

Inversion polymorphisms and natural selection in *Trimerotropis pallidipennis* (Orthoptera)

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Grasshoppers have been much less studied than *Drosophila* when it comes to inversion polymorphisms, despite the occurrence of this rearrangement in several species of grasshoppers. In the present study, 354 males from a natural population of the New World species *Trimerotropis pallidipennis*, polymorphic for 6 pericentric inversions in 4 chromosome pairs, were sampled at the beginning and at the end of the adult life span. This sampling, along with the fact that generations in this grasshopper are annual and discrete, was done to detect differential adult male longevity among karyotypes and departures from formal null models, such as gametic phase equilibrium. These methods allow the detection of natural selection taking place in the wild. The comparison between age classes showed that some inversions were significantly more frequent in one sample, thus revealing the operation of natural selection. Gametic phase disequilibrium was detected in the sample of aged males but not in the sample of young ones. Furthermore, here we aim to detect the phenotypic targets of longevity selection by examining morphometric characters, in order to have a clearer idea of the relation between inversions and natural selection in this species. These results corroborate previous studies that suggested that the inversions are involved in natural selection, and an adaptive model has been proposed for the pattern of inversion frequencies throughout several populations at different altitudes and latitudes.

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In contrast with the considerable amount of information reporting natural selection associated with inversions in several species of *Drosophila* (reviewed by KRIMBAS and POWELL 1992; POWELL 1997), there are far fewer examples in grasshoppers, despite the existence of inversion polymorphisms in many species, especially among the Oedipodinae (JOHN 1984). After the classical work of LEWONTIN and WHITE (1960) and WHITE et al. (1963) in *Warramaba* (formerly *Moraba*) *scurra*, and the far more recent detection of sexual selection in *Sinipta dalmani* (REMIS et al. 2000) no examples of natural selection connected with inversions in grasshoppers were found in the literature.

Trimerotropis pallidipennis (Oedipodinae: Orthoptera) is an excellent species for this kind of studies, since most natural populations from Argentina are polymorphic for 6 different pericentric inversions occurring in chromosome pairs 4, 6, 7 and 8 of the basic complement (DE VAIO et al. 1979; GOÑI et al. 1985) in varying frequencies. Our previous work in this species has demonstrated the existence of altitudinal (CONFALONIERI and COLOMBO 1989) as well as latitudinal and longitudinal (CONFALONIERI 1994) clines for inversion frequencies. This pattern has been explained in terms of adaptation to different climatic variables, especially minimum temperature and humidity (COLOMBO and CONFALONIERI 1996). Despite this purportedly adaptive pattern, thus far there has been no direct detection of natural selection

operating on inversion sequences in this species. This study is precisely aimed to demonstrate the occurrence of natural selection in a wild population through the detection of gametic phase disequilibrium and differential survivorship in different inversion karyotypes of *T. pallidipennis*. Furthermore, in a morphometric study we aim at disclosing the morphological traits that are the direct target of longevity selection.

MATERIAL AND METHODS

In the present study, 354 males of a highly polymorphic population (Uspallata, COLOMBO and CONFALONIERI 1996) of *Trimerotropis pallidipennis* were sampled in January and March 1997, coinciding with the onset and the end of the adult life span in this species. Generations are annual and synchronised, so it can be assumed that all individuals in a sample are of the same age. Collections were made on 5–7 January (80 males) and 18–20 March (267 males) 1997.

Chromosomal analysis

Males were dissected in the field and testes were fixed in 3:1 ethanol: acetic acid and subsequently stored at 4°C. Cytological preparations were made by squashing some follicles in acetic haematoxylin, and karyotypes were determined in meiotic metaphase I.

Differentiation of frequencies between samples and gametic phase disequilibrium, as well as their statistical significance, were assessed by means of the programme "Genepop" (RAYMOND and ROUSSET 1995).

Morphological study

Morphological measurements were obtained with the aid of a graded binocular microscope. The following variables were measured: tegmina length (TgL); total length (TL) (it was taken from the extreme of the head to the articulation between third (3°) coxa and 3° femur); 3° femur length (FL); third tibia length (TiL), thorax length (TxL) and tegmina length (TegL). In the case of paired structures only one was measured.

Statistic procedures

In order to detect the operation of natural selection a multivariate approach was followed. The comparison of mean values between samples was carried out by means of a multivariate analysis of variance (MANOVA). In order to identify the target of longevity selection a multiple regression between all variables (independent variables) and survival (dependent variable) was also performed; as for survival, the young male sample was given the value zero, and the aged male sample was given the value one.

RESULTS

Chromosomal analysis

Gametic disequilibrium between different inversion-bearing chromosome pairs.—Both samples fitted to the Hardy-Weinberg equilibrium for all four inversion-bearing chromosome pairs. With respect to gametic disequilibrium, all inversion-bearing chromosome pairs were compared pairwise (Table 1). In the sample of young males only one comparison rendered significant results, between pairs 4 and 6 ($p = 0.0377$); on the contrary, in the sample of aged males the comparison between pairs 6 and 8 yielded highly significant differences ($P = 0.0089$). The first case can be explained on terms of the number of analyses made, which may render significant results in one of them at random. However, in the second case the significance is very high and this result cannot be so easily explained away.

Differential adult male longevity.—"Genic" (chromosome) differentiation – as opposed to "genotypic", i.e. karyotypic, differentiation- between young males and aged males samples was significant for pairs 7 and 8 (Table 2). In the case of pair 7, the inverted

sequence 7SM2 increased significantly ($P = 0.0009$) its frequency from $q_1 = 0.0731$ to $q_2 = 0.1746$. As for pair 8, the inverted sequence 8SM3 decreased its frequency from $q_1 = 0.3688$ to $q_2 = 0.2566$ ($P = 0.0091$). With respect to pairs 4, the differences between samples were not significant (Table 2); as for pair 6, the difference is significant but not highly so, and so this result could be attributed to random variation. "Genotypic", i.e. karyotypic differentiation was also significant for the same chromosome pairs, but in this case the low frequency of some karyotypes renders this result not reliable (data not shown).

Morphological analysis

Comparison between samples.—The result of the MANOVA comparing the mean values of the morphometric variables was highly significant (Wilks' lambda = 0.7484, $P = 0.00$). In all the cases the sample of aged males showed a decreased value when compared to the sample of young males (Table 3).

Multiple correlation between morphometric variables and survival.—The result of the multiple correlation between all morphometric variables and survival yielded highly significant results for FL, TL and Lteg (Table 4), in that order.

DISCUSSION

Previous work on *Trimerotropis pallidipennis* had already suggested an adaptive role for the inversion polymorphisms of this species, mainly through the correlation between inversion frequency and environmental variables (COLOMBO and CONFALONIERI 1996); as a matter of fact, these correlations help explain the highly variable pattern of inversion frequency distribution along altitudinal, latitudinal and longitudinal clines (CONFALONIERI and COLOMBO 1989; CONFALONIERI 1994). Indeed, this correlation is an indirect indication of natural selection, more direct methods being the deviation from formal null models, such as Hardy-Weinberg or gametic phase equilibria, or, directest of them all, the comparison between age classes (ENDLER 1986; HARTL 1989).

The fact that among grasshoppers generations are discrete and synchronised allows the collection of samples in which the individuals have roughly the same age (COLOMBO 1993). This eases the comparison between different age classes and the assessment of deviations from formal null models, whereas in other organisms with overlapping generations, such as *Drosophila*, the detection of natural selection in the wild is only possible by means of more or less sophisticated schemes (RUIZ et al. 1986). As a result of this study, it is apparent that sequences 7 SM2 and 8 A

Table 1. Pairwise contingency tables for the detection of genotypic disequilibrium by means of the "Genepop" program (RAYMOND and ROUSSET 1995).

Young males sample		Pair 6 karyotypes					
		6 A/6 A	6 M/6 A	6 M/6 M			
Pair 4 Karyotypes	4 A/4 A	16	21	12	49		
	4 A/4 A ¹	7	22	2	30		
	4 SM/4 A	1	1	0	2		
	4 SM/4 A ¹	1	0	0	1		
		25	44	12	82		
$X^2_{(4)} = 10.1665$					P = 0.0377 *		
		Pair 7 karyotypes					
		7 A/6 A	7 SM2/6 A	7 SM2/7 SM2			
Pair 4 Karyotypes	4 A/4 A	42	6	1	46		
	4 A/4 A ¹	26	4	0	32		
	4 SM/4 A	2	0	0	2		
	4 SM/4 A ¹	1	0	0	1		
		66	13	1	82		
$X^2_{(3)} = 2.5576$					P = 0.4649 ns		
		Pair 7 karyotypes					
		7 A/6 A	7 SM2/7 A	7 SM2/7 SM2			
Pair 6 Karyotypes	6 A/6 A	22	3	0	25		
	6 A/6 M	39	5	0	43		
	6 M/6 M	10	2	1	13		
		71	10	1	82		
$X^2_{(2)} = 1.1719$					P = 0.5566		
		Pair 8 karyotypes					
		8 A/8 A	8 SM3/8 A	8 SM3/8 SM3	8 SM4/8 A	8SM3/SM4	
Pair 4 Karyotypes	4 A/4 A	19	16	7	3	4	49
	4 A/4 A ¹	8	12	6	3	1	30
	4 SM/4 A	1	0	1	0	0	2
	4 SM/4 A ¹	1	0	0	0	0	1
		29	28	14	6	5	82
$X^2_{(12)} = 3.2564$							P = 0.9934
		Pair 8 karyotypes					
		8 A/8 A	8 SM3/8 A	8 SM3/8 SM3	8 SM4/8 A	8SM3/SM4	
Pair 6 Karyotypes	6 A/6 A	9	7	5	3	1	25
	6 M/6 A	15	18	6	3	2	44
	6 M/6 M	5	3	3	0	2	13
		29	28	14	6	5	82
$X^2_{(8)} = 5.8544$							P = 0.6635
		Pair 8 karyotypes					
		8 A/8 A	8 SM3/8 A	8 SM3/8 SM3	8 SM4/8 A	8SM3/SM4	
Pair 7 Karyotypes	7 A/7 A	22	27	13	5	4	71
	7 SM/7 A	7	1	1	1	0	10
	7 SM/7 SM	0	0	0	0	1	1
		29	28	14	6	5	82
$X^2_{(4)} = 5.9236$							P = 0.2049

Table 1. (Continued).

a) Aged males sample		Pair 6 karyotypes						
		6 A/6 A	6 M/6 A	6 M/6 M				
Pair 4 Karyotypes	4 A/4 A	66	67	21	154			
	4 A/4 A ¹	37	45	9	91			
	4 SM/4 A	12	7	1	20			
	4 SM/4 A ¹	3	3	1	7			
$X^2_{(6)} = 3.9158$		118	122	32	272 P = 0.6881 ns.			
		Pair 7 karyotypes						
		7 A/6 A	7 SM2/6 A	7 SM2/7 SM2				
Pair 4 Karyotypes	4 A/4 A	104	42	8	154			
	4 A/4 A ¹	61	27	3	91			
	4 SM/4 A	16	4	0	20			
	4 SM/4 A ¹	7	0	0	7			
$X^2_{(6)} = 6.538$		188	73	11	272 P = 0.3657 ns.			
		Pair 7 karyotypes						
		7 A/6 A	7 SM2/7 A	7 SM2/7 SM2				
Pair 6 Karyotypes	6 A/6 A	84	29	5	118			
	6 A/6 M	86	34	2	122			
	6 M/6 M	18	10	4	32			
$X^2_{(4)} = 8.7333$		188	73	11	272 P = 0.0681 ns			
		Pair 8 karyotypes						
		8 A/8 A	8 SM3/8 A	8 SM3/8 SM3	8 SM4/8 A	8SM3/SM4	8 SM4/8 SM4	
Pair 4 Karyotypes	4 A/4 A	57	58	9	23	5	2	154
	4 A/4 A ¹	38	31	8	11	3	0	91
	4 SM/4 A	10	6	0	3	0	0	20
	4 SM/4 A ¹	3	1	0	3	0	0	6
$X^2_{(12)} = 12.3546$		108	96	17	40	9	2	272 P = 0.4180
		Pair 8 karyotypes						
		8 A/8 A	8 SM3/8 A	8 SM3/8 SM3	8 SM4/8 A	8SM3/SM4	8 SM4/8 SM4	
Pair 6 Karyotypes	6 A/6 A	59	33	3	17	6	0	118
	6 M/6 A	33	56	13	16	2	2	122
	6 M/6 M	16	7	1	7	1	0	32
$X^2_{(8)} = 21.8561$		108	96	17	40	9	2	272 P = 0.00889 **
		Pair 8 karyotypes						
		8 A/8 A	8 SM3/8 A	8 SM3/8 SM3	8 SM4/8 A	8SM3/SM4	8 SM4/8 SM4	
Pair 7 Karyot. 7	7 A/7 A	75	68	12	23	9	1	188
	SM2/6 A	28	23	5	16	0	1	73
	6 SM2/7 SM2	5	5	0	1	0	0	11
$X^2_{(8)} = 8.4547$		105	97	15	38	10	2	272 P = 0.3908

Table 2. Contingency tables for “genic” (i.e. chromosome morph) differentiation (as opposed to “genotypic”, – i.e. karyotypic-differentiation). Results are given for both (young and aged males) samples. Ns = not significant; * = significant at the 0.05 level. ** = highly significant at the 0.01 level.

		Pair 4 chromosome morph			
		4 A	4 A ¹	4 SM1	
Sample	Young males	130	31	3	160
	Aged males	419	98	27	534
		549	129	30	708
					P = 0.1811 ns
		Pair 6 chromosome morph			
		6 A	6 M		
Sample	Young males	94	70	164	
	Aged males	358	186	544	
		452	256	708	
					P = 0.0241*
		Pair 7 chromosome morph			
		7 A	7 SM2		
Sample	Young males	152	12	164	
	Aged males	449	95	544	
		584	110	708	
					P = 0.0009***
		Pair 8 chromosome morph			
		8 A	8 SM3	4 SM4	
Sample	Young males	92	61	11	160
	Aged males	352	139	53	544
		444	200	64	708
					P = 0.0091**

Table 3. Mean values and standard deviation (between parenthesis) of the morphometrical variables for both samples. Abbreviations as in Materials and methods. Differences between both samples were highly significant ($p < 0.000001$) for all variables according to the multivariate analysis of variance (MANOVA).

	TL	FL	TiL	TxL	TegL
Young males	9.2637 (0.5076)	10.2915 (0.5528)	9.0691 (1.1941)	3.9924 (0.2426)	19.7643 (2.6034)
Aged males	8.7911 (0.4977)	9.7044 (0.5308)	8.6865 (0.5388)	3.7913 (0.3071)	18.5909 (1.5554)

Table 4. Multiple regression analysis between all morphometric variables (independent variables) and survival (dependent variable). β = regression coefficient.

	TL	FL	TiL	TxL	TegL
β	-0.1489	-0.3328	0.0686	-0.0636	-0.8151
p	0.0057**	<0.001***	0.1068	0.1900	0.0464*

increase significantly its frequency during the adult male life span. It is worth noting that these results would be caused by genes which are linked to the inversions, rather than by the inversions themselves.

In this study it is apparent that the frequency of inversion 7 SM2 increases and that of inversion 8 SM3 decreases significantly over the January–March period (i.e. the adult life span for this species), whereas the standard sequences for both inversion bearing chromosome pairs change in the opposite direction. It had been previously demonstrated that inversion 7 SM2 significantly decreased mean body size for most morphometrical variables, whereas inversions 4 SM1 and 6 M significantly increase them and the inversions of pair 8 have no significant effect on average body size (COLOMBO 2002). In the present study there is a marked decrease of morphometric variables when both the young males and aged males sample were compared, what is consistent with the increase of inversion 7SM2 and the decrease (though not significant, $P = 0.17$) of inversion 6 M.

This kind of studies gives information on adult male longevity, the remaining components of selection remaining obscure. However, the detection of gametic phase disequilibrium, although more indirect, is an indication of natural selection prior to the collection of the sample. In this study, gametic disequilibrium was found among young males, but to be cautious we have established that this trend may be explained away because of the many analyses performed. However, gametic disequilibrium was apparent in aged males beyond any doubt. Coherently with the age classes results, pair 8, which changes its frequency between samples, is involved in gametic phase disequilibrium. Hence it can be stated that natural selection acted on adult males of *T. pallidipennis* favouring some karyotypes and disfavouring others.

The association of colder temperatures with decreased body size is a known feature of Orthopteran populations. Ecological and evolutionary studies showed that grasshoppers from regions with lower minimum temperatures develop faster and are smaller than others from lower altitudes or latitudes (ORR 1996). The same results were obtained in the cricket *A. fasciatus* (MOUSSEAU and ROFF 1989) and in the British grasshoppers *Chorthippus brunneus* (TELFER and HASSALL 1999), *Stenobothrus lineatus*, *Myrmeleotettix maculatus* and *Omocestus viridulus* (WILLOT and HASSALL 1998). We believe that in the case of *T. pallidipennis* body size may be correlated with season length and hence with growing time, and this may have led to the selection of individuals with smaller size in populations with a shorter season length (P. C. Colombo, unpubl.). These smaller indi-

viduals would be carriers of standard (i.e. not inverted, with the exception of inversion 7 SM2) sequences carrying gene complexes that would confer adaptation to lower temperatures. This may be one of the reasons for increased body size in inversion-carrying individuals.

The hypotheses that small size genes are linked with sequences 4 A, 6 A and 7 SM2 may be explicative of the pattern of selection here detected. According to this scheme, the carriers of these sequences may be more resistant to lower temperatures, and this may help explain the differential survival of smaller individuals in colder conditions within a generation. The sampled population is sited at a relatively low latitude (33° S) but at a high altitude (2000 m) and temperatures in autumn may be fairly cool (COLOMBO and CONFALONIERI 1996); it is reasonable to conclude that the differential resistance to cooler temperatures may be conferring the smaller individuals a competitive edge.

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