Effects of B chromosomes on egg fertility and clutch size in the grasshopper \textit{Eyprepocnemis plorans}

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Abstract

We analyse here three components of reproductive success (egg fertility, egg clutch size and embryo clutch size) in several temporal samples from different Spanish and Moroccan populations of the grasshopper \textit{Eyprepocnemis plorans}. The analysis of spatial and temporal variation suggests that egg clutch size, but neither embryo clutch size nor egg fertility, depends significantly on both year of sampling and population of origin. While the former effect could mainly be due to year-to-year variation in food availability (essential to egg production but not to hatching success), the spatial variation may also include population-dependent genetic factors. We also tested the effect of the presence of supernumerary (B) chromosomes carried by many individuals in most natural populations of this species. We found a slight but significant decrease in egg fertility associated with the presence of B chromosomes. We discuss possible causes of the observed variation for these three reproductive traits at both spatial and temporal levels, as well as the effect of B-chromosome presence as a parasitic element disturbing reproduction of carrier females.

Key words

B chromosomes, clutch size, fertility, grasshopper, \textit{Eyprepocnemis plorans}, Orthoptera

Introduction

About 15\% of eukaryote species carry supernumerary (B) chromosomes in addition to members of the standard chromosome complement (Achromosomes). Two models have been put forward to explain B-chromosome maintenance in natural populations: the parasitic (Östergren 1945) and the heterotic (Darlington 1958, White 1973) models. Both assume that B frequency may reach equilibrium as a consequence of the action of two opposite forces, i.e., drive with harmful effects of the B chromosome in the parasitic model, and beneficial effects at low number of Bs, but harmful effects at high B numbers, in the heterotic model.

Most B chromosome systems where transmission and fitness effects have been sufficiently studied, fit the parasitic model (for a recent review, see Camacho 2005). A minority of Bs, however, show characteristics being compatible with the heterotic model, such as providing resistance to pathogens, e.g., \textit{Nectria haematococca} (Miao et al. 1991) (see other examples in Camacho 2005) or being beneficial for survival, as shown for the nondriving B chromosome in the plant \textit{Allium schoenoprasum} (Plowman & Bougourd 1994).

A variant of the parasitic model not assuming equilibrium for B frequency, was built on the basis of population dynamics studies in the grasshopper \textit{Eyprepocnemis plorans}. In this species, the first analyses of B-chromosome transmission showed an absence of drive for the three main B variants (López-León et al. 1992). Subsequent experiments, however, found that one of these variants (B$_1$) showed significant drive when B-carrying females were crossed to males from a B-lacking population, thus suggesting that B drive was suppressed in the B-carrying population (Herrera et al. 1996). This led Camacho et al. (1997) to propose that the same B chromosome may show drive or not, depending on the population evolutionary stage, since parasitic B chromosomes may lose drive due to the evolution of modifier genes in the A chromosomes — a scenario previously suggested by Shaw (1984) and demonstrated by Shaw and Hewitt (1985) and Nur and Brett (1985, 1987).

Therefore, B chromosome frequency in natural populations is not necessarily at equilibrium, but may change as B chromosomes pass through several stages, i.e., parasitic, drive-suppression and neutralized stages (Camacho et al. 1997). At any stage, the B chromosome can mutate to a new variant and when this generates a variant being able to drive, the near-neutral cycle restarts, thus prolonging the life of the B-chromosome polymorphism. Repeated generation of new variants and recovery of drive allow the polymorphism to persist in the populations. B chromosomes in \textit{E. plorans} actually show high mutation rates (López-León et al. 1993, Bakkali & Camacho 2004), which putatively facilitate the substitution of neutralized Bs by derived driving variants. It is worth mentioning that this kind of polymorphism regeneration was evidenced in \textit{E. plorans} (Zurita et al. 1998). Furthermore, the near-neutral model has been considered to explain the long-term evolution of other B-chromosome systems (González-Sánchez et al. 2003, Jones et al. 2008) and other selfish genetic elements (Johnson 1997). Frank (2000) highlighted its importance as a paradigm for the evolution of polymorphisms of attack-defence between parasitic elements and their hosts.

An important aspect of the research on parasitic B chromosomes is to ascertain whether they impose some load on host fitness. At the cytological level, B chromosomes of Spanish \textit{E. plorans} populations were found to increase chiasma frequency (Camacho et al. 1980, Camacho et al. 2002), and the number of active nucleolus organizer regions (NOR) (Cabrero et al. 1987, López-León et al. 1995), although the relationship between these traits and fitness is unknown. Analyses of several exophenotypic traits, however, failed to show any effect of B chromosomes (Camacho et al. 1980, Martín-Alganza et al. 1997). However, B chromosomes in \textit{E. plorans} have been reported to decrease egg fertility (Muñoz et al. 1998, Zurita et al. 1998) and to increase the formation of abnormal spermatids (Suja & Rufás 1989, Teruel et al. 2009), although it is not clear that this latter effect significantly decreases male fertility. In the grasshopper \textit{Myrmeleotettix maculatus}, sperm dysfunction in B-carrying males seems to be associated with a poor transmission of B chromosomes (Hewitt 1973a,b; Hewitt et al. 1987). In high parallelism with Spanish Bs, both parasitic (driving) and neutral-
Table 1. Percentage of B-carrying females, egg fertility and egg and embryo clutch size in eight populations of the grasshopper E. plorans. N= Number of females. SP= Spain, MO= Morocco, SD= Standard deviation.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>% B-carrying</th>
<th>Egg fertility Mean</th>
<th>SD</th>
<th>Egg clutch size Mean</th>
<th>SD</th>
<th>Embryo clutch size Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jete</td>
<td>88</td>
<td>67.05</td>
<td>0.95</td>
<td>0.1</td>
<td>39.92</td>
<td>10.06</td>
<td>38.03</td>
<td>10.61</td>
</tr>
<tr>
<td>Salobréña</td>
<td>69</td>
<td>60.87</td>
<td>0.86</td>
<td>0.18</td>
<td>46.02</td>
<td>14.65</td>
<td>39.34</td>
<td>14.27</td>
</tr>
<tr>
<td>Algarrobo</td>
<td>87</td>
<td>37.93</td>
<td>0.85</td>
<td>0.2</td>
<td>39.39</td>
<td>10.94</td>
<td>33.5</td>
<td>13.21</td>
</tr>
<tr>
<td>Nerja</td>
<td>23</td>
<td>39.13</td>
<td>0.71</td>
<td>0.24</td>
<td>32.69</td>
<td>7.95</td>
<td>23.7</td>
<td>11.16</td>
</tr>
<tr>
<td>Torrox</td>
<td>39</td>
<td>87.18</td>
<td>0.74</td>
<td>0.19</td>
<td>38.72</td>
<td>9.98</td>
<td>28.64</td>
<td>10.87</td>
</tr>
<tr>
<td>Smir</td>
<td>44</td>
<td>40.91</td>
<td>0.88</td>
<td>0.11</td>
<td>34.96</td>
<td>6.77</td>
<td>30.89</td>
<td>7.54</td>
</tr>
<tr>
<td>SO.DE.A</td>
<td>38</td>
<td>31.58</td>
<td>0.82</td>
<td>0.19</td>
<td>29.79</td>
<td>8.83</td>
<td>24.66</td>
<td>9.99</td>
</tr>
<tr>
<td>Mechra</td>
<td>55</td>
<td>41.82</td>
<td>0.85</td>
<td>0.16</td>
<td>36.49</td>
<td>8.35</td>
<td>30.96</td>
<td>9.82</td>
</tr>
<tr>
<td>Total</td>
<td>443</td>
<td>51.92</td>
<td>0.85</td>
<td>0.18</td>
<td>38.5</td>
<td>11.26</td>
<td>33.04</td>
<td>12.41</td>
</tr>
</tbody>
</table>

Results

In four Spanish populations (Jete, Salobréña, Algarrobo and Torrox), we had information on egg fertility and clutch size for two or more samples collected in different years (Fig. 1), which permitted the analysis of temporal variation for the variables analyzed. Two way ANOVA per population, with egg fertility or clutch size (both for eggs and embryos separately) as dependent variable, and year of sampling and B chromosome presence in the female as factors, revealed significant temporal variation for the three dependent variables in the four populations, with the only exception of egg-clutch size in Torrox (Table 2 and Fig. 1). On the contrary, B chromosome effects were observed only in Salobréña, namely for embryo clutch size (Fig. 1). In Torrox, a significant year x B interaction was apparent in egg fertility and embryo clutch size. When the effect of the presence of the B chromosome was tested for each year separately, a significant decrease in egg fertility, egg clutch size and embryo clutch size was apparent in B-carrying females collected in 1992 (Table 3), but no effect was observed in females collected six years later, i.e., in 1998 (not shown).

In Nerja and the three Moroccan populations however, we had samples from a single year, for which reason we only tested for B chromosome presence. One-way ANOVA showed the absence of significant effects of B presence on any of the three variables analyzed (Table 2).

Spatial variation among populations for the three variables analyzed (egg fertility, egg clutch size and embryo clutch size), (see Table 1) was tested by two-way ANOVA, with population and B presence as independent variables. We found significant differences among populations for egg fertility, egg clutch size and embryo clutch size (Table 4). The highest egg fertility was observed in Jete, and the lowest in Nerja and Torrox (Fig. 2A). The highest egg clutch size, however, was observed in Salobréña, and the lowest in Nerja and SO.DE.A., whereas embryo clutch size was highest in Jete and Salobréña and lowest in Nerja and SO.DE.A. (Fig. 2B,C).

In the analysis of egg fertility, a significant effect of B chromosome presence was also apparent (Table 4), due to the lower values shown by B-carrying females in Salobréña, Torrox and SO.DE.A. (see Fig. 2A).

Since data collection was carried out over a wide temporal range (from 1984 in Salobréña to 2005 in Algarrobo), with scarce temporal coincidence among populations, the possibility exists that the spatial variation observed is a by-product of having sampled the populations in different years. A way to test this possibility is by analysing population differences in those cases where two populations were sampled in the same year. This could be done in the Jete and Salobréña populations (samples collected in 1986,

Materials and methods

We scored the number of egg-pods per female, and the number of eggs and embryos per pod in 443 progeny analyses (controlled crosses and gravid females) from eight natural populations of the grasshopper Eyprepocnemis plorans (Table 1). For each egg pod, we obtained three variables: egg fertility (the proportion of eggs carrying an embryo), egg clutch size (the number of eggs per pod) and embryo clutch size (the number of embryos per pod).

Results on egg fertility had previously been reported in seven of these populations: Jete and Salobréña (Granada, Spain) (López-León et al. 1992, 1993), Algarrobo (Málaga, Spain) (Manrique-Poyato et al. 2006), Torrox (Zurita et al. 1998, Perfectti et al. 2004), and Smir, SO.DE.A and Mechra (Morocco) (Bakkali et al. 2002, Bakkali & Camacho 2004). Here we include data on a new population (Nerja, Málaga, Spain) and add new samples of the Algarrobo population (Málaga, Spain). B chromosomes in E. plorans are mitotically stable, which means that all cells within the same individual carry the same number of B chromosomes. In addition to egg fertility, we analyse egg and embryo clutch size, as well as the effects of B chromosome presence on these traits. A simple relationship exists between the three reproductive traits, since embryo clutch size is the product of egg clutch size and egg fertility. Methods for these new samples were similar to those described in the above references. We had data for two or more sampling years in four of the five Spanish populations, which allowed us to investigate temporal variation. The statistical analyses of the data were based on one-way and two-way ANOVA, and Student t test, performed with the Statistica program.
Fig. 1. Temporal dynamics for three reproductive traits in four populations of the grasshopper *E. plorans*. Means (± standard errors) are shown for B-chromosome carrying females (open squares) and 0B females (black-filled circles).

Discussion

B chromosomes have been shown to decrease fertility of B-carrying individuals in many species (for review, see Jones & Rees 1982, Camacho 2005, Jones et al. 2008). In *E. plorans*, a reduction of egg fertility has only been observed for the parasitic B$_{24}$ variant in the Spanish population of Torrox (Zurita et al. 1998) and in some controlled crosses involving the Spanish variant B$_{2}$ and mating frequency limitation (Muñoz et al. 1998). Our present analysis has revealed significant among-year differences in egg fertility and egg and embryo clutch size in the four populations where temporal variation could be tested (see Table 2). This same analysis
also revealed that B chromosome presence does not influence egg fertility in most populations, the only exception being the Torrox population, where the year × B interaction was significant for egg fertility and embryo clutch size.

The analysis of B chromosome effect per year revealed a significant decrease of the three reproductive variables in B-carrying females collected in 1992, in agreement with the results of Zurita et al. (1998), but not in those females collected in 1998, in coincidence with the observations by Perfectti et al. (2004). As discussed by these latter authors, this seems to suggest a rapid neutralization of the harmful effects of this parasitic-B chromosome in this population. In fact, the Nerja population, located toward the east of Torrox, is currently experiencing invasion by the B\(_{xy}\) variant and shows the lowest values for the three reproductive parameters measured here (see Fig. 2). The Algarrobo population is also being invaded by B\(_{xy}\) but does not seem to show such reproductive decrease. Although it is difficult to pinpoint the ultimate causes of this difference from the Nerja population, we may speculate that the B\(_{xy}\) chromosome arrived at Algarrobo earlier than at Nerja, so the modifier genes on the A chromosomes have had more time to neutralize its harmful effects in Algarrobo. The higher frequency of B chromosomes in this population. It also explains why the same analysis showed a slightly significant decrease for these traits in B-carrying females (see Fig. 2). The intensity of this effect is slight, due to a mixture of factors, among which we want to highlight the interpopulation variation in B chromosome evolutionary stage, which determines that B chromosome’s drive and harmful effects can vary both spatially and temporally (Camacho et al. 1997). This explains why, in only six years, the two Torrox samples showed significant differences for egg fertility and embryo clutch size. It also explains why the same B chromosome drives in one Moroccan population (Mechra, the southern, where the B chromosome seems to have arrived later), but not in others (Smir and SO.DE.A., the northern and central, where the B chromosome seems to have arrived earlier) (Bakkali et al. 1999, 2002).

Our analysis of the spatial variation in egg fertility and clutch size showed significant differences among populations (see Table 4 and Fig. 2). In addition, this same analysis showed a slightly significant decrease for these traits in B-carrying females (see Fig. 2). The intensity of this effect is slight, due to a mixture of factors, among which we want to highlight the interpopulation variation in B chromosome evolutionary stage, which determines that B chromosome’s drive and harmful effects can vary both spatially and temporally (Camacho et al. 1997). This explains why, in only six years, the two Torrox samples showed significant differences for egg fertility and embryo clutch size. It also explains why the same B chromosome drives in one Moroccan population (Mechra, the southern, where the B chromosome seems to have arrived later), but not in others (Smir and SO.DE.A., the northern and central, where the B chromosome seems to have arrived earlier) (Bakkali et al. 1999, 2002).

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However, it is unlikely that B chromosome presence is the only explanation for the spatial variation observed in these reproductive traits, since remarkable variation was observed also at the temporal level. In fact, when we compared the three year values between the Jete and Salobrena populations, no significant differences appeared among populations, but there were significant differences among

Table 3. Comparison of three fitness components in standard (0B) and B-carrying (B+) females from Torrox collected in 1992.

<table>
<thead>
<tr>
<th>Item</th>
<th>0B</th>
<th></th>
<th>B+</th>
<th></th>
<th></th>
<th>t</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg fertility</td>
<td>0.95</td>
<td>0.05</td>
<td>0.77</td>
<td>0.14</td>
<td>2.46</td>
<td>20</td>
<td>0.0231</td>
<td></td>
</tr>
<tr>
<td>Egg clutch size</td>
<td>45.63</td>
<td>7.11</td>
<td>35.38</td>
<td>6.30</td>
<td>2.88</td>
<td>20</td>
<td>0.0092</td>
<td></td>
</tr>
<tr>
<td>Embryo clutch size</td>
<td>43.00</td>
<td>5.10</td>
<td>27.52</td>
<td>7.45</td>
<td>3.92</td>
<td>20</td>
<td>0.0009</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Effect of B chromosome presence and year of sampling on egg fertility and clutch size. Four Spanish populations were analysed by two-way ANOVA; Nerja and the three Moroccan populations were only tested for B chromosome presence. Significant tests are highlighted in bold-type.
years for egg and embryo clutch size. This indicates that clutch size seems to be more dependent on year to year environmental changes than fertility does (which did not show temporal changes in these two populations). Indeed, this is a logical result since egg fertility mostly depends on sperm supply and, although *E. plorans* females can lay fertilized eggs during their entire life with just a single mating (López-León et al. 1994), it is also true that this species’ females lay more eggs after multiply mating (Pardo et al. 1995a). Since *E. plorans* is very abundant in Jete and Salobreña, it is unlikely that male availability (i.e., mating frequency) may represent a limiting factor for female fertility in these populations. Previous studies have shown that insect reproductive development and the number of eggs per clutch show plastic responses, depending on food availability (Moehrlin & Juliano 1998, Hatle et al. 2000). It is therefore logically possible that food quality and quantity changing between years could condition the reproductive response of these grasshoppers. For instance, part of the variation observed in egg clutch size could be due to the fact that female grasshoppers resorb some developing oocytes when stressed (Stauffer & Whitman 1997, Sundberg et al. 2001).

Another possible source of error in our data could be that, although all crosses were carried out within just one generation, all egg-pods in *E. plorans* were obtained in the laboratory and might not be representative of field laying: it has been shown in other grasshopper species that egg clutch size is significantly lower in the field than in the laboratory (Stauffer & Whitman 2007). In addition, that egg production in phytophagous insects is generally protein limited (Nijhout 1994), and this appears to apply to grasshoppers (Waskey et al. 2002). Therefore, although our laboratory conditions are largely homogenous between populations and years, and, hence, should have a rather homogenizing effect, the possibility exists that laboratory culture might imply an additional source of variation in egg clutch size, due to potentially limited access to food selected, i.e., preferred, by grasshoppers in their different natural environments.

In all cases where we observed B-chromosome effects on egg fertility and clutch size, in *E. plorans*, B-carrying females showed lower values than 0B females, in consistency with the parasitic nature of these B chromosomes. In the grasshopper *Dichroplus*

Table 4. Two-way ANOVA analysis of the spatial variation in egg fertility and clutch size of pods laid by females of the grasshopper *E. plorans*.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Item</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg fertility</td>
<td>Population</td>
<td>7.51</td>
<td>7, 427</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>4.14</td>
<td>1, 427</td>
<td>0.042533</td>
</tr>
<tr>
<td></td>
<td>Population x B</td>
<td>1.21</td>
<td>7, 427</td>
<td>0.293558</td>
</tr>
<tr>
<td>Egg clutch size</td>
<td>Population</td>
<td>10.23</td>
<td>7, 427</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.72</td>
<td>1, 427</td>
<td>0.395665</td>
</tr>
<tr>
<td></td>
<td>Population x B</td>
<td>1.22</td>
<td>7, 427</td>
<td>0.289740</td>
</tr>
<tr>
<td>Embryo clutch size</td>
<td>Population</td>
<td>10.98</td>
<td>7, 427</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2.95</td>
<td>1, 427</td>
<td>0.086662</td>
</tr>
<tr>
<td></td>
<td>Population x B</td>
<td>1.10</td>
<td>7, 427</td>
<td>0.363069</td>
</tr>
</tbody>
</table>

Table 5. Two-way ANOVA comparison of the spatial and temporal variation in egg fertility and clutch size between the Jete and Salobreña populations of *E. plorans*.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Item</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg fertility</td>
<td>Population</td>
<td>0.377</td>
<td>7, 427</td>
<td>0.540578</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>1.271</td>
<td>2, 108</td>
<td>0.284772</td>
</tr>
<tr>
<td></td>
<td>Population x Year</td>
<td>2.363</td>
<td>2, 108</td>
<td>0.098984</td>
</tr>
<tr>
<td>Egg clutch size</td>
<td>Population</td>
<td>10.23</td>
<td>7, 427</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>4.428</td>
<td>2, 108</td>
<td>0.014177</td>
</tr>
<tr>
<td></td>
<td>Population x Year</td>
<td>0.426</td>
<td>2, 108</td>
<td>0.654307</td>
</tr>
<tr>
<td>Embryo clutch size</td>
<td>Population</td>
<td>10.98</td>
<td>7, 427</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>3.783</td>
<td>2, 108</td>
<td>0.025824</td>
</tr>
<tr>
<td></td>
<td>Population x Year</td>
<td>1.388</td>
<td>2, 108</td>
<td>0.254060</td>
</tr>
</tbody>
</table>
Elongatus, B-carrying females displayed higher number of ovarioles and embryo clutch size than B-lacking females (Rosetti et al. 2007), with no significant differences among years. The beneficial effect of the B chromosome on D. elongatus females contrasts with its harmful effect on male mating success, through a decreased body size (Rosetti et al. 2007).

These sexually antagonistic effects of B chromosomes might have something to do with B chromosome transmission, with Bs being more detrimental in the sex where they show drive. In D. elongatus, B chromosomes are mitotically unstable, i.e., their number varies among cells within the same individual. It has been shown that mitotic instability of B chromosomes during embryo development leads to B-chromosome drive in males of other grasshopper species (Nur 1963, 1969, Kayano 1971, Viseras et al. 1990, Pardo et al. 1995b). It would be highly interesting to ascertain whether mitotic instability of B chromosomes in D. elongatus leads to B drive in males, but not in females, to test for a possible relationship between B transmission and effects in this species. In the case of E. plorans, scarce effects have been found in males at the level of formation of a low proportion of aberrant spermatids (Teruel et al. 2009). However, B chromosomes in this species are more detrimental in females, decreasing egg fertility, especially when B chromosomes are in a driving stage (see Zurita et al. 1998).

Acknowledgments

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