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Parallel effects of a B chromosome and a mite that decrease female fitness in the grasshopper *Eyprepocnemis plorans*

Estefanía Muñoz, Francisco Perfectti, Ángel Martín-Alganza† and Juan Pedro M. Camacho*

Departamento de Genética, Universidad de Granada, E-18071 Granada, Spain

The effects of a genomic parasite (a B chromosome) and an ectoparasite (a mite) on the fitness of the host (the grasshopper *Eyprepocnemis plorans*) have been analysed in 60 experimental females. These two parasites differ in their infectious transmission mode. B chromosomes are vertically transmitted from host-parents to offspring, but mites are horizontally transmitted from one grasshopper to another within the same generation. The transmission mode can influence the virulence of these parasites, so that it should be expected that B chromosomes would be less virulent than mites. However, as mite transmission is linked to host mobility, some attenuation is also expected. Four egg pods were analysed from each female, the first two egg pods were laid after a mating and the remaining two were not preceded by a mating. The results show that B chromosomes severely decrease the proportion of eggs containing an embryo (egg fertility), mainly from the second egg pod onwards. Mites also decrease egg fertility but, in addition, they produced a decrease in the rate of embryo production over time (embryo productivity), which might be derived from both the fertility decrease and a slight delay in egg production. The analysis of the relative effect of both parasites suggests that they have a synergistic effect on embryo clutch size and egg fertility. Possible mechanisms for the observed effects are discussed.

Keywords: ectoparasitism; mites; B chromosomes; fitness; *Eyprepocnemis plorans*

1. INTRODUCTION

Parasites are a major controlling factor in the dynamics of natural populations of many animal and plant species (Anderson & May 1978; Hamilton *et al*. 1990; Price 1980; Marcogliese & Cone 1997). The virulence of a parasite, i.e. the effect of a parasite on host fitness, is strongly influenced by its mode of transmission (Anderson & May 1982; Lipsitch *et al*. 1995). Theory predicts that parasites transmitted vertically, i.e. whether the parasite moves between a parent and offspring generation, will be less virulent than those parasites transmitted horizontally, i.e. whether the parasite moves between two members of a population other than a parent–offspring pair. Parasites with vertical transmission have their fitness linked to the fitness of their hosts, and therefore decreasing host reproductive success will reduce parasite fitness. In fact, several comparative (Ewald & Schubert 1989; Herre 1993; Clayton & Tompkins 1994) and experimental (Bull *et al*. 1991) studies have suggested that the degree of vertical transmission in nature is positively correlated with benignity. However, if the horizontally transmitted parasite depends on the mobility of its host for its transmission, a reduction in virulence is also expected (Ewald 1994).

Macroparasites are usually horizontally transmitted. Although they are not typically involved in marked oscillations in host abundance, their effects on host fitness can be pronounced (Lehmann 1993). However, little information is available about how and to what extent parasitic arthropods, such as mites, can influence host fitness and population dynamics (Polak 1996).

B chromosomes are selfish genetic elements (Östergren 1945; Nur 1969). The presence of B chromosomes in the genome of many organisms depends on the presence of drive mechanisms that enhance their transmission to values higher than those of standard (A) chromosomes, despite being detrimental to the individuals carrying them (Jones 1985, 1991; Beukeboom 1994). Thus they behave as genomic parasites, although their deleterious effects are weak in many cases owing to coevolution with the host genome, which leads to selection of the best genotypes in B tolerance (Shaw and Hewitt 1990; Castro *et al*. 1998). Because the only mechanism of transmission of B chromosomes is from parent to progeny, they can be considered as ultimately vertically transmitted parasites.

In the present work, we analyse the effects and potential interactions of these genomic parasites (B chromosomes) and an ectoparasite (a mite: *Podapolipus* sp.) on female fitness in the grasshopper *Eyprepocnemis*

*Author for correspondence (jpmcamac@goliat.ugr.es).
†Present address: Max-Planck-Institut fur Verhaltensphysiologie, D-82319 Seewiesen (Post Starnberg), Germany.

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plorans. The B chromosome is vertically transmitted but the mite is most likely to be horizontally transmitted, and its transmission is probably related to the mobility and reproductive contacts of its host. If both parasites have some components of their fitness linked to host fitness, a reduction in virulence is expected. Both parasites similarly decreased female fitness during late reproduction, but the aetiology of the two parasites on hosts is different. In addition, we assess the potential effect of ectoparasites on the dynamics of the B chromosomes.

2. MATERIALS AND METHODS

(a) *The host*

The grasshopper *Eyprepocnemis plorans* is very common in wet places and croplands located close to rivers along the Mediterranean and southern Atlantic coasts in the Iberian peninsula. Although females can lay up to nine pods (5.4 on average; López-León *et al*. 1994) with a single mating, they usually show multiple matings (López-León *et al*. 1995), most likely because of the nutritional benefits obtained from the ejaculate (Pardo *et al*. 1995).

A sample of males and females was collected in Salobreña (Granada, Spain) during September–October 1995. The females were caught as last-instar nymphs and were isolated from males until adulthood. Each female was mated twice (once before each of the first two pods) and a total of four egg pods was obtained from each female individually maintained in culture cages. The laying period was divided into two different periods: (i) early reproduction, including the first and second pods and characterized by a mating before each laying; and (ii) late reproduction, including the third and fourth pods and characterized by laying without any additional mating. These periods had a mean duration of 25.6 days and 21.5 days, respectively. Individuals were allowed to feed *ad libitum*. We analysed a total of 60 females and the 240 egg pods laid.

Egg pods were incubated for 10 days at 28 °C and then the eggs were individually counted. Each egg was then dissected to check for the presence of a viable embryo. After laying the fourth egg pod, each female was injected with 0.05% colchicine in insect saline solution 6 h before the fixation of ovarioles in 3:1 ethanol–acetic acid.

To estimate host fitness, we quantified the following parameters: clutch size (the number of eggs or embryos per egg pod), egg fertility (the proportion of eggs containing an embryo), productivity (clutch size divided by the number of days elapsed between mating and laying or between layings; e.g. egg productivity in the fourth pod was the number of eggs contained in the fourth ootheca divided by the number of days elapsed between the laying of the third and fourth pods), and experimental reproductive period (days from first mating to last laying).

(b) *The parasites*

The grasshopper *E. plorans* harbours a very widespread polymorphism for B chromosomes. Almost all populations hitherto analysed in Spain, Italy and North Africa carried B chromosomes, the effects of which on carrier fitness appear to be slight (Camacho *et al*. 1997). Every individual shows the same number of B chromosomes in all its cells. The number of B chromosomes per individual in natural populations ranges from zero to three, whereas individuals with four or more are rarely found (López-León *et al*. 1992*a*; Camacho *et al*. 1997).

Mites attached to *E. plorans* individuals have been frequently observed in the Salobreña population. These mites attach to the abdominal pleural intersegments at the base of wings and hind legs, where they may suck grasshopper haemolymph. The mites found in *E. plorans* belong to the genus *Podapolipus*.

The number of B chromosomes in each female was scored on gonad tissue by the C-banding technique described in Camacho *et al*. (1991). Female bodies were stored in 70% ethanol and the number of mites attached to each female was counted under a stereomicroscope.

To estimate the frequency of parasites in the natural population, and to compare this with the experimental population, the number of mites and B chromosomes were scored in a sample of 258 adult *E. plorans* females caught in Salobreña in 1992 (Martín-Alganza *et al*. 1997). These females had been stored in ethanol on the collection day, so that any increase of mite number under the laboratory conditions was prevented.

(c) *Statistics*

Because some variables (mainly fertility) did not have normal distributions, and because both types of parasites increased the variance of most variables affected by their presence (data not shown), the statistical tests used were mainly non-parametric (Kruskal–Wallis ANOVA).

Whereas B chromosomes do not vary in number over an individual's ontogeny, mites reproduce much faster than the grasshopper host and thus the number of grasshoppers presumably varies considerably with their age. Therefore, mite effects were investigated only in the last egg pod (the fourth) laid immediately before scoring the number of mites.

For a joint analysis of B chromosome and mite effects, we performed an ANCOVA with the number of B chromosomes as an independent variable and the number of mites (transformed to $\log(x+1)$) as a covariate. Each of several fitness traits measured in the fourth egg pod constituted the dependent variables (transformed to $\log(x+1)$). To estimate the interaction between the two parasites, we performed a two-way ANOVA with the number of B chromosomes and the number of mites as independent variables. A categorizing variable was thus generated for the number of mites by using the median number of mites per grasshopper, transformed to log $(x+1)$. This yielded two groups of grasshoppers, each comprising 30 females: those above, and those falling below the median number of mites. Each group of females was thus characterized as being lightly or heavily parasitized. Four groups of female grasshoppers were established depending on their number (0, 1, 2 and 3) of B chromosomes.

3. RESULTS

B-chromosome frequency in the experimental sample fit a Poisson distribution (goodness-of-fit $\chi^2=0.28$, d.f. $=1$, $p=0.594$), ranging from zero to three. The mean was 1.07 and the variance 0.98. Thus the coefficient of dispersion $(s^2/\overline{x})$ was nearly one (0.92), as in random distributions. The prevalence (percentage of individuals 'infected') was 66.6%. The B-chromosome frequency in the field-population sample fit a binomial distribution ($\chi^2=0.22$, d.f. $=1$, $p=0.641$) with a mean of 0.829 and variance of 0.570. The range was the same as in the experimental population. The coefficient of dispersion was 0.69, slightly less than that in the experimental population. The prevalence was 63.2%, which was not significantly different from that in the experimental

Table 1. *Effects of B chromosomes on female grasshopper fitness measured by the number of eggs per pod (egg clutch size), the number of embryos per pod (embryo clutch size), the proportion of eggs containing an embryo (egg fertility), the number of eggs per day (egg productivity), the number of embryos per day (embryo productivity) and the experimental reproductive period (exp. reprod. period)*

(p_b, probability corrected by means of the sequential Bonferroni method.)

variable	egg-pod number	0B ($n=20$) mean	s.e.	1B ($n=23$) mean	s.e.	2B ($n=10$) mean	s.e.	3B ($n=7$) mean	s.e.	Kruskal–Wallis ANOVA H_2	p	p_b
egg clutch size	1	43.05	2.21	41.52	2.04	42.65	2.05	40.29	3.61	0.33	0.849	—
	2	44.35	2.01	46.52	1.73	49.82	1.85	46.86	3.61	3.32	0.190	—
	3	44.15	1.95	46.74	1.70	45.29	2.58	42.14	4.00	0.87	0.648	—
	4	44.40	2.41	47.17	2.13	45.82	2.38	40.00	2.71	0.80	0.669	—
embryo clutch size	1	40.85	2.24	39.61	2.16	39.30	2.69	24.86	7.44	4.89	0.180	—
	2	42.90	2.28	43.26	2.24	47.50	1.59	29.29	8.49	4.59	0.205	—
	3	42.75	1.96	43.61	2.61	33.20	7.24	21.29	8.07	6.17	0.104	—
	4	42.50	2.35	43.70	2.70	38.20	5.64	21.86	7.15	8.79	0.032	0.128
egg fertility	1	0.948	0.019	0.951	0.014	0.889	0.041	0.589	0.152	8.67	0.0340	0.0340
	2	0.963	0.023	0.929	0.032	0.921	0.031	0.612	0.156	12.97	0.0047	0.0094
	3	0.968	0.010	0.918	0.042	0.659	0.124	0.486	0.160	16.38	0.0009	0.0027
	4	0.961	0.020	0.919	0.039	0.757	0.094	0.514	0.157	22.50	0.0001	0.0004
egg productivity	1	7.67	1.56	6.34	1.18	7.478	1.777	4.544	1.443	2.43	0.488	—
	2	7.50	1.42	6.01	0.46	7.150	1.201	4.470	0.886	2.26	0.521	—
	3	4.80	0.29	4.48	0.30	5.388	0.615	4.544	0.670	1.44	0.696	—
	4	4.93	0.39	4.73	0.38	6.063	0.573	4.301	0.749	4.89	0.180	—
embryo productivity	1	7.24	1.45	6.00	1.14	6.677	1.775	3.618	1.549	2.98	0.394	—
	2	7.00	1.31	5.56	0.48	6.578	1.028	2.983	1.066	5.73	0.126	—
	3	4.65	0.29	4.15	0.35	3.622	0.898	2.460	1.042	6.24	0.101	—
	4	4.76	0.40	4.25	0.36	4.842	0.857	1.999	0.606	9.17	0.027	0.108
exp. reprod. period	4	39.80	3.07	43.09	3.86	36.80	3.49	53.57	9.42	2.01	0.570	—

sample (comparison between the two percentages, $p=0.664$).

Mite frequency in the experimental sample fit an exponential distribution ($\chi^2=1.48$, d.f.$=2$, $p=0.477$), ranging from zero to 1335, with a mean of 158.68 and variance of 6.49×10^4. The coefficient of dispersion (409.0) was greater than unity, implying an overdispersed (i.e. contagious) distribution. The prevalence was 91.7%. The number of mites in the field population did not fit an exponential distribution or any other distribution with clear biological meaning. The range (0–157) was lower than in the experimental population. The number of mites was significantly lower in the field population (mean$=$13.69, variance$=$744.58) than in the experimental population (Mann–Whitney U-test$=2596$, $p<0.001$), and the coefficient of dispersion (54.4) was also greater than unity. The prevalence (51.2%) was significantly less than in the experimental population (comparison between two percentages, $p<0.001$).

The number of mites was not significantly different between females with different numbers of B chromosomes in either the experimental (Kruskal–Wallis ANOVA, $H=1.53$, d.f.$=3$, $p=0.675$) or the field populations ($H=0.77$, d.f.$=3$, $p=0.856$), suggesting that grasshopper susceptibility to mites is independent of the number of B chromosomes.

(a) *Effects of the B chromosome*

Egg fertility was significantly lower in the four egg pods from females with three B chromosomes (3B females) (table 1), with a reduction of 46.5%. The same effect was noted in the third and fourth pods laid by 2B females (table 1). This suggests that B chromosomes are harmful to 3B females, independently of mating frequency, and for 2B females in the absence of matings (third and fourth pods).

(b) *Effects of mites*

The effect of mite number was analysed only in the last egg pod (the fourth) because it was obtained soon before the number of mites was scored. The ANCOVA in table 2 shows that mite number was significantly negatively correlated with embryo clutch size ($r=-0.331$), egg fertility ($r=-0.366$), egg productivity ($r=-0.279$), embryo productivity ($r=-0.478$) and experimental reproductive period ($r=0.498$), although the effect on egg productivity became non-significant after the sequential Bonferroni test. Therefore, the mite–grasshopper relationship appears not to be simply phoretic, because mites are clearly harmful to grasshoppers, decreasing egg fertility and delaying the production of embryos and pods.

(c) *Joint effect*

We performed several ANCOVAs to study the effects of both the numbers of B chromosomes and mites in the fourth pod. This analysis showed that embryo clutch size, egg fertility and embryo productivity were affected by both parasites (table 2), which implied a general reduction in the production of embryos. Neither the numbers of mites nor B chromosomes affected the number of eggs laid by the female grasshopper.

To analyse the possible interaction of these parasites, we performed two-way ANOVAs (table 2). These analyses suggested a synergistic effect of B chromosomes and mites

Table 2. *ANCOVA and two-way ANOVA with the data observed in the fourth egg pod*

(All dependent variables were transformed to $\log(x+1)$.)

		egg clutch size		embryo clutch size		egg fertility		egg productivity		embryo productivity		exp. reprod. period	
	d.f.	MS	*p*	MS	*p*	MS	*p*	MS	*p*	MS	*p*	MS	*p*
ANCOVA													
B	3	0.076	0.252586	2.579	0.000542	0.147	0.000202	0.202	0.130495	0.910	0.001611	0.149	0.171427
mite	1	0.038	0.409404	2.551	0.011988	0.161	0.005101	0.476	0.035887	2.557	0.000171	1.556	0.000081
error	55	0.054		0.378		0.019		0.103		0.157		0.106	
ANOVA													
B	3	0.093	0.184283	3.763	<0.000001	0.204	<0.000001	0.178	0.176610	1.252	0.000105	0.171	0.154778
mite	1	0.123	0.142452	8.857	<0.000001	0.457	<0.000001	0.121	0.287621	3.462	0.000011	0.776	0.005745
inter-action	3	0.022	0.750839	2.700	0.000003	0.114	0.000048	0.112	0.371860	0.308	0.111729	0.188	0.123663
error	52	0.056		0.218		0.012		0.105		0.147		0.094	

on embryo clutch size and egg fertility. However, no significant interaction was observed for the other variables analysed. The effect of these parasites on the number of embryos was strong (figure 1*a*) and, consistently, the impact on egg fertility was also very pronounced (figure 1*b*). Females with no B chromosomes and a low number of mites had a mean fertility of 0.987 embryos per egg, a high value when compared with the 0.090 embryos per egg of 3B females with many mites. This change represents a reduction of more than 90% in fertility. Therefore, the main effect of these parasites on *E. plorans* female reproductive fitness was a reduction in the production of viable embryos.

4. DISCUSSION

The genomic parasite and the mite produced parallel harmful effects on several fitness components in female grasshoppers, *E. plorans*. Both mites and B chromosomes primarily reduced the number of eggs that developed into embryos, in the extreme case decreasing egg fertility by more than 90%. Effects of parasites on fertility have also been reported in other arthropods such as *Tribolium confusum* infected by cestodes (Keymer 1980), *Drosophila putrida* infected by nematodes (Jaenike 1992), *Sceliodes cordalis* infected with protozoans (Mercer & Wigley 1987) or, more recently, *Drosophila nigrospiracula* parasitized by mites (Polak 1996).

Several factors may have influenced our measure of parasite effects on host fitness. The first is that females were fed *ad libitum*, counteracting the nutrient deprivation that mites and other ectoparasites provoke (e.g. Lehmann 1993; Polak 1996).

The second factor is that the negative impact of the parasite may be reduced under the controlled laboratory conditions. Factors such as climate or predators may affect the observed virulence of the parasite (Lehmann 1993), and are usually avoided under laboratory conditions.

The last factor works in the opposite direction. The large number of mites in the experimental sample may increase the parasite effects to levels not usually found in natural populations. However, *E. plorans* populations strongly fluctuate in the studied area from year to year (J. P. M. Camacho, personal observation), as would be expected from a high pressure of parasitism. In any case, this subject demands further research.

Although B chromosomes are not parasites *sensu stricto*, some B chromosomes show clear parasitic behaviour (e.g. Nur 1969; for a recent review, see Beukeboom 1994) causing deleterious effects and maintaining themselves by drive mechanisms (Jones 1991). B chromosomes of *E. plorans* mainly affected egg fertility, but this effect was weak in the first pod. Previous analyses in this grasshopper species had reported no effects of B chromosomes on egg fertility in the first egg pod (Camacho *et al*. 1997). The difference with the previous analysis is that we have now analysed egg fertility in 2B and 3B females separately. In fact, had our present analysis been performed pooling the 2B and 3B female data, as we have done previously (Camacho *et al*. 1997), no significant effect would have been noticed in the first pod (Kruskal–Wallis ANOVA, $H=5.32$, d.f.$=2$, $p=0.070$). The present results show that fertility decreased significantly in the four egg pods laid by 3B females, and in the third and fourth pods laid by 2B females. This suggests that the presence of two or more B chromosomes may disturb the use of the sperm stored in the spermatheca. In 2B females, if the egg pod is laid with a previous mating, then the effect is not apparent. Mating scarcity in *E. plorans* populations with low population density might increase the negative effect of B chromosomes on the fertility of females carrying two or more B chromosomes. This effect on fertility may contribute to the low frequency of B chromosomes reported in low-density populations of *E. plorans* (Henriques-Gil *et al*. 1984). In 3B females, fertility decreases independently of the existence of a previous mating. This suggests that other processes (e.g. egg fertilization, resource allocation to the eggs, early development, etc.) might also be disturbed in females carrying a high number of B chromosomes. Zurita *et al*. (1998) have recently reported that egg fertility decreases significantly in *E. plorans* females carrying a new B variant possessing drive. Moreover, Martín *et al*. (1996) found in laboratory experiments that B chromosomes of *E. plorans* delayed the occurrence of the first mating, presumably because of a

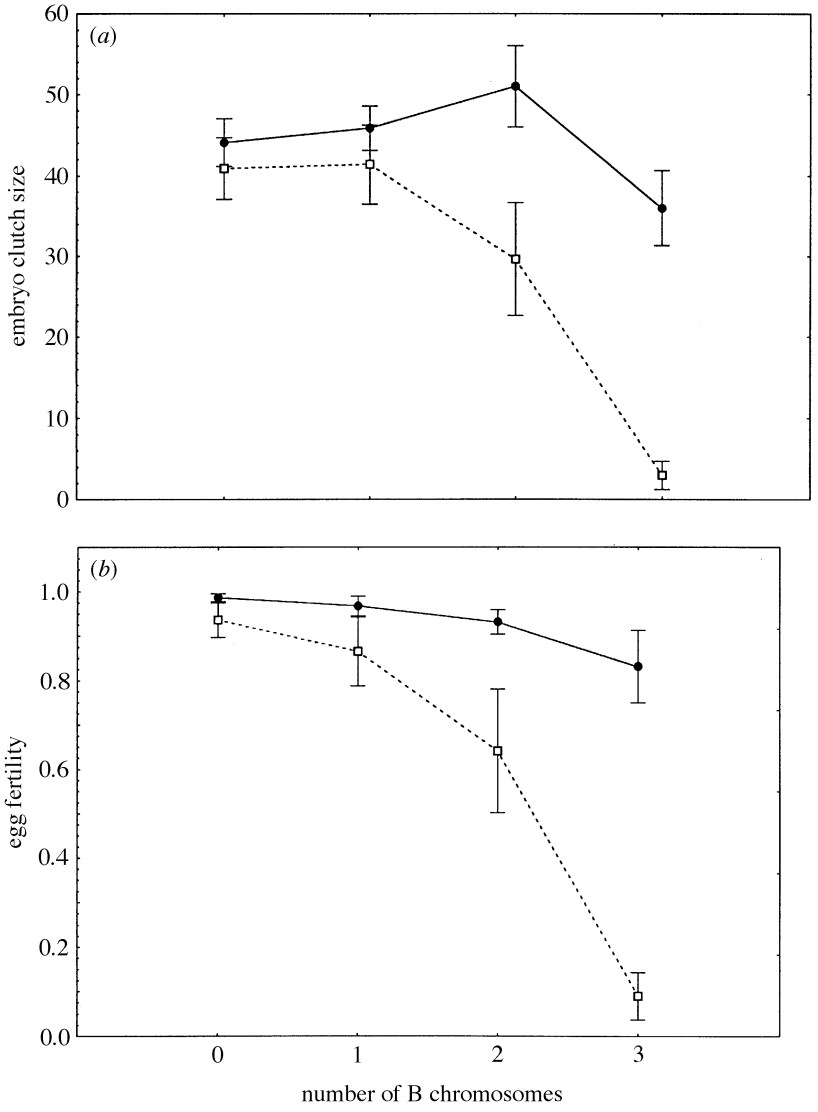

Figure 1. (*a*) Embryo clutch size (number of embryos), and (*b*) egg fertility (embryos/egg ratio per clutch) observed in the fourth pod of *Eyprepocnemis plorans* females. Females with high numbers of mites (broken line) had a lower production of embryos and lower fertility than females with low numbers of mites (solid line). This effect was more intense in females with two or three B chromosomes. Values are means and error bars represent one standard error.

slower development of B carriers, and provoked a reduction in the number of matings. However, mating frequency in the field, as deduced from the analysis of mating pairs, did not differ between individuals with different numbers of B chromosomes (López-León *et al.* 1992*b*).

B chromosomes affected embryo clutch size, egg fertility and embryo productivity in the fourth egg pod. Mites also reduced these variables and, in addition, they increased the time from the first mating to the fourth pod. The effects on fertility and the delay in the production of progeny are usual outcomes of other ectoparasites (e.g. Polak 1996). The lack of effect of mites on the number of eggs laid by grasshoppers could be caused by the unrestricted diet provided. Because the effects of ectoparasitism may be similar to the effects of starvation (Polak 1996), the *ad libitum* availability of food could obscure the effect of mites on egg production. An alternative possibility is that mites transfer some pathogen, and that this pathogen which produced the phenotypic effects. At the moment, this last possibility cannot be ruled out, but the clear correlation of effects and mite number suggests a harmful effect of mites *per se*.

The decrease in the grasshopper's egg fertility caused by both parasites might be provoked in three different, but not mutually exclusive, ways: sperm shortage, egg refractoriness to fertilization, and early-development failure. Both parasites may harm the quality of the stored sperm, which could decrease the amount of spermatozoa available for fertilizing eggs. Because ectoparasites have been reported to cause desiccation in host tissues (Lehmann 1993), mites could cause this supposed loss of sperm quality by desiccation. However, the causal mechanism of B-chromosome effect is not obvious.

The remaining possibility is that egg fertility and embryo productivity decrease in presence of B chromosomes and mites because both interfere with resource allocation to the eggs, leading to decreased egg quality (in terms of energy and regulatory substances provided by the female). This would cause a fertilization failure in some eggs, or else certain zygote mortality during early development. Incomplete provisioning of oocytes could result in a failure to shift from one developmental stage to the next (Parson 1962).

The different mode of transmission of B chromosomes (vertical) and mites (horizontal) may affect the virulence shown by these parasites. In theory, vertically transmitted parasites should be less virulent than parasites with horizontal transmission (Anderson & May 1982). Thus, vertically transmitted parasites are often benign (Ewald 1994;

Lipsitch *et al*. 1995). However, the fitness of horizontally transmitted parasites, such as ectoparasites, is not always linked to the fitness of their hosts, and many ectoparasites may increase their fitness by increasing their virulence (Lehmann 1993). The exception lies with parasites showing a transmission mode dependent on host mobility or contact, for which fitness should partly be linked to their host fitness (Ewald 1994). In a comparative analysis of the virulence of different parasites, Clayton & Tompkins (1994, 1995) showed that mites (horizontally transmitted) were clearly more virulent than lice (vertically transmitted) to their host, the rock dove *Columbia livia*. Our data have shown that B chromosomes are less virulent than mites. This result fits the standard theory but might also be the result of a reduction in the virulence of both the vertical (B chromosome) and the horizontal (mite) parasites, if in both their fitness is linked to host fitness. This reduction in virulence could be manifested with different intensity on the different components of host fitness. B chromosomes are expected to be less virulent on components of host fitness associated with reproduction. Mites could be less virulent on components of host fitness associated with host mobility and interaction. The analysis of additional fitness components, mainly those related to growth and mobility, would complete the picture of the interaction between these parasites and their host.

The best-studied B chromosomes seem to be parasitic, e.g. those in *Pseudococcus affinis*, which produce deleterious effects on male viability (Nur 1966); those in the grasshopper *Myrmeleotettix maculatus*, which slow down development (Harvey & Hewitt 1979); or those in *Nasonia vitripennis*, which eliminate the paternal genome and thereby enhance their own transmission (Werren 1991). B chromosomes in all three species show drive mechanisms, which counteract the negative effects on host fitness and therefore permit their maintenance in the host population in spite of their harmful effects. However, the B chromosome of *E. plorans* has very low or absent drive (López-León *et al*.1992*a*), and there is no evidence for sexual selection or assortative mating based on B chromosomes (López-León *et al*. 1992*b*). Without drive, a high virulence may negatively affect B-chromosome fitness and thus its own evolutionary fate. Empirical and theoretical analyses suggest that the B system of *E. plorans* shows non-equilibrium dynamics. The B chromosome passes through successive stages: a first phase with strong drive and increase in prevalence; a second phase with selection for B drive-suppression in the A chromosomes; and a third phase where the B chromosome does not show drive and has the final fate of extinction unless new B variants with enhanced drive can appear (Camacho *et al*. 1997).

The possible synergistic effect of mites and B chromosomes on egg fertility may also influence the dynamics of the B chromosome, even more than mite population dynamics influences that of the host. Because mites can spread horizontally, the greater cost in host fitness inflicted on 2B or 3B females probably does not have an important cost for mite fitness. Nevertheless, this cost may be high for the B chromosome, because its fitness is inevitably linked to host fitness. What effect this interaction may have in nature is an unsolved question. The number of mites per grasshopper female in the field sample was lower than in the experimental sample. Fluctuations in natural populations of this mite are unknown, preventing us from assessing a more realistic comparison between field and laboratory conditions. Moreover, other factors such as climate, food deprivation or population structure may also influence the outcome of this interaction in a natural environment. Nevertheless, the parallel effects of B chromosomes and mites on *E. plorans* fitness may be clearly asymmetric. Mites can find other individual hosts, but B chromosomes cannot.

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