et al. 1998; Bakkali et al. 2002). Three of these species show all A chromosomes acrocentric, but *M. maculatus* and *O. burri* have, like many Old World gomphocerines, three biarmed pairs arisen by the fixation of three centric fusions (see

Hewitt 1979), one of which probably involved the autosome carrying the histone genes, i.e. the eighth in order of decreasing size in the ancestral karyotype (Cabrero et al. 2009). On the basis of the theory of centromeric drive, this suggests a polarity reversal of the centromere number rule after the fixation of these three fusions in the ancestor species, and thus suggests that B chromosomes in these species are probably young. In fact, whereas B chromosome age in *M. maculatus* was dated at less than 8,000–10,000 years (Hewitt and Ruscoe 1971), *Locusta migratoria* Bs seem to be at least 750,000 years old (Teruel et al. 2010).

Centromeric drive may also help to explain other aspects of chromosomal diversity in the Orthoptera. For example, Castillo et al. (2010) propose that asymmetry favouring the polar body pole has led to the establishment of a large number of Robertsonian fusions and neo-sex chromosomes in Neotropical melanopline grasshoppers. This reversal of the direction of centromeric drive may have originated via selection against individuals carrying B chromosomes (Palestis et al. 2004a; Castillo et al. 2010). In addition, the applicability of centromeric drive at the intraspecific level has also been shown in a grasshopper, *Dichroplus pratensis* (Bidau and Martí 2004). They found a negative correlation between the frequency of B chromosomes and the frequency of banded A chromosomes in 53 populations being polymorphic for Robertsonian translocations involving several A chromosome pairs. As predicted by centromeric drive theory, B chromosomes were more frequently found in populations with a higher proportion of acrocentric A chromosomes.

Our logistic regression analysis has shown a negative relationship between B chromosome presence and A chromosome number in Orthoptera, consistent with flowering plants where B chromosomes are more frequent in species with few A chromosomes (Trivers et al. 2004). There is no clear explanation for this association, except a possible strengthening of meiotic control evolved in species with more chromosomes to guarantee equal assortment of more chromosomes. However, no relationship between chromosome number and B presence has been found in mammals (Palestis et al. 2004b). A negative correlation between B presence and chromosome number could occur indirectly, via the positive correlation between Bs and genome size and the negative correlation between genome size and chromosome number, but Trivers et al. (2004) showed significant, independent effects of both chromosome number and genome size. We also find evidence for a peak in B prevalence at a chromosome number of $2n = 24$ (see Fig. 3). However, examination of Table 1 suggests that this peak may result from the numbers being skewed by the family Acrididae, which is by far the largest orthopteran taxon, has a modal chromosome number of 24,

and has a relatively high proportion of species harbouring Bs.

Genome size has been determined in only 39 orthopteran species. Although data are scarce, they show a tendency in the same direction as in flowering plants (Trivers et al. 2004). This correlation was explained by these authors through relaxed selection against large genome size, implying weaker selection against Bs. For the same reason, species with small genomes may be less able to tolerate B effects (Palestis et al. 2004b). This question would merit the analysis of genome size in a high number of orthopteran species. Interestingly, Orthoptera is the only insect order known to contain species with large genomes (Gregory 2010).

We also found a relationship between A and B chromosome shape, as has been previously reported for mammals (Palestis et al. 2004a). This would be expected under the intraspecific mode of B chromosome origin (see Camacho 2005) but also under White's principle of karyotypic orthoselection (White 1973), which produces an order and symmetry in the karyotypes resulting from the operation of the same type of structural change affecting most chromosomes within karyotypes. As possible explanations, White (1973) suggested orthoselection imposed by the environment, orthoselection caused by the need for chromosomal shapes and sizes to remain harmonious with spindle and cell dimensions, and orthoselection resulting from the need for a similar chiasmata distribution among the chromosomes. The second type clearly predicts that B chromosome shape is expected to be a reflection of A chromosome shape, independently of whether Bs have arisen intra- or interspecifically.

Centromeric drive can provide additional explanations for the principle of karyotypic orthoselection, as proposed by Pardo-Manuel de Villena and Sapienza (2001a). If the unequal centromere number rule (UCNR) operates in a group of species, it is clear that certain types of chromosome changes will be permitted and others will be forbidden, and this will be valid for changes affecting all chromosomes in the karyotype. This necessarily leads to the symmetry patterns and interchromosome resemblances typical of many karyotypes, which constitute the basis for the principle of karyotypic orthoselection. The longer the same UCNR is operating, without reversal, the higher the expected prevalence for a given type of chromosome change among the chromosomes within a karyotype. As pointed out by White (1973), "in many groups of grasshoppers where the chromosomes are acrocentric, fusions seem to be incapable to establish themselves". A long evolutionary persistence for a higher capability of the oocyte to capture centromeres easily explains why most acridoid grasshoppers have acrocentric chromosomes and also why Bs have found an easy pathway to prosper in

grasshopper karyotypes. It would be interesting to know if the percentage of acrocentrics across various insect orders also correlates with the frequency of species with B chromosomes.

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