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Authors: Guzman, Noelia Verónica, and Confalonieri, Viviana Andrea

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The evolution of South American populations of *Trimerotropis pallidipennis* (Oedipodinae: Acrididae) revisited: dispersion routes and origin of chromosomal inversion clines

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NOELIA VERÓNICA GUZMAN AND VIVIANA ANDREA CONFALONIERI

Grupo de Investigación en Filogenias Moleculares y Filogeografía, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Intendente Güiraldes y Costanera Norte s/n., 4to. Piso Pabellón II, Ciudad Universitaria, C1428EHA Ciudad Autónoma de Buenos Aires, Argentina.
(VAC) Email: bibilu@ege.fcen.uba.ar

Abstract

In the past few years large-scale genome sequencing has demonstrated that inversion rearrangements are more frequent than initially hypothesized. Many examples suggest they may have played an important function in delineating the evolution of biodiversity. In particular, clinal patterns of variation for this type of rearrangement point out its significance in relation to the evolution and adaptation of organisms to changing environments. Within grasshoppers, *Trimerotropis* and *Circotettix* show high incidence of inversion rearrangements, either in fixed or polymorphic state, according to which several hypotheses about phylogenetic relationships have been proposed. Herewith we review the evolutionary significance of inversions in *Trimerotropis pallidipennis*, whose South American populations show clinal variation for pericentric inversions along environmental gradients. We report a new phylogeographic analysis that includes populations of this species from North and South America, and also other species of *Trimerotropis* and *Circotettix*. This study was performed in order to gain further insights into the phylogenetic relationships of this group of species, the dispersion routes followed by *T. pallidipennis* in South America and the evolutionary significance of inversion clines.

Key words

polymorphic rearrangements, phylogeography, grasshoppers, gradients

Introduction

The past few years have seen a revival of interest in inversions since genomic comparisons have highlighted the large number of inverted regions that distinguish species (Catchen *et al.* 2009, Luo *et al.* 2009, Trick *et al.* 2009, Wu & Tanksley 2010), and because new mapping efforts have indicated the presence of inversion polymorphisms in many organisms (Bansal *et al.* 2007, Hoffmann & Rieseberg 2008). Large-scale genome-sequencing methods have recently been designed in order to detect structural variants (Korbel *et al.* 2007), through which it may be possible to demonstrate that the number of polymorphic rearrangements is much larger than initially hypothesized, and that many of them potentially affect gene function. In fact, six human diseases have been recently associated with inversion polymorphisms (Antonacci *et al.* 2009).

Contrasting these detrimental effects, there is an overwhelming number of examples in the scientific literature supporting the idea that inversions have played an important function in delineating the evolution of biodiversity (see Hoffman & Rieseberg 2008 for a review); this includes their possible role in preserving the newly arisen genetic differences that differentiated the human lineage from its sister species (Hey 2003). Inversions have been considered as responsible for maintaining species integrity, impeding the homog-

enization between related species in geographic regions of sympatry. Indeed, inversions can create genomic regions of low recombination that may provide a means to create "islands of differentiation" between species (Brown *et al.* 2004; Butlin 2005; Slotman *et al.* 2006, 2007). This pattern of localized differentiation should be particularly strong if regions of low recombination also harbor loci with divergently selected alleles or alleles conferring reproductive isolation (Feder & Nosil 2009), a hypothesis that has recently been tested on a genome-wide scale (Kulathinal *et al.* 2009).

Clinal patterns of variation for inversion frequencies observed in natural populations of many organisms also point out the significance of this particular type of rearrangement in relation to the evolution and adaptation of organisms to changing environments. These patterns can reflect variable selection in space and are thought to be maintained by a trade-off between dispersal and local adaptation. Although clines by themselves are not sufficient to infer adaptive divergence, strong evidence of selection is obtained if those patterns can be independently replicated and understood in terms of likely fitness effects (Endler 1986). As chromosomal inversions reduce recombination in heterokaryotypes, they can help to preserve particular combinations of alleles within the inverted segment, mainly near inversion breakpoints (Andolfatto *et al.* 2001, Laayouni *et al.* 2003). If the new array of alleles results in adaptive (epistatic) effects, then these polymorphisms may be selected for and retained in populations.

Drosophila inversion polymorphisms were among the first genetic markers quantified in natural populations (Dobzhansky 1937, Powell 1997, Hoffmann *et al.* 2004). For instance, research on the inversion polymorphisms of *Drosophila subobscura* demonstrated that these vary clinally along this species' entire native Palearctic range. Following the recent introduction of the species into the New World, several inversions converged independently in North and South America to their respective clines in the Old World (Balanya *et al.* 2006). The persistence of these spatiotemporal patterns despite continent-wide gene flow, further attests that they are adaptive (Balanya *et al.* 2006).

Inversion rearrangements in grasshopper species

Almost a century ago, the earlier workers on orthopteran cytology (E. Carothers, R. Helwig and R.L. King), were the first to report "centromeric shifts" in grasshoppers (Carothers 1917, 1921; King 1923; Helwig 1929), which were later considered in most of the species discovered as true inversion rearrangements. Since these pioneering works, many researchers have intended to disentangle the evolutionary significance of this kind of chromosomal variation in natural populations. But it was the famous cytogeneticist M.J.D

White that contributed most to this task (White 1949, 1951a,b, 1956, 1957, 1961, 1973, 1977; White *et al.* 1963, 1964, 1969). Further work was developed in several species by a number of researchers (Nur 1968; Weissman 1976; Shaw *et al.* 1976, 1988; Moran 1979; Moran & Shaw 1977; Fletcher & Hewitt 1978; Vaio *et al.* 1979; Goñi *et al.* 1985; Confalonieri 1988, 1992, 1994, 1995; Confalonieri & Colombo 1989; Colombo & Confalonieri 1996, 2004; Colombo 2002, *etc.*).

White (1949) and Coleman (1948) found that within-Orthoptera pericentric inversions were mainly observed in three genera, *Trimerotropis*, *Circotettix* and *Aerochoreutes* (band-winged grasshoppers, Oedipodinae), referring to them as “trimerotropines” to reflect their relatedness [*Aerochoreutes* was later removed from synonymy to *Circotettix* (Otte 1984)]. White (1949) proposed that “it would be worthwhile to reinvestigate the cytology of the natural populations of these trimerotropine grasshoppers, to determine the frequencies of the various chromosomal types, whether significant differences in respect to these frequencies exist between geographically remote or isolated populations, and whether these frequencies are determined by the same general principles as seem to govern the distribution of inversions in *Drosophila* populations. Such a task will necessarily involve several years’ work....”. In this way, M.J.D. White established the starting point for research that continues today.

Within trimerotropine species, the genus *Trimerotropis* is by far the largest (Rentz & Weissman 1980, Weissman & Rentz 1980, Otte 1984, Eades *et al.* 2010). With the exception of a few species, such as *T. pallidipennis* and *T. ochraceipennis*, it is confined to arid regions of North America.

On cytological grounds, White (1948) divided the genus *Trimerotropis* into sections A and B. In the first of these he included those species in which there are $2n = 23$ chromosomes and all of them, including the X, are acrocentric (rod-shaped). Since this is the condition which is almost universal in the Acrididae (except for a few genera), White (1948) proposed that these are the primitive species of the genus, in which the centromeres of all the chromosomes still retain an almost terminal position. This section includes the more eastern North American species *T. maritima*, and the western species *T. fontana* and *T. pistrinaria*, to mention only a few (White 1945, 1948; Coleman 1948; Weissman & Rentz 1980).

Section B comprises those species of *Trimerotropis* in which some of the chromosomes have become metacentric (V-shaped), as a consequence of pericentric inversions. The exact number of metacentric elements varies within group B from species to species, and in some from individual to individual. In all species hitherto studied however, the X chromosome is metacentric. According to White (1948) it must therefore have undergone a centromere shift early in the phylogenetic history of Section B.

Weissman and Rentz (1980) subdivided section B into three subsections. The first includes species showing no polymorphism in any chromosome and the ancestral $2n = 23$ number: *T. diversellus*, *T. huroniana*, *T. lauta*, *T. saxatilis* and *T. schaefferi*. Other species were assigned to subsection two because they always have some autosomes floating (polymorphic), while others are fixed metacentrics; the chromosome number is reduced to $2n = 21$ in four of these species. This subsection includes, among many others, *T. ochraceipennis* and *T. pallidipennis*, both with $2n = 23$ chromosomes, and *T. gracilis* and *T. verruculata*, both with $2n = 21$ chromosomes. Finally, only one species, *T. occidentalis*, which has a telocentric X (similar to section A species), but autosomal inversion polymorphism (as in section B), is placed in subsection three because this species is most closely related to other section-B species, as gauged by the reduced $2n = 21$ chromosome number, by its ability to produce viable hybrids in

natural crosses with another section-B member (John & Weissman 1977), and by its possession of blue color in the hind wing disk (Weissman & Rentz, 1980).

The genus *Circotettix* includes nine species (Eades *et al.* 2010). Its affinities are clearly with section B of *Trimerotropis* rather than with section A, because all species that have been investigated present the X chromosome and some of the autosomes, metacentric in polymorphic or fixed state and $2n = 21$ chromosomes, excepting for *C. undulatus* whose chromosome numbers show intraspecific variation from $2n = 21$ to $2n = 23$ (White 1951, 1973) and *C. carlinianus*, that has $2n = 21$ (White 1951, 1973; Weissman & Rentz 1980).

Apparently, there are no morphological characters that specifically unite phylogenetically the genera *Trimerotropis* and *Circotettix* (Weissman & Rentz 1980). However, White (1973) and Weissman and Rentz (1980) presented general arguments to support the tenet that the chromosomally diverse section-B *Trimerotropis* species evolved from the chromosomally uniform section-A taxa, and that the former subsequently gave rise to the montane *Circotettix*. If this hypothesis were true, then the genus *Trimerotropis* would not be monophyletic, because it would also include the derived taxa *Circotettix*. In fact, in a recent paper that analyzed the phylogenetic relationships among members of the subfamily Oedipodinae (Fries *et al.* 2007), the two species of *Trimerotropis* included are not grouped together. Herewith, we present new data supporting this idea.

Inversion rearrangements in *Trimerotropis pallidipennis*: chromosomal clines

From the beginning of the last century, the Darwinian Theory encouraged many researchers to seek morphological, chromosomal or biochemical features maintained in populations by natural selection and in recent decades, statistics were developed that permitted its evaluation directly on the DNA molecule.

Unlike positive or negative selection, which usually reduce genetic variability, increasing or decreasing the frequency of a particular genetic variant, many types of selection maintain polymorphisms, especially when overdominance, spatially heterogeneous environments, temporally varying environments, or epistasis are occurring. A sensitive method to detect selection of this sort is to search for correlation between gene or genotype frequencies and particular environmental factors. When the same correlations are found in several independent groups of populations, we can be reasonably sure that natural selection is operating (Clarke 1975).

Trimerotropis pallidipennis is an American grasshopper whose South American populations are polymorphic for pericentric inversions (Mesa 1971, Vaio *et al.* 1979, Goñi *et al.* 1985, Confalonieri 1988), North American ones being monomorphic (White 1951). Goñi *et al.* (1985) observed for this species a geographical pattern of chromosomal polymorphisms not associated with phytogeographical or climatic characteristics. However, Confalonieri and Colombo (1989) later reported a marked clinal variation in frequencies of six chromosomal sequences along an altitudinal gradient, suggesting that altitude (or other correlated environmental variables) may exert a differential selective pressure on co-adapted gene blocks in the mutually inverted sequences. This hypothesis claims the existence of several arrays of alleles (*i.e.*, supergenes) more adapted each to different micro-environments across the ecological gradient. It relies on the assumption that chromosomal rearrangements are true pericentric inversions, instead of three-break transpositions of autosomes, because this last type of rearrangement would not necessarily lead to any difference in genetic sequences between homologous regions of chromosomes, except for the position of the

centromeric region. In fact, cytological evidence from C-banding techniques demonstrates that rearranged chromosomes of *T. pallidipennis* show inverted patterns of Cbands (Sanchez & Confalonieri 1993).

Several lines of evidence supported the hypothesis that these inversions are special sequences maintained by deterministic forces: 1) Three inversions [4 AI (Inverted Acrocentric morphology involving chromosome four), 7 SM2 (submetacentric morphology involving chromosome seven) and 8 SM4 (submetacentric morphology involving chromosome 8)] correlate simultaneously with altitude, latitude, and minimum temperature, and two other inversions [6M (metacentric morphology involving chromosome 6) and 8 SM3 (submetacentric morphology involving chromosome 8)] correlate with longitude and humidity, in such a way that the frequency distribution of these chromosome rearrangements could be predicted for unstudied regions (Confalonieri 1994, Colombo & Confalonieri 1996); *i.e.*, they follow similar patterns among independent geographical areas with similar ecological gradients. 2) Gradual variation of inversion frequencies is correlated with environmental gradients, and was maintained stable over more than 10 y (Confalonieri 1994). 3) A linkage disequilibrium between two esterase loci and two chromosome sequences could be demonstrated, whose alternative alleles were linked to different sequences of medium chromosome 8 (Matrajt *et al.* 1996); both enzymatic loci also showed allelic frequencies which correlated with environmental conditions, even in populations not sited on an altitudinal gradient (Matrajt *et al.* 1996).

Although all this evidence favors the selection hypothesis, additional studies were performed in order to gain further insight into the origin of these clinal variations, including a new analysis that will be reported in the following sections.

Phylogeographic studies and the origin of chromosomal clines: hybrid zones or geographically variable selection coefficients?

As previously mentioned, the frequent association of inversion polymorphisms with particular environments is sometimes explained on the basis of the ability of some inversions to generate co-adapted supergenes which are maintained by selection (Dobzhansky *et al.* 1980). Chromosomal clines observed in *T. pallidipennis* might be the consequence of secondary contacts, as occurs in other grasshopper species (Hewitt 1988, Shaw *et al.* 1980), or of geographically variable selection. The former process requires that all populations at lower altitudes had remained isolated and fixed for the same inverted sequences of chromosomes 4, 6, 7 and 8, either by chance or by deterministic forces. Selection could be acting against recombinant genotypes based on inversion types. However, as the populations are all polymorphic, except for some sited at the very extremes of the distribution of the species along arid and semiarid regions of Argentina (Goñi *et al.* 1985, Confalonieri 1994), the width of the hybrid zone would seem to be too extensive.

In order to disentangle this enigma, Confalonieri *et al.* (1998) performed a phylogeographic study of RFLP (Restriction Fragment Length Polymorphisms) variation of the entire mitochondrial DNA genome in several populations along an altitudinal cline. Phylogeography has introduced a phylogenetic-historical perspective to investigate the evolution of populations, contributing to the drawing of conclusions regarding sequences of colonization, diversification, and extinction of genetic lineages in certain areas (Avice 2000, Lanteri & Confalonieri 2003, Scataglini *et al.* 2006). This study was based on the assumption that, whereas natural selection will differ in its

effects on individual loci or gene clusters with epistatic effects, the influence of history should be more or less similar on all genetic markers (Hale & Singh 1991). Therefore, if the chromosomal clines were the result of secondary contacts, this historical process should have affected all genetic markers in the same way.

The analysis of 69 individuals belonging to four populations along an altitudinal cline in Mendoza and San Luis Provinces (Argentina) and another two geographically isolated, revealed the presence of seventeen mitochondrial DNA haplotypes for RFLP variation (Confalonieri *et al.* 1998). The unrooted tree showed that haplotypes were not connected according to their geographical vicinity. Moreover, many of them were present in most of the populations, indicating no evidence of differentiation between both extremes of the clines or between chromosomally differentiated populations, and that a high level of gene flow was occurring. Therefore, gradual variation in chromosomal frequencies would not be the consequence of secondary contacts between populations that historically diverged as a result of some demographic event, only by chance, because this process should have affected all genetic markers in the same way. Instead, gradual variation in selection coefficients acting on the chromosomal sequences along the environmental gradient would have originated and maintained the chromosomal clines. The selection hypothesis was again reinforced.

A new analysis of *T. pallidipennis* populations: origin of South American populations and their phylogenetic relationships

According to Carbonell (1977) the neotropical acridomorph fauna can be roughly divided into three strata, taking into account mainly their probable origin and history, the present distribution, and the assumed length of their presence in the Neotropical region. The subfamily Oedipodinae and four other subfamilies within Acrididae, belong to the most recent faunal strata, which probably invaded South America during Late Cenozoic (*i.e.*, Pliocene and post Pliocene) times.

The most common oedipodine species in South America is *Trimerotropis pallidipennis*. As previously mentioned it is endemic to western North America, but has also dispersed southwards. Its basic requirement appears to be the prevalence of arid and semi-arid conditions (Vaio *et al.* 1979), such as are characteristic of the western regions of America. According to White (1973) colonization of southern latitudes by *T. pallidipennis* occurred from North American populations with basic chromosome arrangements, presumably along the high Andean lands of South America. Here, it then extended its distribution to lower altitudes (Vaio *et al.* 1979) where inversion sequences appeared in polymorphic or fixed states.

Herewith we present a new phylogenetic and phylogeographic analysis that includes *T. pallidipennis* samples from North and South America, collected in Argentina, Peru and USA (Table 1), and the related species *Trimerotropis pristinaria*, *Circotettix carlinianus* and *Oedaleus decorus*. The sample from Peru was identified as *Trimerotropis pallidipennis andeana* (M.M. Cigliano, pers. com.), and the rest of the samples from South America as *T. pallidipennis pallidipennis*. Samples from Argentina were collected in January, 2010, along an altitudinal gradient in Mendoza and San Luis Provinces, from 534 to 2,740 m above sea level, and in other locations geographically distant from the cline, in Buenos Aires Province (Table 1, Fig. 1). This preliminary analysis was performed in order to gain further insight into: i) the hypothesis proposed by White (1973) and Carbonell (1977) about the Nearctic origin of South American Oedipodinae, and ii) the hypothesis proposed by Confalonieri (1994) and Confa-

Table 1. Geographical position and haplotype (H1-H11) identification of *T. pallidipennis* samples analyzed from North and South America for COI sequences. N = number of individuals.

Source Location	Lat, Long	Altitude (in m above sea level)	Collector	N	Haplotypes
Argentina, Mendoza, Pte. del Inca	32° 49' 0" S, 69° 55' 0" W	2740	N. Guzman & V. Confalonieri	3	H2
Argentina, Mendoza, Uspallata	32° 35' 0" S, 69° 20' 0" W	2039	N. Guzman & V. Confalonieri	11	H1 (5), H2 (2), H3 (1), H4 (1), H5 (1), H6 (1)
Argentina, Mendoza, Cacheuta	33°02' 19"S, 69°06' 04"W	1245	N. Guzman & V. Confalonieri	1	H1
Argentina, Mendoza, San Martín Park	32° 53' 25" S, 68° 50' 50" W	746,5	N. Guzman & V. Confalonieri	6	H7 (4), H8 (1), H9 (1)
Argentina, San Luis, Trapiche	33° 7' 0" S, 65° 5' 0" W	1107	N. Guzman & V. Confalonieri	1	H9
Argentina, San Luis, Chosmes	33° 24' 0" S, 66° 49' 0" W	534	N. Guzman & V. Confalonieri	2	H9
Argentina, Buenos Aires, Villarino	38°49' 0" S, 62°42' 0" W	3	M. M. Cigliano	1	H1
Perú, Anchash	09°46' 49.5" S, 77°25' 08" W	3571	M. M. Cigliano	1	H10
USA, Texas	29° 15' 0" N, 103° 15' 0" W	ca 1500	M. Husemann & D. Ferguson	1	H11
USA, Arizona	31° 44' 2" N, 109° 49' 47" W	1501	Ted Cohn	1	H11

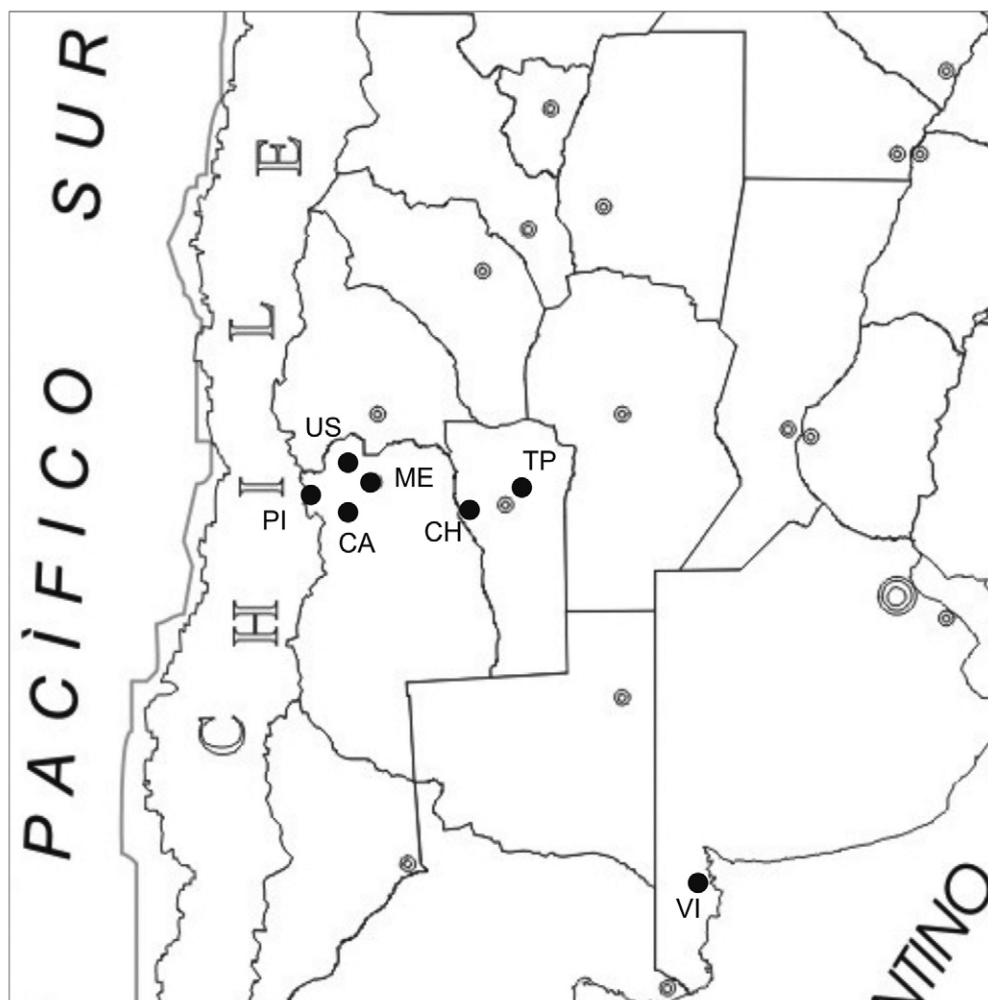
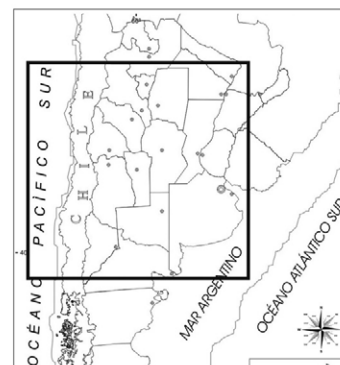


Fig. 1. Populations sampled of *T. pallidipennis* from Argentina. PI: Puente del Inca. US: Uspallata. CA: Cacheuta. ME: San Martín Park. CH: Chosmes. VI: Villarino.



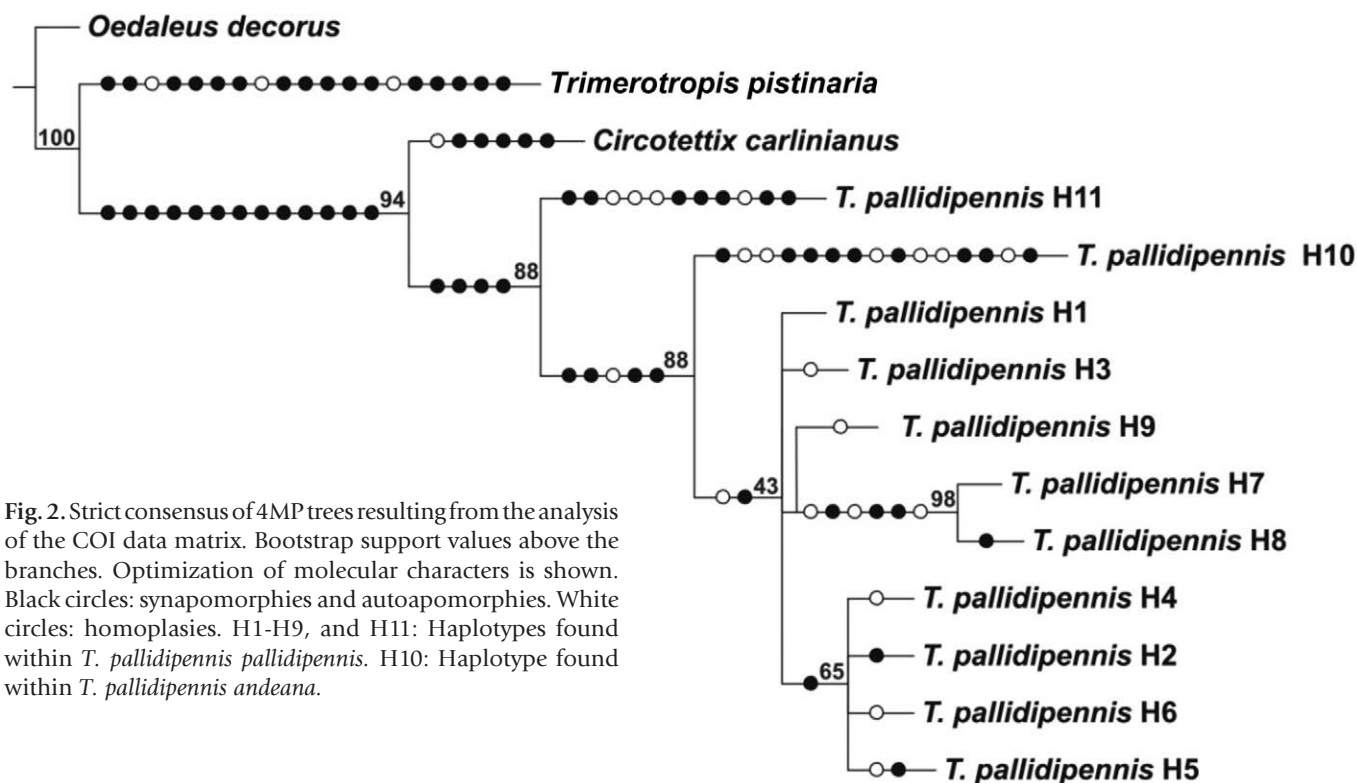


Fig. 2. Strict consensus of 4MP trees resulting from the analysis of the COI data matrix. Bootstrap support values above the branches. Optimization of molecular characters is shown. Black circles: synapomorphies and autoapomorphies. White circles: homoplasies. H1-H9, and H11: Haplotypes found within *T. pallidipennis pallidipennis*. H10: Haplotype found within *T. pallidipennis andeana*.

lonieri *et al.* (1998) about the deterministic origin of chromosomal clines.

With these purposes, we sequenced cytochrome oxidase I (COI) mitochondrial gene in 28 individuals, following procedures of DNA extraction, PCR amplification, and sequencing described elsewhere (Colombo *et al.* 2005, Dinghi *et al.* 2009). Sequences from *T. pristinaria*, *Circotettix carlinianus* and *Oedaleus decorus* were obtained from GenBank (Accession numbers EF151848.1; EF151845.1; EF151834.1, respectively). Alignment of sequences from *T. pallidipennis* yielded 11 different haplotypes arbitrarily named as H1-H11. The distribution of these haplotypes in the different populations is shown in Table 1.

T. pristinaria and *C. carlinianus*, along with the eleven haplotypes of *T. pallidipennis*, were used as terminal taxa for the phylogenetic analysis, which was performed under maximum parsimony using TNT (Goloboff *et al.* 2003). *Oedaleus decorus* was used as outgroup. An exact search of the aligned COI sequences (601 characters, 34 parsimony-informative and 54 singletons) yielded four most-parsimonious trees (Length = 299; Consistency Index = 82; Retention Index = 66). The consensus tree obtained is shown in Fig. 2.

T. pristinaria emerges basally with respect to the rest of the species, and *T. pallidipennis* is more related to *C. carlinianus* than to the other species of the same genus. This last relationship is supported by a high bootstrap value. Besides, all haplotypes within *T. pallidipennis* are grouped together within a monophyletic clade, also with high bootstrap support. The North American haplotype H11, found in both individuals analyzed from USA, occupies the most basal position within this species. All South American haplotypes are grouped together with a high support value, and *T. pallidipennis andeana* is the most basal within this clade. The branch conductive to this last subspecies and to the sample from North America, shows higher numbers of autoapomorphies, compared to branches conductive to the rest of the haplotypes of *T. pallidipennis pallidipennis*. Fig. 3 shows the unrooted phylogenetic network obtained from *T. pal-*

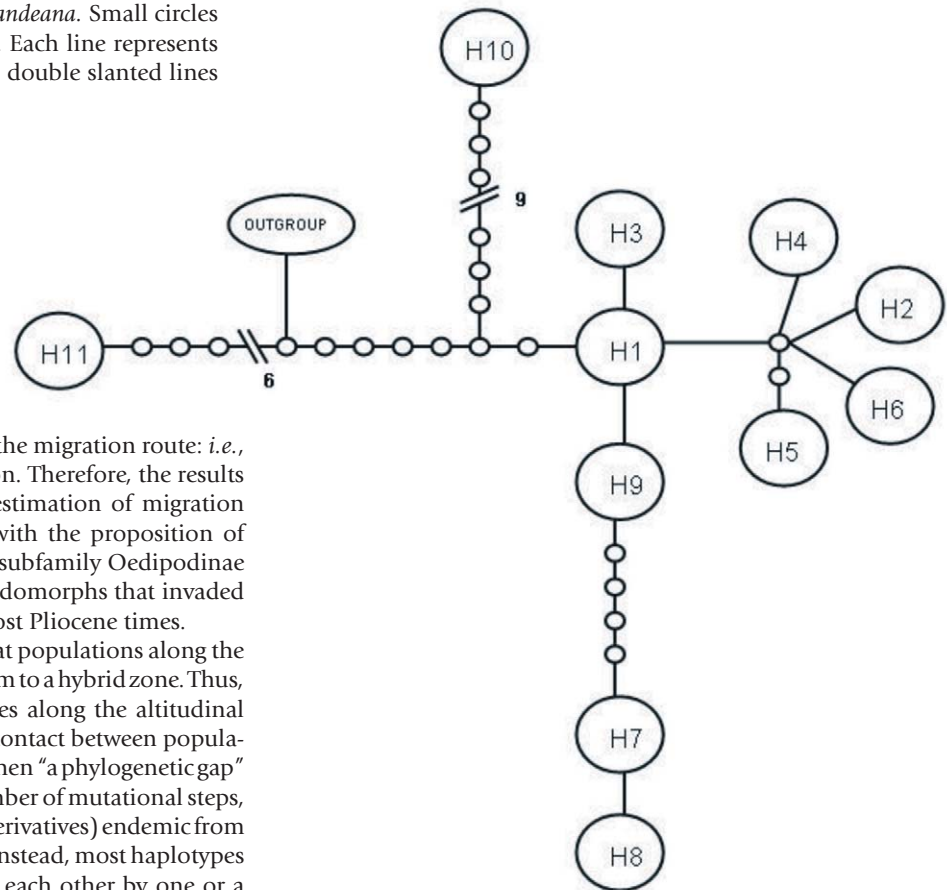
lidipennis haplotypes.

H1 occupies a central position within the network, from which H9, H3 and the ancestor of haplotypes H2, H4, H5, H6, emerge, separated by only one mutational step. H1 is the Argentinean haplotype more related to the Peruvian one and is the most frequent in Uspallata (Mendoza Province), a location sited at almost the highest extreme of the altitudinal cline (Table 1, Fig. 1); it also appears in Cacheuta (Mendoza Province). The rest of the populations, sited with respect to Uspallata at higher (Puento del Inca) or lower (San Martin, Trapiche and Chosmes) altitudes along the cline, bear haplotypes separated by only one mutational step from H1 (*i.e.*, H2 and H9).

The present results, although preliminary, give further support to the hypothesis proposed by Carbonell (1977) of the Nearctic origin of South American oedipodines, and by White (1973) about the colonization of South America by *T. pallidipennis* populations from North America. The basal position of USA individuals within the monophyletic clade that includes all specimens from this species (Fig. 2) is consistent with these hypotheses, by indicating that they are older than South American populations. Furthermore, the basal position of the Peruvian individuals within the South American clade, agrees with the proposed colonization route that would have been followed by this grasshopper: the Peruvian sample is older than those sited in southern latitudes, where the species supposedly arrived in more recent times.

In a previous paper, Confalonieri *et al.* (1998) estimated through a molecular clock analysis that mean divergence time among all RFLP mitochondrial haplotypes found in South American populations of *T. pallidipennis* was 3 My, and proposed that this result could be supporting the idea that this species invaded South America during late Pliocene- early Pleistocene, after the emergence of the land bridge connecting both subcontinents took place. However, this analysis did not include samples from North America which could have

Fig. 3. Phylogenetic network connecting haplotypes from *T. pallidipennis pallidipennis* and *T. pallidipennis andeana*. Small circles represent absent intermediate haplotypes. Each line represents one mutational step and numbers next to double slanted lines indicate numbers of mutational steps.



given stronger support to the direction of the migration route: *i.e.*, from the Nearctic to the Neotropical region. Therefore, the results herewith reported, along with previous estimation of migration times (Confalonieri *et al.* 1998) agrees with the proposition of Carbonell (1977) according to which the subfamily Oedipodinae belongs to the most recent stratum of acridomorphs that invaded the neotropical region during Pliocene- Post Pliocene times.

Present results also support the idea that populations along the altitudinal cline in Argentina do not conform to a hybrid zone. Thus, if clinal variation for inversion frequencies along the altitudinal cline were the consequence of secondary contact between populations fixed for alternative rearrangements, then "a phylogenetic gap" (Avice 2000), *i.e.*, a branch with a high number of mutational steps, should be separating haplotypes (or their derivatives) endemic from populations at both extremes of the cline. Instead, most haplotypes emerge from H1, and are separated from each other by one or a few mutational steps (Fig. 3), indicating that in the past, no barrier against gene flow has apparently existed among populations of this cline. Furthermore, H1 was also found in Villarino, a population sited at only 3 m above sea level and separated by a distance of *ca* 900 km from the Mendoza-San Luis cline (Table 1). H1's central position in the phylogenetic network, its frequency in populations, and its extended geographic distribution in Argentina, favor the hypothesis that this haplotype is the most ancient for these southern latitudes; from it probably derived other haplotypes that appeared during the colonization of new environments at lower altitudes.

Finally, the phylogenetic tree herewith obtained (Fig. 3) demonstrates that the genus *Trimerotropis* is not monophyletic. Similar results can be deduced from the tree reported by Fries *et al.* (2007). However, the phylogenetic position of *Trimerotropis pristinaria*, a species classified on cytological grounds to section A, of *Circotettix carlinianus* and *T. pallidipennis*, both classified to section B, supports the hypothesis proposed by White (1948): species belonging to the last section would derive from the first section, and thus the genus *Trimerotropis* would be paraphyletic. Within *T. pallidipennis* species, it is clearly seen that branches conductive to North American individuals of *T. pallidipennis pallidipennis* and to *T. pallidipennis andeana* are genetically more divergent compared to those connecting samples from Argentina. This result questions the species status of *T. pallidipennis*: *i.e.*, the genetic cohesion of samples from North and South America.

A more thorough analysis including more trimerotropine species, especially those from South America, and more individuals and populations of *T. pallidipennis* and its different subspecies, will be necessary to give stronger support to these results, a task that is currently being undertaken.

Conclusion

The karyotype is evolutionarily conservative, changes slowly with time, and its nature may be a better reflection of phylogeny than morphological (exophenotypic) characters (Weissman & Rentz 1980). In agreement with this idea, more than 50 y ago, the brilliant cytogeneticist M.J.D. White was able to propose several hypotheses about the phylogenetic relationships of the Oedipodine, and colonization routes followed by *T. pallidipennis*, based solely on microscopic observations and his knowledge of the distribution of the species. Today we have far more sophisticated tools that allow us to support White's proposals.

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References

- Andolfatto P., Depaulis F., Navarro A. 2001. Inversion polymorphisms and nucleotide variability in *Drosophila*. *Genetical Research* 77: 1-8.
- Antonacci F., Kidd J.M., Marques-Bonet T., Ventura M., Siswara P., Jiang Z., Eichler E.E. 2009. Characterization of six human disease-associated inversion polymorphisms. *Human Molecular Genetics* 18: 2555-2566.
- Avise J.C., 2000. *Phylogeography: the History and Formation of Species*. Harvard University Press, Cambridge, MA.
- Balanya J., Oller J. M., Huey R. B., Gilchrist G. W., Serra L. 2006. Global genetic change tracks global climate warming in *Drosophila subobscura*. *Science* 313: 1773-1775.
- Bansal V., Bashir A., Bafna V. 2007. Evidence for large inversion polymorphisms in the human genome from HapMap data. *Genome Research* 17: 219-30.
- Brown K.M., Burk L.M., Henagan L.M., Noor M.A. 2004. A test of the chromosomal rearrangement model of speciation in *Drosophila pseudoobscura*. *Evolution* 58: 1856-1860.
- Butlin R.K. 2005. Recombination and speciation. *Molecular Ecology* 14: 2621-2635.
- Carbonell C.S. 1977. Origin, evolution and distribution of the neotropical acridomorph fauna (Orthoptera): a preliminary hypothesis. *Revista de la Sociedad Entomológica Argentina* 36: 153-175.
- Carothers E. 1917 The segregation and recombination of homologous chromosomes as found in two genera of Acrididae (Orthoptera). *Journal of Morphology* 28: 445-494.
- Carothers E. 1921 Genetical behavior of heteromorphic homologous chromosomes of *Circotettix* (Orthoptera). *Journal of Morphology* 35: 457-483.
- Catchen J.M., Conery J.S., Postlethwait J.H. 2009. Automated identification of conserved synteny after whole-genome duplication. *Genome Research* 19: 1497-1505.
- Clarke B. 1975. The contribution of ecological genetics to evolutionary theory: detecting the direct effects of natural selection on particular polymorphic loci. *Genetics* 79 (Supplement): 101-113.
- Coleman L.C. 1948. The cytology of some Western species of *Trimerotropis* (Acrididae). *Genetics* 33: 519-528.
- Colombo P.C. 2002. Chromosome inversion polymorphisms influence morphological traits in *Trimerotropis pallidipennis* (Orthoptera). *Genetica* 114: 247-252
- Colombo P.C., Confalonieri V.A. 1996. Adaptive pattern of inversion polymorphism in *Trimerotropis pallidipennis*. Correlation with environmental variables: an overall view. *Hereditas* 125: 289-296.
- Colombo P.C., Confalonieri V.A. 2004. Cytogeography and the evolutionary significance of B-chromosomes in relation to inverted rearrangements in a grasshopper species. *Cytogenetics and Genome Research* 106: 351-358.
- Colombo P., Cigliano M.M., Sequeira A., Lange C., Vilardi J.C., Confalonieri V.A. 2005. Phylogenetic relationships in *Dichroplus* Stal (Orthoptera: Acrididae: Melanoplinae) inferred from molecular and morphological data: testing karyotype diversification. *Cladistics* 21: 375-389.
- Confalonieri V.A. 1988. Effects of centric-shift polymorphisms on chiasma conditions in *Trimerotropis pallidipennis* (Oedipodinae: Acrididae). *Genetica* 76: 171-179.
- Confalonieri V.A. 1992. B-chromosomes of *Trimerotropis pallidipennis* (Oedipodinae: Acrididae): new effects on chiasma conditions. *Caryologia* 45: 145-153.
- Confalonieri V.A. 1994. Inversion polymorphism and natural selection in *Trimerotropis pallidipennis*: correlations with geographical variables. *Hereditas* 121: 79-86.
- Confalonieri V.A. 1995. Macrogeographic patterns in B-chromosomes and inversion polymorphisms of the grasshopper *Trimerotropis pallidipennis*. *Genetic Selection Evolution* 27: 305-311.
- Confalonieri V.A., Colombo P. 1989. Inversion polymorphisms in *Trimerotropis pallidipennis* (Orthoptera): clinal variation along an altitudinal gradient. *Heredity* 62: 107-112
- Confalonieri V.A., Sequeira A.S., Todaro L., Vilardi J.C. 1998. Mitochondrial DNA phylogeography of the grasshopper *Trimerotropis pallidipennis* in relation to clinal distribution of chromosome polymorphisms. *Heredity* 81: 444-452.
- Delprat A., Negre B., Puig M., Ruiz A. 2009. The transposon Galileo generates natural chromosomal inversions in *Drosophila* by ectopic recombination. *PLoS ONE* 4(11): e7883. doi:10.1371/journal.pone.0007883.
- Dinghi P., Confalonieri V.A., Cigliano M.M. 2009. Phylogenetic studies in the South American tribe Dichroplini (Orthoptera: Acrididae: Melanoplinae): is the Paranaense-Pampeano informal genus group a natural clade? *Zootaxa* 2174: 51-62.
- Dobzhansky T. 1937. *Genetics and the Origin of Species*. Columbia University Press, New York.
- Dobzhansky T., Ayala F., Stebbins G.L., Valentine I.W. 1980. *Evolución*. Ediciones Omega, S.A. Barcelona.
- Eades D.C., Otte D., Cigliano M.M., Braun H. 2010. Orthoptera Species File Online. Version <http://orthoptera.speciesfile.org>.
- Endler J.A. 1986. *Natural selection in the wild*. Princeton University Press, Princeton, New Jersey.
- Feder J.L., Nosil P. 2009 Chromosomal inversions and species differences: when are genes affecting adaptive divergence and reproductive isolation expected to reside within inversions? *Evolution* 63: 3061-3075.
- Fletcher H.L., Hewitt G.M. 1978 Non-homologous synaptonemal complex formation in a heteromorphic bivalent in *Keyacris scurra* (Morabinae, Orthoptera). *Chromosoma* 65: 271-281.
- Fries M., Chapco W., Contreras D. 2007. A molecular phylogenetic analysis of the Oedipodinae and their intercontinental relationships. *Journal of Orthoptera Research* 16: 115-125.
- Goñi B., de Vaio E., Beltrami M., Leira M., Crivel M., Panzera F., Castellanos P., Basso A. 1985. Geographic patterns of chromosomal variation in populations of the grasshopper (*Trimerotropis pallidipennis*) from southern Argentina. *Genome* 27: 259-271.
- Hale L.R., Singh R.S. 1991. A comprehensive study of genetic variation in natural populations of *Drosophila melanogaster*. IV. Mitochondrial DNA variation and the role of history vs selection in the genetic structure of geographic populations. *Genetics* 129: 102-117.
- Helwig R. 1929 Chromosomal variations correlated with geographical distribution in *Circotettix verruculatus* (Orthoptera). *Journal of Morphology* 47: 1-36.
- Hey J. 2003. Speciation and inversions: chimps and humans. *Bioessays* 25: 825-828.
- Hewitt G.M. 1988. Hybrid zones: natural laboratories for evolutionary studies. *Trends in Ecology and Evolution* 3: 158-167.
- Hoffmann A.A., Rieseberg L.H. 2008. Revisiting the impact of inversions in evolution: from population genetic markers to drivers of adaptive shifts and speciation. *Annual Reviews Ecology Evolution and Systematics* 39: 21-42.
- Hoffmann A.A., Sgro C.M., Weeks A.R. 2004. Chromosomal inversion polymorphisms and adaptation. *Trends in Ecology and Evolution* 19: 482-488.
- John B., Weissman D.B. 1977 Cytogenetic components of reproductive isolation in *Trimerotropis thalassica* and *T. occidentalis*. *Chromosoma* 60: 187-203.
- King R.L., 1923. Heteromorphic homologous chromosomes in three species of *Pseudotrimerotropis* (Orthoptera: Acrididae). *Journal of Morphology* 38: 19-63.
- Kirsten M. Brown, Lisa M. Burk, L.M. Henagan, Noor M.A.F. 2004. A test of the chromosomal rearrangement model of speciation in *Drosophila pseudoobscura*. *Evolution* 58: 1856-1860.
- Korbel J.A., Ekehart Urban A., Affourtit J. P., Godwin B., Grubert F., Simons J.F., Kim P. M., Palejev D., Carriero N. J., Lei Du, Taillon B. E., Chen Z., Tanzer A., Saunders A. C. E., Chi J., Yang F., Carter N. P., Hurles M. E., Weissman S.M., Harkins T. T., Gerstein M.B., Egholm M., Snyder M. 2007. Paired-end mapping reveals extensive structural variation in the human genome. *Science* 318: 420-426.

- Kulathinal R.J., Stevison L.S., Noor M.A.F. 2009. The genomics of speciation in *Drosophila*: diversity, divergence, and introgression estimated using low-coverage genome sequencing. *PLoS Genetics* 5(7): e1000550. doi: 10.1371/journal.pgen.1000550.
- Laayouni H., Hasson E., Santos M., Fontdevila A. 2003. The evolutionary history of *Drosophila buzzatii*. XXXV. Inversion polymorphism and nucleotide variability in different regions of the second chromosome. *Molecular Biology. Evolution* 20: 931-44.
- Lanteri A.A., Confalonieri V.A. 2003. Filogeografía: objetivos, métodos y ejemplos. In: Llorente Bousquets J., Morrone J.J. (Eds) *Introducción a la Biogeografía en Latinoamérica: Conceptos, teorías, métodos y aplicaciones*. vol II, pp 185-193 (Facultad de Ciencias, UNAM, México).
- Lee J., Han K., Meyer T.J., Kim H-S, Batzer M.A. 2008. Chromosomal inversions between human and chimpanzee lineages caused by retrotransposons. *PLoS ONE* 3(12): e4047. doi:10.1371/journal.pone.0004047.
- Luo M.C., Deal K.R., Akhunov E.D., Akhunova A.R., Anderson O.D., Anderson J.A., Blake N., Clegg M.T., Coleman-Derr D., Conley E.J., Crossman C.C., Dubcovsky J., Gill B.S., Gu Y.Q., Hadam J., Heo H.Y., Huo N., Lazo G., Ma Y., Matthews D.E., McGuire P.E., Morrell P.L., Qualset C.O., Renfro J., Tabanao D., Talbert L.E., Tian C., Toleno D.M., Warburton M.L., You F.M., Zhang W., Dvorak J. 2009. Genome comparisons reveal a dominant mechanism of chromosome number reduction in grasses and accelerated genome evolution in Triticeae. *PNAS* 106: 15780-15785.
- Matrajt M., Confalonieri V.A., Vilardi J. 1996. Parallel adaptive patterns of allozyme and inversion polymorphisms on an ecological gradient. *Heredity* 76: 346-354.
- Mesa A. 1971. Polimorfismo cromosómico en *Trimerotropis pallidipennis* (Orthoptera-Acridoidea-Oedipodinae). *Revista Peruana de Entomología* 14: 2.
- Moran C., Shaw D.D. 1977. Population cytogenetics of the genus *Caledia* (Orthoptera: Acrididae). III. Chromosomal polymorphism, racial parapatry and introgression. *Chromosoma* 63: 181-204.
- Moran C. 1979. The structure of a narrow hybrid zone in *Caledia captiva*. *Heredity* 42: 13-32.
- Nur U. 1968. Synapsis and crossing over within paracentric inversions in the grasshopper *Camnula pellucida*. *Chromosoma* 25: 198-214.
- Otte D. 1984. *The North American Grasshoppers*, volume II, Acrididae, Oedipodinae. Harvard Press.
- Powell J.R. 1997. *Progress and Prospects in Evolutionary Biology: the Drosophila Model*. Oxford University Press, New York.
- Rentz D.C.F., Weissman D.B. 1980. An annotated checklist of the grasshopper species of *Aerchoreutes*, *Circotettix*, and *Trimerotropis* (Orthoptera: Acrididae: Oedipodinae). *Transactions American Entomological Society* 106: 223-252.
- Sanchez V., Confalonieri V.A. 1993. Chromosome banding pattern in *Trimerotropis pallidipennis* (Orthoptera: Acrididae). *Cytobios* 73: 105-110.
- Scatagliani M.A., Lanteri A.A., Confalonieri V.A. 2006. Diversity of boll weevil populations in South America: a phylogeographic approach. *Genetica* 126: 353-362.
- Shaw D.D., Webb G.C., Wilkinson P. 1976. Population cytogenetics of the genus *Caledia* (Orthoptera: Acrididae). II. Variation in the pattern of C-banding. *Chromosoma* 56: 169-190.
- Shaw D.D., Coates D.J., Arnold M.L. 1988. Complex patterns of chromosomal variation along a latitudinal cline in the grasshopper *Caledia captiva*. *Genome* 30: 108-117.
- Shaw D.D., Moran C., Wilkinson P. 1980. Chromosomal rearrangements, geographic differentiation and the mechanism of speciation in the genus *Caledia*, pp 171-194. In: Blackman R.L., Hewitt G.M., Ashburner M. (Eds) *Insect Cytogenetics*. Blackwell Scientific Publications, Oxford.
- Slotman M.A., Reimer L.J., Thiemann T., Dolo G., Fondjo E. 2006. Reduced recombination rate and genetic differentiation between the M and S forms of *Anopheles gambiae* s.s. *Genetics* 174: 2081-2093.
- Slotman M.A., Tripet F., Cornel A. J., Meneses C. R., Lee Y., Reimer L. J., Thiemann T. C., Fondjo E., Fofana A., Traore S.F. and Lanzaro G.C. 2007. Evidence for subdivision within the M molecular form of *Anopheles gambiae*. *Molecular Ecology* 16: 639-649.
- Trick M., Kwon S., Ryun Choi S., Fraser F., Soumpourou E., Drou N., Wang Z., Lee S.Y., Yang T., Mun J., Paterson A.H., Town C.D., Pires J.C., Lim Y.P., Park B., Bancroft I. 2009. Complexity of genome evolution by segmental rearrangement in *Brassica rapa* revealed by sequence-level analysis. *BMC Genomics* 10: 539.
- Vaio E. S., Goñi B., Rey C. 1979. Chromosome polymorphism in populations of the grasshopper *Trimerotropis pallidipennis* from Southern Argentina. *Chromosoma* 71: 371-386.
- Weissman D.B. 1976. Geographical variability in the pericentric inversion system of the grasshopper *Trimerotropis pseudofasciata*. *Chromosoma* 55: 325-347.
- Weissman D.B., Rentz D.C.F. 1980. Cytological, morphological, and crepitational characteristics of the trimerotropine (*Aerchoreutes*, *Circotettix*, and *Trimerotropis*) grasshoppers (Orthoptera: Oedipodinae). *Transactions American Entomological Society* 106: 253-272.
- White M.J.D. 1945. *Animal Cytology and Evolution* (1st ed.) Cambridge University Press, New York.
- White M.J.D. 1949. A cytological survey of wild populations of *Trimerotropis* and *Circotettix* (Orthoptera: Acrididae) I. The chromosomes of twelve species. *Genetics* 34: 537-563.
- White M.J.D. 1951a. Cytogenetics of orthopteroid insects. *Advances in Genetics* 4: 267-328.
- White M.J.D. 1951b. Structural heterozygosity in natural populations of the grasshopper *Trimerotropis sparsa*. *Evolution* 5: 376-394.
- White M. J. D. 1956. Adaptive chromosomal polymorphism in an Australian grasshopper. *Evolution* 10: 298-313.
- White M.J.D. 1961. Cytogenetics of the grasshopper *Moraba scurra*. VI. A spontaneous pericentric inversion. *Australian Journal of Zoology* 9: 784-790.
- White M.J.D. 1969. Chromosomal rearrangements and speciation in animals. *Advances in Genetics* 3: 75-98.
- White M.J.D. 1973. *Animal Cytology and Evolution*, 3rd edit. Cambridge University Press, London.
- White M.J.D. 1977. *Modes of Speciation*. San Francisco: W.H. Freeman, 1977.
- White M.J.D., Lewontin C., Andrew L.E. 1963. Cytogenetics of the grasshopper *Moraba scurra*. VII. Geographic variation of adaptive properties of inversions. *Evolution* 17: 147-162.
- White M.J.D., Carson H.L., Cheney J. 1964. Chromosomal races in the Australian grasshopper *Moraba viatica* in a zone of geographic overlap. *Evolution* 18: 417-429.
- White M.J.D., Chinnick L. 1957. Cytogenetics of the grasshopper *Moraba scurra*. III. Distribution of the 15- and 17-chromosome races. *Australian Journal of Zoology* 5: 338-347.
- White M.J.D., Key K.H.L., Andrew M., Cheney J. 1969. Cytogenetics of the viatica group of Morabine grasshoppers. II. Kangaroo Island populations. *Australian Journal of Zoology* 17: 313-328.
- Wu F., Tanksley S.D. 2010. Chromosomal evolution in the plant family Solanaceae. *BMC Genomics* 11: 182.