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Original article

Esterase variation among Argentine populations of *Trimerotropis pallidipennis* (Orthoptera)

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Summary – Isozyme studies were carried out in 3 populations of *Trimerotropis pallidipennis* from Tucumán and San Luis Provinces (TU, SL_1 and SL_2) (Argentina). The purposes of this study were: determination of the numbers of genes and alleles involved in the production of the isozymes and their frequencies, detection of diagnostic loci for population identification and measuring the genetic variability and the degree of genetic differentiation among populations. With this aim, the esterase (*EST*) isozyme system was studied and 6 loci were analysed (*Est 1* to *Est 6*). The *EST* patterns allowed population samples from different biogeographic provinces to be distinguished. Qualitative differences between populations were observed in the expression of some loci (*Est 1, Est 2* and *Est 3*) and the presence of additional alleles for other ones (*Est 5* and *Est 6*), characterizing TU, were also detected. Therefore, this differentiation among populations refers to both gene expression and allelic frequencies which may represent 2 distinct responses to environmental differences. Possible correlations of genetic and chromosomal variation are also discussed.

orthoptera / isozyme / esterase / *Trimerotropis* / genetic variation

Résumé – Variabilité des estérases dans des populations argentines de *Trimerotropis pallidipennis* (Orthoptères). *Trois populations de* Trimerotropis pallidipennis *situées dans les provinces de Tucumán et San Luis (TU, SL_1 et SL_2) (République argentine) ont été étudiées afin de déterminer le nombre de gènes et d'allèles responsables de la synthèse des isoenzymes estérasiques, d'identifier les locus pouvant caractériser des populations, et d'évaluer la variabilité et le degré de différentiation génétique entre les populations. Six locus probables (*Est 1 *à* Est 6*) ont été reconnus. Les enzymogrammes estérasiques ont permis de distinguer les échantillons des différentes provinces biogéographiques, tandis que ceux de la même province ne pouvaient pas être distingués. Il existe, entre les populations, des différences qualitatives dans l'expression des locus* Est 1, Est 2 *et* Est 3 *et ainsi que dans la présence d'allèles différentiels aux locus* Est 5 *et* Est 6. *Cette différence entre les populations se manifeste aussi bien dans l'expression des gènes que dans les fréquences alléliques, ce qui pourrait représenter 2 types de réponses adaptatives. Les corrélations entre la variabilité génétique et chromosomique sont discutées.*

orthoptère / isozyme / estérase / *Trimerotropis* / variabilité génétique

INTRODUCTION

Grasshopper species of the genus *Trimerotropis* are of interest from an evolutionary genetics standpoint because they show chromosomal variation with respect to the position of the centromere (White, 1973), usually considered as produced by pericentric inversions (Hewitt, 1979; John, 1983).

Most of the species of this genus inhabit arid regions from Western North America; *T pallidipennis* is one of the few Trimerotropines to have successfully extended its distribution to Andean South America (White, 1973). Argentine populations studied are polymorphic for pericentric inversions (Mesa, 1971; Vaio *et al*, 1979; Goñi *et al*, 1985; Confalonieri, 1988; Confalonieri and Colombo, 1989).

Goñi *et al* (1985) observed for this species a geographical pattern of chromosomal polymorphisms associated with neither phytogeographical nor climatic characteristics. Clinal variation along an altitudinal gradient of these polymorphisms was recently found (Confalonieri and Colombo, 1989). There is, therefore, a need for a more exhaustive ecological approach. This requires a parallel study of chromosomal, genetical and ecological variation.

In spite of the known usefulness of isozymal studies to population genetics, only a few studies (Moran *et al*, 1980; Gill, 1981; Halliday *et al*, 1983; Nevo *et al*, 1984; Chapco and Bidochka, 1986) have been performed dealing with allozyme variation in grasshopper species.

In this work, isozyme studies were carried out in *T pallidipennis* with the purposes of: a) estimating the number of responsible genes and their allelic frequencies; b) detecting diagnostic loci for populations; c) determining genetic variability and the degree of differentiation between populations; d) correlating genetic and chromosomal variation.

This paper reports the results obtained from the study of the esterase (*EST*) isozyme system in 3 populations of *T pallidipennis*.

MATERIALS AND METHODS

Samples of adult grasshoppers were collected from Tucumán (TU) and San Luis (SL_1 and SL_2, República Argentina. TU is located at Amaicha del Valle (2 040 m above sea level); SL_1 is situated on the National Road No 7, km 792.5, and SL_2 on the same road at km 816 (560 and 440 m above sea level respectively). The populations sampled belong to 2 different biogeographic provinces of the Chaco Dominion, Neotropical Region (Cabrera and Willink, 1973). TU is in the Prepuna while both SL populations are situated in the Chaco Biogeographic Province (fig 1).

Twenty-four individuals from TU, 29 from SL_1 and 20 from SL_2 were collected. After removal of the testes for chromosomal analysis, the grasshoppers were frozen and stored in liquid air until enzyme assays were done.

Extracts were obtained from the eviscerated head and thorax. Each individual was thoroughly homogenised in approximately 0.6 ml of 0.1 mol/l Tris-HCl, pH 7.1. The homogenate was centrifuged at 7 000 rpm for 10 min in a refrigerated centrifuge.

The electrophoresis was conducted in 7% polyacrylamide horizontal gels according to the technique of Cordeiro (1974). The system of gel and tray buffers was discontinuous Poulik, pH 7.9, modified by Schaal and Anderson (1974).

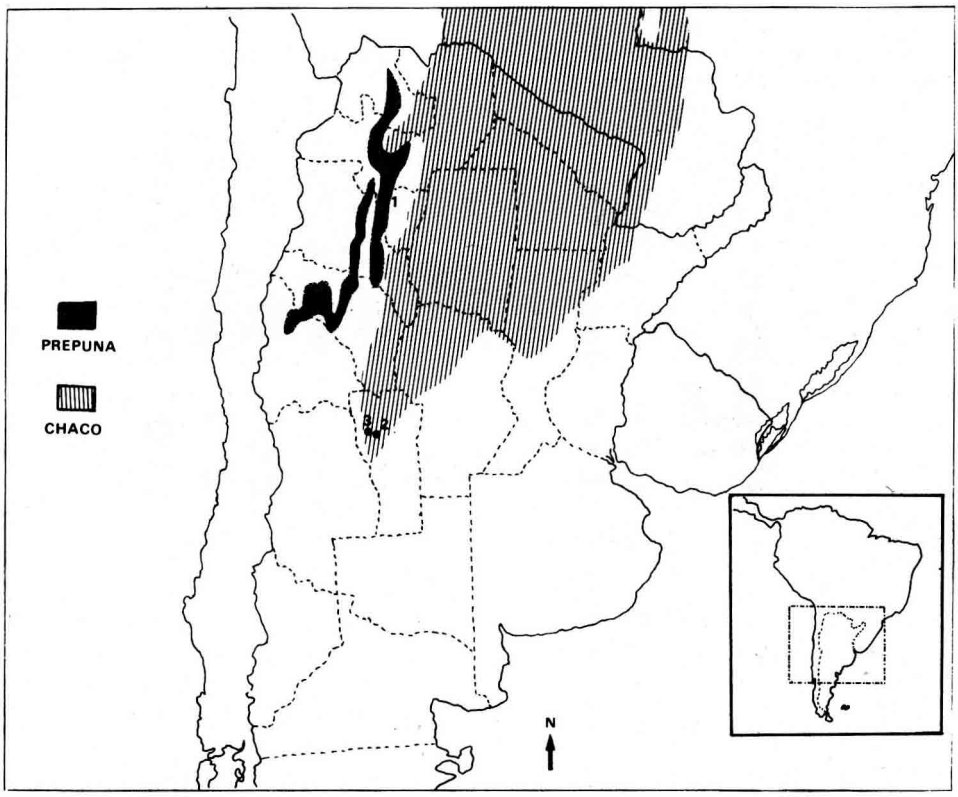

Fig 1. Map showing the 3 localities from Argentina where specimens of *Trimerotropis pallidipennis* were collected. Biogeographic Provinces according to Cabrera and Willink (1973). Tucumán: 1; San Luis 1: 2; San Luis 2: 3.

Staining techniques are described by Cladera (1981). Different substrates (esters) were used to characterize the different bands: α-naphthyl acetate (α-NA), β-naphthyl acetate (β-NA), α-naphthyl laurate (α-NL), α-naphthyl butyrate (α-NB), α-naphthyl oleate (α-NO), α-naphthyl propionate (α-NP) and naphthol AS-D acetate (AS-D).

The interpretation of the genetic variation in electrophoretic patterns is inferential according to Saidman and Naranjo (1982).

Bands are labelled in capital letters and numbered according to decreasing electrophoretic mobilities. Loci are named with an initial capital letter followed by lowercase letters and numbered according to decreasing mobilities of isoenzymes. Alleles are named with the symbol of their loci and an index according to decreasing mobilities of allozymes.

RESULTS

Considering the 3 populations, 17 anodic bands were observed but only 12 (*EST 1* to *EST 12*) could be analysed (fig 2).

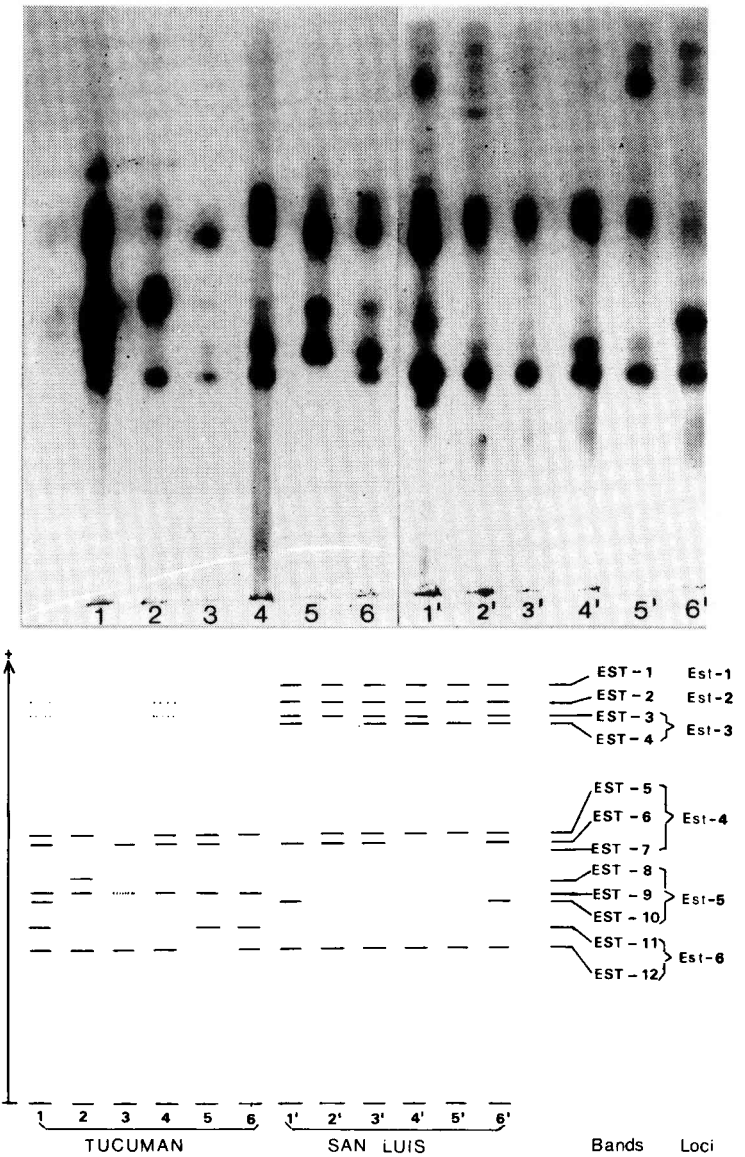

Fig 2. a) Some of the observed allozyme phenotypes for the esterase system. b) Schematic representation of bands from (a) and their possible genetic determination.

The results obtained with different substrates are presented in table I. Bands could be grouped according to their ability to react with different esters: *EST 1* - *EST 2* - *EST 3*; *EST 4* - *EST 5*; *EST 6*; *EST 7* - *EST 8*; *EST 9*; *EST 10* - *EST 11*; *EST 12*. The probable loci and alleles implicated in the genetic determination of bands were inferred taking into account the combinations of the differential substrate affinities.

Table I. Comparisons of staining affinities of bands with esterase activity.

Bands	*α-Na*	*β-NA*	*α-NL*	*α-NS*	*α-NA + β-NA*
EST 1	+++	+−−	++−	− − −	+++ (α-NA)
EST 2	+++	++−	+−−	+−−	+++ (α-NA)
EST 3	+++	++−	++−	/−−	+++ (α-NA)
EST 4	+++	++−	++−	/−−	+++ (α-NA)
EST 5	+++	+++	++−	− − −	+++ (α-NA)
EST 6	+++	+++	++−	− − −	+++ (α-NA)
EST 7	+++	+++	++−	− − −	+++ (α-NA)
EST 8	++−	+++	NT	NT	+++ (β-NA)
EST 9	++−	+++	− − −	/−−	+++ (β-NA)
EST 10	++−	+++	− − −	/−−	+++ (β-NA)
EST 11	+++	++−	/−−	+−−	+++ (β-NA)
EST 12	+++	++−	/−−	+−−	+++ (β-NA)

Bands	*α-NM*	*α-NB*	*AS-D*	*α-NP*	*α-NO*
EST 1	− − −	+++	− − −	+++	− − −
EST 2	+−−	− − −	/−−	+++	− − −
EST 3	/−−	+++	+−−	− − −	− − −
EST 4	/−−	+++	+−−	− − −	− − −
EST 5	/−−	+−−	++−	+++	− − −
EST 6	/−−	+−−	++−	+++	− − −
EST 7	/−−	+−−	++−	+++	− − −
EST 9	− − −	/−−	+−−	++−	− − −
EST 10	− − −	/−−	+−−	++−	− − −
EST 11	+−−	++−	++−	+++	− − −
EST 12	+−−	++−	++−	+++	− − −

References: Staining intensities: +++ = very intense; ++− = intense; +−− = slight; /−− = slight or absent; − − − = absent. NT = not tested.

EST 1 and *EST 2* are invariable and may be produced by 2 monomorphic loci: *EST 1* and *EST 2* (fig 2). *EST 3* and *EST 4* show similar affinities and migrate very close to each other. Three kinds of patterns were observed: *EST 3*, *EST 4* and *EST 3* with *EST 4*. On these grounds the bands were considered as allozymes coded by 1 locus with 2 alleles, *EST* 3^1 and *EST* 3^2 respectively.

EST 1 band was never observed in the TU population, and the expression of bands *EST 2* to *EST 4* in this sample was very poor or absent, showing some degree of individual variation.

EST 5, *EST 6* and *EST 7* are also considered as allozymes. They appeared in the following combinations: *EST 5*; *EST 6*; *EST 7*; *EST 5* with *EST 6*; *EST 5* with *EST 7*; *EST 6* with *EST 7* (fig 2). Since the *EST 7* band is at a low frequency, it is absent in the individuals depicted in figure 2a; in any case, its relative mobility is

also indicated in 2b. The corresponding alleles were named; *EST* 4^1, *EST* 4^2 and *EST* 4^3.

EST 8, *EST 9* and *EST 10* react similarly to $\alpha-$ and $\beta-$NA. *EST 9* and *EST 10* also gave similar staining affinities with the rest of the substrates employed. Because of its low frequency, *EST 8* was absent in the individuals assayed with the latter substrates, and therefore its affinities could not be confirmed. Despite this fact, these results along with the combinations observed (*EST 8*; *EST 9*, *EST 10*; *EST 8* with *EST 9*; *EST 8* with *EST 10*; *EST 9* with *EST 10*; absence of bands) may correspond to those expected for a locus with 4 alleles, 3 codominants, *EST* 5^1, *EST* 5^2, *EST* 5^3 and 1 null *EST* 5^0. The latter in a homozygous condition is characterized by the absence of bands. All phenotypes are shown in figure 2 except the combination *EST 8* - *EST 10* which appeared in other gels not depicted in this figure.

EST 11 and *EST 12* varied showing the following combinations: *EST 11*; *EST 12*; *EST 11* with *EST 12*. They were considered as produced by 1 locus with 2 alleles: *EST* 6^1 and *EST* 6^2 (fig 2). The bands corresponding to *EST 6* could not be properly ascertained in 1 individual from TU and therefore it was excluded from the analysis of this locus.

Five additional bands, 4 between *EST 4* and *EST 5* and 1 between *EST 10* and *EST 11* were also found. Their respective affinities differentiated them from those of *EST 1* to *EST 12*, but their patterns were not constant enough to be unequivocally analysed.

Assuming that this genetic interpretation of bands is correct, phenotype and/or genotype frequencies for each locus were determined (table II). Then, allelic frequencies were estimated by counting or, in the case of the *EST 5* locus which has a null allele, by the method of Neimann-Sorensen (1956) (table III). In addition, mean frequencies of heterozygotes per locus were also estimated and are indicated in table III.

From the 6 loci analysed, 4 displayed qualitative differences between TU and both SL populations. Thus, *EST 1*, *EST 2* and *EST 3*, clearly expressed SL_1 and SL_2, were either absent or almost non detectable in TU. *EST 6* was polymorphic in the latter and monomorphic in the other 2 populations.

The allelic frequencies at *EST 3*, *EST 4* and *EST 5* were compared among populations by means of contingency tables (table III). Allelic frequencies of *EST 3* from both SL populations were not statistically different and, at the *EST 4* locus, differences were also not significant when all populations were considered (though in this case the X^2 for SL_1 *vs* SL_2 was significant at the 5% level). However, *EST 5* locus significantly differentiates TU from both SL populations. This is due to the fact that *EST* 5^0, the most frequent allele in SL_1 and SL_2, was absent in TU. Also, the *EST* 5^2 frequency was high in TU and low in the other samples.

The average expected heterozygosity was also similar for SL_1 and SL_2 (about 0.24) and clearly different from that of TU (0.505) (table III).

In all populations, observed genotypic frequencies were compared with Hardy-Weinberg expectations by means of chi-square tests for the loci *EST 3*, *EST 4* and *EST 6* (table II). These statistics were further applied only to the data of *EST 5* from TU because some classes were too small or absent in the other samples. In every case differences were not significant.

Table II. Genotype frequencies of Esterase loci observed in 3 populations of *T pallidipennis* from San Luis (SL_1 and SL_2) and Tucumán (TU). X^2 statistics test for Hardy-Weinberg equilibrium (except for those cases where some genotypic classes were too small or absent) (the degrees of freedom are indicated in brackets).

	SL_1	SL_2	TU
$EST\ 1^{1/1}$	29	20	not expressed
$EST\ 2^{1/1}$	29	20	absent or poorly expressed
$EST\ 3^{1/1}$	5	2	absent or poorly
$EST\ 3^{1/2}$	16	10	
$EST\ 3^{2/2}$	8	8	expressed
X^2	0.388 (1)	0.196 (1)	–
$EST\ 4^{1/1}$	9	6	7
$EST\ 4^{1/2}$	12	8	7
$EST\ 4^{1/3}$	1	4	2
$EST\ 4^{2/2}$	6	1	4
$EST\ 4^{2/3}$	1	0	3
$EST\ 4^{3/3}$	0	1	1
X^2	0.270 (1)	1.424 (2)	1.607 (2)
$EST\ 5^{1/1+1/0}$	0	0	2
$EST\ 5^{1/2}$	1	0	5
$EST\ 5^{2/2+2/0}$	1	1	8
$EST\ 5^{2/3}$	5	1	7
$EST\ 5^{3/3+3/0}$	8	5	2
$EST\ 5^{1/3}$	2	0	0
$EST\ 5^{0/0}$	12	13	0
X^2	–	–	4.061 (2)
$EST\ 6^{1/1}$	0	0	3
$EST\ 6^{1/2}$	0	0	6
$EST\ 6^{2/2}$	29	20	14
X^2	–	–	2.405 (1)

DISCUSSION

The genetic interpretation of bands studied was inferential, but the hypotheses put forward agree with the fact that in all cases where statistical comparisons could be performed, observed genotypic frequencies fit very well with those expected according to the Hardy-Weinberg law.

Since 5 of the 6 loci analysed showed marked differences in either gene expression on allelic frequency, esterase zymogrames could be used to identify populations from different (but not from the same) provinces.

An additional difference between TU and SL populations is the level of variability of these loci. In the former, H has more than twice the value it has in the latter.

The virtual lack of expression of *EST 1* to *EST 3* loci in TU may be explained by different (but not necessarily mutually exclusive) hypotheses:

Table III. Allelic frequencies of esterase loci and expected frequencies of heterozygotes (H) estimated in 3 populations of *T pallidipennis* from San Luis (SL_1 and SL_2) and Tucumán (TU). (Degrees of freedom of chi square tests in brackets).

Gene	SL_1		SL_2		*TU*			*Contingency* X^2
	freq	*H*	*freq*	*H*	*freq*	*H*	*Total*	SL_1 *vs* SL_2
EST 1^1	1.000	0.000	1.000	0.000	not expressed		–	–
EST 2^1	1.000	0.000	1.000	0.000	absent or poorly expressed		–	–
EST 3^1	0.448	0.494	0.350	0.455	"		–	0.95
EST 3^2	0.552		0.650		"		–	(1)
EST 4^1	0.535		0.600		0.480			
EST 4^2	0.430	0.528	0.250	0.555	0.375	0.608	7.11	6.21
EST 4^3	0.035		0.15		0.145		(4)	(2)
EST 5^0	0.540		0.785		0.000?			
EST 5^1	0.052	0.609	0.000	0.354	0.190	0.575	76.58 **	6.84
EST 5^2	0.122		0.051		0.580		(6)	(3)
EST 5^3	0.286		0.164		0.230			
EST 6^1	0.000	0.000	0.000	0.000	0.260	0.385	–	–
EST 6^2	1.000		1.000		0.740			
average		0.272		0.227		0.523		

*: $0.05 < P < 0.01$; **: $P < 0.001$.

– 1) Mutations can produce changes in the amino acid sequence of polypeptide chains affecting the specific activity (null alleles or alleles with a very slight expression).

– 2) Lower amounts of enzymatic molecules with the same structure are possibly a consequence of:

a) a regulatory gene;

b) different factors in the environment which produce the activation (or inactivation) of certain regulatory gene(s);

c) the existence of chromosome inversions fixed in this population that silence these loci by position effects;

The first hypothesis implies that alternative alleles for 3 out of 6 loci analysed are fixed in different populations. However, the most common situation among conspecific populations is that 1 allele is the most frequent in almost all of them (Lewontin, 1979). Besides, this hypothesis seems to be untenable according to the parsimony principle.

The hypothesis 2a implies a mutation affecting only a single gene (controlling *EST 1* to *EST 3* loci) which seems to be a simpler and more likely explanation, though 2b and 2c are also acceptable.

The hypothesis 2b is plausible because esterases are enzymes frequently acting on external substrates proceeding from ingestion. The precise plant species that constitutes this grasshopper's food in Argentina is not known. Possible candidates are annual plants associated with species of the genus *Larrea* (creosote bush) (Otte and Joern, 1975; Wallner, 1987). As TU and both SL populations are located in different phytogeographic provinces, it is expected that the food supply exploited

will not be the same. In fact, *L divaricata* is widely distributed over the arid and semiarid regions in Argentina, but morphological differences are readily observed between plants at different altitudes. This may be a consequence of both genetical and physiological adaptations (Hunziker, personal communication). The plants associated with the creosote bush which constitue *T pallidipennis* food are also expected to adapt to the corresponding environment. Therefore, distinct enzyme expression between the orthopterans feeding upon them is not surprising.

The hypothesis 2c is based on the high frequency of inversion polymorphisms and chromosomal differentiation among *T pallidipennis* populations (Vaio *el al*, 1979; Goñi *et al*, 1985; Confalonieri, 1988; Confalonieri and Colombo, 1989). A similar kind of differentiation between the populations reported here was also evident when chromosome investigation was carried out (Confalonieri *et al*, submitted). The 3 populations were polymorphic for 3 centric shifts which involved 4 medium chromosomes; when they were statistically compared, differences with respect to inversion frequencies were highly significant among all populations except SL ones. Moreover, some chromosome arrangements were at high frequencies in TU and absent or at very low frequencies in SL_1 and SL_2, respectively. Therefore, these results are in agreement with the last hypothesis (2c) because if 2 populations have different fixed inversions, or are of very different frequencies, position effects are not an unexpected phenomena. Finally, inversion frequencies from 15 populations (including TU, SL_1 and SL_2 from Argentina) were correlated in a very significant fashion with altitude (Confalonieri and Colombo, 1989; Confalonieri, in preparation), suggesting the action of natural selection in the maintenance of these polymorphisms. Therefore, as TU and both SL populations have very different inversion frequencies, most probably as a result of living at different altitudes, their chromosomal variation might also be related to genetical differentiation.

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