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The B chromosomes of the grasshopper *Eyprepocnemis plorans* and the intragenomic conflict

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Abstract

The grasshopper *Eyprepocnemis plorans* harbours an extremely widespread polymorphism for supernumerary (B) chromosomes, which is found in almost all circum-Mediterranean and Caucasian populations hitherto analysed. B chromosomes in this species have been shown to evolve through several stages of parasitic and near-neutral nature, presumably because of an arms race between the standard (A) and B chromosomes. This intragenomic conflict can either be solved with the extinction of the neutralised B chromosome or, more interestingly, with the replacement of the neutralised B by a mutant version being parasitic again and thus prolonging B chromosome life. This species thus provides a complete view of the long-term life-cycle of parasitic B chromosomes.

B chromosomes and the genetic conflict

A genome might be considered as a perfect mutualism resulting from the cooperation among its constituent genes. But genomes may harbour genetic elements that evolve only for their own transmission (acquiring some mechanism of drive), usually at the expense of cooperative genes. Among these selfish genetic elements, the B chromosomes were the first ones uncovered (Wilson, 1907), but their parasitic nature (Östergren, 1945; Jones, 1985) was recognised later. Paternal Sex Ratio in *Nasonia vitripennis* (Werren, 1991, this issue) and *Trichogramma kaykai* (Stouthamer et al., 2001) are the most parasitic B chromosomes hitherto known, since they cause the disintegration of the chromosomes accompanying them in the spermatozoa, thus reducing to zero the fitness of the host genome. B chromosomes in many other species are not so harmful, but some decrease of host fitness is frequently observed (Jones & Rees, 1982).

The host genome frequently evolves resistance to B drive. This arms race between A and B chromosomes is a manifestation of genetic conflict between these two genomic components. Multiple solutions are conceivable for this conflict, (i) an equilibrium between the increase in B frequency derived from its drive and the decrease caused by the low fitness of individuals carrying Bs (*parasitic* model) (Östergren, 1945; Nur, 1977; Jones, 1985) leading to frequency stability over years, (ii) a non-equilibrium solution, where the B chromosomes change from parasitic to neutralised (Camacho et al., 1997), setting up a situation where the neutralised B can be replaced by a newly parasitic variant (Zurita et al., 1998). Under the latter model, B frequency is not necessarily stable over years, although it might appear to be stable when the B is in the near-neutral stage because changes in B frequency are mainly stochastic and may be small, and inversely related to population size (Camacho et al., 1997).

There is another model of B chromosome evolution, that is, the *heterotic* model (White, 1973), which proposes an equilibrium between the increase in B frequency derived from its beneficial effects on host fitness at low numbers and the frequency decrease caused by detrimental effects at high numbers. This model does not imply a genetic conflict between the A and B chromosomes, but assumes all selective effects at individual level.

The non-equilibrium model has filled an important gap in the understanding of the long run dynamics of B chromosome evolution; it may be relevant to other selfish genetic elements (Johnson, 1997), and provide a paradigm for the study of polymorphisms of attack and defence at genomic level (Frank, 2000). This model has been developed on the basis of the study of the B chromosome system of the grasshopper *Eyprepocnemis plorans*. The present paper is a review of the state of knowledge of this polymorphism.

The parasite

The B chromosomes of *E. plorans* are heterochromatic and adopt a multitude of different morphologies, with more than 50 different B-types described on the basis of size, centromere position and chromosome banding (Henriques-Gil, Santos & Arana, 1984; López-León et al., 1993; Bakkali, 2001). Of them, B_1 is the most widespread variant in natural populations of Spain, which led to Henriques-Gil, Santos and Arana (1984) to propose B_1 as the ancestor of all remaining B types. The most widespread B in Moroccan *E. plorans* populations is very similar to the Iberian B_1 (Bakkali et al., 1999), which is consistent with the hypothesis that B_1 is ancestral.

Two types of C-bands are usually found on the Bs, differing in staining intensity. The darker bands (proximally located on B_1) contain a 180 bp tandem DNA repeat and the lighter ones (located distally) contain ribosomal DNA (López-León et al., 1994) (Figure 1). Remarkably, all B types seem to be mainly composed of these two repetitive DNAs (Cabrero et al., 1999). Although the sequence of the 180 bp tandem DNA repeat does not include transcribable open reading frames (López-León et al., 1995), its amount might have a role in B drive (Cabrero et al., 1999), as has been recently shown in maize for some tandem DNA repeats involved in neocentromere formation (Hiatt, Kentner & Dawe, 2002). Ribosomal DNA on the B is usually inactive, with the single exception of a mutant male carrying the B_2 fused to the longest autosome, which showed an extra nucleolus associated to the rDNA of the B in about half of spermatocytes (Cabrero, Alché & Camacho, 1987). A possible relationship between the inactivity of the B rDNA and DNA methylation was observed (López-León, Cabrero & Camacho, 1991), although this is not the only inactivating mechanism since B chromosomes in embryos are not methylated but do not have active rDNA (López-León, Cabrero & Camacho, 1995).

The two repetitive DNAs constitute almost the entire long arm of the B. The minute short arm, which is sometimes difficult to observe and led us to mistake it for a pericentric inversion in a small autosome in our first paper on this species (Camacho, Carballo & Cabrero, 1980), presumably consists of other DNAs, which might be also intercalated between the already known sequences. We are currently analysing a B_2 library obtained by microdissection which, we hope, is going to fill the remaining gaps in our knowledge of B chromosome DNA.

Distribution and frequency

Four subspecies have been described in *E. plorans* (Dirsh, 1958), but only *E. plorans plorans* seems to carry B chromosomes (Camacho, Carballo & Cabrero, 1980). Cytological studies in the other subspecies did not report the presence of B chromosomes (John & Lewis, 1965). *E. plorans plorans* is an abundant grasshopper along the coasts of the Mediterranean area, the Caucasus, Turkey, Turkmenistan, Iran and the south-west of Arabia (Dirsh, 1958). B chromosomes are extremely abundant in this subspecies. In Spain, almost all natural populations hitherto analysed (from Tarragona to Huelva, Mallorca and Menorca) have shown the presence of B chromosomes (Camacho, Carballo & Cabrero, 1980; Henriques-Gil, Santos & Arana, 1984; Henriques-Gil & Arana, 1990; E. Petitpierre, personal communication; Cabrero et al., unpublished). The only known exception is an inland region at the head of the Segura river basin, where there are several populations lacking Bs isolated from the B-carrying regions by geographical barriers (Cabrero et al., 1997). We think these populations could be remnants of ancient populations never reached by the Bs.

B chromosomes are also very prevalent in other geographical regions. For example, all nine populations analysed by Bakkali et al. (1999) in Morocco carried B chromosomes. B chromosomes are also present in Sardinia (López-Fernández, Mezzanotte & Gosálvez, 1992) and, recently, they have been found in the most Eastern population known, in Dhaguestan (North Caucasus) (Bugrov, Warchalowska-Sliwa & Vysotskaya, 1999). B chromosomes might therefore have reached the entire geographical distribution of the *E. plorans plorans* subspecies, although the

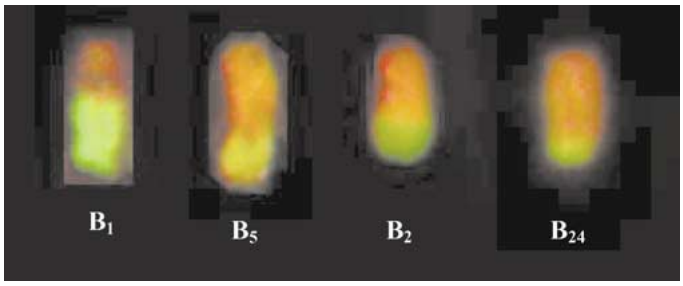

Figure 1. Main types of B chromosomes in the grasshopper *E. plorans*.

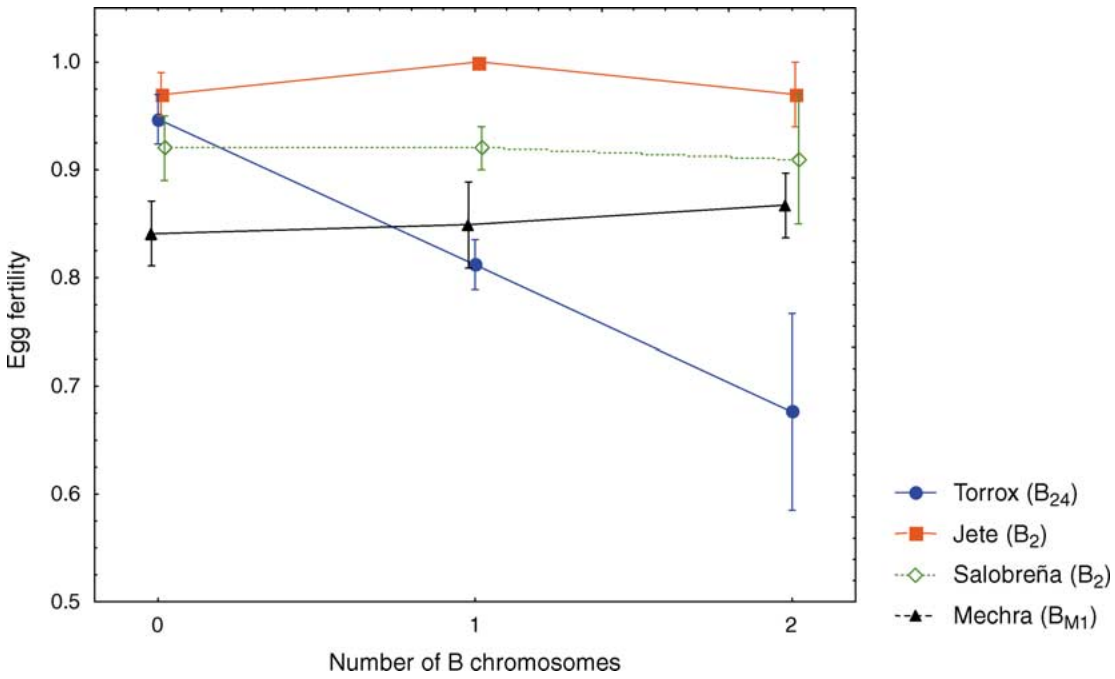

Figure 3. Reaction norm of different genetic backgrounds in the face of several B chromosomes. Only the B_{24} chromosome showed significant drive and virulence (data from López-León et al., 1992a,b; Camacho et al., 1997; Zurita et al., 1998; Bakkali, 2001).

analysis of eastern Mediterranean populations may help to clarify the origin and distribution of this polymorphism.

The frequency with which Bs are found in natural populations varies much from place to place, presumably depending on the stage of the life cycle at which the B is in the moment of study. Camacho, Sharbel and Beukeboom (2000) proposed that there are four factors determining B frequency: (i) its average transmission ratio, (ii) its harmful effects on host fitness, (iii) stochastic changes if the B is in the near-neutral stage, and (iv) time from B invasion. The highest mean number of Bs per individual ever found in *E. plorans* (1.53) was shown in the Torrox population in 1994, when the new parasitic B, B_{24}, was completing its invasion of this population in substitution of the former neutralised B, B_2 (see below).

The inoffensive appearance of a parasite

The two main attributes of a parasite are its transmission mode and its harmful effects on the host. Our first analyses of B chromosome transmission in *E. plorans* showed that the three B chromosome variants most widespread in the Iberian Peninsula (B_1, B_2 and B_5) lack drive (López-León et al., 1992a). In addition, our analyses showed an absence of any effects, at low B number, on body size or weight-based somatic condition (Martín-Alganza et al., 1997), or on several fitness

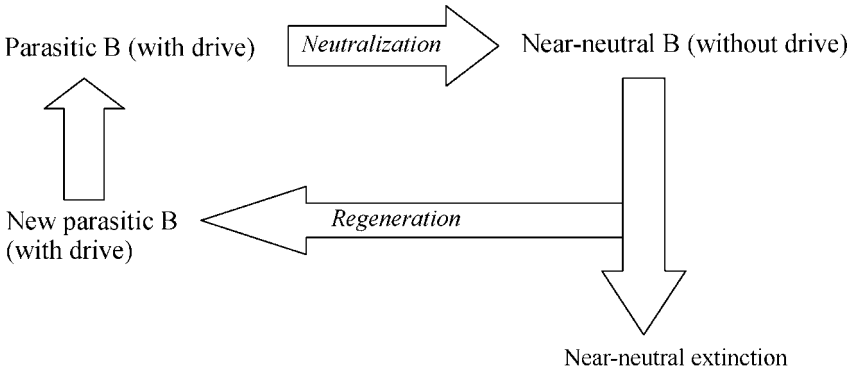

Figure 2. Long-term evolution of B chromosomes in *E. plorans*.

components such as mating frequency (López-León et al., 1992b), clutch size, egg fertility and viability from embryo to adult (Camacho et al., 1997). The absence of drive and significant effects on fitness of these B chromosomes rendered this polymorphism inexplicable under the two predominant models of that time, that is, they did not seem to be either parasitic, because the absence of drive and significantly harmful effects, or heterotic, because the absence of beneficial effects on fitness that might explain their prevalence in the absence of drive. This suggested us the existence of a new kind of B chromosomes that we named *near-neutral*, and are characterised by the absence of drive and fitness effects, at least at low B number. The finding that B_2 (one of these near-neutral Bs) is able to drive in crosses involving B-carrying females and males from a population lacking Bs suggested that, in natural populations, near-neutral Bs could have their drive through females suppressed by a mechanism in which males appear to be involved (Herrera et al., 1996). This led us to think that near-neutral Bs are in fact parasitic Bs that have been neutralised by the host genome (Camacho et al., 1997). We later obtained additional evidence supporting this conclusion. First, we were able to show that B-carrying females show egg fertility significantly lower than 0B ones when mating frequency is restricted to a single mating (Muñoz et al., 1998). This could explain why B chromosomes tend to be less frequent in low-density populations (Henriques-Gil, Santos & Arana, 1984), since mating frequency is expected to be low in these populations. The other evidence for parasitic B chromosomes in *E. plorans* was the finding of a B chromosome type in Torrox (Málaga, Spain) (B_{24}) showing drive and decreasing egg fertility of B-carrying females (Zurita et al., 1998). Recently, we have found that a B chromosome very similar to the Iberian B_1 shows drive in some Moroccan populations but not in others (Bakkali, Perfectti & Camacho, 2002), providing additional evidence that B chromosomes in *E. plorans* pass through successive parasitic and near-neutral stages.

Less direct evidence for the parasitic nature of B chromosomes in *E. plorans* is the effects they have on chiasma frequency and thus recombination. According to the Theory of the Inducible Recombination (Bell & Burt, 1990), chiasma effects exerted by B chromosomes are reminiscent of their parasitic nature. As suggested by these authors, parasitic B chromosomes evoke a response in the host to increase recombination (through chiasma effects) as an evolved response, favoured by selection, because it creates recombinant progeny, some of which are resistant to the accumulation of Bs in the germ line (Bell & Burt, 1990). Chiasma frequency effects of B chromosomes in *E. plorans* were firstly reported by Camacho, Carballo and Cabrero (1980), who observed an increase in mean cell chiasma frequency in five populations analysed. Later, Henriques-Gil, Santos and Giráldez (1982) showed that the B effect depends on the genetic background of the individuals carrying it. Moreover, Cano et al. (1986) found significant effects of both the familial and the individual level on chiasma frequency. In addition, Cano, Jones and Santos (1987) analysed chiasma frequency in males and females from four natural populations containing B chromosomes and showed the absence of B effects on chiasma frequency in either sex. Recently, we have reanalysed this subject in males from eight natural populations, and concluded that B chromosomes increase chiasma frequency, but the intensity of the effect depends on the evolutionary status of the polymorphism: the more selfish the B chromosome, the more intense the increase in chiasma frequency

(Camacho et al., 2002). These observations also suggest that B chromosomes in *E. plorans* are of parasitic nature.

Long run of the A–B chromosome arms race

Building a mathematical model and a computer program including all characteristics observed in our previous studies of the *E. plorans* B chromosome system threw much light on the long run evolution of parasitic B chromosomes (see Camacho et al., 1997, for details). Simulation experiments showed that a near-neutral B cannot invade a population, thus giving support to the idea that these kind of Bs were, in fact, parasitic Bs whose drive had been suppressed by the host genome. Simulations also showed that near-neutral Bs are condemned to extinction by a combination of stochastic loss and selection against individuals with many Bs (near-random loss). After analysing the dynamics of drive suppression, the life cycle of a B chromosome resulted to consists of three successive stages: (1) invasion by virtue of drive, (2) drive-suppression by the evolution of suppressor genes in the host genome, and (3) the near-random loss (see Figure 2 in Camacho et al., 1997). The complete life cycle of a B might last hundreds or thousands of generations, mainly depending on population size (which might shorten the near-random loss if small) and deleterious effects of Bs (shortening the last stage if large).

The finding that B_{24}, a new parasitic B, was substituting B_2 in Torrox (Zurita et al., 1998) led us to consider drive regeneration as a possible detour on the previous scheme and to propose the following scenario for B chromosome evolution (Figure 2). Bs are more likely to begin being parasitic, thus showing drive and deleterious effects on the host. Any gene variant on A chromosomes being able to suppress B drive is favoured by natural selection. The parasitic B is thus neutralised becoming a near-neutral B that might follow two pathways. The least interesting possibility is the near-random loss, since it would mean the disappearance of the B. The second one is to become, by mutation, in a different B variant rescuing drive ability and thus beginning a new invasion and re-starting a cycle that might operate again and again. The high mutability of *E. plorans* B chromosomes (0.35% frequency of apparition of *de novo* B types in crosses, and with more than 50 described B variants, López-León et al., 1993; Bakkali, 2001) explains the relative ease for polymorphism regenerations in this species. Accepting that the most widespread B (B_1) is the ancestral one in the Iberian Peninsula (which is the most parsimonious hypothesis), the B polymorphism seems to have regenerated in the provinces of Granada and Eastern Málaga, where the predominant variant is B_2, and in the Fuengirola (Málaga) area, where the most frequent B, in a number of populations surrounded by populations carrying B_1, is B_5 (Henriques-Gil & Arana, 1990). In addition, a secondary substitution has recently taken place in the Torrox (Málaga) area, where B_{24} has replaced B_2 (Henriques-Gil & Arana, 1990; Zurita et al., 1998). Next section analyses this case in some detail.

Monitoring the life cycle of a parasitic B

In a sample collected at Torrox (Málaga) in 1984, Henriques-Gil and Arana (1990) noticed the predominance of B_{24} (with 0.344 Bs per individual, on average) over B_2 (0.09). Populations located east and west carried B_2 only. These authors thus proposed that a substitution of B_2 for B_{24} was taking place in this population. Cytologically, B_{24} is similar to B_2 with a duplication of the interstitial dark C-band and a deletion of part of the distal less-dark band (see Fig. 1). Therefore, B_{24} contains more 180 bp repetitive DNA, which could be related with its ability to drive (see Hiatt, Kentner & Dawe, 2002), but less ribosomal DNA than B_2 (Cabrero et al., 1999).

In 1992, we collected specimens at this locality and found that B_{24} frequency had increased dramatically (0.975), which might suggest that B_{24} was in the invasion stage (Zurita et al., 1998). We performed controlled crosses that showed significant drive through females (0.7). We did not find, however, B_2 in this year, and the same result, but an even higher frequency of B_{24} (1.533), was found 2 years later. This is evidence that the replacement of B_2 (a B which is neutralised in nearby populations) by B_{24} had been completed at that time and occurred by virtue of B_{24} drive through females.

We have now evidence of the suppression of B_{24} drive. In 1998, we performed new controlled crosses and observed an average transmission ratio of 0.51, indicating a 28.6% decrease in drive in only 6 years (Corral et al., in preparation). Such a speed is not compatible with the polygenic model we assumed in our previous simulations, which would predict an order of magnitude more generations (Camacho et al., 1997).

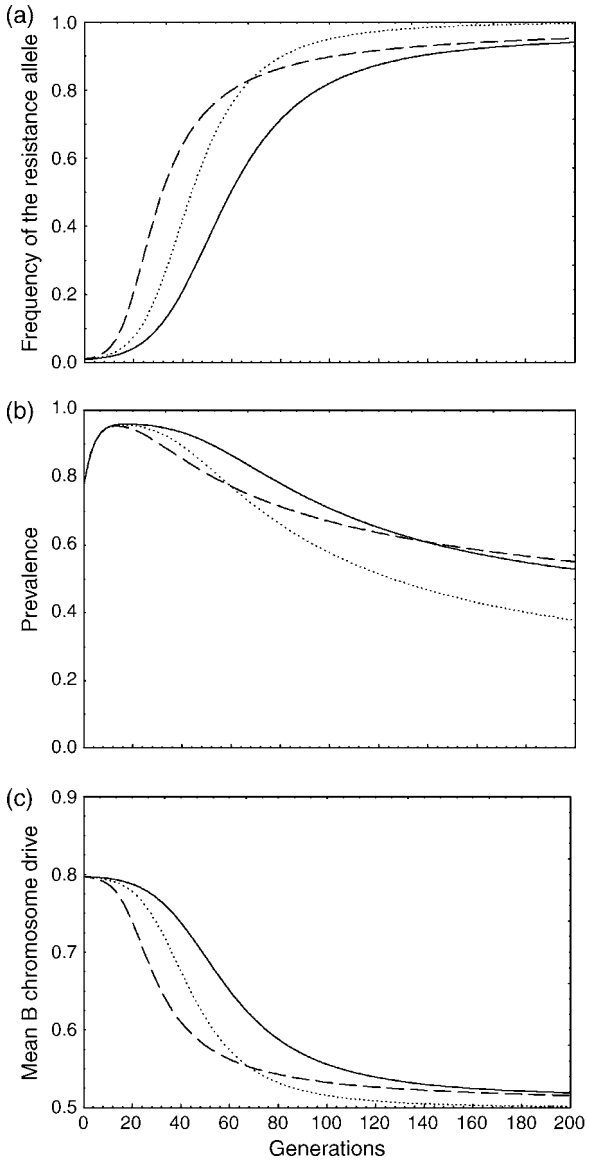

Figure 4. Evolution of the frequency of a drive-suppressor (resistance) allele (a), prevalence (b) and mean drive (c) of a B chromosome. A resistance allele that reduce both drive and virulence of a B-chromosome is positively selected in a population with parasitic B-chromosomes. Analyses were done assuming codominance in drive reduction, B chromosome drive = 0.8, initial frequency of Bs = 1.5; initial modifier frequency = 0.01, no drive through males, B chromosome pairing of 20% and no reproduction for individuals with more than three B chromosomes. B chromosomes reduce egg fertility of non-resistant females according to the following fertility distribution: 0B = 1.0, 1B = 0.85, 2B = 0.70 and 3Bs = 0.50. Broken lines represent the case for dominant resistance with no cost, solid lines represent a resistance cost of 0.1 and dot lines represent no resistance costs but codominant effects (intermediate reduction in virulence for heterozygous individuals).

This might suggest that drive suppression, in this case, might be based on few or even a single gene.

Evolution of resistance to B chromosomes

Shaw (1984) and Camacho et al. (1997) modelled the evolution of B chromosomes under several circumstances, and mainly showed that the elimination of a parasitic B chromosome is a long-term process because selection for a drive modifier is a relatively slow process. But if the drive mechanism of the B is associated with deleterious effects on host fitness, a modifier of drive could confer an immediate advantage to its carriers. We think this might be the case in *E. plorans*. As Figure 3 shows, non-driving B chromosomes produce no detectable effects on egg fertility, at least at low B number, but B_{24}, the variant showing the highest drive, caused significant decrease on this trait. It is therefore conceivable that increased transmission is associated with high virulence (deleterious effects on host fitness), as has been observed in other host–parasite systems (Lehmann, 1993; Sorci, Møller & Boulinier, 1997). In such a case, the attenuation of a B chromosome (in order to decrease the fitness cost to the host) would not be expected to evolve, because the parallel lost of drive would greatly diminish B survival ability (and competitiveness with other more parasitic Bs). In this situation, an equilibrium between drive and fitness costs could lead to a temporally stable B frequency, such as is predicted by the parasitic model (Nur, 1977). But these fitness costs could promote the evolution of defence strategies in the host genome (see Frank, 2000). In theory, it could imply both host resistance to drive and/or host tolerance (a reduction in B fitness costs).

Level of tolerance can be viewed in terms of the reaction norm of a genotype exposed to several degrees of parasitism (i.e., number of B chromosomes). A tolerant genotype would be able to carry B chromosomes with less reduction in fitness, compared with a non-tolerant genotype. If we represent host fitness in respect to B chromosome number (e.g., Figure 3), the slope of the function will represent the tolerance. A negative slope indicates *intolerance*, a flat slope implies *tolerance* and a positive slope represents *overtolerance*, a circumstance that would imply beneficial effects of B chromosomes, and has never been observed in *E. plorans*. It is also possible that a same genotype is tolerant at low parasite number but not at high numbers (*incomplete tolerance*).

The evolution of tolerance could depend on several factors, including the fitness cost of tolerance itself. The evolution of incomplete tolerance would imply that B chromosome effects on fitness would be mani-

fested only in individuals with high number of Bs. If drive is not suppressed, incomplete tolerance would simply imply a new equilibrium with higher number of B chromosomes per individual.

Tolerance is expected to be a plastic trait, and its expression could be influenced by both biotic and abiotic factors (Stowe et al., 2000). In *E. plorans*, parasitic mites and sperm shortage (by limiting the number of copulations) have been shown to change tolerance, promoting a larger effect of B chromosomes on female fertility (Muñoz et al., 1998). Unfortunately, we do not know whether, in experimental conditions, the increased B chromosome virulence was linked to increased drive. Future experiments should approach this question.

Evolution of resistance (drive suppression) is clearly a better option for A chromosomes. If drive and virulence are associated, putative suppressor genes would reduce both drive and B virulence. Resistant genotypes would be selected for because, compared to susceptible genotypes, they would get a gain in fertility and their progeny will bear less B chromosomes. The spread of resistant alleles will depend on dominance and is proportional to the difference in fitness between genotypes (which depend on B virulence and resistance cost). Standard evolutionary models of resistance evolution assume a cost for resistance (Rausher, 2001). But that is not necessarily applicable to all cases. With the present data, we cannot rule out the possibility of negligible costs for resistance in *E. plorans* and, presumably, the existence of a polymorphism for resistant loci.

Assuming a monogenic control for resistance and low or no costs, a fast increase in frequency is expected for the resistant allele. If a new driving B chromosome invades a population being polymorphic for resistant alleles (at low frequency), the selection of resistant genotypes will provoke a fast decrease in mean B drive (a fact actually observed for B_{24} in Torrox), but not a pronounced reduction in B prevalence (Figure 4). If there is a cost for resistance, an equilibrium is expected but the initial dynamics is very similar (see Figure 4).

In some B chromosome systems, resistance alleles that promote elimination of the B chromosome have been found (M.J. Puertas, personal communication). The B chromosome polymorphism in *E. plorans* could be favoured by the absence of resistance alleles provoking rapid B chromosome elimination by means of transmission rates below Mendelian predictions. In these conditions, a long life is expected for neutralised Bs such as, for instance, B_1 and B_2 (see Camacho et al., 1997).

Because of the high mutability of B chromosomes in *E. plorans* (López-León et al., 1993; Bakkali, 2001), several B variants are expected to coexist, at least for a short time lapse. This has been observed in most *E. plorans* populations (Henriques-Gil, Santos & Arana, 1984; Henriques-Gil & Arana, 1990; López-León et al., 1993; Bakkali, 2001). In this situation, soft selection between Bs could be an important factor in the evolution of virulence (see Chao et al., 2000 for a review of parasites and soft/hard selection). If different Bs compete for some limited resources needed for drive, the possibility of interference between them needs to be considered. B chromosome interference would probably encompass a reduction in the fitness costs produced by the Bs. The rapid replacement of B_2 by B_{24} in *E. plorans* (Zurita et al., 1998), however, does not seem to have implied a high drive interference between these two B chromosome variants, presumably because B_2 was already neutralised when B_{24} appeared.

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References

Bakkali, 2001. Evolución de los cromosomas B del saltamontes *Eyprepocnemis plorans* en Marrueccos. PhD Thesis, Universidad de Granada, Granada, Spain.

Bakkali, M., F. Perfectti & J.P.M. Camacho, 2002. The B-chromosome polymorphism of the grasshopper *Eyprepocnemis plorans* in North Africa. II. Parasitic and neutralized B_1 chromosomes. Heredity 88: 14–18.

Bakkali, M., J. Cabrero, M.D. López-León, F. Perfectti & J.P.M. Camacho, 1999. The B chromosome polymorphism of the grasshopper *Eyprepocnemis plorans* in North Africa. I. B variants and frequency. Heredity 83: 428–434.

Bell, G. & A. Burt, 1990. B-chromosomes: germ-line parasites which induce changes in host recombination. Parasitology 100: S19–S26.

Bugrov, A., E. Warchalowska-Sliwa & L. Vysotskaya, 1999. Karyotypic features of Eyprepocnemidinae grasshoppers from Russia and Central Asia with reference to the B chromosomes in *Eyprepocnemis plorans* (Charp.). Folia biol. (Kraków) 47: 97–104.

Cabrero, J., J.D. Alché & J.P.M. Camacho, 1987. Effects of B chromosomes of the grasshopper *Eyprepocnemis plorans* on nucleolar organiser regions activity. Activation of a latent NOR on a B chromosome fused to an autosome. Genome 29: 116–121.

Cabrero, J., M.D. López-León, R. Gómez, A.J. Castro, A. Martín-Alganza & J.P.M. Camacho, 1997. Geographical distribution of B chromosomes in the grasshopper *Eyprepocnemis plorans*, along a river basin, is mainly shaped by non-selective historical events. Chromosome Res. 5, 194–198.

Cabrero, J., M.D. López-León, M. Bakkali & J.P.M. Camacho, 1999. Common origin of B chromosome variants in the grasshopper *Eyprepocnemis plorans*. Heredity 83: 435–439.

Camacho, J.P.M., M. Bakkali, J.M. Corral, J. Cabrero, M.D. López-León, I. Aranda, A. Martín-Alganza & F. Perfectti, 2002. Host recombination is dependent on the degree of parasitism. Proc. R. Soc. Lond. Ser. B 269: 2173–2177.

Camacho, J.P.M., A.R. Carballo & J. Cabrero, 1980. The B chromosome system of the grasshopper *Eyprepocnemis plorans* sub. *plorans* (Charpentier). Chromosoma 80: 163–166.

Camacho, J.P.M., T.F. Sharbel & L.W. Beukeboom, 2000. B chromosome evolution. Phil. Trans. R. Soc. Lond. B 355: 163–178.

Camacho, J.P.M., M.W. Shaw, M.D. López-León, M.C. Pardo & J. Cabrero, 1997. Population dynamics of a selfish B chromosome neutralized by the standard genome in the grasshopper *Eyprepocnemis plorans*. Am. Nat. 149: 1030–1050.

Cano, M.I., N. Henriques-Gil, P. Arana & J.L. Santos, 1986. The relationship between chiasma frequency and bivalent length: effects of genotype and supernumerary chromosomes. Heredity 56: 305–310.

Cano, M.I., G.H. Jones & J.L. Santos, 1987. Sex differences in chiasma frequency and distribution in natural populations of *Eyprepocnemis plorans* containing B chromosomes. Heredity 59: 237–243.

Chao, L., K.A. Hanley, C.L. Burch, C. Dahlberg & P.E. Turner, 2000. Kin selection and parasite evolution: higher and lower virulence with hard and soft selection. Quart. Rev. Biol. 75: 261–275

Dirsh, V.M., 1958. Revision of the genus *Eyprepocnemis* Fieber, 1853 (*Orthoptera*:*Acridoidea*). Proc. Roy. Ent. Soc. Lond. B 27: 33–45.

Frank, S.A., 2000. Polymorphism of attack and defence. Trends Ecol. Evol. 15: 167–171.

Henriques-Gil, N. & P. Arana, 1990. Origin and substitution of B chromosomes in the grasshopper *Eyprepocnemis plorans*. Evolution 44: 747–753.

Henriques-Gil, N., J.L. Santos & P. Arana, 1984. Evolution of a complex polymorphism in the grasshopper *Eyprepocnemis plorans*. Chromosoma 89: 290–293.

Henriques-Gil, N., J.L. Santos & R. Giráldez, 1982. Genotype-dependent effect of B-chromosomes on chiasma frequency in *Eyprepocnemis plorans* (*Acrididae*: *Orthoptera*). Genetica 59: 223–227.

Herrera, J.A., M.D. López-León, J. Cabrero, M.W. Shaw & J.P.M. Camacho, 1996. Evidence for B chromosome drive suppression in the grasshopper *Eyprepocnemis plorans*. Heredity 76: 633–639.

Hiatt, E.N., E.K. Kentner & K. Dawe, 2002. Independently regulated neocentromere activity of two classes of tandem repeat arrays. The Plant Cell 14: 407–420.

John, B. & K.R. Lewis, 1965. Genetic speciation in the grasshopper *Eyprepocnemis plorans*. Chromosoma 16: 548–578.

Johnson, N.A., 1997. Selfish genetic elements: long-range dynamics predicted by non-equilibrium models. Trends Ecol. Evol. 12: 376–378.

Jones, R.N., 1985. Are B chromosomes selfish? pp. 397–425 in The Evolution of Genome Size, edited by T. Cavalier-Smith, Wiley, London.

Jones, R.N. & H. Rees, 1982. B Chromosomes. Academic Press, New York.

Lehmann, T., 1993. Ectoparasites: direct impact on host fitness. Parasitol. Today 9: 8–13.

López-Fernández, C., R. Mezzanotte & J. Gosálvez, 1992. Autosomal, sex and B chromosomes in *Eyprepocnemis plorans* (Orthoptera) viewed with restriction endonuclease *in situ* digestion. Heredity 68: 365–372.

López-León, M.D., J. Cabrero & J.P.M. Camacho, 1991. A nucleolus organizer region in a B chromosome inactivated by DNA methylation. Chromosoma 100: 134–138.

López-León, M.D., J. Cabrero & J.P.M. Camacho, 1995. Changes in DNA methylation during development in the B chromosome NOR of the grasshopper *Eyprepocnemis plorans*. Heredity 74: 296–302.

López-León, M.D., J. Cabrero, J.P.M. Camacho, M.I. Cano & J.L. Santos, 1992a. A widespread B chromosome polymorphism maintained without apparent drive. Evolution 46: 529–539.

López-León, M.D., M.C. Pardo, J. Cabrero & J.P.M. Camacho, 1992b. Random mating and absence of sexual selection for B chromosomes in two natural populations of the grasshopper *Eyprepocnemis plorans*. Heredity 69: 558–561.

López-León, M.D., J. Cabrero, M.C. Pardo, E. Viseras, J.P.M. Camacho & J.L. Santos, 1993. Generating high variability of B chromosomes in the grasshopper *Eyprepocnemis plorans*. Heredity 71: 352–362.

López-León, M.D., N. Neves, T. Schwarzacher, T.S. Heslop-Harrison, G.M. Hewitt & J.P.M. Camacho, 1994. Possible origin of a B chromosome deduced from its DNA composition using double FISH technique. Chromosome Res. 2: 87–92.

López-León, M.D., P. Vázquez, G.M. Hewitt & J.P.M. Camacho, 1995. Cloning and sequence analysis of an extremely homogeneous tandemly repeated DNA in the grasshopper *Eyprepocnemis plorans*. Heredity 75: 370–375.

Martín-Alganza, A., J. Cabrero, M.D. López-León, F. Perfectti & J.P.M. Camacho, 1997. Supernumerary heterochromatin does not affect several morphological and physiological traits in the grasshopper *Eyprepocnemis plorans*. Hereditas 126: 187–189.

Muñoz, E., F. Perfectti, A. Martín-Alganza & J.P.M. Camacho, 1998. Parallel effect of a B chromosome and a mite decreasing female fitness in the grasshopper *Eyprepocnemis plorans*. Proc. R. Soc. Lond. Ser. B. 265: 1903–1909.

Nur, U., 1977 Maintenance of a 'parasitic' B chromosome in the grasshopper *Melanoplus femur-rubrum*. Genetics 87: 499–512.

Östergren, G., 1945. Parasitic nature of extra fragment chromosomes. Bot. Notiser 2: 157–163.

Rausher, M.D., 2001. Co-evolution and plant resistance to natural enemies. Nature 411: 857–864.

Shaw, M.W., 1984. The population genetics of the B-chromosome polymorphism of *Myrmeleotettix maculatus* (Orthoptera: Acridae). Biol. J. Linn. Soc. 23: 77–100.

Sorci, G., A.P. Møller & T. Boulinier, 1997. Genetics of host–parasite interactions. Trends Ecol. Evol. 12: 196–200.

Stouthamer, R., M. van Tilborg, J.H. de Jong, L. Nunney & R.F. Luck, 2001. Selfish element maintains sex in natural populations of a parasitoid wasp. Proc. R. Soc. Lond. B. 268: 617–622.

Werren, J.H., 1991. The paternal-sex-ratio chromosome of *Nasonia*. Am. Nat. 137: 392–402.

Werren, J.H. & R. Stouthamer. PRS (Paternal Sex Ratio) chromosomes: the ultimate selfish genetic element. Genetica, this issue.

White, M.J.D., 1973. Animal Cytology and Evolution. 3rd edn. Cambridge University Press, London.

Wilson, E.B., 1907. The supernumerary chromosomes of Hemiptera. Science 26: 870–871.

Zurita, S., J. Cabrero, M.D. López-León & J.P.M. Camacho, 1998. Polymorphism regeneration for a neutralized selfish B chromosome. Evolution 52: 274–277.