POPULATION DYNAMICS OF A SELFISH B CHROMOSOME NEUTRALIZED BY THE STANDARD GENOME IN THE GRASSHOPPER EYPREPOCNEMIS PLORANS

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Abstract.—Effects of the B chromosome polymorphism of the grasshopper Eyprepocnemis plorans were analyzed in two natural populations. Postmating sexual selection, female fertility, and survival were studied. The B chromosome lacks drive and has no detectable effects on fitness. A neutral B cannot invade a population and establish a polymorphism, but the confidence limits on our estimates cannot exclude the possibility that the polymorphism is maintained by a balance between weak drive and weak selection against individuals with two and three B’s. However, other lines of evidence favor the following model of the dynamics of the B in E. plorans. In a newly invaded population, the B has substantial drive, but the evolution of drive suppressor genes in the A chromosomes neutralizes the B drive so that it becomes near-neutral and begins a random walk toward extinction by stochastic loss. Because the B is common by the time drive disappears, the random walk is likely to continue for a long time. If in the course of the random walk a variant B with greater drive appears, then it will displace the original variant, and a new cycle of drive suppression and drift to extinction occurs. A simulation model of this process suggested that the mean time to extinction is proportional to the two-thirds power of the population size; it is much less affected by subpopulation size or the number of populations in a subdivided population.

The B chromosomes are very large pieces of dispensable independently segregating DNA. This makes them interesting probes of the evolutionary forces acting on noncoding DNA. Two very general models of how B chromosomes are maintained in populations have been proposed. These are the parasitic (Östergren 1945) or selfish (Jones 1985) model, which states that B’s are maintained by meiotic drive despite deleterious effects on carrier fitness (Jones 1991), and the heterotic model (White 1973), which suggests that B’s lacking drive might be maintained because of beneficial effects on individuals carrying small numbers of B’s, while large numbers were detrimental. Both models implicitly assume that an equilibrium exists. However, it is also possible that some popula-
tions polymorphic for B chromosomes are in metastable states far from the general equilibrium.

It is essential to know the effects of B chromosomes and how they are inherited to understand their presence in the genome. Some B chromosomes are genetically inactive (Fox et al. 1974; Ishak et al. 1991); others show some evidence of transcription, for example, those in the frog *Leiopelma hochstetteri* (Green 1988) and the blackfly *Simulium juxtacrenobium* (Brockhouse et al. 1989). Some B chromosomes contain ribosomal RNA genes (for a review, see Green 1990; Mabuchi 1991; Beukeboom 1994a), while others affect gene expression in standard chromosomes (Ruiz Rejón et al. 1980; Oliver et al. 1982). In general, the dispensability of B’s is presumably related to their having evolved from the A complement (Jones and Rees 1982) and therefore at first containing only transcribed DNA sequences present also elsewhere in the genome.

Many attempts to estimate the effects of B chromosomes on fitness have been made (Jones and Rees 1982; Shaw and Hewitt 1990; Beukeboom 1994b). In most organisms, the conclusions point toward B chromosomes as selfish genomic elements. The only exception known so far is in the chive *Allium schoenoprasum*, in which seeds carrying small numbers of B chromosomes survived from seed to seedling better than those lacking them (Holmes and Bougourd 1989) and there was no evidence of B chromosome drive (Bougourd and Parker 1979).

The B chromosome polymorphism in *Eyprepocnemis plorans* closely resembles that in *Allium* by lacking detectable drive for the three main types of B’s (B1, B2, and B3) (López-León et al. 1992b). The B chromosomes do not detectably influence exophenotypic characters (Camacho et al. 1980). The B2 chromosome in *E. plorans* had no detectable effect on mating success in either sex in two natural populations, nor was there detectable departure from random mating (López-León et al. 1992a). (Despite these results, recent work shows that in certain laboratory settings, the B chromosome might affect mating success slightly; Martin et al. 1996).

Crosses between populations with and without B’s have suggested that the absence of drive for *E. plorans* B’s is due to drive suppressor genes in the A chromosomes (Herrera et al. 1996). The average transmission ratio shown by one-B females was 0.63 when crossed to a zero-B male from a non-B population but 0.48 when crossed to a zero-B male from their own population. The existence of such genes has also been demonstrated in the grasshopper *Myrmeleotettix maculatus* (Shaw and Hewitt 1985) and the mealy bug *Pseudococcus affinis* (Nur and Brett 1987). Furthermore, new B chromosome variants appear frequently in natural populations of *E. plorans* (López-León et al. 1993a), and replacement of one B variant by another (Henriques-Gil and Arana 1990) seems to be associated with detectable meiotic drive of the variant that is increasing in frequency (S. Zurita, J. Cabrero, M. D. López-León, and J. P. M. Camacho, unpublished data).

In this article, we show that the B2 chromosome of *E. plorans* has at most
slight effects on fitness when present in one or two copies in an individual and that temporal trends in frequency are weak. We hypothesize that B’s were initially more strongly driven but were neutralized by the host genome through the evolution of drive suppressor genes and that they persist indefinitely because they are eliminated very slowly once common, and new driven B variants emerge from time to time, further prolonging the polymorphism. The distinctive feature of this hypothesis is that the system is presumed not to be seen at equilibrium. We develop a mathematical model of B chromosome evolution in *E. plorans* under this hypothesis and show that the idea is quantitatively plausible and accounts for all the relevant observations, while alternative hypotheses either require remarkable coincidences or are incompatible with the data.

**Material and Methods**

Two natural populations were studied, Jete and Salobreña in the province of Granada (Spain). Samples of adults were collected from each population on eight (Jete) and five (Salobreña) occasions between 1977 and 1993. In 1990, mating pairs, nonmating individuals and gravid females were collected from each population to analyze mating behavior, as described elsewhere (López-León et al. 1992a). Because there is very strong second male sperm precedence in *Eyprepocnemis plorans* (López-León et al. 1993b), it was important to avoid bias toward first matings. We therefore only considered as gravid those females with a very enlarged abdomen, because these were likely to be ready to lay and unlikely to mate again before laying. Each gravid female was placed alone in a cage with fresh grass supplied daily and a tube with moist vermiculite for laying. After the first egg pod was obtained, the female and the embryos contained in the egg pod were cytologically analyzed following the procedures described elsewhere (López-León et al. 1992b).

During 1991, samples of adult males and females from each population were collected and karyotyped. Comparison of the B frequency in these samples with that in the embryos from the gravid females in 1990 allowed us to estimate the effects of B’s on survival.

**Results**

**Evolution of B Frequency**

The B frequency at Jete and Salobreña during the study period is shown in figure 1. In Salobreña there were no significant differences between karyotypic frequencies in different years ($\chi^2_3 = 10.6, P = .23$), but in Jete there were ($\chi^2_4 = 28.1, P = .014$), although no overall trend was apparent.

**Effects of B Chromosomes on Fitness**

Karyotype frequencies were estimated in adults collected in 1990 and 1991 and in the embryos produced by gravid females from 1990 (table 1).

*Analysis of postmating selection.*—The fitness component operating between
Fig. 1.—Short-term evolution of B chromosome frequency in two natural populations of *Eyprepocnemis plorans*: A, Jete; B, Salobreña. Bars represent 2 SEs on either side of each mean.

TABLE 1

<table>
<thead>
<tr>
<th>Population and Sample</th>
<th>Zero-B</th>
<th>One-B</th>
<th>Two-B</th>
<th>Three-B</th>
<th>Four-B</th>
<th>Total</th>
<th>$\bar{x}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jete:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult females 1990</td>
<td>57</td>
<td>51</td>
<td>22</td>
<td>2</td>
<td>0</td>
<td>132</td>
<td>.765</td>
</tr>
<tr>
<td>Adult males 1990</td>
<td>83</td>
<td>95</td>
<td>43</td>
<td>5</td>
<td>0</td>
<td>226</td>
<td>.867</td>
</tr>
<tr>
<td>Gravid females 1990</td>
<td>24</td>
<td>21</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>55</td>
<td>.782</td>
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<tr>
<td>Embryos 1990</td>
<td>834</td>
<td>858</td>
<td>313</td>
<td>80</td>
<td>6</td>
<td>2,091</td>
<td>.874</td>
</tr>
<tr>
<td>Adults 1991</td>
<td>80</td>
<td>83</td>
<td>27</td>
<td>3</td>
<td>0</td>
<td>195</td>
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<td>Salobreña:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult females 1990</td>
<td>55</td>
<td>62</td>
<td>14</td>
<td>2</td>
<td>0</td>
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<td>.722</td>
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<td>Adult males 1990</td>
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<td>92</td>
<td>33</td>
<td>7</td>
<td>0</td>
<td>206</td>
<td>.869</td>
</tr>
<tr>
<td>Gravid females 1990</td>
<td>23</td>
<td>24</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>51</td>
<td>.647</td>
</tr>
<tr>
<td>Embryos 1990</td>
<td>870</td>
<td>652</td>
<td>187</td>
<td>54</td>
<td>2</td>
<td>1,765</td>
<td>.699</td>
</tr>
<tr>
<td>Adults 1991</td>
<td>59</td>
<td>54</td>
<td>15</td>
<td>9</td>
<td>1</td>
<td>138</td>
<td>.833</td>
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</table>

**Note.**—Mean number of B’s is indicated by $\bar{x}$.
TABLE 2
Karyotypes of the Offspring of Gravid Females Allowed to Lay in the Laboratory

<table>
<thead>
<tr>
<th>Population of Gravid Females and Number of B's</th>
<th>N</th>
<th>Zero-B</th>
<th>One-B</th>
<th>Two-B</th>
<th>Three-B</th>
<th>Four-B</th>
<th>Total</th>
<th>$\bar{x}_B$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jete:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>24</td>
<td>568</td>
<td>332</td>
<td>44</td>
<td>9</td>
<td>...</td>
<td>953</td>
<td>0.472</td>
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<td>1</td>
<td>21</td>
<td>241</td>
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<td>159</td>
<td>19</td>
<td>1</td>
<td>802</td>
<td>0.982</td>
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<tr>
<td>2</td>
<td>8</td>
<td>20</td>
<td>127</td>
<td>88</td>
<td>40</td>
<td>1</td>
<td>276</td>
<td>1.515</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>5</td>
<td>17</td>
<td>22</td>
<td>12</td>
<td>4</td>
<td>60</td>
<td>1.993</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>834</td>
<td>858</td>
<td>313</td>
<td>80</td>
<td>6</td>
<td>2,091</td>
<td>0.874</td>
</tr>
<tr>
<td>Salobreña:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>23</td>
<td>580</td>
<td>204</td>
<td>2</td>
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<td>...</td>
<td>786</td>
<td>0.269</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>287</td>
<td>417</td>
<td>119</td>
<td>26</td>
<td>...</td>
<td>849</td>
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<td>1.904</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>...</td>
<td>3</td>
<td>10</td>
<td>10</td>
<td>...</td>
<td>23</td>
<td>2.304</td>
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<tr>
<td>Total</td>
<td>51</td>
<td>870</td>
<td>652</td>
<td>187</td>
<td>54</td>
<td>2</td>
<td>1,765</td>
<td>0.699</td>
</tr>
</tbody>
</table>

Note.—Mean number of B’s is indicated by $\bar{x}_B$.

Mating and zygote formation may be assessed by comparing karyotypic frequencies among the 10-d-old embryos produced by gravid females with the frequencies expected from the proportions of different kinds of mating pairs observed in the field (López-León et al. 1992a) and the karyotypic composition of each of these crosses in laboratory matings (López-León et al. 1992b).

The data for this comparison are shown in table 2 and the cited references. The statistical method described in the appendix showed no significant differential postmating selection associated with the B chromosome in either of the two populations analyzed.

Differential zygotic mortality in the first 10 d of embryo development must be fairly slight because almost all eggs (98% in Jete and 92% in Salobreña) contained live embryos (see table 3).

Average transmission frequencies: Jete.—With assumed random mating, the mean number of B’s among the embryos from zero-B females ($\bar{x}_B = 0.472$; see table 2) represents the frequency of B’s transmitted through males. The ratio of this to $\bar{x}_B$ in all adult males collected in the 1990 season (0.867; see table 1) suggests a male transmission ratio (0.544) very close to that observed in controlled crosses (López-León et al. 1992b).

Females with one B yielded progeny with $\bar{x}_B = 0.982$. If we subtract the B frequency transmitted through males (0.472), based on the zero-B results, this implies a female B transmission ratio of 0.510, again very close to the results of controlled crosses.

Finally, two-B females produced embryo offspring with $\bar{x}_B = 1.515$, which implies that the B transmission ratio through these females was equal to $(1.515 - 0.475)/2 = 0.520$, a figure that is also consistent with controlled crosses. In
TABLE 3
Egg and Embryo Production per Pod in 55 Females from Jete and 51 from Salobreña Collected in 1990

<table>
<thead>
<tr>
<th>Population of Females and Karyotype</th>
<th>LEVENE TEST ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Jete:</td>
<td></td>
</tr>
<tr>
<td>Eggs/pod:</td>
<td></td>
</tr>
<tr>
<td>Zero-B</td>
<td>24</td>
</tr>
<tr>
<td>One-B</td>
<td>21</td>
</tr>
<tr>
<td>Two-B'</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
</tr>
<tr>
<td>Embryos/pod:</td>
<td></td>
</tr>
<tr>
<td>Zero-B</td>
<td>24</td>
</tr>
<tr>
<td>One-B</td>
<td>21</td>
</tr>
<tr>
<td>Two-B'</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
</tr>
<tr>
<td>Embryos/eggs:</td>
<td></td>
</tr>
<tr>
<td>Zero-B</td>
<td>24</td>
</tr>
<tr>
<td>One-B</td>
<td>21</td>
</tr>
<tr>
<td>Two-B'</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
</tr>
<tr>
<td>Salobreña:</td>
<td></td>
</tr>
<tr>
<td>Eggs/pod:</td>
<td></td>
</tr>
<tr>
<td>Zero-B</td>
<td>23</td>
</tr>
<tr>
<td>One-B</td>
<td>24</td>
</tr>
<tr>
<td>Two-B'</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
</tr>
<tr>
<td>Embryos/pod:</td>
<td></td>
</tr>
<tr>
<td>Zero-B</td>
<td>23</td>
</tr>
<tr>
<td>One-B</td>
<td>24</td>
</tr>
<tr>
<td>Two-B'</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
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<tr>
<td>Embryos/eggs:</td>
<td></td>
</tr>
<tr>
<td>Zero-B</td>
<td>23</td>
</tr>
<tr>
<td>One-B</td>
<td>24</td>
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<tr>
<td>Two-B'</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
</tr>
</tbody>
</table>

Note.—A Levene test of homogeneity of variances indicated no significant differences in the variances between pods for number of eggs or embryos between the different female karyotypes. It was therefore valid to apply a parametric ANOVA to test for differences in means between karyotypes. In no case were there significant differences among zero-B, one-B, and two-B' (two or more B's) females. The embryo/egg ratio in Jete was so close to 1 that ANOVA was inappropriate.

Conclusion, the data from gravid females and their offspring in Jete are completely consistent with the very low or absent drive demonstrated in controlled crosses (López-León et al. 1992b).

Average transmission frequencies: Salobreña.—Only zero- and one-B females may be used for inferring populational B transmission, because there were too few gravid females with two and three B’s (see table 1). The frequency of B chromosomes transmitted through males, as deduced from $x_B$ in the progeny of zero-B females (0.269), was conspicuously lower than half of $x_B$ in adult
males in the same season \((0.869 \times 0.5 = 0.435)\), suggesting undertransmission of B’s through males. This could be due to a postmating fertilization advantage of zero-B males over one- and two-B ones, but the analysis shown in the appendix shows that these differences were very far from being significant. The B transmission ratio through one-B females \((0.893 - 0.269 = 0.624)\) seemed to be higher than the Mendelian one and inconsistent with that observed in controlled crosses, suggesting that for whatever reason the strong undertransmission estimated for B-carrying males via zero-B females does not apply to one-B females. This suggests that the apparent undertransmission in zero-B’s may be a chance event.

Female fertility.—Table 3 shows the mean number of eggs per pod, the mean number of embryos per pod, and the proportion of eggs containing an embryo in the gravid females collected in 1990. These three components of female fertility were independent of the presence of B chromosomes in both populations.

Viability from embryo to adult.—To test for differences in viability related to the presence of B chromosomes, we compared karyotype frequencies in embryos produced by gravid females in the 1990 season with adults of the same generation, collected in 1991 (table 4). The karyotypes of the eggs in a pod are correlated, but not completely. The sample size associated with estimates of the variance in estimates of karyotype frequency therefore lies between the number of eggs karyotyped and the number of pods examined. Since the frequency of B’s varies greatly between egg pods, the appropriate binomial variance is that based on the number of gravid females examined. The difference in the frequency of B’s in the egg sample and the adults was tested against a normal distribution with the sum of the two variances. There were no significant differences in viability from embryo to adult between zero-B, one-B, and two-B\(^+\) karyotypes in either of the two populations analyzed.

### Table 4

**Analysis of Viability from Embryo to Adult by Comparing the Frequencies of Zero-B, One-B, and Two-B\(^+\) Embryos Yielded by Gravid Females in 1990 with Those of Adults of the Same Generation Collected in 1991**

<table>
<thead>
<tr>
<th>Population and Stage</th>
<th>N</th>
<th>Mean</th>
<th>Binvar</th>
<th>Mean</th>
<th>Binvar</th>
<th>Mean</th>
<th>Binvar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jete:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryos</td>
<td>2,091</td>
<td>.399</td>
<td>.0044</td>
<td>.410</td>
<td>.0044</td>
<td>.191</td>
<td>.0028</td>
</tr>
<tr>
<td>Adults</td>
<td>193</td>
<td>.415</td>
<td>.0013</td>
<td>.430</td>
<td>.0013</td>
<td>.155</td>
<td>.0007</td>
</tr>
<tr>
<td>Z-test</td>
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<td>.27 NS</td>
<td></td>
<td>.61 NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salobreña:</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Embryos</td>
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<td>.0049</td>
<td>.369</td>
<td>.0046</td>
<td>.138</td>
<td>.0023</td>
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<tr>
<td>Adults</td>
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<td>.428</td>
<td>.0018</td>
<td>.391</td>
<td>.0017</td>
<td>.181</td>
<td>.0011</td>
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<td>Z-test</td>
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<td>.28 NS</td>
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<td>.74 NS</td>
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</table>

**Note.**—Binvar = binomial variance.
Models of the Dynamics of the B Chromosome

The B chromosome in these populations appears to have at most small effects on fitness and to be subject to weak meiotic disturbances. An adequate theory must explain the forces maintaining the B in the population and how such a genetic element could ever come to be widespread. There appear to be four possibilities. First, perhaps the B was always without tendency to increase or decrease and has reached and remains at its present frequency by random drift. Second, the B might have become common and be maintained by a balance between very weak meiotic drive and very weak selective forces, too small to be detected with the margins of error in the measurements so far. Third, the B might have been initially subject to meiotic drive, which has been suppressed by evolution in the rest of the genome. Fourth, the B might have been initially advantageous but have lost this advantage after becoming common. In the third and fourth cases, it is necessary to explain what now maintains the B in the population separately from what initially made it common. Both models imply that the B is now observed in an unstable state, on a slow passage to extinction.

Mathematical model.—To compare these ideas quantitatively with the data, it seemed useful to construct a mathematical model of the dynamics of the *E. plorans* B chromosome. Feldman and Otto (1991) have reviewed models of the evolution of systems of segregation distortion within the A genome. None of these are precisely appropriate to the present setting, because they are all deterministic and because in a B system there is no limit to the number of B’s an individual might in principle contain. We therefore built a simulation model.

First, we constructed a general description of the probabilities of gametes with different numbers of B’s arising from males and females with given numbers of B’s. A proportion \(d\) of the B’s pair and are distributed one to each pole of the gametocyte at meiosis 1; the remainder are distributed according to a binomial distribution with a parameter \(t\) that describes the drive. In principle, sperm might also then be subject to selection, but there is no evidence for this in *E. plorans*. If the notation \(\binom{l}{k}\) denotes the binomial coefficient for \(l\) successes in \(k\) trials, this scheme gives the following general expression for the probability that an adult with \(i\) B’s will give rise to a gamete with \(j\) B’s:

\[
\Pr\{j|i\} = \sum_{k=0}^{i} \left[ \prod_{l=0}^{k-1} \{1 - (1 - d)^{\binom{C_{i-2l}}{l}}\} \right] \binom{i-2l}{l} C_{i-2l}(1 - t)^{t(j-\binom{k}{2})} t^{j-k},
\]  

subject to \(\binom{C_0}{l} = 1\), but \(\binom{C_0}{l} = 0\) for \(l < 0\).

The zygotic array representing the next generation before selection is then obtained for each class of mating by multiplying the gamete arrays from each parent and adding the probabilities that result in the same number of B’s in the offspring:

\[
\Pr\{i|j \times k\} = \sum_{u=0}^{j} \Pr\{i - u|j\} \Pr\{u|k\}.
\]
Then the initial expected B frequencies in the next generation are obtained by multiplying each of these arrays by the probability of the appropriate class of mating and adding up over all the mating classes. Finally, each B frequency is multiplied by the appropriate zygotic fitness, and the postselection frequencies are calculated by dividing by the average fitness.

There is some evidence in *E. plorans* for a remarkable sort of assortative fertilization in which one-B sperm preferentially fertilize zero-B eggs and zero-B sperm preferentially fertilize one-B eggs (López-León et al. 1996). Without details of how this effect operates in all classes of mating, it is not possible to model populations in which two-B's are not lethal. In a population in which two-B's are lethal, the effect is to raise the B frequency at which any transmission bias is balanced by selection against two-B's. The qualitative dynamics are little changed.

Drift was included in the model discussed here by using these frequencies as the probabilities in a multinomial distribution based on the effective population size and by picking a set of actual offspring numbers using standard techniques based on pseudo-random numbers.

Eshel (1985) demonstrates that suppressors freely recombining with an unfavorable but driven element will always be favored. Selection against drive was modeled as a polygenic process. It should be slow by comparison with the changes in frequency resulting from drive, because the selective forces are produced by differences in the extent of drive rather than the drive itself. It was therefore modeled using the approach of Lande (1982). The way fitness changes with drive was determined by differentiating equation (1) with respect to drive, and the rate of selection was calculated from this by multiplying by the genetic variance in drive. The influence of drift on selection against drive was neglected, as justified elsewhere (Shaw 1984).

Migration between subpopulations was modeled by using a binomial distribution to choose the number of migrants of each karyotype emigrating into a central pool as a random variable controlled by the average migration rate and population size. Individuals from this pool were then assigned at random to populations in the metapopulation.

Using this model, we studied the probability of initial invasion in the absence of drive (which confirmed the more abstract argument above, although because of the tiny probabilities involved only very partial experiments could be done); the equilibrium B frequencies with weak drive and selection; the rate at which selection reduces drive and therefore the equilibrium B frequency; and the length of time a “neutralized” B chromosome would be expected to survive in a large population or set of populations.

**Pure drift.**—This first possibility is extremely unlikely. An exactly neutral element would reach a frequency of 50% in about $2/N_e$ of events creating the B. (Neutrality implies that the frequency of an element does not change averaged over a very large ensemble of identical evolving populations, so the probability of fixation must be $1/N_e$. Symmetry implies that half of all neutral elements reaching 50% frequency must fix, so the frequency of elements reaching 50% is double the fixation frequency, or $2/N_e$. The B cannot actually fix, since it does
not undergo regular disjunction, but this does not affect its dynamics until it becomes common.) Since the B is widespread throughout the range of *E. plorans* in Spain, \( N_e \) refers to the effective size of the entire population, which is likely to be on the order of \( 10^4 \)–\( 10^7 \), since the animal is a common inhabitant of most moist riverside environments. The creation of a large variant chromosome with negligible effects on fitness is unlikely to be common but must have a probability proportional to \( N_e \). This means that the probability of a common, neutral B arising in any generation is just twice the proportion of mutations carrying, de novo, a neutral B chromosome, independent of population size. The creation of a new neutral B is clearly improbable, but exactly how improbable is impossible to determine.

If the newly arising B were disadvantageous to an extent greater than roughly the reciprocal of the population size, the probability of reaching a substantial frequency would be greatly reduced. Thus, for example, a B in a population of \( 10^4 \) with a disadvantage of one part in \( 10^4 \) has effectively no chance of reaching a frequency of 50%. We conclude that neutrality of the B from the start is extremely improbable.

**Very weak drive and selection.**—The deterministic equilibrium for various combinations of drive, disjunction, and fitness of two- and three-B animals was determined. Male drive was set to 0.5; female drive was 0.52, 0.53, or 0.54. Disjunction in both males and females was 0, 0.5, or 0.7. Fitness of one-B relative to zero-B was set to 1, so that the B could in principle invade even with the very weak drive. Fitness of two-B was 0.7, 0.8, or 0.9 and of three-B was 0.3, 0.5, 0.7, or 0.9. Even in this very restricted part of the parameter space, deterministic equilibria close to those observed in wild *E. plorans* populations (i.e., about 40% zero-B, 40% one-B, 15% two-B) were possible for many parameter combinations. All the parameter combinations shared the characteristic that the fitness of two-B's was close to 1.

This demonstrates that in any one population the *E. plorans* B polymorphism could simply be maintained by a balance between weak selection and weak drive. Unfortunately, since plausible equilibria were possible even with female drive of 0.51, provided two-B individuals had a fitness of nearly 1 and population sizes were large enough to make the small deterministic force dominant over drift, it would be extremely hard to rule out this explanation by observations on fitness and drive alone. However, this explanation sheds no light on the diversity of B chromosome types observed or on the differences in drive observed in crosses between populations with and without B’s (López-León et al. 1993a; Herrera et al. 1996). If correct, the B should be lost by drift more often in small populations, so there should be a correlation between the frequency of populations without the B and population size, which might be empirically testable.

**Nonequilibrium models.**—The implication of the third or fourth hypothesis is that most populations are in a meta-stable state and that the results of observations on populations will vary with the time since the B invaded the population.

We argue that a model with an initial selective advantage to one-B’s is unlikely. The difficulty is to understand what evolutionary force could remove a
selective advantage initially accruing to animals containing B’s. The force cannot be internal selection, because the hypothesis is of an initial advantage, and animals with a reduced advantage would by definition be underrepresented in subsequent generations. The only mechanism, therefore, is environmental change. However, the B has very small effects on fitness in two populations in very different environments but with similar B frequencies, and the B is widespread over the range of the grasshopper, across a great diversity of habitats (Henriques-Gil et al. 1984). Furthermore, the hypothesis does not explain populations in which a novel variant B is increasing in frequency with substantial drive present.

To assess further the model of initially substantial drive requires us to know the timescales of the different phases considered.

Selection against drive.—The typical pattern of frequency change and change in drive is illustrated in figure 2. The time until drive reaches 0.53 was used as a measure of the time taken for the ‘‘neutralization’’ process. As expected from the construction of the model, this time was proportional to the variance in drive. If the standard deviation in drive were about 0.03—for example, 95% of individuals between 0.74 and 0.86—it would take about 3,000 generations to reach a drive of 0.53. The effect of the disadvantage to animals carrying two B’s is relatively large; the larger this disadvantage, the slower selection for reduced drive proceeds, because at equilibrium fewer individuals have the B. The effect of drive is relatively weak, because the higher the drive, the more rapidly
EVOLUTION OF AN APPARENTLY NEUTRAL B

Fig. 3.—The average time between the introduction of a single B into a large population and the reduction of female drive to 0.53, as a function of the initial female drive and the fitness of individuals containing two B's. The time units are inversely proportional to the variance in drive. Thus, if the actual variance were 0.01, the times shown would be between 1,000 and 300 generations; if the actual variance were 0.001, the times would be between 10,000 and 3,000 generations. The other parameters used were zero- and one-B fitness, 1; male transmission, 0.5; male and female disjunction, 0.5; population, 10,000. The times are almost invariant with three-B fitness between 0 and 0.5. The contours are based on a nonlinear fit to the output of simulations at parameter values 0.05 apart over each relevant interval. The fit accounted for 99% of the variance in the data.

selection proceeds but the farther it has to go. Figure 3 shows the time from the emergence of the B until drive reaches 0.53 as a function of the initial drive level and the disadvantage to two-B animals. Within the range 0–0.5, the fitness of three-B animals has no influence on the lifetime of the B.

Lifetime of the B as a near-neutral element.—Once a B is not subject to drive (or its selective advantage has disappeared), it still suffers a slight disadvantage from the occasional generation of individuals with large numbers of B’s. This disadvantage increases the commoner it becomes, which means that the element cannot become fixed. It must therefore eventually become extinct through random drift. This process is slower the larger the population (fig. 4), with the mean lifetime being roughly proportional to the two-thirds power of the population size up to the largest population sizes studied of 1,000,000. The variance in this lifetime is proportional to the lifetime, with a coefficient of variation of about 60%.

Subdivision of the population was studied with three levels of gene flow inversely proportional to the size of each subpopulation, so that the number of migrants per generation, \( N_e m \), was 0.1, 1, or 10. Figure 5 shows that for moderate numbers of populations, the lifetime of the B increases only logarithmically with
numbers of populations, being proportional to about the one-fifth power of the number of subpopulations, while remaining proportional to the two-thirds power of the subpopulation size. For large populations, the actual migration rate makes little difference; this is presumably because a small number of B’s introduced into a population without them are likely to become extinct quickly. To have any substantial effect, genetic exchange has to be large enough to successfully reintroduce the B into subpopulations from which it had nearly or completely vanished. Thus, B’s in subpopulations of size 100 exchanging 10% of individuals every generation do show a marked increase in lifetime (fig. 5). Therefore, the lifetime of the neutralized B in populations the size of the Iberian *E. plorans* will depend rather more on local than on total population size. If we assume the peninsula to contain 10,000 populations of average size 100, but there are actually only 1,000 or 100,000, we make an error of only 60% in estimating the average time until extinction of the B. For the purposes to which we wish to put the model, this is more or less irrelevant.

Typical lifetimes of a neutralized B will therefore lie between a few hundred and a few thousand years; it is not plausible that the average effective population size is more than a few thousand. This time is comparable to the time taken to move from a driven element to a neutral element.

**Further observations.**—One prediction of the fourth scenario is that the B chromosome should be driven if introduced to a population in which it has not previously existed. Experiments to test this have been set up but will take several years to give results. The hypothesis that the B is maintained by an equilibrium between very slight meiotic drive and differences in fitness among phenotypes is inconsistent with this observation, provided it is general.

![Graph showing the relationship between time to extinction of a neutral B and the population size in a single panmictic population.](image)
Fig. 5.—The relationship between the number of subpopulations making up a metapopulation and the time until final extinction of a "neutral" B, starting from a frequency of 0.5 one-B's. Fitnesses of animals with zero, one, two, or three B’s were identical; male and female disjunction was 0.5. Male and female transmission were both 0.5. Each point represents the mean of nine replications. Three subpopulation sizes are shown on each graph. The lowest line has $N_e = 100$, the middle line has $N_e = 1,000$, and the uppermost line has $N_e = 10,000$. Each graph corresponds to a different migration rate: A, $N_e m = 0.1$; B, $N_e m = 1$; and C, $N_e m = 10$. 
The model used polygenic modification of the drive rate, but drive suppression might also be primarily caused by alternative alleles at few loci. In this case, breeding experiments might be able to discover them. If found, sequence studies of these alleles, compared with studies on the B chromosome, could reveal the relative lengths of time that the B and the suppressor had been common. The “nonequilibrium” scenario above implies that these lifetimes should be similar.

**DISCUSSION**

In natural populations, the frequency of most plant and animal B’s that have been studied over several years has remained stable (Jones and Rees 1982; Cano and Santos 1989; Parker et al. 1991). The only exceptions are the grasshoppers *Atractomorpha bedeli* and *Acrida lata* inhabiting places that are highly disturbed by human activity (Sannomiya and Kayano 1968) and the cline movement in the grasshopper *Myrmelotettix maculatus* in East Anglia (Shaw 1983). Our results in *Eyprepocnemis plorans* show that B frequency changed little at Salobrêa but that fluctuations were larger in Jete, with a possible but nonsignificant downward trend. The Salobrêa population is dense and extends over a large area of sugar cane plantations. The Jete population is rather smaller and found along the banks of a river (Rio Verde) that is sometimes subjected to severe flooding that produces population bottlenecks. These differences in population characteristics are consistent with a lower effective population size and the more variable frequency at Jete.

We have not measured the effect of B’s on the rate of egg and embryo production in females. Although this is a very difficult trait to measure in natural conditions, we have some data indirectly indicating that B’s have little effect on female fecundity. For example, the rate of egg and embryo production by *E. plorans* females is directly related to mating frequency because males transfer with the ejaculate proteinaceous nutrients that are incorporated into the eggs (Pardo et al. 1995). We do not know whether B’s influence the quantity of nutrients contributed by the male. However, the absence of assortative mating based on B’s and the similar mating frequency of *E. plorans* females with a different number of B’s in natural conditions (López-León et al. 1992a) suggest that female fecundity is unaffected in these ways by the presence of B’s.

We suggest that the population dynamics of the B chromosome of *E. plorans* has three phases: an initial rapid increase to moderate frequencies in at most a few tens of generations; then a period of stability with drive balanced by selection, longer or shorter according to the variance in drive present; and, finally, a period of quasi-neutrality.

The B chromosome of *E. plorans* appears to be very mutable. The many different B types described (Henriques-Gil et al. 1984; Henriques-Gil and Arana 1990), the occasional translocations between A and B chromosomes (Henriques-Gil et al. 1983; Cabrero et al. 1987), and the frequent generation of new B variants (López-León et al. 1993a) all indicate that the B polymorphism in this species is very dynamic. A new B variant showing drive would increase in fre-
frequency, increasing the average number of B’s per individual. If only large numbers of B’s are assumed to be detrimental, this decreases the average fitness of individuals containing B’s of either the old or the new type. This will cause the relative frequency of the old B, lacking drive, to decrease.

Thus, although a ‘‘neutralized’’ B cannot be maintained indefinitely in the same form in natural populations, nonetheless a long-lived polymorphism may be regenerated by the appearance of new B variants, some of which are selfish and temporarily restore a drive-selection balance, before themselves being neutralized. The most widespread and presumably ancestral B in the Iberian Peninsula is B1, but it has been supplanted by other variants in some Spanish regions, namely, B2 in Granada and part of Málaga and B5 in Fuengirola (Málaga) (Henriques-Gil et al. 1984; Henriques-Gil and Arana 1990). In Torrox (Málaga), B2 has been substituted by B33, which is accumulated through the female (mean B transmission ratio = 0.696; S. Zurita, J. Cabrero, M. D. López-León, and J. P. M. Camacho, unpublished data). We interpret this as evidence for at least two successive regenerations of the B polymorphism.

The hypotheses put forward here to explain the B chromosome polymorphism in E. plorans could apply in other systems also. However, most well-studied B’s seem to be parasitic—for example, those in rye, in which there is drive in both sexes counteracting large effects on fertility (Jones 1985; Puertas et al. 1985; Romera et al. 1991); in the lily Lilium callosum, in which B’s accumulate only through the female side but are detrimental to pollen and seed fertility (Kimura and Kayano 1961); in the mealy bug Pseudococcus affinis (formerly obscursus), in which B’s accumulate during male transmission and have deleterious effects on male viability (Nur 1966a, 1966b, 1969a); in the grasshopper Myrmeleotettix maculatus, in which B’s accumulate during oogenesis (Hewitt 1976) but slow down development (Hewitt and East 1978; Harvey and Hewitt 1979) and produce sperm dysfunction (Hewitt et al. 1987); and in the parasitic wasp Nasonia vitripennis, in which the B eliminates the paternal genome, thereby enhancing its own transmission (Werren 1991). If these have similarities to the E. plorans case, they are all in the first stage of evolution of the polymorphism.

During this first stage, there is a coevolutionary arms race between the B and the host genome during which B variants with increased drive or decreased deleterious phenotypic effects are favored, while gene variants in the host genome that reduce B drive are also favored (Shaw and Hewitt 1990). Evidence of suppressors of B drive genes in the A genome has been put forward in the grasshopper M. maculatus (Shaw and Hewitt 1985; Shaw et al. 1985), the mealy bug P. affinis (Nur and Brett 1985, 1987, 1988), and E. plorans (Herrera et al. 1996). Furthermore, some kind of genetic control of B transmission has been shown in maize Zea mays (Carlson 1969), rye Secale cereale (Romera et al. 1991; Jiménez et al. 1995), and the diploid wheat Aegilops speltoides (Cebriá et al. 1994).

We suggest that the B chromosome polymorphism in the two natural populations of E. plorans analyzed here is in a second stage of B evolution, in which drive of the B has been lost along with most of the detrimental effects on the host. During this stage, the main changes in frequency occur through genetic drift, although there may be a slight long-term trend. This stage has some gen-
eral evolutionary interest, because it suggests that although the evolutionary equilibrium will eventually be reached, what we actually observe is a meta-stable, transitory state.

In a third stage of $B$ evolution, newly emerging variants less affected by the drive suppressors may substitute for the original neutralized form. Both the generation of $B$ variants (López-León et al. 1993a) and the substitution of old by new variants through intragenomic selection (S. Zurita, J. Cabrero, M. D. López-León, and J. P. M. Camacho, unpublished data) have been found in $E. plorans$. This stage completes a cycle that may take place over and over again.

For most of the time that a $B$ of this type is present, if this hypothesis is correct, it would be more or less neutral. This would allow time for the polymorphism to become heterotic, in the sense of White (1973), if suitable variation occurred in the environment or the genome. The idea that a $B$ chromosome could change from parasitic to heterotic was first proposed by Kimura and Kayano (1961). The main problem with this view is to explain how a near-neutral $B$ could evolve a beneficial effect if the genes on it are inactivated. However, some $B$ chromosomes harbor active rRNA genes (for review, see Green 1990; Mabuchi 1991; Beukeboom 1994a), so some regions of some $B$’s are not inactivated. The comparison of these genes (and spacer regions) between $A$ and $B$ chromosomes would throw much light on this matter and, perhaps, also on the origin of $B$’s.

In conclusion, we suggest that the $B$ polymorphism in $E. plorans$ may be giving us a glimpse of very long-term processes in the evolution of $B$ chromosomes.

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APPENDIX

Analysis of Postmating Selection

We have estimates of the $B$ frequencies in the offspring of some gravid females mated in the wild, of the $B$ frequencies in the adult wild population, of the frequency of the different mating pairs established in the field, and of the transmission rates observed in laboratory mated pairs. If we assume that the output of laboratory matings represents the output of field matings, the sample frequencies of types of mating observed in the field are an unbiased representation of the whole population, and there is no differential sperm displacement correlated to the presence or absence of the $B$, then the array of $B$’s in the offspring of gravid females collected from the field should be the same as that expected from adding up the output of laboratory matings, weighted by the field frequency of each type of mating. If this equality fails, then one of the assumptions underlying it must be false, which implies either that some kind of selection is occurring or that transmission rates in laboratory matings differ systematically from those in gravid females. Because we have independent evidence that transmission rates in gravid females whose broods have clear parentage are the same as those in laboratory matings, the comparison is a test for selection. However, all the estimates are subject to error, and the final comparisons that we wish to make are complicated
functions of the original observations. To make the comparison valid, we need estimates of their expected variance.

Initially, consider only one B chromosome class, say $j$. The estimated variance in the frequency of this among the gravid females collected from the wild is not less than

$$\text{var}(\hat{p}_g) \leq \frac{pq}{N_g}.$$  \hfill (A1)

Because the B composition of eggs from any single female is strongly but not perfectly intercorrelated, the population size, $N_g$, in this estimate lies between the number of gravid females and the number of eggs. Depending on the statistical type of error one wishes to avoid, it will be safer to use one extreme or the other.

The variance of the estimate based on offspring of artificial matings is the variance of a sum of products of the probabilities of each type of mating, indexed by $i$, with the probabilities of producing $j$'s from that mating, $z_{ij}$.

$$\text{var}(\hat{p}_j) = \text{var}\left( \sum_i p_i z_{ij} \right).$$  \hfill (A2)

Now $i$ has a fairly wide range, so the estimates of $p_i z_{ij}$ are only weakly correlated for different values of $i$. It is therefore safe to neglect terms of the type $p_i z_{ij} p_k z_{kj}$ on the right-hand side of equation (A2). We then have, roughly,

$$\text{var}\left( \sum_i p_i z_{ij} \right) = \sum_i \text{var}(p_i z_{ij}).$$  \hfill (A3)

Because $p_i z_{ij}$ is typically 0.1 or substantially less, this expression should be within 10% or so of the correct one. This error is probably negligible by comparison with the effects of the major approximation that we shall make during this reasoning, which is the usual statistical one of replacing expectations by their estimates.

The values of $p_i$ and $z_{ij}$ come from quite distinct sets of observations and should be completely uncorrelated, so

$$\text{var}(p_i z_{ij}) = \text{var}(p_i) \text{var}(z_{ij}) + \beta_i^2 \text{var}(z_{ij}),$$  \hfill (A4)

where expectations have been replaced by their estimates. Now each of the variances in equation (A4) is a binomial variance of the form $p_i (1 - p_i)/N$, so, substituting equation (A4) into equation (A3), we can calculate the approximate variance of our estimate of frequency expected on the basis of our assumptions. Equation (A1) gives the variance of the observed frequencies. The variance of the difference between expected and observed is the sum of the two variances, because the estimates are independent.

Since we have several phenotypic classes, we actually have a vector of differences $\mathbf{d}$, whose components necessarily sum to 0. This means that $\text{var}(\mathbf{1}^T \mathbf{d}) = 0$. We can use this to deduce the variance covariance matrix $\mathbf{V}$ for $\mathbf{d}$, given the variances of the components of $\mathbf{d}$ ($v_0$, $v_1$, $v_2$), which we have from the previous paragraph. We obtain

$$\mathbf{V} = \begin{bmatrix} v_0 & -(v_0 + v_1 + v_2)/2 & -(v_0 - v_1 + v_2)/2 \\ -(v_0 + v_1 + v_2)/2 & v_1 & -(v_1 - v_0 - v_2)/2 \\ -(v_0 - v_1 + v_2)/2 & -(v_1 - v_0 - v_2)/2 & v_2 \end{bmatrix}.$$  \hfill (A5)

Now we can calculate the variance of independent combinations of the components of $\mathbf{d}$. The obvious choices are the two contrasts:

$$\mathbf{c}_1 = (2 \ -1 \ -1)$$  \hfill (A6)

and

$$\mathbf{c}_2 = (0 \ -1 \ 1).$$
These test how the difference $d$ is distributed over its components. The first is a test of the difference in zero-B frequency, while the second is a test of differences in the predicted distribution of individuals with B’s between one- and two-B classes. The scalars $c_1^T d$ and $c_2^T d$ are formed and compared with their approximate standard deviations, $(c_i^T V c_i)^{0.5}$. For the data in the Results section, the first estimate for the Salobren a population are

$$c_1^T d = \begin{pmatrix} -0.1801 \\ -0.0744 \\ 0.1057 \end{pmatrix} = 0.1801,$$

$$c_1^T V c_1 = 0.0583,$$  \hspace{1cm} (A7)

and

$$Z = \frac{0.1801}{\sqrt{0.058}} = 0.7 \text{ NS}.$$

The second comparison gives $Z = 0.3$, even farther from significance. The significance levels for the Jete population were lower still.

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