Causes of B chromosome variant substitution in the grasshopper *Eyprepocnemis plorans*

M.I. Manrique-Poyato[1], A.J. Muñoz-Pajares[1], V. Loreto[1,2], M.D. López-León[1], J. Cabrero[1] & J.P.M. Camacho[1*]

[1]*Departamento de Genética, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain; Tel.: +34 958248925; Fax: +34 958244073; E-mail: jpmcamac@ugr.es;*

[2]*Unidade Acadêmica de Garanhuns, Universidade Federal Rural de Pernambuco, Garanhuns, Brazil*

[*]*Correspondence*

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Abstract

We have analysed B chromosome frequency for three consecutive years, B transmission rate at population and individual levels, clutch size, egg fertility and embryo-adult viability in a natural population of the grasshopper *Eyprepocnemis plorans* containing two different B chromosome variants, i.e. B_2 and B_{24}, the second being derived from the first and having replaced it in nearby populations. From 2002 to 2003, the relative frequency of both variants changed, although the differences did not reach significance. A mother-offspring analysis showed no significant effect of any of the two B variants on clutch size, egg fertility and embryo-adult viability, but B_{24} was more efficiently transmitted than B_2 through males from the 2002 season, which explains the observed frequency change. Controlled crosses, at individual level, showed significant drive through some females for B_{24} but not for B_2, suggesting that this difference in transmission rate might also be important for the substitution process. The analysis of relative fitness for B_2 and B_{24} carriers for all fitness components, as a whole, showed a significantly better performance of B_{24}-carrying individuals, suggesting that the cumulative effect of these slight differences might contribute to the replacement process of B_2 for B_{24}.

Introduction

Parasitism is one of the most widespread life styles at all levels, from genes to species. Among them, B chromosomes, also known as accessory or supernumerary, are extra chromosomes which, in most cases, behave as parasitic elements within eukaryote genomes (Östergren 1945; for recent reviews, see Camacho 2004, 2005). B chromosome systems may be at about stable frequencies in natural populations due to equilibrium between two main forces, viz. the increase in frequency derived from accumulation (drive) mechanisms and the decrease caused by their harmful effects on B carriers. But, as indicated by our studies in the B chromosome system of the grasshopper *Eyprepocnemis plorans*, such an equilibrium may be broken by B chromosome neutralization through selection for A chromosome gene variants suppressing B drive. This converts the system into a dynamic one in which B chromosomes pass through successive stages of parasitism and near-neutrality (Camacho *et al.* 1997). This non-equilibrium model includes the possibility that a neutralized B, which is condemned to extinction through stochastic loss plus selective loss of individuals with high number of Bs, mutates into a new B variant possessing drive which regains the parasitic status. Such a substitution of one B variant for another has been documented in the Torrox population (Zurita *et al.* 1998), but the timing of such a replacement is one of the most interesting open questions of B chromosome evolution. Recent research has shown that B drive suppression may take place in only a few generations since B_{24} in Torrox did show drive in 1992 (Zurita *et al.* 1998) but not in 1998 (Perfectti *et al.* 2004), suggesting that drive suppression might be caused by a single A gene of major effect (Perfectti *et al.*, 2004). In Torrox, the B_2 variant had been replaced by the B_{24} variant in 1992, the first time we sampled this population (Zurita *et*

al. 1998), but a sample from 1984 indicated the presence of both B_2 and B_{24} in this population (Henriques-Gil & Arana 1990), with B_2 showing a very low frequency. The absence of B_2 in 1992 suggested that B variant replacement had already been completed, and the significant drive showed by B_{24} in controlled crosses (Zurita *et al.* 1998) suggested that the replacement occurred because of the more efficient transmission rate for B_{24}, since B_2 did not show drive in several Spanish populations close to Torrox (López-León *et al.* 1992). The fact that B_{24} was harmful for egg fertility in Torrox whereas B_2 was not in the other populations analyzed, could add some uncertainty to the former conclusion, since it could imply a lower selective load for B_2 carriers in Torrox, but we could not analyze the effect of both B variants on this fitness component in the same population. In an attempt to uncover possible causes for B variant replacement, we analyze here B frequency evolution through three consecutive years, as well as several fitness components and B chromosome transmission rate, in a natural population of the grasshopper *Eyprepocnemis plorans* showing the presence of the two B chromosome variants (B_2 and B_{24}) at about similar frequency, indicating that it is at an intermediate status of the substitution process.

Materials and methods

In 2002, 2003 and 2004, we collected adult males and females of the grasshopper *Eyprepocnemis plorans* in Algarrobo (Málaga, Spain), a population located at only 14 Km from Torrox. In 2002, we also collected gravid females which were carried to the laboratory to get one egg-pod, from each female, which was incubated for ten days to obtain embryo offspring. All individuals were fixed for cytological analysis as indicated in Camacho *et al.* (1997). All individuals were analyzed by the C-banding technique

(described in Camacho *et al.* 1991) to differentiate B chromosome variants. A mother-offspring analysis was performed to the 2002-2003 data, following the method described in Camacho *et al.* (1997), based on Christiansen & Frydenberg (1973). It allowed inferring the average B transmission rate through both sexes and the differential effect of both B chromosome variants on three fitness components, viz. clutch size, egg fertility and embryo-adult viability. This procedure includes the following calculations: 1) The mean B frequency among the progeny of 0B females indicates the net B transmission rate through males since all Bs in these females' progeny is from paternal origin. 2) The mean B frequency in the offspring of B-carrying females minus that in the offspring of 0B females (i.e. that from paternal origin) allows calculating the mean transmission rate through B carrying females. A comparison between the frequency of karyotypes (0B, B_2-carrying, B_{24}-carrying and B_2B_{24}-carrying individuals) observed in the 2003 embryo sample and those expected from the 2002 adult sample (assuming random mating, Mendelian transmission and independent meiotic behaviour of both B variants) provided a means of testing significance of transmission efficiency of both B variants. 3) The number of eggs per pod is a measure of clutch size. 4) Egg fertility was calculated as the embryos/eggs ratio for each egg-pod and female analyzed. 5) Embryo-adult viability was inferred from an intra-generation comparison between B frequency in the 2003 embryo sample (yielded by the 2002 gravid females) and the 2003 adult field sample.

B frequency was measured by two parameters: the mean number of B chromosomes per individual (mean) and the proportion of individuals carrying Bs (prevalence). Given the low frequency of individuals with more than 1B, both parameters were very similar in most samples, for which reason we used prevalence for contingency tests in order to improve statistical power.

In 2004, we also sampled some female nymphs which were bred isolated from males to preserve virginity. Once adult, they were crossed to 0B males which had previously been vivisected to extract several testis follicles which were immediately analyzed cytologically to detect B chromosome presence. Transmission rate (k_B) of both B chromosome variants through females was deduced from these crosses. In one case, B transmission through a B-carrying male was also analyzed.

Contingency tables for B frequency comparisons among years (to analyze temporal evolution) and different life-cycle stages (to analyze reproductive and viability fitness components) were analyzed by the *RXC* program, which employs the Metropolis algorithm to obtain an unbiased estimate of the exact *P*-value (Rousset & Raymond 1995). In all cases, 20 batches of 2500 replicates were performed. Population B transmission efficiency was tested by the goodness-of-fit chi square test. B effects on clutch size and egg fertility were analyzed by two-way ANOVA, and transmission rate in controlled crosses was analyzed by the *Z*-test described in López-León *et al.* (1992). *Z* was calculated as the quotient between (observed transmission rate minus 0.5) and the square root of the quotient between 0.25 and the number of embryo progeny analyzed. *Z* values higher than 1.96 indicate significant drive if positive or drag if negative.

Results

B chromosome frequency

The total prevalence of B chromosomes, including the two observed variants, B_2 and B_{24}, was rather stable in the three years analyzed, with about 40% of individuals carrying B chromosomes (Table 1). Although the relative frequency of the two B

variants changed from 2002 to 2003, with B_2 decreasing in prevalence from 0.28 to 0.19 and B_{24} increasing from 0.23 to 0.31, these changes, however, did not reach statistical significance (contingency tests: P= 0.229±0.007 for B_2 prevalence and P= 0.242±0.008 for B_{24} prevalence). B frequency was almost the same in 2003 and 2004.

Transmission rate (k_B) of B chromosomes at population level

Table 2 shows a summary of the mother-offspring analysis performed to 23 gravid females lacking B chromosomes and 17 carrying them, collected in 2002 (for further details, see Supplementary Material). The average frequency of B_2 and B_{24} chromosomes found in the offspring of the 0B females was 0.12 and 0.14, respectively (Table 2). This corresponds to the effective B transmission rate through males at population level. The mean frequency of B_2 and B_{24} in adult males of the 2002 season was 0.31 and 0.24, respectively, (see Table 1) which implies average transmission rates (k_B) through males equal to 0.12/0.31= 0.39 for B_2 and 0.14/0.24= 0.58 for B_{24}. This suggests a better performance of B_{24} carrying males than B_2 carrying ones.

The average frequency of B_2 and B_{24} found in the embryo progeny yielded by $1B_2$ and $1B_{24}$ females was 0.56 and 0.60, respectively. Subtracting, in each case, the B frequency transmitted through males (0.12 and 0.14, respectively) we obtained the average transmission rate for each B variant through 1B females. It was 0.55-0.12= 0.43 for B_2 and 0.60-0.14= 0.46 for B_{24}, i.e. close to the Mendelian rate (0.5).

To test for significance of these differences, we calculated the expected frequencies of progeny belonging to four categories, i.e. 0B, B_2, B_{24} and B_2B_{24}, from the corresponding frequencies of adults observed in 2002 (with females being the mothers of the embryos analysed) assuming random mating, Mendelian transmission and

random meiotic behaviour of B chromosomes (see summary in Table 3). Goodness-of-fit chi square tests showed parallel significant excess of B_{24}-carrying and deficiency of B_2-carrying embryos in the total sample, the 0B-mothers sample and the +B-mothers sample (Table 4). Since all B chromosomes in the embryo progeny of 0B mothers were necessarily inherited from the father, these results suggest some kind of advantage of B_{24} over B_2 during male transmission.

Clutch size, egg fertility and embryo-adult viability

Table 2 shows that the average number of eggs per pod (clutch size) was 47.22 (*SE*= 2.44) in the 23 females lacking B chromosomes, 37.00 (*SE*=0.79) in the 7 females with $1B_2$ and 42.71 (*SE*= 4.66) in the 7 females carrying $1B_{24}$. A two-way ANOVA showed that the difference, with respect to 0B females, was close to significance for B_2 (*P*= 0.051) but not for B_{24} (*P*= 0.34). No significant effect was either observed for egg fertility, which was 0.92 (*SE*= 0.02) in 0B females, 0.91 (*SE*= 0.04) in B_2 females and 0.96 (*SE*= 0.01) in B_{24} females (two-way ANOVA: *P*= 0.58 for B_2 and *P*= 0.96 for B_{24}). A comparison of B frequency between the embryos obtained in the lab from the gravid females collected in 2002 (thus belonging to the 2003 generation) and the adults collected in 2003 showed no B effects on embryo-adult viability (Table 5).

Controlled crosses

To investigate B chromosome transmission at individual level, we performed several controlled crosses with specimens collected in 2004. Of them, we obtained useful data from eight crosses (Table 6) leading to the following conclusions : i) B_2 did not show

significant drive or drag through the three females carrying them, and the weighed mean transmission rate (0.47) was only slightly higher than the value estimated at population level (0.43) and not significantly different from the Mendelian rate (0.5). ii) B_{24} showed significant drive in two of the six females carrying it, and the weighed mean transmission rate (0.60) was also significantly higher than 0.5 and than the value estimated at population level (0.46). iii) The only male carrying $1B_{24}$ showed a high transmission rate (0.65), but the *Z*-test did not show significance due to low number of progeny. This figure, however, is not very different from the value estimated at population level (0.58).

Discussion

The present results have permitted to analyze the relative efficiency of two B chromosome variants in some reproduction and viability fitness components. The relative frequency of B_2 and B_{24} slightly changed (not significantly) between 2002 and 2003 but remained stable in 2004. It suggests that B frequency changes at this population are currently slight from generation to generation, as is characteristic of the near-neutral stage of the B chromosome life cycle (Camacho *et al.* 1997). In consistency with the 2002-2003 frequency change, the analysis of transmission at population level showed that B_{24} was transmitted more efficiently than B_2 through males from the 2002 season. In addition, controlled crosses showed that B_{24} still drives in some females, which also points to a better performance for B_{24} than B_2 in female transmission. However, the mean transmission rate (k_B) through B_{24}-carrying females in controlled crosses (0.6), although still significantly higher than the Mendelian value, indicates that B_{24} is in the process of neutralization. The observation that one third of the crossed

females carrying B_{24} showed significant drive for it suggests, with the logical reservations of the small number of crosses performed, that this B in the Algarrobo population sampled in 2004 is at an intermediate situation between B_{24} in Torrox analyzed in 1992 (Zurita *et al.* 1998) and 1998 (Perfectti *et al.*, 2004) where 61% and 19% of females, respectively, showed significant drive for this B chromosome. Consistently, the average transmission rate, deduced from controlled crosses, was also intermediate in Algarrobo (0.6) with respect to the values estimated in Torrox in 1992 (0.696) and 1998 (0.523).

A remarkable difference in Algarrobo is the absence of harmful effects of B_{24} on egg fertility, the fitness component which had been shown to be the most sensitive to parasitic Bs in this species (Zurita *et al.* 1998; Muñoz *et al.* 1998). Whereas egg fertility in Torrox decreased with increasing number of B_{24} chromosomes, even after drive neutralization in 1998 (Perfectti *et al.*, 2004), in Algarrobo, however, B_{24} does not decrease significantly egg fertility. A possible explanation is that the Torrox samples were taken in a cultivated area where insecticide treatment may be stronger than in Algarrobo, an abandoned field besides a road. Muñoz *et al.* (1998) showed that the B_2 chromosome does not influence egg fertility in populations from the Granada province, but it highly decreases it in laboratory experiments performed in conditions of mating scarcity. Likewise, the Algarrobo population might be submitted to a less stressful environment than the Torrox one, although this is not consistent with the fact that B frequency was three times higher in Torrox, and this would not be expected under the parasitic theory predicting that Bs should be more frequent in less stressful environments.

Alternatively, B_{24} might have begun to be neutralized in Algarrobo at even higher speed than in Torrox, and this would have impeded it to reach comparable

frequencies to those in Torrox. B_{24} seemed to originate from B_2 in Torrox and, when the A genome responded suppressing the strong B_{24} drive, it had already reached extremely high frequency (about 1.5 Bs per individual) (Zurita *et al.* 1998). By 1998, B_{24} had almost completely been neutralized in Torrox. It is conceivable that when the B_{24} chromosome expanded from Torrox towards nearby populations (e.g. Algarrobo), the migrant individuals carried suppressor gene variants in the A genome in addition to B_{24}. It is thus likely that, when B_{24} invasion reached Algarrobo, it was accompanied by the entry of the A gene variants specifically suppressing its drive, thus impeding this B variant to increase very much in frequency (see mean B_{24} frequency in Table 1). Perfectti *et al.* (2004) argued that drive resistance for B_2 in Torrox could serve as a pre-adaptation facilitating a rapid drive suppression of B_{24}. Thus the entry of the specific B_{24} drive suppressors in parallel to B_{24} invasion in Algarrobo (and perhaps other populations close to Torrox) could provide an even faster suppression acting from the mere invasion and thus impeding the invading B to reach a high frequency.

We cannot rule out the possibility that some fitness effects differentially acting on B_2 and B_{24} carriers can contribute to the substitution process, but we have found only slight differences for clutch size, egg fertility and embryo-adult viability, none reaching statistical significance separately. However, it is remarkable that, for all components combined, including B transmission, B_{24} carriers showed a significantly better performance than B_2 carriers (Table 7). The most apparent difference between B_2 and B_{24} concerns transmission through males, which highly contrasts with Zurita *et al.* (1998) conclusion that replacement of B_2 by B_{24} was due to difference in transmission efficiency through females. The advantage of B_{24} over B_2 during male transmission could be achieved through mechanisms such as meiotic drive, mating preference of B_{24}-carrying males, assorted mating, preferential fertilization or assorted fertilization. With

the available data we cannot choose among these possibilities, but the most parsimonious hypothesis should yield both the excess of B_{24} and the deficiency of B_2. A transmission analysis of these B variants through males in controlled crosses would throw much light on this subject.

The absence of B_{24} effects on egg fertility in Algarrobo is in high contrast to the significant effect observed by Zurita *et al.* (1998) for this same B variant in Torrox. This is a sign of lower virulence for B_{24} in Algarrobo. Neutralization of B chromosomes in *E. plorans* has been suggested for the B_1 and B_2 variants (Camacho *et al.* 1997) and has directly been witnessed for B_{24} in the Torrox population, since B drive and harmful effects on host egg fertility were still present in 1992 (Zurita *et al.* 1998) but had vanished in 1998 (Perfectti *et al.*, 2004). In Algarrobo, it seems that B_{24} is evolving toward a lower virulence, and this could also contribute to the substitution process once B_{24} drive is close to suppression.

As remarked by Ebert & Mangin (1997), single-factor explanations for the evolution of virulence can lead to wrong predictions. Multiple infections and high parasite intensities frequently lead to increased levels of virulence, because within-host competition of parasite mutants favors higher host exploitation rates (for review, see Frank 1996). It is thus intriguing why the two B chromosome variants in Algarrobo do not seem to behave this way, since effects on fitness were slight. The A genome could have evolved high tolerance to B_2 since it presumably was present in this population since long. The low frequency of both B variants in this population, each one showing prevalence about 20-30%, implies a low intensity of parasitism, with scarce individuals carrying both B variants, which might help to explain this departure from expectations.

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Table 1. Frequency of B_2 and B_{24} chromosomes in adult specimens of the grasshopper *Eyprepocnemis plorans* in the Algarrobo population for three consecutive years. Prevalence= Proportion of B carriers.

		Adults with								Prevalence for			Mean for		
Year	Sex	0B	$1B_2$	$1B_{24}$	$2B_2$	$2B_{24}$	$1B_2+1B_{24}$	$1B_2+2B_{24}$	Total	B_2	B_{24}	Total	B_2	B_{24}	Total
2002	m	40	12	5	0	1	9	0	67	0.31	0.22	0.4	0.31	0.24	0.55
	f	24	6	8	1	0	1	1	41	0.22	0.24	0.41	0.24	0.27	0.51
	Total	64	18	13	1	1	10	1	108	0.28	0.23	0.41	0.29	0.25	0.54
2003	m	28	5	5	0	2	3	0	43	0.19	0.23	0.35	0.19	0.28	0.47
	f	16	4	11	0	0	3	0	34	0.21	0.41	0.53	0.21	0.41	0.62
	Total	44	9	16	0	2	6	0	77	0.19	0.31	0.43	0.20	0.34	0.53
2004	m	25	8	9	0	2	1	0	45	0.2	0.27	0.44	0.2	0.31	0.51
	f	16	3	6	0	2	2	0	29	0.17	0.34	0.45	0.17	0.41	0.59
	Total	41	11	15	0	4	3	0	74	0.19	0.30	0.45	0.19	0.35	0.54

Table 2. Mother-offspring analysis to 40 gravid females lacking B chromosomes and 1,233 embryo offspring obtained from them. Fert.= egg fertility= embryos/eggs.

Female					Embryo progeny						
					With					Mean number of	
Type	Number	Eggs	Embryos	Fert.	0B	B_2	B_{24}	B_2+B_{24}	Total	B_2	B_{24}
0B	23	47.22	43.57	0.92	530	59	73	17	679	0.12	0.14
$1B_2$	7	37.00	33.43	0.91	92	95	8	10	205	0.56	0.08
$1B_{24}$	7	42.71	41.29	0.96	98	20	119	23	260	0.20	0.60
$2B_2$	1	42	42	1	5	10	9	6	30	0.83	0.70
$2B_{24}$	1	37	34	0.92	8	0	23	2	33	0.06	0.88
$1B_2$+$2B_{24}$	1		28		2	0	12	12	26	0.46	1.50

Table 3. Expected frequencies of progeny from the 2002 adult males and females, assuming random mating, absence of selection, Mendelian transmission rate and random meiotic behaviour of both B variants.

		Expected offspring			
Mothers	Freq.	0B	B_2	B_{24}	B_2B_{24}
0B	0,585	0,446	0,072	0,048	0,020
B-carrying	0,415	0,142	0,109	0,113	0,051
$1B_2$	0,146	0,056	0,071	0,009	0,011
$1B_{24}$	0,195	0,074	0,012	0,090	0,019
All	1,000	0,587	0,181	0,161	0,071

Table 4. Goodness-of-fit chi square test comparing the observed frequencies in embryos from 2003 (shown in Tables 2 and 3) to the expected from the adults observed in 2002 (shown in Table 4).

Mothers	Item	0B	B_2	B_{24}	B_2B_{24}	Total
0B	Observed (o)	530	59	73	17	679
	Expected (e)	516.85	83.61	55.74	22.80	
	$((o-e)^2)/e$	0.33	7.24	5.35	1.48	14.40
	P					0.0024
+B	Observed (o)	205	125	171	53	554
	Expected (e)	189.15	145.19	151.51	68.16	
	$((o-e)^2)/e$	1.33	2.81	2.51	3.37	10.01
	P					0.0185
$1B_2$	Observed (o)	92	95	8	10	205
	Expected (e)	78.02	99.82	11.86	15.30	
	$((o-e)^2)/e$	2.504	0.233	1.254	1.835	5.826
	P					0.1204
$1B_{24}$	Observed (o)	98	20	119	23	260
	Expected (e)	98.96	16.01	120.30	24.74	
	$((o-e)^2)/e$	0.009	0.996	0.014	0.122	1.141
	P					0.7672
All	Observed (o)	735	184	244	70	1233
	Expected (e)	723.94	222.86	199.07	87.13	
	$((o-e)^2)/e$	0.17	6.77	10.14	3.37	20.46
	P					0.0001

Table 5. Analysis of B chromosome effects on viability from ten-day-old embryo to adult. Contingency tests were performed by the *RXC* program.

	Presence of					Prevalence			B_2			B_{24}		
	0B	B_2	B_{24}	B_2+B_{24}	total	B_2	B_{24}	Total	-	+	Total	-	+	Total
Embryos 2003	741	184	244	70	1239	0.205	0.253	0.402	985	254	1239	925	314	1239
Adults 2003	44	9	18	6	77	0.195	0.312	0.429	62	15	77	53	24	77
Total	785	193	262	76	1316				1047	269	1316	978	338	1316
P					0.617						1.000			0.287
SE					0.014						0.000			0.008

Table 6. Controlled crosses performed with specimens collected in 2004. Average transmission rate for B_2 and B_{24} through females (last row) was weighed because of the high differences in the number of embryo offspring analyzed among crosses. The Z test was performed according to López-León *et al.* (1992), and indicates B chromosome drive when positive and drag when negative, indicating significant drive or drag when Z is higher than 1.96 in absolute value. Significant Z-tests are indicated in bold-type letter. Note the presence of two females showing significant drive for B_{24}, the same as the weighed mean transmission for B_{24}.

									Female transmission						Male transmission		
	Number of Bs in parent		Number of embryo offspring with						B_2			B_{24}			B_{24}		
Cross	Female	Male	0B	$1B_2$	$1B_{24}$	$1B_2+1B_{24}$	$2B_{24}$	Total	mean	k_B	*Z*	mean	k_B	*Z*	mean	k_B	*Z*
1	$2B_{24}$	0	3	0	10	0	2	15	0	-	-	0.93	0.47	-0.26	-	-	-
2	$1B_{24}$	0	1	0	11	0	0	12	0	-	-	0.92	**0.92**	**2.89**	**-**	**-**	**-**
3	$1B_{24}$	0	9	0	6	0	0	15	0	-	-	0.40	0.40	-0.77	-	-	-
4	$1B_2$	$1B_{24}$	6	3	9	8	0	26	0.42	0.42	-0.78	0	-	-	0.65	0.65	1.53
5	$1B_{24}$	0	5	0	29	0	0	34	0	-	-	0.85	**0.85**	**4.12**	-	-	-
6	$1B_{24}$	0	22	0	20	0	0	42	0	-	-	0.48	0.48	-0.31	**-**	**-**	**-**
7	$1B_2+1B_{24}$	0	1	4	2	1	0	8	0.63	0.63	0.71	0.38	0.38	-0.71	-	-	-
8	$1B_2$	0	2	2	0	0	0	4	0.50	0.50	0.00	0	-	-	-	-	-
Total								156	0.47	0.47	-0.66	0.66	**0.60**	**2.58**	-	-	-

Table 7. Summary of relative fitness for B_2 and B_{24} carriers, deduced from the mother-offspring analysis. A Wilcoxon Matched Pairs Test showed significant tendency of B_{24} carriers to show higher relative fitness than B_2 carriers for these traits (Z= 2.02, P= 0.04).

	Absolute fitness		Relative fitness	
Issue	B_2	B_{24}	B_2	B_{24}
Male transmisión (k_B)	0.39	0.58	0.67	1
Female transmission (k_B)	0.43	0.46	0.93	1
Clutch size	37.00	42.71	0.87	1
Egg fertility	0.90	0.96	0.94	1
Embryo-adult viability	0.95	1.23	0.77	1

Supplementary material 1. Mother-offspring analysis to 23 gravid females lacking B chromosomes and 679 embryo offspring obtained from them. Fert.= egg fertility= embryos/eggs. In order to avoid useless work without compromising the reliability of the data, we assumed that those 0B females whose first eight embryo offspring was 0B had actually mated to a 0B male. That was the case in mothers no. 3, 5, 6, 12, 23, 28, 29, 30, 33, 35, 47, 50 and 51. For calculations, in these cases, we used 30 embryo offspring when the number of available embryos was 30 or higher, and the number of available embryos if it was lower than 30. Note that the likelihood of finding 8 lacking B embryos, in a 0B female fertilized by a 1B male, is equal to $0.5^8 = 0.004$, and even lower if the male carried 2B or more.

Mother				Embryo offspring with								Mean		
code	Eggs	Embryos	Fert.	0B	$1B_2$	$1B_{24}$	$2B_2$	$2B_{24}$	$1B_2+1B_{24}$	$2B_2+1B_{24}$	Total	B_2	B_{24}	Total
1	52	50	0.96	6	1	21	0	2	0	0	30	0.03	0.83	0.87
3	31	30	0.97	30	0	0	0	0	0	0	30	0	0	0
5	76	73	0.96	30	0	0	0	0	0	0	30	0	0	0
6	44	38	0.86	30	0	0	0	0	0	0	30	0	0	0
8	48	45	0.94	14	8	3	1	0	3	1	30	0.5	0.23	0.73
12	40	38	0.95	30	0	0	0	0	0	0	30	0	0	0
20	62	60	0.97	17	0	13	0	0	0	0	30	0	0.43	0.43
21	54	42	0.78	18	12	0	0	0	0	0	30	0.4	0	0.4
23	49	46	0.94	30	0	0	0	0	0	0	30	0	0	0
28	51	47	0.92	30	0	0	0	0	0	0	30	0	0	0
29	31	30	0.97	30	0	0	0	0	0	0	30	0	0	0
30	51	47	0.92	30	0	0	0	0	0	0	30	0	0	0
32	56	53	0.95	10	2	13	0	0	3	0	28	0.18	0.57	0.75
33	44	43	0.98	30	0	0	0	0	0	0	30	0	0	0
35	36	32	0.89	30	0	0	0	0	0	0	30	0	0	0
41	45	44	0.98	14	0	16	0	0	0	0	30	0	0.53	0.53
45	51	45	0.88	20	9	0	0	0	0	0	29	0.31	0	0.31
46	32	30	0.94	5	7	5	1	0	9	1	28	0.71	0.54	1.25
47	27	27	1	27	0	0	0	0	0	0	27	0	0	0
50	49	27	0.55	27	0	0	0	0	0	0	27	0	0	0
51	38	38	1	30	0	0	0	0	0	0	30	0	0	0
53	63	63	1	13	17	0	0	0	0	0	30	0.57	0	0.57
55	56	54	0.96	29	1	0	0	0	0	0	30	0.03	0	0.03
Total	47.2	43.6	0.92	530	57	71	2	2	15	2	679	0.12	0.14	0.26

Supplementary material 2. Mother-offspring analysis on 17 gravid females carrying B chromosomes in the Algarrobo population. Embr.= embryos; Fert.= egg fertility= embryos/eggs.

	Mother with						Embryos with											Mean number of B chromosomes in embryo offspring					
																		B_2 in mothers with			B_{24} in mothers with		
Code	B_2	B_{24}	Any B	Eggs	Embr	Fert.	0B	$1B_2$	$1B_{24}$	$2B_2$	$2B_{24}$	$1B_2$+$1B_{24}$	$3B_2$	$2B_2$+$1B_{24}$	$1B_2$+$2B_{24}$	$1B_2$+$3B_{24}$	TOTAL	$0B_2$	$1B_2$	$2B_2$	$0B_{24}$	$1B_{24}$	$2B_{24}$
7	1	0	1	38	31	0.82	16	13	2	0	0	0	0	0	0	0	31		0.42		0.06		
11	1	0	1	35	34	0.97	18	15	0	0	0	0	0	0	0	0	33		0.45		0		
26	1	0	1	34	31	0.91	8	14	1	5	0	2	0	0	0	0	30		0.87		0.1		
27	1	0	1	38	37	0.97	8	16	0	4	0	2	0	0	0	0	30		0.87		0.07		
31	1	0	1	36	36	1	9	14	0	0	0	0	0	0	0	0	23		0.61		0		
44	1	0	1	38	28	0.74	17	8	2	0	0	0	0	0	0	0	27		0.3		0.07		
54	1	0	1	40	37	0.93	16	6	3	0	0	6	0	0	0	0	31		0.39		0.29		
4	0	1	1	34	33	0.97	14	7	2	0	0	5	0	0	0	0	28	0.43				0.25	
9	0	1	1	56	55	0.98	31	0	24	0	0	0	0	0	0	0	55	0				0.44	
15	0	1	1	61	60	0.98	27	0	28	0	0	1	0	0	0	0	56	0.02				0.52	
24	0	1	1	30	27	0.9	7	0	19	0	0	0	0	0	0	0	26	0				0.73	
42	0	1	1	38	38	1	7	7	14	0	3	5	0	0	0	0	36	0.33				0.69	
43	0	1	1	32	30	0.94	7	0	17	0	5	0	0	0	0	0	29	0				0.93	
48	0	1	1	48	46	0.96	5	6	7	0	0	12	0	0	0	0	30	0.6				0.63	
22	2	0	2	42	42	1	5	4	5	5	4	2	1	2	2	0	30			0.83	0.7		
39	0	2	2	37	34	0.92	8	0	19	0	4	2	0	0	0	0	33	0.06					0.88
17	1	2	3		28		2	0	9	0	3	2	0	0	8	2	26		0.46				1.5