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Effects and maintenance of a pericentric inversion polymorphism in the grasshopper *Aiolopus strepens*

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Seven natural populations of the grasshopper *Aiolopus strepens* were analysed to investigate the effects of a pericentric inversion in the smallest chromosome (S_{11}) on chiasma formation and nucleolus organizer regions (NORs) activity. This polymorphism was stable in one population after comparing the frequencies of standard and inverted S_{11} chromosomes in two samples taken 5 years apart. Furthermore, mean cell chiasma frequency in this population tended to decrease over these 5 years. Males homozygous for the S_{11} inversion showed a higher frequency of inactivity of one of the NORs located on the L_3 chromosome than that found in standard homozygous and heterozygous males. The results suggest that this stable polymorphism is maintained by heterosis, and that inversion homozygotes are less fit than standard homozygotes, which explains the low frequency of inverted chromosomes in all populations analysed. However, the possibility of recurrence of the inversion cannot be ruled out.

Keywords: *Aiolopus strepens*, chiasma frequency, NOR activity, pericentric inversion.

Introduction

Chromosomal inversions in natural populations of grasshoppers seem to show a perfect equilibrium since the frequencies of the different chromosomal types do not change significantly from generation to generation (White, 1958; Lewontin & White, 1960; White *et al.*, 1963). In *Trimerotropis gracilis*, the composition of natural populations which were polymorphic for pericentric inversions in chromosomes 5 and 7 did not change between the years 1949 and 1957 (White, 1958). In *Keyacris scurra*, the frequencies of inversions remain stable for years, although populations only 5 miles apart may differ considerably in cytogenetic composition (Lewontin & White, 1960).

Inversions in grasshoppers are considered as absolute crossover suppressors since heterozygotes often show asynapsis or straight non-homologous pairing, but not inversion loops, between standard and inverted sequences (White, 1973; Hewitt, 1979). As Hewitt (1979) pointed out, the crossover suppression produced by the inversions, which prevents free recombination of genes within the sequence, is probably a crucial selective attribute, since a new inversion may arise a 'supergene'. This could explain the maintenance of stable polymorphisms in perfect equilibrium by heterosis in the above mentioned cases of *T. gracilis* and *K. scurra*, among other species.

We investigated other causes which help to maintain pericentric inversion polymorphism in natural populations of the grasshopper *Aiolopus strepens*. We analysed the effects of this inversion on chiasma formation and NOR activity in a population, and compared the former character in two samples collected in 1980 and 1985. Furthermore, we analysed six other natural populations to investigate the incidence of polymorphism and to compare chromosomal frequencies in space (between populations) and time (samples from 1980 and 1985 in a specific population).

Materials and methods

A total of 415 adult males and females of *A. strepens* were caught at seven natural populations in the province of Granada (southeastern Spain, Fig. 1).

The testes were removed from each male through a small cut in the dorsal abdomen, and fixed in 3:1 ethanol-acetic acid. The cut was sealed with paraffin and each male was injected with 0.05 per cent colchicine in isotonic insect saline solution. After 6 h, males

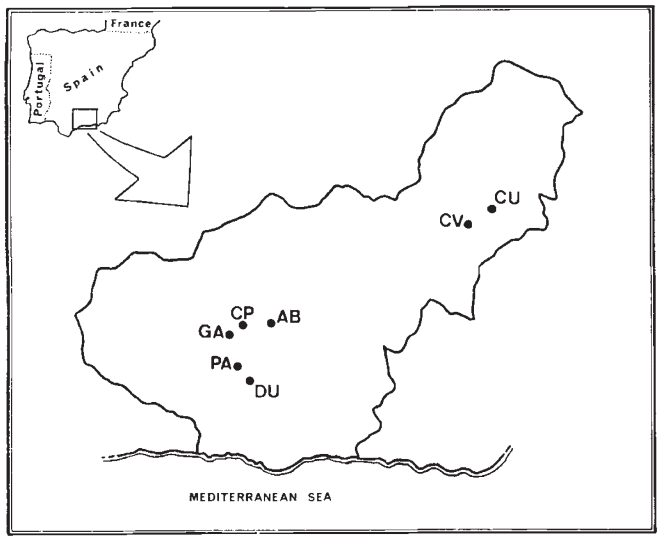

Fig. 1 Map of the Province of Granada showing the location of the seven populations sampled. CU, Cúllar; DU, Dúrcal; GA, Gabias; AB, Río Aguas Blancas; CP, Camino de Purchil; CV, Cañada de las Ventanas; PA, Padul.

were dissected to fix the gastric caeca. Females were injected with 0.05 per cent colchicine 6 h prior to fixation of the ovarioles and gastric caeca.

A female from population CP which was gravid when caught was maintained in the laboratory in a culture cage until several egg pods were obtained. The pods were incubated at 27°C for 10 days, after which the eggs were dissected and the embryos were immersed in 1 ml 0.05 per cent colchicine in isotonic insect saline solution for 1 h. Then 1 ml of distilled water was added for hypotonic shock and the embryos were subsequently fixed in 3:1 ethanol-acetic acid.

The S_{11} chromosomes were characterized in all individuals by studying mitotic chromosomes in the gastric caeca (males and females) and ovarioles (females), by squashing the organ in lacto-propionic orcein. The effects of S_{11} inversion on chiasma frequency and NOR activity were investigated in male meiocytes stained with orcein and silver nitrate. Silver impregnation was done according to the technique described by Rufas *et al.* (1982).

Results

The standard individuals of *A. strepens* show a chromosome complement consisting of $2n = 22 + XO/XX$ subtelocentric chromosomes, with three long (L_1-L_3), five medium (M_4-M_8) and three short (S_9-S_{11}) autosomal pairs, the X chromosome being shorter than L_3 but longer than M_4 (Cabrero & Camacho, 1982).

All seven natural populations of *A. strepens* showed variation for the S_{11} chromosome caused by a pericentric inversion which gave rise to a metacentric S_{11} from a standard subtelocentric chromosome (Fig. 2a and b). We analysed the effects of the inversion in S_{11} on two endophenotypic characters: chiasma frequency and NOR activity in diplotene cells. The number of chiasmata per cell was scored in eight standard homozygous (SS) and eight heterozygotes (SI) males from population CU (Table 1). Mean chiasma frequency per cell in SI males (17.35) was lower than in SS males (17.88), but the difference was not statistically significant ($t = 1.73$, d.f. = 14, $P = 0.10$–0.30).

Since *A. strepens* shows only one active NOR located on L_3 chromosomes, the only possible way for variation to occur is by the occasional inactivity of the NOR in one of both L_3. Our analysis of 20 diplotene cells in each of eight SS males, eight SI males and two II males from population CU gave the results shown in Table 2. A contingency chi-squared test demonstrated dependence of NOR inactivity on the S_{11} inversion ($\chi^2_{(2)} = 9.27$, $P = 0.001$–0.01). While the frequency of NOR inactivity was similar in SS (14.4 per cent) and SI (17.5 per cent) males, II males showed twice this figure (35 per cent), and this difference was statistically significant, as deduced from a contingency chi-squared

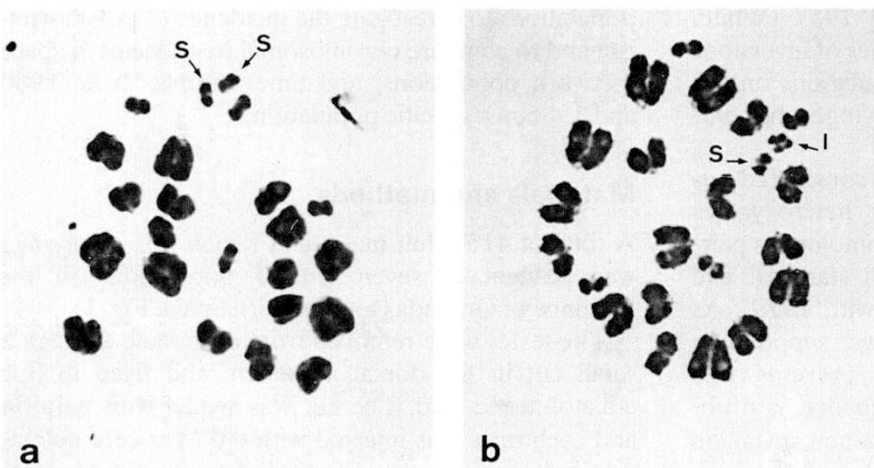

Fig. 2 Gastric caeca mitotic metaphase cells from (a) a standard homozygous individual (SS) (b) an inversion heterozygous individual (SI). Arrows show standard (S) and inverted (I) S_{11} chromosomes.

Table 1 Chiasma frequency in eight SS and eight SI males from population CU

Male type	Male no.	Number of cells with 14–22 chiasmata per cell									Total cells	$\bar{x}$	ε
		14	15	16	17	18	19	20	21	22			
SS	6	—	—	—	4	10	5	—	1	—	20	18.20	
	23	—	—	2	3	14	1	—	—	—	20	17.70	
	26	—	1	—	5	14	—	—	—	—	20	17.60	
	27	—	—	3	3	14	—	—	—	—	20	17.55	
	32	—	—	—	—	19	1	—	—	—	20	18.05	
	33	—	1	—	8	6	4	—	1	—	20	17.80	
	42	—	—	—	2	14	4	—	—	—	20	18.10	
	78	—	—	—	1	17	2	—	—	—	20	18.05	
Total		—	2	5	26	108	17	—	2	—	160	17.88	0.088
SI	10	—	—	2	3	11	2	2	—	—	20	17.95	
	13	—	3	3	6	4	4	—	—	—	20	17.15	
	18	—	—	—	1	9	5	1	2	2	20	19.00	
	35	—	1	—	4	10	—	—	—	—	15	17.53	
	39	—	2	5	7	5	1	—	—	—	20	16.90	
	53	—	2	—	9	8	—	—	—	—	19	17.21	
	63	1	5	3	6	4	—	1	—	—	20	16.55	
	66	2	3	3	7	4	1	—	—	—	20	16.55	
Total		3	16	16	43	55	13	4	2	2	154	17.35	0.288

S = Standard S_{11}. I = Inverted S_{11}.

Table 2 NOR activity in three types of males according to genotype for the S_{11} inversion

Male type	No. of diplotene cells with			
	Both NORs active	One inactive NOR	Percentage NOR inactivity	Total
SS	137	23	14.4	160
SI	132	28	17.5	160
II	26	14	35.0	40
Total	295	65		360

test grouping SS and SI males ($\chi^2_{(2)} = 7.49$, $P = 0.001$–0.01). Thus, the S_{11} inversion influences NOR activity on L_3 chromosomes since, at least in the homozygous state, the frequency of cells showing an inactive NOR is significantly increased.

The frequencies of individuals of both sexes and of the three S_{11} karyotypes (SS, SI and II) are summarized in Table 3. The frequencies of standard and inverted S_{11} chromosomes did not differ between males and females, as demonstrated by contingency chi-squared tests in the five populations in which such analysis was possible (Table 4). A second approach to this feature was used by analysing segregation of S_{11} unequal bivalents with respect to the X chromosome in heterozygous males. For this purpose, 173 second metaphase II cells from SI males were analysed, of which 39 possessed a standard (S) S_{11} chromosome plus the X chromosome, 43 showed an inverted (I) S_{11} and the X, and the remaining lacked the X chromosome. Of this last group 50 carried a standard S_{11} and 41 an inverted S_{11}. A chi-squared test demonstrated that S_{11} unequal bivalents of SI males segregate at random with respect to the X chromosome at the first meiotic division ($\chi^2_{(3)} = 1.59$, $P = 0.50$–0.70).

Inverted S_{11} chromosomes were present in all seven wild populations analysed. Their frequency was always low, and there were only slight differences between populations. A contingency chi-squared test demonstrated no significant differences between populations for the frequencies of S and I chromosomes ($\chi^2_{(6)} = 6.57$, $P = 0.30$–0.50) (Table 5).

To test the Hardy–Weinberg equilibrium, given the absence of inversion homozygotes in most populations

Table 3 Karyomorph and chromosomal frequencies for S_{11} polymorphism in seven natural populations of *A. strepens*

	Males				Females				Total			
Population	SS	SI	II	q	SS	SI	II	q	SS	SI	II	q
CU	82	12	2		48	10	0		130	22	2	
	0.854	0.125	0.021	0.083	0.828	0.172	0	0.086	0.844	0.143	0.013	0.084
DU	20	3	0		14	3	0		34	6	0	
	0.870	0.130	0	0.065	0.824	0.176	0	0.088	0.850	0.150	0	0.075
GA	27	6	0		20	1	0		47	7	0	
	0.818	0.182	0	0.091	0.952	0.048	0	0.024	0.878	0.130	0	0.065
AB	21	1	0		16	3	0		37	4	0	
	0.955	0.045	0	0.023	0.842	0.158	0	0.079	0.902	0.098	0	0.049
CP	20	0	0		19	1	0		39	1	0	
	1.000	0	0	0	0.950	0.050	0	0.025	0.975	0.025	0	0.013
CV	—	—	—		35	4	0		35	4	0	
	—	—	—	—	0.897	0.103	0	0.051	0.897	0.103	0	0.051
PA	22	4	0		20	1	0		42	5	0	
	0.846	0.154	0	0.077	0.952	0.048	0	0.024	0.894	0.106	0	0.053
Total									364	49	2	
									0.877	0.118	0.005	0.064

S = Standard S_{11}, I = inverted S_{11}, q = frequency of inverted chromosomes.

Table 4 Comparison of S_{11} chromosome frequencies between sexes

	Number of S_{11} chromosomes					
	Males		Females			
Population	S	I	S	I	$\chi^2_{(1)}$	P
CU	176	16	106	10	0.015	0.90–0.95
DU	43	3	31	3	0.002	0.95–0.98
GA	60	6	41	1	0.960	0.70–0.80
AB	43	1	35	3	0.442	0.50–0.70
PA	48	4	41	1	0.460	0.30–0.50

Table 5 Comparison of S and I S_{11} chromosome frequencies between populations

	Number of S_{11} chromosomes			
Population	S type	I type	Total	q
CU	282	26	308	0.084
DU	74	6	80	0.075
GA	101	7	108	0.065
AB	78	4	82	0.049
CP	79	1	80	0.013
CV	74	4	78	0.051
PA	89	5	94	0.053

q = Frequency of inverted chromosomes.

we normalized karyotypic frequencies by angular transformation (Table 6). These analyses demonstrated that the observed karyotypic frequencies fit those expected under the Hardy–Weinberg equilibrium.

Finally, a female that was gravid when caught in the field laid several egg pods in the laboratory, allowing the analysis of 98 embryos cytologically. This female was heterozygous for the S_{11} inversion, thus the transmission of S and I chromosomes through this specimen could be analysed. Keeping in mind that this female was collected at population CP, which showed the lowest frequency of inverted S_{11} chromosomes (all 20 males were SS and only this female out of 20 analysed carried an I chromosome), it is reasonable to assume that she had been fertilized by an SS male. Out of 98 embryos, 47 were SS and 51 were SI, which implies a rate of transmission for the inverted S_{11} chromosome equal to 0.52, a figure which does not differ significantly from the expected Mendelian value (0.50) ($\chi^2_{(1)} = 0.16$, $P = 0.50$–0.70).

Table 6 Tests for Hardy–Weinberg equilibrium with karyotypic frequencies normalized by angular transformation*. Note that none of the normalized karyotypic frequencies is higher than the theoretical value (± 1.96)

Population		SS	SI	II	q_i
CU	Observed	0.844	0.143	0.013	0.084
	Equilibrium	0.840	0.154	0.007	
	Normalized	0.096	−0.270	0.535	
DU	Observed	0.850	0.150	0.000	0.075
	Equilibrium	0.856	0.138	0.006	
	Normalized	−0.076	0.153	−0.694	
GA	Observed	0.870	0.130	0.000	0.065
	Equilibrium	0.874	0.122	0.004	
	Normalized	−0.062	0.125	−0.658	
AB	Observed	0.902	0.098	0.000	0.048
	Equilibrium	0.906	0.091	0.002	
	Normalized	−0.061	0.108	−0.405	
CP	Observed	0.975	0.025	0.000	0.013
	Equilibrium	0.974	0.025	0.001	
	Normalized	0.028	−0.028	−0.125	
CV	Observed	0.897	0.103	0.000	0.051
	Equilibrium	0.901	0.096	0.003	
	Normalized	−0.059	0.103	−0.484	
PA	Observed	0.894	0.106	0.000	0.043
	Equilibrium	0.916	0.082	0.002	
	Normalized	−0.364	0.399	−0.434	

*Normalized karyotypic frequency:

$$\mathrm{fr} = \frac{\arcsin\sqrt{P\mathrm{obs.}} - \arcsin\sqrt{P\mathrm{exp.}}}{\sqrt{820.8(2/N)}}.$$

Discussion

The S_{11} pericentric inversion in *A. strepens* is an absolute crossover suppressor between standard and inverted sequences in the heterozygous S_{11} bivalents. It is also a partial crossover suppressor in the S_9 and S_{10} bivalents, whose only chiasma is distally located at a higher frequency in SI males than in SS males, although mean cell chiasma frequency was not significantly different (Cabrero & Camacho, 1982). In this study this latter finding was re-examined in order to test the possible tendency of variation of chiasma frequency over time in a sample of the population CU caught 5 years after the initial study. Our results confirm previous observations: although mean cell chiasma frequency in SI males was lower than in SS males, the difference was not statistically significant. However, when mean cell chiasma frequency in SS males was compared between the first sample in 1980 ($\bar{x} = 18.53$) and the present sample, caught in 1985 ($\bar{x} = 17.88$), there was a significant decrease after five generations (Table 7). In SI males, on the other hand, there was no

Table 7 Comparisons of mean cell chiasma frequencies in SS and SI males sampled in 1980 and 1985

	SS males			SI males			SS + SI males		
Year	$\bar{x}$	ε	n	$\bar{x}$	ε	n	$\bar{x}$	ε	n
1980	18.53	0.143	54	17.60	0.484	7	18.43	0.142	61
1985	17.88	0.088	8	17.35	0.288	8	17.62	0.161	16
	$t = 3.87$			$t = 0.44$			$t = 3.76$		
	$P < 0.001$			$P = 0.50$–0.70			$P < 0.001$		

significant difference between the 1980 ($\bar{x} = 17.60$) and 1985 ($\bar{x} = 17.35$) samples. As a whole, mean cell chiasma frequency decreased significantly in population CU between 1980 ($\bar{x} = 18.43$) and 1985 ($\bar{x} = 17.62$). Thus, it seems that the total amount of genetic variation caused by meiotic crossing-over tends toward reduction, which is more apparent in SS males with a higher mean chiasma frequency. This means that natural selection favours genetic variants with reduced amounts of genetic recombination. In this context, SI males could be considered more fit than SS males on the basis of the crossover suppression exerted by the S_{11} inversion.

Population CU passed through a bottleneck in 1973 due to flooding which presumably killed most grasshoppers. After this event, genotypes releasing large amounts of genetic variation by means of meiotic recombination may have been favoured by natural selection among individuals which recolonized population CU. After several generations, the progressive adaptation to the new habitat may have favoured low levels of genetic variation, which would explain the tendency observed in our sample from 1980 to 1985.

Our analyses of the effects on NOR expression suggest that II males have significantly less NOR activity than SS and SI males. Similar interchromosomal effects on NOR activity in the grasshoppers *Eyprepocnemis plorans* (Cabrero *et al.*, 1987) and *Locusta migratoria* (Salcedo *et al.*, 1988) were related to the presence of B chromosomes. Although, however, the mechanism for these effects is presently unknown, it must involve a decrease in the capacity of II males to synthesize ribosomal RNA, since the expression of r-RNA genes is reduced. Consequently, II males could be less fit than SS and SI males; this could explain the low frequency of I chromosomes in natural populations.

Our frequency analysis demonstrated, however, that S_{11} inversion polymorphism is broadly distributed in the Iberian Peninsula, since in all populations in which a high number of individuals have been analysed, at

least one individual appeared with the S_{11} inversion. This is true for all seven populations studied in the province of Granada (this article) and for two populations from the provinces of Alicante and Avila studied by Suja *et al.* (1986). It is remarkable that inverted chromosomes are equally rare in all populations, the mean frequency being 6.4 per cent. The maintenance of similar rates of polymorphism in wild populations merits some consideration. Firstly, the frequency of polymorphism seems to be stable over time, since in population CU the frequency of S and I chromosomes did not change significantly from 1980 to 1985 (Table 8). Secondly, a SI female transmitted S and I chromosomes at Mendelian rates (0.5). Thirdly, since *A. strepens* shows only one chromosome pair with NOR activity, any reduction in the level of NOR expression may be critical, as it would dramatically decrease the potential for protein synthesis. In species with more than one active NOR this is not necessarily so, since minimal levels of r-RNA production are guaranteed by the number of existing NORs. Thus, II males may be at a disadvantage with respect to SS and SI males due to the formers' reduced capacity for protein synthesis. We could quantify this disadvantage by counting the frequency with which both NORs are active in the different types of males. SS and SI males showed very similar figures (average 84 per cent), while in II males 65 per cent of the cells showed NOR activity maximum, making this component of fitness in II males 0.77 times that of SS and SI males.

The stability of S_{11} polymorphism, the disadvantage of II males in ribosomal RNA production, and the plausible advantage of SI males versus SS males through the maintenance of a supergene, suggest the possibility that polymorphism is maintained in natural populations by heterosis. The absence of a significant excess of heterozygotes with respect to Hardy–Weinberg equilibrium could be explainable if the heterozygous advantage depended on an increase in fecundity rather than in viability (White, 1973). Alternatively, inbreeding may generate more homozygotes (Hewitt, 1979). In fact, inbreeding has been reported in grasshopper populations (Cabrero & Camacho, 1987). The lower fitness of II males versus SS males may explain the low frequencies of I chromosomes in all natural populations analysed, since equilibrium frequencies caused by heterosis are determined exclusively by the selective coefficients of both homozygotes (see White, 1973).

Another way in which S_{11} inversion polymorphism may be maintained is suggested by its broad distribution and the low frequencies of inverted chromosomes in all populations, as the result of a mutation-selection equilibrium. The possibility of recurrence of the S_{11} inversion, assuming that the neotelomeric zone represents a fragile point of breakage in the standard S_{11} chromosome, was pointed out by Suja *et al.* (1986). If this were the case, the rate of mutation can be calculated by the formula of mutation-selection equilibrium in the case of selection against only one homozygote ($S = 0.23$) and given the mean value of all seven populations analysed in this article ($\hat{q} = 0.064$). Thus, a mutation rate of 9.42×10^{-4} would be necessary to reach an equilibrium frequency of inverted chromosomes equal to 0.064 with a selection coefficient of 0.23 acting against II individuals. Although this is actually a high frequency, it is not very different from that observed in *Keyacris scurra*, where at least one individual in a thousand seems to be heterozygous for some kind of unique, and presumably newly arisen, chromosomal rearrangement usually a translocation (White, 1961, 1963).

The recurrence of the S_{11} inversion is testable in the laboratory by controlled crosses to determine whether two parents lacking this rearrangement produce descendants with the S_{11} inversion. This analysis would, however, require a large numbers of crosses. Alternative methods must therefore be developed; direct analysis of the fragility of the neotelomeric zone by inducing chromosomal breakage with radiation to adult individuals, or cell culture, in the laboratory are two possibilities. At present we are trying to culture cells for further studies of the frequency of the S_{11} inversion in *Aiolopus strepens*.

Table 8 Frequency of S_{11} polymorphism in population CU 5 years apart

	Number of individuals with karyomorph				Number of chromosomes			Frequency of I		
Year	SS	SI	II	Total	S	I	Total	chromosome	$\chi^2_{(1)}$	P
1980	97	12	0	109	206	12	218	0.055	1.23	n.s.
1985	130	22	2	154	282	26	308	0.084		

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