Chromosome Research **8**: 425–433, 2000.

Fitness effect analysis of a heterochromatic supernumerary segment in the grasshopper *Eyprepocnemis plorans*

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Received 7 January 2000; received in revised form and accepted for publication by Pat Heslop-Harrison 24 March 2000

Key words: *Eyprepocnemis plorans*, fitness, heterochromatin, population dynamic, supernumerary chromosome segment

Abstract

Several components of fitness were analysed in relation to the presence of a supernumerary chromosome segment (SCS) in two natural populations of the grasshopper *Eyprepocnemis plorans*, including clutch size, egg fertility, egg and embryo productivity and survivability from embryo to adult, and SCS transmission through males. The results have shown the absence of a significant relationship between SCS presence and these fitness components, with the single exception of egg fertility which decreases significantly in SCS females with mating shortage. This fertility decrease is thus expected to be relevant for the population dynamics of the SCS only in low-density populations, those in which it is difficult for females to find a male to copulate with before each egg-batch is ready to be laid. The analysis of the SCS transmission through males showed no significant differences between expected and observed SCS frequencies. The SCS polymorphism seems to be at a status close to neutrality in respect to fitness, but its slight disadvantage in transmission through females carrying B chromosomes predicts that the polymorphism should tend to disappear, unless SCS recurrent amplification, or another undiscovered force, counteracts this tendency.

Introduction

Supernumerary chromosome segments (SCS) constitute one of the most frequent polymorphisms in natural populations of grasshoppers (Cabrero 1985). Much is known about their cytological properties, for example most are heterochromatic but heterogeneous in their response to C-banding (Camacho *et al*. 1984). Several mechanisms can lead to their origination (Camacho & Cabrero 1987), the most important being the amplification of repetitive DNA sequences. Some SCSs influence chiasma frequency (Schroeter & Hewitt 1974), most of them affect chiasma distribution on the carrier chromosome (John 1981, Camacho *et al.* 1984, Navas-Castillo *et al.* 1985, de la Torre *et al.* 1986, Navas-Castillo *et al.* 1987), and some of them influence the activity of nucleolus organising regions (Cabrero *et al.* 1986). It has been shown that some contain satellite DNA (John *et al.* 1986) while others contain ribosomal DNA (Cabrero *et al.* 1998). However, less is

known regarding their maintenance in natural populations, i.e. the existence of accumulation mechanisms and fitness effects on the individuals carrying them. The few cases where transmission of supernumerary segments has been analysed indicate that accumulation mechanisms may be as common for them as for B chromosomes (Rhoades 1978, Ainsworth *et al.* 1983, Wilby & Parker 1988, Ruiz-Rejón *et al.* 1988, López-León *et al.* 1992a, Garrido-Ramos *et al.* 1998). Effects of supernumerary segments on fitness are even less known, but their investigation is necessary to ascertain their biological role in natural populations. Two cases analysed in the Liliaceae, *Tulipa australis* (Ruiz-Rejón *et al*. 1988) and *Scilla autumnalis* (Jamilena *et al*. 1995), have shown superior fitness for heterozygous bulbs for the presence of SCS in progeny germination proportion and seed weight, respectively.

Complementary analyses on the evolution of SCS polymorphism in natural populations for several years must also be performed to predict any long-term dynamics. This, however, has been achieved for few supernumerary segments, and the results are somewhat contradictory. For example, whilst supernumerary segments on the three smallest autosomes of the grasshopper *Phaulacridium vittatum* showed frequency stability (Westerman 1975), a supernumerary segment in the plant *Tulipa australis* doubled its frequency within a single generation (Ruiz-Rejón *et al.* 1988).

Eyprepocnemis plorans is a grasshopper species very abundant along the Mediterranean and South Atlantic Spanish coasts, where it shows extensive polymorphisms for B chromosomes and heterochromatic supernumerary segments on several chromosomes (Camacho 1980, Henriques-Gil & Arana 1990, López-León *et al.* 1991). The most frequent supernumerary segment in natural populations from the province of Granada is located proximally on the smallest autosome (S_{11}), and it is the subject of the present study. It is composed of a 180-bp repetitive DNA motif (Cabrero *et al.* unpublished) which is also present in both A and B chromosomes (López-León *et al.* 1994a). This supernumerary segment shows a Mendelian transmission ratio through males (López-León *et al.* 1991) but its transmission ratio through females is influenced by the presence of B chromosomes. Thus, while heterozygous females lacking B chromosomes transmit the supernumerary segment to half of their offspring, those possessing B chromosomes undertransmit the supernumerary segment (López-León *et al.* 1991, 1994b). The only analysis of SCS effects on fitness performed to date, influence on mating frequency (López-León *et al.* 1995), has failed to show any significant effect of SCS presence. Nevertheless, the possibility still remains that the maintenance of this polymorphism in natural populations could be based on beneficial effects on other fitness traits. Here we analyse the effects of the supernumerary segment on several other fitness components, specifically male and female fertility, clutch size and survivability from embryo to adult. The results have shown the absence of significant effects of the SCS on these fitness components, with the exception of female fertility with mating shortage that decreases significantly in females carrying the SCS.

Materials and methods

Adult males and females of the grasshopper *Eyprepocnemis plorans* were collected in two natural populations, Jete (J) and Salobreña (S), in the province of Granada (Spain), in 1990 and 1991. Furthermore, a sample of gravid females was collected from each population in 1990. Because there is very strong second male sperm precedence in *E. plorans* (López-León *et al.* 1993), it was important to avoid any bias towards first matings. We therefore only considered as gravid those females with a very enlarged abdomen, as these were likely to be ready to lay and unlikely to mate again before laying. Each gravid female was placed alone in a cage with fresh grass supplied daily, and a tube with moist vermiculite for oviposition. After obtaining the first egg-pod, the eggs were incubated at 28ºC for ten days, and then the female and the embryos contained in the eggs were cytologically analysed following the procedures described in López-León *et al.* (1992b). *Eyprepocnemis plorans* specimens possess $2n = 22$ X0 ♂/XX ♀ chromosomes. Three different karyotypes were observed with respect to the supernumerary segment on the S_{11} chromosome: normal homozygotes (*NN*) with both S_{11} chromosomes lacking the supernumerary segment,

segmented homozygotes (*SS*) with both S_{11} chromosomes possessing the supernumerary segment, and heterozygotes (*NS*).

In addition, several fitness-related characteristics were analysed: clutch size (the number of eggs or embryos per egg pod), early female fertility (the proportion of eggs containing an embryo in the first pod), and survivability from embryo to adult (by comparing SCS frequency in embryos from 1990 and adults from 1991).

We have also performed an indirect estimation of overall SCS transmission through males, by comparing the observed SCS frequency transmitted through males (q_{tm}) with the frequency of the SCS in the males of the population (q_{pm}). The latter was calculated with data from 1990 samples at Jete and Salobreña (López-León *et al.* 1995). Q_{tm} was calculated as twice the observed SCS frequency in the progeny of *NN* females (q_{off}). The population and transmitted male q_s were compared by means of Student's *t*-test, after estimating the binomial variance for each parameter:

$$V(x_i) = \frac{x_i(1 - x_i)}{2n}$$

To avoid pseudoreplication due to parentage among embryos from the same clutch, we used the number of females (one clutch per female) as the *n* value.

To calculate q_{tm} from the progeny of *NS* females, we assumed all transmissional properties previously shown for this system (López-León *et al.* 1991, 1994b): a Mendelian transmission ratio through males and females lacking B chromosomes, and undertransmission through females with one or two B chromosomes. The values of k_s (transmission ratio of the SCS) employed for females from these populations were those reported previously by López-León *et al.* (1994b). The specific values for the Jete population were 0.408 for 1B females and 0.326 for 2B females. In the Salobreña population, k_s was 0.452 for 1B females and 0.265 for 2B females. The population and transmitted male q_s were also compared by means of Student's *t* test.

To analyse long-term clutch size, fertility and egg and embryo productivity (clutch size divided by the number of days elapsed between mating and laying or between two consecutive layings), an additional sample of adult males and last-instar nymph females (1995 Salobreña sample) was collected in Salobreña during September and October 1995. Female nymphs were isolated from males until becoming virgin adults. Then each female was mated twice (once before each of the two first pods) and permitted to lay four egg-pods. The laying period was thus divided into two clearly different periods: (1) early reproduction, including the first and second pods and characterised by a mating before each laying, and (2) late reproduction, including the third and fourth pods and characterised by laying without any additional mating. To estimate the possible effect on fitness of the SCS, we needed to remove the effect of B chromosomes and mites (*Podapolipus* sp.) which parasitised these females (Muñoz *et al.* 1998). We performed an ANCOVA with SCS as an independent factor and B chromosome frequency as covariate for the first to the third pod. For the fourth pod, we added as covariate the number of mites (transformed to $\log(x + 1)$) present in each female, as performed by Muñoz *et al.* (1998). All these fitness-related variables were transformed to $\log(x + 1)$. Finally, we used a repeated measures one-way ANOVA to analyse the possible effect of pod order on these fitness-related variables.

Results

Clutch size and egg fertility in the first pod laid by gravid females

Table 1 shows egg and embryo clutch size and egg fertility observed in 55 gravid females collected at Jete and 51 others collected at Salobreña in 1990. Student's *t*-test showed the absence of significant differences between females lacking the SCS (*NN*) and those carrying it (*NS* + *SS*). Thus, the presence of the SCS appears not to influence any of these components of early female fertility.

SCS transmission through males

Table 2 shows the results of a mother–offspring analysis in 106 gravid females collected in the field. The embryo offspring carrying the SCS yielded by *NN* females necessarily inherited it from the male

Table 1. Clutch size (eggs or embryos per pod) and egg fertility (embryos/egg) in the first pod laid by gravid females collected at Jete ($n = 55$) and Salobreña ($n = 51$), during the 1990 season.

		NN females			*NS* + *SS* females			Student's *t*-test		
Population	Item	Mean	SE	*n*	Mean	SE	*n*	*t*	df	*p*
Jete	Eggs/pod	40.08	1.47	40	45.27	2.24	15	1.87	53	0.07
	Embryos/pod	39.83	1.52	40	43.13	2.82	15	1.09	53	0.28
	Embryos/egg	0.99	0.01	40	0.95	0.04	15	333.5*	1	0.11
Salobreña	Eggs/pod	44.74	2.42	27	39.63	2.06	24	1.59	49	0.12
	Embryos/pod	40.89	2.11	27	36.42	1.63	24	1.65	49	0.11
	Embryos/egg	0.92	0.02	27	0.93	0.02	24	0.29	49	0.77

SE = standard error.
n = number of females.
* This value corresponds to the Mann–Whitney U-test which was applied because the Levene test rejected the hypothesis of homogeneity of variances, and none of the usual transformations corrected this situation.

Table 2. Mother–offspring analysis of SCS transmission through 106 gravid females collected in the field in the 1990 season.

			Embryo offspring						
Population	Female genotype	*n*	*NN*	*NS*	*SS*	Total	q_{offs}	q_{tm}	q_{pm}
Jete	*NN*	40	1383	115	—	1498	0.038 ± 0.021	0.077 ± 0.030	
	NS	13	265	241	34	540	0.286 ± 0.089	0.121 ± 0.064	
	SS	2	—	53	—	53	0.500 ± 0.250	—	
	Total	55	1648	409	34	2091	0.114 ± 0.030	0.085 ± 0.027	0.121 ± 0.045
Salobreña	*NN*	27	739	244	—	983	0.124 ± 0.045	0.248 ± 0.059	
	NS	23	270	385	119	774	0.402 ± 0.072	0.343 ± 0.070	
	SS	1	—	22	—	22	0.500 ± 0.354	—	
	Total	51	1009	651	119	1779	0.250 ± 0.043	0.286 ± 0.045	0.234 ± 0.021

n = Number of females.
q_{offs} = SCS frequency in the offspring (± standard error).
q_{tm} = deduced SCS frequency among male parents (± standard error).
q_{pm} = SCS frequency among adult males in 1990 (± standard error).

parent. Therefore, the frequency of the SCS among the offspring of these females (q_{offs}) reflects the actual SCS frequency transmitted via males (q_{tm}), which should be equal to two-fold q_{offs}. In Jete, q_{offs} was 0.038, so that q_{tm} was 0.077 (see Table 2). The SCS frequency among the adult males (q_{pm}) was 0.121 in 1990 (López-León *et al.* 1995). These values were not significantly different ($t = 1.316$, $p = 0.189$). The same calculations in Salobreña gave $q_{tm} = 0.248$ and $q_{pm} = 0.234$, with no significant differences between them ($t = 0.224$, $p = 0.823$).

The analysis in *NS* females gave q_{tm} and q_{pm} values not differing significantly between them in both Jete ($t = 0.004$, $p = 0.997$) and Salobreña ($t = 1.496$, $p = 0.136$; Table 2). These results indicate that SCS males transmit SCS in the rate expected from its frequency in the population.

The net result of sexual transmission of the SCS in the 1990 generation was a slight but non-significant difference in SCS frequency between adults and embryos in both populations (Table 3). It appears that the frequency of the SCS does not change significantly in a single generation of sexual transmission.

Survivability from embryo to adult

A comparison between genotypic frequencies observed in embryos from the 1990 season and those observed in adults from the same generation

Table 3. Inter- and intra-generation comparative analysis of SCS frequency.

Population	Item	NN	NS	SS	Total	q_s	χ^2	*p*
Jete	1990 Adults	269	70	8	347	0.124	0.918[a]	0.632
	1990 Embryos	1648	409	34	2091	0.114	1.876[b]	0.391
	1991 Adults	150	42	1	193	0.114		
Salobreña	1990 Adults	174	117	13	304	0.235	2.881[a]	0.237
	1990 Embryos	1010	637	119	1766	0.248	1.142[b]	0.565
	1991 Adults	76	55	7	138	0.250		

The inter-generation comparison was performed between adults and embryos from a same year, and reflected the net result of sexual SCS transmission[a]. The intra-generation comparison, however, was carried out by comparing SCS frequency between embryos from one year and adults from the following year, thus indicating the viability from embryo to adult[b].

collected in 1991, showed the absence of significant effects of the SCS on the survivability from embryo to adult in both populations (Table 3).

Analysis of clutch size, egg productivity, fertility and embryo productivity in females from the 1995 Salobreña sample

Since there were only two *SS* females, we established two groups for comparisons, i.e. females lacking SCS (*NN*) and those possessing it (*NS* + *SS,* i.e. *S*_). After discarding the effects of B chromosomes and mites (previously reported by Muñoz *et al.* 1998), the ANCOVA showed that SCS presence significantly reduces egg fertility (See Table 4) in *NS* + *SS* females. Embryo number was also slightly reduced in the third and fourth egg-pods layed by these females. SCS presence seems not to affect any other fitness-related variable analyzed (see Table 4).

A repeated measures one-way ANOVA was performed to analyze possible differences among pods (Table 5). This analysis was carried out separately for each kind of female (*NN* or *S*_). In *NN* females, significant differences among pods were found in both egg and embryo clutch size. The differences were a result of low egg (and consequently embryo) number in the first pod laid by *NN* females. In *S*_ females, three variables showed significant differences among pods: embryo number, egg fertility and embryo productivity. Interestingly, the differences among pods were produced by a reduction in the number of embryos in the third and fourth pods (see Tables 4 and 5), those not preceded by a mating.

Discussion

Previous research on S_{11}-SCS in *E. plorans* has shown that: (1) SCS frequency has remained stable over years (three in Salobreña, six in Jete) in the two populations analyzed in the present paper (López-León *et al.* 1995), (2) SCS is undertransmitted through heterozygous females carrying B chromosomes (López-León *et al.* 1991, 1994b), and (3) SCS does not influence mating frequency (López-León *et al.* 1995). Our present data have furthermore shown that: (1) SCS males transmit SCS in the proportion expected from their frequency in the population, (2) the presence of SCS does not affect viability from embryo to adult, clutch size and egg productivity, and (3) there are no apparent effects of SCS on egg fertility measured by the proportion of eggs containing an embryo in the first pod laid by gravid females. The same result was obtained for the first two pods laid by the Salobreña females crossed in the laboratory, although the presence of SCS in these females caused a significant decrease in egg fertility and embryo productivity in late reproduction, with mating scarcity, i.e. in the third and fourth pods that were not preceded by mating.

All these observations suggest the following scenario for the population dynamics of this polymorphism. Frequency stability could suggest that

Table 4. Effects of SCS 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) and the number of embryos per day (embryo productivity).

		NN ($n = 37$)		*S-* ($n = 23$)		ANCOVA		SCS effect		
Variable	Egg-pod	Mean	SE	Mean	SE	B effect	Mite effect	*F*	*p*	p_b
Egg clutch size	1	41.46	1.51	43.78	2.01	–		1.093	0.3	–
	2	45.05	1.35	49.43	1.76	–		2.146	0.148	–
	3	44.86	1.37	46.43	2.11	–		0.355	0.554	–
	4	45.54	1.53	46.39	2.44	–	–	0.102	0.751	–
Embryo clutch size	1	39.43	1.59	36.35	3.20	**		0.298	0.587	–
	2	42.95	1.44	41.04	3.57	*		0.448	0.506	–
	3	43.35	1.44	31.96	4.68	**		6.365	0.01	0.058
	4	43.86	1.55	33.35	4.17	**	*	2.435	0.124	–
Egg fertility	1	0.948	0.013	0.8165	0.057	**		2.129	0.15	–
	2	0.953	0.015	0.8207	0.062	**		2.178	0.146	–
	3	0.964	0.008	0.6427	0.083	***		12.536	<0.001	0.003
	4	0.963	0.010	0.6917	0.075	**	*	6.227	0.02	0.047
Egg productivity	1	7.408	1.037	5.728	1.077	–		1.374	0.246	–
	2	6.484	0.624	6.568	1.072	–		0.052	0.82	–
	3	4.763	0.268	4.717	0.288	–		0	0.955	–
	4	5.135	0.320	4.7	0.352	–	–	0.04	0.842	–
Embryo productivity	1	6.997	0.982	5.039	1.062	–		2.068	0.156	–
	2	6.050	0.518	5.684	1.127	*		0.235	0.63	–
	3	4.601	0.269	3.110	0.467	*		6.933	0.01	0.043
	4	4.938	0.301	3.167	0.422	*	**	3.645	0.06	–

p_b = Probability corrected by means of the sequential Bonferroni method.
– = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 5. Repeat measures one way ANOVA testing pod order effects on several fitness-related traits.

Variable	Females	MS	df	F	*p*	p_b
Egg clutch size	*NN*	0.076	3, 108	3.585	0.016	0.032
	*S_*	0.065	3, 66	0.036	0.158	—
Embryo clutch size	*NN*	0.1007	3, 108	3.057	0.031	0.031
	*S_*	1.422	3, 66	3.373	0.023	0.046
Egg fertility	*NN*	0	3, 108	0.505	0.68	—
	*S_*	0.083	3, 66	4.725	0.005	0.01
Egg productivity	*NN*	0.4165	3, 108	2.639	0.053	—
	*S_*	0.1997	3, 66	1.328	0.273	—
Embryo productivity	*NN*	0.3503	3, 108	2.277	0.084	—
	*S_*	0.9843	3, 66	4.015	0.011	0.022

the polymorphism is at a stable equilibrium. However, the temporal scope is too narrow (six years in Jete and three in Salobreña) for being conclusive (López-León *et al.* 1994b). Nevertheless, the present evidence implies that frequency changes over consecutive generations are slight (see Table 3). The dynamics of this polymorphism seems to be rather similar in the two populations analyzed; SCS has a disadvantage in transmission, although it only operates in *NS* females carrying B chromosomes. The selection coefficient (s) for SCS depends on transmission through *NS* females

carriyng B chromosomes. Therefore, the reduction in SCS frequency in each generation is proportional to both the frequency of NS females and s:

$$\Delta_q = q - \frac{q - 0.5pqs}{1 - 0.5pqs}$$

q being the frequency of the SCS chromosome and s the coefficient of selection (0.1785 in Jete and 0.1447 in Salobreña). Without accounting for the effects of SCS on fertility, which are expected to occur in low-density populations although this is not the case in the two populations analysed (see below), deterministic analyses show that this gametic loss should decrease to half the SCS frequency observed in Jete in 1990 (to reach the value 0.06) in only ten generations, and would subsequently reach 1% after 30 generations. In Salobreña, SCS frequency would be halved (0.1175) in only 14 generations and would be 1% after 50 generations. In small populations, the time to SCS extinction would be even shorter.

In order for the SCS to be not eliminated from the population, these losses through NS carrying B females must be counteracted by some SCS advantage at another level. However, our analyses have failed to find such an advantage after analyzing many fitness components, and thus unless an undiscovered force leads to an increase in SCS frequency, SCS is fated to extinction. Since the basic S_{11} chromosome also carries the 180-bp tandem repeat DNA of which the SCS is composed, the most plausible explanation for SCS origin is the amplification of this repeat. Therefore, recurrent amplification of this DNA repeat could counteract the transmission loss through females carrying B chromosomes, in which case the SCS could even reach an equilibrium.

The biological meanings of repetitive DNA contained in heterochromatin vary from the functional role at the level of centromeres and telomeres, to the apparently functionless role of most satellite DNAs. This does not rule out the possibility of positive fitness effects correlated with satellite DNA presence, as has been reported for *Drosophila* in which viability reduction is associated with the deletion of the Responder sequences (Wu *et al.* 1989).

Taken together, it would predict that the negative effect of SCS presence on egg fertility, in conjunction with the transmissional loss through females, should lead to the disappearance of the SCS. However, since the reduction in fertility was only significant in the third and fourth clutches, those laid with no additional mating since the previous clutch, we think that this is a factor of minor importance, operating only in low-density populations where mating scarcity is frequent. *E. plorans* females mate many times before initial oviposition and once more before each subsequent laying, a behavior that enhances female egg productivity through the receipt of ejaculate nutrients with each mating (Pardo *et al.* 1995).

The effect of the heterocromatic SCS decreasing egg fertility could be explained supposing SCS operates through a position effect on 'fertility genes' (i.e. genes playing a role in these aspects of the female reproductive physiology) located on the S_{11} chromosome. Since the basic S_{11} chromosome in *E. plorans*, lacking the SCS, also contains a small amount of the same repetitive DNA contained in the SCS (Cabrero *et al.*, in preparation), these putative fertility genes would probably be 'heterochromatic sensitive genes' susceptible to a dose effect. This kind of effect would be reminiscent of position effect variegation (PEV) that has been extensively described in *Drosophila* (for review, see Weiler & Wakimoto 1995). There is ample evidence that heterochromatin and euchromatin are functionally different domains in terms of gene expression, so that when a gene is moved from its normal location in one domain to a site within the other domain, it is often subject to mosaic inactivation (PEV). Genetic studies have provided evidence that the organization of chromosomes and the position of genes in the nuclei may affect gene expression (Donaldson & Karpen 1997).

The fertility decrease should be proportional to the number of genes silenced or to the reduction in expression of these genes, and therefore to the amount of heterochromatin in the genome. In addition, the effects of heterochromatin over other phenomena, such as chiasma positions and distribution, could also produce disruption of coadapted gene complexes or influence chromosome segregation with a subsequent influence over fitness components. In *Drosophila*, it has been

suggested that, if interphase nucleus compartmentalization in heterochromatic and euchromatic domains is important for PEV, then the size of the heterochromatic block inducing PEV might be expected to affect the degree of silencing (Dorer & Henikoff 1997; see also Donaldson & Karpen 1997). These effects may be the result of gene silencing caused by the repetitive DNA contained in the heterochromatic domains. Such repeat-induced gene silencing has been observed in plants, fungi and *Drosophila* (Flavell 1994, Rossignol & Faugeron 1995, Dorer & Henikoff 1997). In *E. plorans*, by extension, such an influence could be correlated with the amount of repetitive DNA contained in the SCS, that is also present in centromere regions of most chromosomes, including B chromosomes. Furthermore, the B_2 type contains the largest amount of this DNA repeat in the genome and is also harmful for egg fertility with mating scarcity (Muñoz *et al.* 1998). Remarkably, another B variant (B_{24}), found in the Torrox population, contains an even larger amount of repetitive DNA (Cabrero *et al.* 1999) and decreases egg fertility even without mating limitation (Zurita *et al*. 1998). These results suggest that egg fertility in *E. plorans* may be inversely proportional to the amount of repetitive DNA found in the genome.

Acknowledgements

We thanks Tim Sharbel for improving comments. This study was supported by grants from the Spanish Dirección General de Enseñanza Superior (no. PB96-1433) and Plan Andaluz de Investigación, Grupo no. CVI-165 (Spain).

References

Ainsworth CC, Parker JS, Horton DM (1983) Chromosome variation and evolution in *Scilla autumnalis*. *Kew Chrom Conf* **2**: 261–268.

Cabrero J (1985) Estudios citogenéticos en saltamontes de la subfamilia gomphocerinae: heterocromatina, reordenaciones cromosómicas y actividad nucleolar. *PhD Thesis*, Universidad de Granada, Spain.

Cabrero J, Navas-Castillo J, Camacho JPM (1986) Effects of supernumerary chromosome segments on the activity of nucleolar organiser regions in the grasshopper *Chorthippus binotatus*. *Chromosoma* **93**: 375–380.

Cabrero J, López-León MD, Camacho JPM (1998) Ribosomal DNA in a supernumerary chromosome segment of the grasshopper *Oedipoda fuscocincta* confirms its origin by translocation. *Hereditas* **129**: 15–18.

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

Camacho JPM (1980) Variabilidad cromosómica en poblaciones naturales de Tettigonioidea, Pamphagoidea y Acridoidea. *PhD. Thesis*, Universidad de Granada, Spain.

Camacho JPM, Cabrero J (1987) New hypotheses about the origin of supernumerary chromosome segments in grasshoppers. *Heredity* **58**: 341–343.

Camacho JPM, Viseras E, Navas J, Cabrero J (1984) C-heterochromatin content of supernumerary chromosome segments of grasshoppers: detection of an euchromatic extra segment. *Heredity* **53**: 167–175.

Donaldson KM, Karpen GH (1997) Trans-suppression of terminal deficiency-associated position effect variegation in a *Drosophila* minichromosome. *Genetics* **145**: 325–337.

Dorer DR, Henikoff S (1997) Transgene repeat arrays interact with distant heterochromatin and cause silencing in cis and trans. *Genetics* **147**: 1181–1190.

Flavell RB (1994) Inactivation of gene-expression in plants as a consequence of specific sequence duplication. *Proc Nat Acad Sci USA* **91**: 3490–3496.

Garrido-Ramos MA, Jamilena M, de la Herrán R, Ruiz-Rejón C, Camacho JPM, Ruiz-Rejón M (1998) Inheritance and fitness effects of a pericentric inversion and a supernumerary chromosome segment in *Muscari comosum* (Liliaceae). *Heredity* **80**: 724–731.

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

Jamilena M, Martínez F, Garrido-Ramos MA *et al.* (1995) Inheritance and fitness effects analysis for a euchromatic supernumerary chromosome segment in *Scilla autumnalis* (Liliaceae). *Biol J Linn Soc* **118**: 249–259.

John B (1981) Heterochromatin variation in natural populations. *Chromosomes Today* **7**: 128–137.

John B, Appels R, Contreras N (1986) Population cytogenetics of *Atractomorpha similis*. II. Molecular characterisation of the distal C-band polymorphisms. *Chromosoma* **94**: 45–58.

López-León MD, Cabrero J, Camacho JPM (1991) Meiotic drive against an autosomal supernumerary segment promoted by the presence of a B chromosome in females of the grasshopper *Eyprepocnemis plorans*. *Chromosoma* **100**: 282–287.

López-León MD, Cabrero J, Camacho JPM (1992a) Male and female segregation distortion for heterochromatic supernumerary segments on the S_8 chromosome of the grasshopper *Chorthippus jacobsi*. *Chromosoma* **101**: 511–516.

López-León MD, Cabrero J, Camacho JPM, Cano MI, Santos JL (1992b) A widespread B chromosome polymorphism maintained without apparent drive. *Evolution* **46**: 529–539.

López-León MD, Cabrero J, Pardo MC, Viseras E, Camacho JPM (1993) Paternity displacement in the grasshopper *Eyprepocnemis plorans*. *Heredity* **71**: 539–545.

López-León MD, Neves N, Schwarzacher T, Heslop-Harrison JS, Hewitt GM, Camacho JPM (1994a) Possible origin of a B chromosome deduced from its DNA composition using double FISH technique. *Chromosome Res* **2**: 87–92.

López-León MD, Pardo MC, Cabrero J, Camacho JPM (1994b) Undertransmission of a supernumerary chromosome segment through heterozygous females possessing B chromosomes in the grasshopper *Eyprepocnemis plorans*. *Genome* **37**: 705–709.

López-León MD, Martín-Alganza A, Pardo MC, Cabrero J, Camacho JPM (1995) Temporal frequency stability and absence of effects on mating behaviour for an autosomal supernumerary segment in two natural populations of the grasshopper *Eyprepocnemis plorans*. *Genome* **38**: 320–324.

Muñoz E, Perfectti F, Martín-Alganza A, Camacho JPM (1998) Parallel effects of a B chromosome and a mite that decrease female fitness in the grasshopper *Eyprepocnemis plorans*. *Proc R Soc Lond B* **265**: 1903–1909.

Navas-Castillo J, Cabrero J, Camacho JPM (1985) Chiasma redistribution in bivalents carrying supernumerary chromosome segments in grasshoppers. *Heredity* **55**: 245–248.

Navas-Castillo J, Cabrero J, Camacho JPM (1987) Chiasma redistribution in presence of supernumerary chromosome segments in grashopper: dependence of the size of the extra segment. *Heredity* **58**: 409–412.

Pardo MC, López-León MD, Hewitt GM, Camacho JPM (1995) Female fitness is increased by frequent mating in grasshoppers. *Heredity* **74**: 654–660.

Rhoades MM (1978) Genetic effects of heterochromatin in maize. In: Walker DB, ed. *Maize Breeding and Genetics*. New York: Wiley Interscience, pp 641–671.

Rossignol JL, Faugeron G (1995) MIP – An epigenetic gene silencing process in *Ascobolus immersus*. *Curr Topics Microbiol Inmunol* **197**: 179–191.

Ruiz-Rejón C, Lozano R, Ortega-Nieto FJ, Ruiz-Rejón M (1988) B chromosomes and supernumerary chromosome segments in Liliaceae: selfish or heterotic DNA? *Kew Chrom Conf* **3**: 141–149.

Schroeter G, Hewitt GM (1974) The effects of supernumerary chromatin in three species of grasshopper. *Can J Genet Cytol* **16**: 285–296.

de la Torre J, López-Fernández C, Nichols R, Gosálvez J (1986) Heterochromatin readjusting chiasma distribution in two species of the genus *Arcyptera*: The effects amomg individuals and populations. *Heredity* **56**: 177–184.

Weiler KS, Wakimoto BT (1995) Heterochromatin and gene-expression in *Drosophila*. *Ann Rev Genet* **29**: 577–605.

Westerman M (1975) Population cytology of the genus *Phaulacridium* III. *P. marginale* (Walker); polymorphisms for extra heterochromatin. *Heredity* **34**: 11–27.

Wilby AS, Parker JS (1988) The supernumerary segment system of *Rumex acetosa*. *Heredity* **60**: 109–117.

Wu CI, True JR, Johnson N (1989) Fitness reduction associated with the deletion of a satellite DNA array. *Nature* **341**: 248–251.

Zurita S, Cabrero J, López-León MD, Camacho JPM (1998) Polymorphism regeneration for a neutralized selfish B chromosome. *Evolution* **52**: 274–277.