Transient Microgeographic Clines during B Chromosome Invasion

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abstract: The near-neutral model of B chromosome evolution predicts that the invasion of a new population should last some tens of generations, but the details on how it proceeds in real populations are mostly unknown. Trying to fill this gap, we analyze here a natural population of the grasshopper *Eyprepocnemis plorans* at three time points during the last 35 years. Our results show that B chromosome frequency increased significantly during this period and that a cline observed in 1992 had disappeared in 2012 once B chromosome frequency reached an upper limit at all sites sampled. This indicates that, during B chromosome invasion, transient clines for B chromosome frequency are formed at the invasion front on a microgeographic scale. Computer simulation experiments showed that the pattern of change observed for genotypic frequencies is consistent with the existence of B chromosome drive through females and selection against individuals with a high number of B chromosomes.

Keywords: cline, computer simulation, *Eyprepocnemis plorans*, near-neutral model, parasitic model.

Introduction

A huge variety of selfish genetic elements populate eukaryotic genomes, frequently causing genetic conflicts with other genomic elements that fuel evolutionary change and innovation (Werren 2011). Among them, B chromosomes are one of the easiest to detect and follow, and they have contributed much to our understanding of intragenomic co-evolutionary patterns. B (also known as supernumerary or accessory) chromosomes are extra dispensable chromosomes found in addition to the set of standard (A) chromosomes in about 15% of eukaryotes (Camacho 2005). B chromosomes prosper in natural populations because of some selective advantage either in their favor or in the favor of the host individual as a whole, thus being considered either parasitic (Östergren 1945) or heterotic (Darlington 1958; White 1973) elements, respectively. Parasitic B chromosomes usually show a transmission advantage (drive) and can be harmful to the host individuals. Heterotic B chromosomes, however, would confer a selective advantage to individuals carrying few B chromosomes but can be harmful at high numbers. A conceivable destiny of both parasitic and heterotic B chromosomes is to reach an equilibrium frequency across the years. But in the case of parasitic B chromosomes, this equilibrium can be disrupted by drive-suppressor genes turning the B chromosomes into near-neutral chromosomes (Camacho et al. 1997).

Theoretical and empirical studies have revealed four main stages in the life history of B chromosomes: invasion, resistance, near neutrality, and regeneration (Camacho et al. 1997; Zurita et al. 1998). Once a driving B chromosome arrives at or arises in a natural population (primary invasion), it rapidly increases in frequency in only some tens of generations. The resistance stage begins as soon as a harmful B chromosome becomes a burden for the individuals carrying it and proceeds through selection for any gene variant in the host genome able to suppress B chromosome drive. Whereas these first two stages are expected to be relatively fast, the near-neutral stage is expected to be a very long, near-random walk lasting thousands of generations, with B chromosome frequency showing random variation by drift or even a slight decrease due to negative selection against individuals with high numbers of B chromosomes. Since this is the longest stage, B chromosomes appear as nearly neutral elements most of the time. Finally, the regeneration stage depends on mutation of the B chromosome and the appearance of new B chromosome variants that are able to drive again and replace the former (neutralized) B chromosome. This kind of secondary invasion may restart the B chromosome cycle, thus prolonging its evolutionary life further still.
All four stages have been empirically shown for the B chromosomes in *Eyprepocnemis plorans*: Riera et al. (2004) provided an example of primary invasion, Herrera et al. (1996) showed evidence of drive suppression, López-León et al. (1992) and Camacho et al. (1997) reported examples of neutralized B chromosomes, and Zurita et al. (1998) reported an example of secondary B chromosome invasion.

The existence of drive-suppressor genes was first postulated by Shaw (1984), and it was subsequently experimentally demonstrated by Shaw and Hewitt (1985) and by Nur and Brett (1985). The near-neutral model has also been shown to be valid for other B chromosome systems, such as those in rye and maize (Puertas 2002; González-Sánchez et al. 2003; Jones et al. 2008). Under this model, the dynamics of B chromosome systems is viewed as a coevolutionary arms race between A and B chromosomes, implying a series of mechanisms of attack and defense (Frank 2000), the main weapons being drive for the B chromosome and drive suppression for the A chromosomes, with B chromosome harmfulness simply being a collateral effect.

The experimental observation of even the shortest of the four stages (i.e., invasion) necessarily requires long-term monitoring of the same population for tens of generations. In samples of the grasshopper *E. plorans* collected in 1977 at several Spanish localities, we observed that B chromosome frequency in Otívar (0.26) was four times lower than that in Jete, located only 3 km away (Camacho et al. 1980). Since this suggested the possibility that B chromosomes had recently invaded the population, we planned its long-term monitoring with a minimum impact on the natural invasion process. For this purpose, we resampled this population in 1992 and 2012, thus obtaining a temporal scope of 35 years, the longest monitoring ever performed for intrapopulation B chromosome frequency. Values of drive and selection against B carriers derived from a simulation of the invasion are comparable with experimentally derived values.

### Material and Methods

Adult males of the grasshopper *Eyprepocnemis plorans* were collected at populations neighboring a Spanish village (Otívar, province of Granada) during the months of October and November in 1992 and 2012, respectively (table 1). The 1992 samples were collected at four sites along a transect from the Rio Verde Valley to the village, located at 180 m (site 1), 210 m (site 2), 250 m (site 3), and 270 m (site 4) altitude (fig. 1). With only a 322-m map distance between the two farthest sites, the average slope in the transect was about 28%. In 2012, we could resample only sites 1–3 because site 4 had been swallowed by urban growth.

Males were anesthetized with ethyl-acetate vapors and dissected under a stereomicroscope to take out the testes, which were fixed in 3:1 ethanol-acetic acid and stored at 4°C. Cytological preparations of testis follicles were performed by squashing them in lactopropionic orcein. The number of B chromosomes in each male was determined by observation of primary spermatocytes in these preparations under an Olympus microscope.

We used the data from the three temporal points for which we have information (1977, 1992, and 2012) to estimate the plausible values of parameters controlling frequency changes in 0B, 1B, 2B, and 3B genotypes. For this purpose, we employed the Metatree program described in Camacho et al. (1997). For each simulation, starting frequencies were chosen at random from a binomial distribution with sample size and observed B chromosome frequencies matching the 1977 data; the discrepancy due to

<table>
<thead>
<tr>
<th>Year, site</th>
<th>Proportion of individuals with indicated no. of chromosomes</th>
<th>N</th>
<th>Prevalence</th>
<th>Mean</th>
</tr>
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<tbody>
<tr>
<td>1977: a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>.74 .26 .00 .00</td>
<td>19</td>
<td>.26</td>
<td>.26</td>
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<tr>
<td>1992:</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>.67 .24 .07 .02</td>
<td>45</td>
<td>.33</td>
<td>.44</td>
</tr>
<tr>
<td>2</td>
<td>.59 .32 .07 .02</td>
<td>44</td>
<td>.41</td>
<td>.52</td>
</tr>
<tr>
<td>3</td>
<td>.43 .46 .09 .03</td>
<td>35</td>
<td>.57</td>
<td>.73</td>
</tr>
<tr>
<td>4</td>
<td>.37 .56 .07 .00</td>
<td>27</td>
<td>.63</td>
<td>.7</td>
</tr>
<tr>
<td>Mean</td>
<td>.54 .37 .07 .02</td>
<td>151</td>
<td>.46</td>
<td>.57</td>
</tr>
<tr>
<td>2012:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>.40 .37 .18 .05</td>
<td>38</td>
<td>.61</td>
<td>.88</td>
</tr>
<tr>
<td>2</td>
<td>.36 .45 .17 .02</td>
<td>42</td>
<td>.64</td>
<td>.85</td>
</tr>
<tr>
<td>3</td>
<td>.43 .34 .23 .00</td>
<td>35</td>
<td>.57</td>
<td>.8</td>
</tr>
<tr>
<td>Mean</td>
<td>.39 .39 .19 .03</td>
<td>115</td>
<td>.61</td>
<td>.86</td>
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</tbody>
</table>

* The 1977 data are from Camacho et al. (1980).
this was not included in the goodness-of-fit measure. Frequencies in subsequent years were estimated allowing for non-Mendelian transmission (male and female transmission and disjunction: \( t_m, t_f, d_m, \) and \( d_f \)), the fitness of 2B (\( w_2 \)) and 3B (\( w_3 \)) individuals relative to that of 0B and 1B individuals (both assigned a fitness of 1), and polygenic change in female transmission due to the selection imposed on B-containing individuals by unfit progeny. The cost of drive suppression was set to 0 because this would be a very weak force on the timescales and amount of change estimated in pilot runs, and migration was similarly assumed to be negligible. Male transmission of the B chromosome, \( t_m \), was set to 0.5 in all simulations, in accordance with observations. Two hundred replicates of each simulation were run for each combination of parameter values used. The deviation of predicted frequencies from the ob-

\[ \text{Figure 1: Geographical location of the four sites sampled in the Otivar population.} \]
erved values in 1992 and 2012 in each simulation run was measured as a conventional contingency $\chi^2$ value (merging categories as necessary). The results are summarized as counts of simulations in which the $\chi^2$ test would lead to acceptance of the parameter values with a critical $\chi^2$ $P$ value of .05. (This amounts to approximate Bayesian computation of the posterior parameter distribution starting from uniform priors for all parameters between the upper and lower limits imposed by the model structure; see chapter 8 of Marin and Robert 2014.) The procedure was checked with a data set resulting from a known parameter set (e.g., fig. A1; figs. A1–A3 are available online).

Basic data analysis was done using Genstat (ver. 16; VSN International, Oxford, United Kingdom). The simulation model was written in Borland Pascal and run under Delphi (ver. 4) as a console application. Parameter fitting was done using Mathematica (ver. 10; Wolfram Research, Champaign, IL) using the function ListInterpolation to fit the grid of counts of acceptable goodness-of-fit values obtained from the simulations. Initially, the fit was to a grid of parameters covering female drive, male and female disjunction, 2B and 3B fitness, and effective population size causing drift. On the basis of the shape of the hypersurface fitting these data, a second fit was done using a finer grid and 200 replicates covering female drive (0.54–0.7), 2B fitness (0–1), 3B fitness (0 to the 2B fitness), male and female disjunction (0.7 and 0.3, respectively; pooling the data for these values increased the sample size to 800 replicates per parameter set for the remaining parameters), and additive genetic variance in transmission (0, 0.05, and 0.1). Preliminary testing confirmed that drift did not alter the general patterns observed. The effective population size, $N_e$, was set to infinity for the results reported here.

Results

The B chromosome found in the studied population is about one-third the size of the X chromosome and is mostly composed of ribosomal DNA and a 180-bp repetitive DNA in 1:2 proportion, similar to the B chromosome variant in other nearby populations (Cabreró et al. 2014).

Table 1 shows the proportion of individuals with 0–3 B chromosomes found in the samples collected in 1992 and 2012, also including a 1977 sample previously published by Camacho et al. (1980).

The frequency of B-carrying individuals increased smoothly over the study period (1B: 2%/year; 2B: 6% /year; df = 2; $P = .001$, generalized linear model with multinomial error and log link function; linear change $P < .001$, quadratic deviation $P > .2$), and there was some evidence for a linear increasing trend in B chromosome frequency from site 1 near the river to site 4 at the top of the hill (df = 2; $P = .05$, generalized linear model using site differences described by contrasts representing linear and higher-degree polynomial contrasts). The change in this gradient between 1992 and 2012, though striking, was not significant ($P = .5$, comparing only those sites present in both years).

We fitted a simulation of the evolution expected during an invasion to the data. Using a relatively coarse grid over the whole parameter space, we established that the goodness of fit was weakly affected by male and female disjunction frequency. Measured data for meiotic metaphase I and II cells in 21 Otivar individuals gave a male disjunction, $d_m$, of $0.65 \pm 0.2$ (2 SEM), and for subsequent simulations either male disjunction was fixed at 0.7 and female disjunction, $d_f$, was fixed at 0.3, consistent with previous reports (López-León et al. 1992), or the average of 0.3 and 0.7 was used for both. The best-fitting drive and 2B fitness parameters were strongly negatively correlated, and there was weak negative correlation between the best-fitting fitness of 2B and 3B individuals (fig. 2; see also figs. A2, A3).

With no variance in transmission rate (and therefore no evolution in transmission rate), plausible fits were obtained in small but elongated regions of parameter space centered around $t_1 = 0.62$ and $w_1 = 0.8$, but with very strong negative correlation between the two parameters. This is intrinsic to the system. Stronger drive increases the proportion of 2B individuals while lower 2B fitness reduces it, so they tend to compensate for each other close to an optimum. Support for $w_1$ and $\sigma$ was very broad, with almost all values plausible. The highest plausible transmission rates are consistent with but a little lower than the drive observed in Torrox (Zurita et al. 1998). The 2B fitness implied is consistent with available measurements (Camacho et al. 1997), being within the error limits of those estimates. There was weak evidence for evolutionary reduction in transmission with time, with the best-fitting parameter sets including $\sigma$ values greater than and equal to 0. The inclusion of drift in the simulation, with $N_e = 100$ or 500, increased the median discrepancy between the observed and simulated data, as expected, but did not alter the best-fitting regions of parameter space.

Discussion

Most studies of B chromosome frequency have focused on spatial variation, but only a few have monitored the same population for periods longer than 5 years. In some of these cases (e.g., Nur 1966; Jackson and Cheung 1967; Kayano et al. 1970), B chromosome frequency remained stable across years. In the grasshopper *Eyprepocnemis plorans*, for instance, B frequency remained stable for 9 and 16 years in two Spanish populations (Camacho et al. 1997), for 2 or 3 years in nine Moroccan populations (Bukkali et al. 1999), and for up to 9 years in tens of populations to the east of the Iberian Peninsula (Perfetti et al. 2004). In other cases, rapid increase in B chromosome frequency has occurred, as
in the cline movement noted by Shaw (1983) for the grasshopper *Myrmeleotettix maculatus*. In the grasshopper *E. plorans*, the B<sub>2</sub> variant rapidly replaced the B<sub>0</sub> one in a Spanish population (Zurita et al. 1998). Other cases of B chromosome invasion were later reported in the fish *Prochilodus lineatus* (Cavallaro et al. 2000), the Brazilian wasp *Trypoxylon albitarse* (Araujo et al. 2002), and a Mallorca Island population of *E. plorans* (Riera et al. 2004). In the mouse *Apodemus peninsulae*, the mean number of B chromosomes per individual in a Russian population increased from 2.3 to 6.5 over 22 years (Borisov 2008), probably representing the early phase of increase after introduction of the B chromosome to a new population.

Our present results in the Otivar population of *E. plorans* represent the longest series of natural B chromosome frequencies available. The 1977 sample had a B chromosome prevalence (0.26) three times lower than that (0.76) in Jete, 3 km away (Camacho et al. 1980). B chromosome prevalence did not change in Jete from 1977 to 1993 (Camacho et al. 1997) but doubled in Otivar (reaching 0.57 at site 3 in 1992), with smaller increases over the next 20 years, reaching values close to the stable B prevalence in the Jete population. This suggests that B chromosomes had entered the Otivar population only a few years prior to our 1977 sampling and were approaching a drive-selection balance consistent with weak B drive (0.6) and selection against 2B and 3B individuals. The fitnesses inferred in this article from model fitting at Otivar are consistent with effects on female fecundity observed directly (Muñoz et al. 1998).

The only means by which a B chromosome can expand its geographical range is through host gene flow into B-lacking populations, with drive subsequently increasing B frequency in the new population. As suggested by our data, B chromosomes, like epidemic parasites, can infest new territories through human activities, thus expanding their geographical range. Several local invasions of B chromosomes into *E. plorans* populations have been reported. That B chromosome invasion occurred later in the populations studied in this work than in other downstream populations along the Verde River indicates that B chromosomes first invaded the coastal populations, from which they then spread toward inland populations. Additional evidence in favor of this scenario is the existence of B-lacking populations in the headwaters of another river basin (Segura River) due to the presence of geographical barriers impeding the upstream advance of B-carrying individuals (Cabrero et al. 1997). Recent molecular population genetic analysis provides additional support for this conclusion (Manrique-Poyato et al. 2015).

The cline observed in 1992, with B chromosome frequency being higher at the two up-mountain samples (sites 3 and 4), had disappeared by 2012 due to increased frequency at the lower sites. Although the change was not significant, it is a plausible example of the formation and disappearance of transient frequency clines on microgeographic scales during B chromosome invasion. This transience is consistent with the rapid spatial movement of a cline for B chromosome frequency reported in the grasshopper *M. maculatus* (Shaw 1983). In addition, that B chromosome frequency at the sites at higher altitude (3 and 4) was already close to the upper limit in 1992 while it was not at sites 1 and 2 suggests that B chromosome invasion of this population began close to the village and advanced downward. *Eyprepocnemis plorans* lives among and feeds on a variety of plants in humid places by rivers or small water courses, including agricultural areas. Since B chromosomes entered the Iberian Peninsula through the coast (see above), the natural upstream pathway for B chromosome invasion of the Otivar popula-

![Figure 2: Combinations of 2B fitness (vertical axis) and female drive (horizontal axis) resulting in model agreement with the observed frequencies of animals with varying numbers of B chromosomes at Otivar. Contours show the proportion of simulations that agreed sufficiently well with the data that a standard χ² test would not reject the parameter set. In effect, this is proportional to the likelihood of the parameter set. a, No coevolution in female drive. b, Standard deviation of drive = 0.05. c, Standard deviation of female drive = 0.1. t<sub>f</sub> = female transmission, w<sub>1</sub> = fitness of 2B individuals, w<sub>2</sub> = fitness of 3B individuals.](image-url)
tion would have been from the river (site 1) upward, that is, the pattern opposite to that suggested by the 1992 cline. The observed pattern is therefore more consistent with a scenario of B chromosome spread aided by human activities. In fact, the twentieth century witnessed an intensive reforestation of the studied zone with subtropical trees (cherrymoya, avocado, etc.), which by 1990 had reached maximum productivity (Cambil-Rodriguez and Pretel-Aneas 2006). A resident of the area (Asunción Fajardo Aneas) who was born and grew up in the town of Ótivar remembered that around 1970 her father planted cherimoya trees in his field (site 4), which probably came from nurseries on the coast of Almuñecar or Motril. If this was the route by which B-containing E. plorans entered the Ótivar population, it would explain the cline in frequency seen in 1992.

The short-term dynamics of a B chromosome invasion, as illustrated here, are the result of conflicting interests of parasitic B chromosomes (showing transmission advantage but being harmful) and the host A chromosomes (Shaw 1984; Frank 2000). On a much longer (∼10-fold) timescale, the resulting coevolutionary arms race is expected to decrease drive and increase the fitness of 2B and 3B individuals. The near-neutral model of B chromosome evolution predicts that B chromosome frequency will be roughly stable for many years in Ótivar and that B chromosomes will persist for an even longer period. The general framework of the model seems likely to be appropriate for most B chromosome systems.

Acknowledgments

We dedicate this work to the memory of Prof. Godfrey Hewitt (1940–2013), whose work on B chromosomes was our main source of inspiration and who was our role model. We thank T. López for technical assistance. S. Proulx provided exceptionally useful comments during the review process. M.B. was supported by a Ramón y Cajal Fellowship from the Spanish Ministerio de Ciencia e Innovación.

Literature Cited


"Similar in interest are some specimens of Neuroptera from the Carboniferous beds of Morris, Illinois; they occurred in small flattened iron-stone concretions, like the clay-stones in clay banks of the present day. These Neuroptera also represent families distinct from any now living, and, like many of the Devonian insects, are synthetic in character; that is, combine in one and the same form features which, in after ages, are distributed among the members of different families." From "The Insects of Ancient America" by S. H. Scudder (*The American Naturalist*, 1868, 1:625–631).