

Against the central-marginal model: Three cases in chromosomally polymorphic grasshoppers (Orthoptera: Acrididae)

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Key words. Orthoptera, Acrididae, central-marginal model, chromosomal polymorphisms, grasshoppers, recombination, environmental gradient

Abstract. The central-marginal model is widely accepted in chromosomally polymorphic species of *Drosophila*. In fact, geographically and ecologically central populations of *Drosophila* show higher levels of polymorphism for paracentric inversions, whereas marginal populations tend to be monomorphic. This fact has been variously explained. Chromosomal polymorphisms in grasshoppers have also been attributed to show such geographical structuring, as in the case of the South-American grasshopper *Dichroplus pratensis* Bruner (Orthoptera: Acrididae). However, in three other cases involving Acrididae – *Leptysma argentina* Bruner, *Trimerotropis pallidipennis* (Burmeister) and *Cornops aquaticum* (Bruner), it is clear that chromosomal polymorphisms (sometimes with a wide extension over the Argentine area) do not conform to this pattern, and show instead clear correlations with environmental variables, especially minimum temperature, showing low or null frequencies of the rearrangements at one extreme of the environmental gradient and with high or fixed frequencies at the other. Furthermore, this correlation with temperature might also be true in the case of *D. pratensis*. These aforementioned examples emphasise the dangers of over-generalization when discussing chromosomal polymorphisms, and suggests that such polymorphisms should be considered very much in a case-specific manner in terms of the particular genetic system under study.

INTRODUCTION

The central-marginal model was proposed in the 1950s in order to explain the distribution of pericentric inversion polymorphisms in different species of *Drosophila* (Carson, 1955, 1959; da Cunha et al., 1959). It relates to the fact that ecologically and geographically marginal populations of *Drosophila* are less polymorphic – or indeed monomorphic – whereas central populations display a wealth of chromosomal variability. In organisms other than *Drosophila*, such as the grasshopper *Dichroplus pratensis* Bruner (Orthoptera: Acrididae), with a complex system of Robertsonian translocations whose effect resembles an inversion polymorphism in the sense that they reduce recombination in carriers of such rearrangements, the authors also claim the existence of a central-marginal pattern related to geographical-based structuring in several populations over a wide area in Argentina (Bidau & Marti, 2002, 2005).

However, according to my experience with polymorphic rearrangements in grasshoppers, I have never found a central-marginal pattern; rather, clinal variation was observed in all cases, a situation which may rather be correlated with environmental variables. In the present study, I provide examples of New World grasshoppers of the family Acrididae, namely *Leptysma argentina* Bruner, *Trimerotropis pallidipennis* (Burmeister) and *Cornops aquaticum* (Bruner), all of which display polymorphisms for different recombination-reducing chromosome rearrangements, and where the central-marginal pattern is

clearly not applicable. *L. argentina* is a semiaquatic grasshopper that lives and lay its eggs in Cyperaceae (it is a Leptysmine grasshopper and thus its oviposition is endophytic). Its habitat extends from Central Argentina and Uruguay to Southern Brazil (Amedegnato, 1974; Roberts, 1978). *T. pallidipennis* is an Oedipodinae grasshopper with a New World distribution (White, 1951). It inhabits desert and semidesert areas in North and South America. Lastly, *C. aquaticum* is another Leptysmine grasshopper whose distribution ranges from Southern Mexico to Central Argentina and Uruguay (Adis & Junk, 2003; Adis et al., 2004, 2008). It feeds and lays eggs exclusively in water-hyacinths (Pontederiaceae) and hence has been considered and studied as a potential control agent for water-hyacinths growing plague proportions in other tropical and subtropical regions of the world, mainly in Africa (e.g. Oberholzer & Hill, 2001). The comparative study of chromosome polymorphisms of these ecologically widely different species is important because, despite their differences, similar features emerge when their genetic systems are being compared, especially the relationship between recombination reducing chromosome polymorphisms and environmental variables.

It is known that clinal patterns may be indirect evidence of natural selection taking place in wild populations (Endler, 1977, 1986). A remarkable case was that of *Drosophila subobscura* Collin & Gordon, where clinal patterns of inversion distribution correlated with temperature were detected (Powell, 1997); later, this pattern was

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repeatedly found in two other continents in South (Brncic & Budnik, 1980) and North America (Beckenbach & Prevosti, 1986), further suggesting an involvement of temperature adaptation in this cline. Finally, Rego et al. (2009) provided evidence of associations between inversion polymorphisms related to thermal preferences and heat stress resistance in this species. In our case, the kind of evidence presented in order to suggest temperature adaptation is a primary one, namely a correlation of rearrangement frequency with environmental variables. No matter how indirect this evidence may be, it is an indication that clinal variation is a widespread phenomenon; further research is needed in order to more firmly link the environmental variables with the chromosomal clines here described.

The present study has two main aims: (1) to show examples of clinal variation in Acridid grasshoppers and demonstrate that this pattern may result from adaptation to environmental factors, and (2) to compare two different patterns in these animals, i.e. the clinal variation and the central-marginal model, which is not always the best fitting explanation for chromosome polymorphism cytogeography in natural populations.

MATERIAL AND METHODS

In *Leptysma argentina*, 248 male individuals from 9 populations from central-eastern Argentina that bore different frequencies for the 3/6 Robertsonian translocation (i.e., fusion between acrocentrics 3 and 6 of the basic complement) were previously (karyologically analysed Colombo, 1989) (Table 1, Fig. 1). In *Cornops aquaticum*, 160 individuals from 6 populations along a latitudinal gradient (Colombo, 2008) were chromosomally analysed and the frequencies of the 1/6, 2/5 and 3/4 Robertsonian translocations recorded in an earlier work (Colombo, 2008) (Table 3, Fig. 2). In *Trimerotropis pallidipennis* (see Note added in proof following Discussion), 366 individuals from 20 populations along latitudinal and altitudinal gradients in the arid and semiarid regions in Argentina were cytologically studied and the seven different pericentric inversions karyotyped in earlier studies (see maps and frequencies in Confalonieri & Colombo, 1989; Confalonieri, 1994; Colombo & Confalonieri, 1996) (Fig. 3). The cytological studies in the published work were conducted in all cases through squashes in propionic haematoxylin or lactopropionic orcein and karyotypes determined at metaphase I. Statistical analyses in the present study were performed using the STATISTICA 6.0 package (Statistic Statsoft Inc., Tulsa, OK, USA).

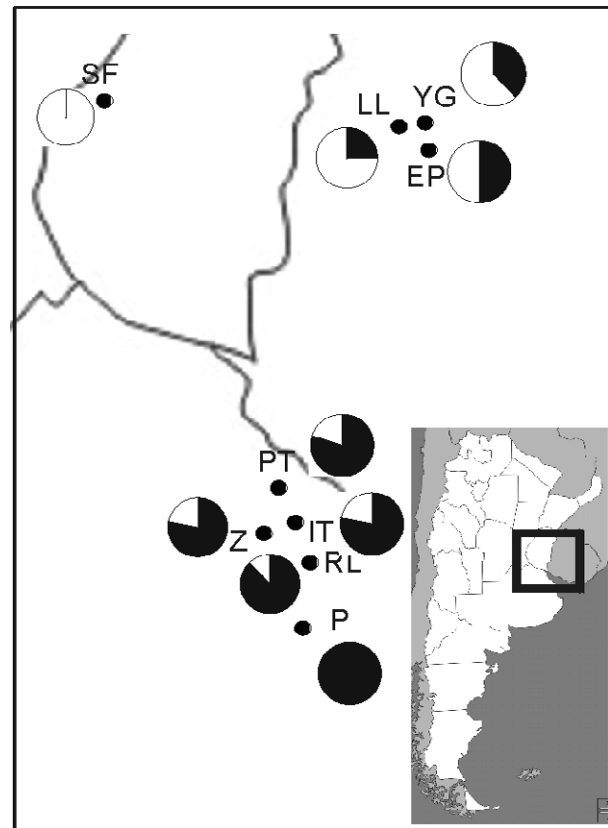


Fig. 1. Map of the distribution and fusion 3/6 frequency of the *Leptysma argentina* populations studied in this work. Pie diagrams illustrate (in black) the frequencies (see Table 1). SF – Santa Fe; LL – Los Loros; YG – Yará Guazú; EP – El Palmar; PT – Puerto Talavera; IT – Isla Talavera; Z – Zárte; RL – Río Luján; P – Pilar.

RESULTS

Leptysma argentina

L. argentina has a standard karyotype of $2n = 21$ in males, with an X0/XX sex determination system (Bidaú & Hasson, 1984). In almost all populations so far studied there is a polymorphism for a Robertsonian translocation (= centric fusion) between chromosome 3 and 6 of the standard complement (fusion 3/6) (Colombo, 1989). In the present study, 248 male individuals were analysed for the frequency of this rearrangement in nine Argentine populations. The frequencies, along with coordinates,

TABLE 1. Populations sampled, latitude, longitude, frequencies of 3/6 chromosome fusion (F), average annual minimum temperature (Tmin), and average annual rainfall in 9 populations of *Leptysma argentina*.

Population	Latitude	Longitude	F	Tmin	Rainfall
Santa Fe	31°40'S	60°46'W	0.00	12.11	981
Los Loros	31°52'S	58°17'W	0.25	11.93	1193.64
Yará Guazú	31°54'S	58°15'W	0.38	11.93	1193.64
El Palmar	31°57'S	58°19'W	0.50	11.93	1193.64
Zárte	34°02'S	59°12'W	0.78	11.06	1044
Puerto Talavera	33°46'S	58°36'W	0.80	11.06	1167
Isla Talavera	34°05'S	59°00'W	0.80	11.06	1167
Río Luján	34°17'S	58°53'W	0.88	10.08	1152.6
Pilar	34°27'S	58°54'W	1.00	9.38	1044

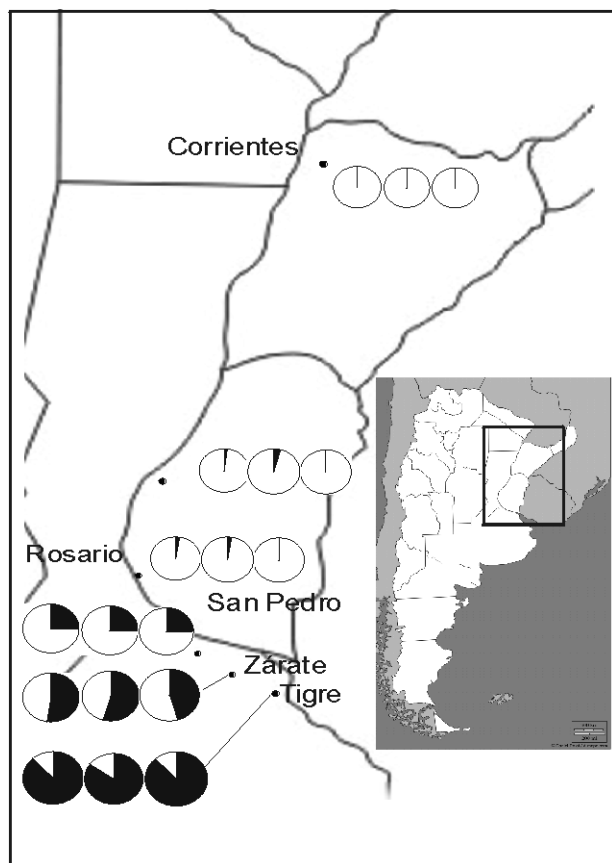


Fig. 2. Map of the distribution of fusion frequencies of the *Cornops aquaticum* populations studied in this work. For each population the three pie diagrams illustrate (in black) the fusion frequencies of fusions 1/6, 2/5 and 3/4 (from left to right, respectively) (see Table 3).

minimum temperature and rainfall, are shown in Table 1 and Fig. 1. Minimum temperature (T_{min}) and rainfall were chosen as environmental variables due to our own experience in organisms such as *T. pallidipennis*, where these two variables are the only ones to correlate with inversion frequencies. Furthermore, Powell (1997) has stated that in *Drosophila* these are the variables that mostly correlate with inversion frequencies.

This seems to be the case in *L. argentina* too (Table 2), only that in this case rainfall shows a marginal significance ($P = 0.08$) in the regression between T_{min} and rainfall (independent variables) with fusion 3/6 frequency (dependent variable). Instead, regression with T_{min} is negative and highly significant ($P = 0.0016$). In fact, populations with the highest temperatures have a low frequency for the centric fusion or are outright monomorphic (Table 1, Fig. 1). The 3/6 fusion has a high

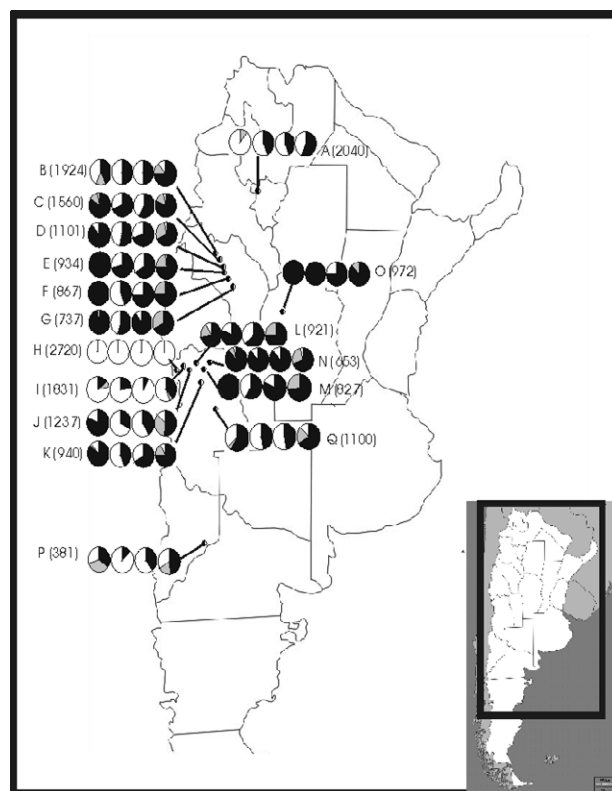


Fig. 3. Map of the distribution and inversion frequencies of the *Trimerotropis pallidipennis* populations studied in the work. For each population the pie diagrams illustrate, in white, the frequency of the standard acrocentric arrangements, in black that of the most frequent inversion and in grey that of other arrangements, in chromosomes 4, 6, 7 and 8 (from left to right, respectively). The altitude in each population is given between parentheses. A – Ampimpa; B – Las Gredas; C – Famatina; D – Chilecito; E – Nonogasta; F – Vichigasta; G – Catanzaco; H – Puente del Inca; I – Uspallata; J – Cacheuta; K – San Carlos; L – Chacras de Coria; M – Mendoza; N – San Martín; O – Capilla del Monte; P – El Chocón; Q – Cañón del Atuel.

frequency in southern populations, where the temperature is lower. In the southernmost population (Pilar) fusion 3/6 is fixed.

Cornops aquaticum

Cornops aquaticum has a standard karyotype of $2n = 23$ acrocentric chromosomes in males, with an X0/XX sex determination system (Rocha et al., 2004; Colombo, 2008). Mesa (1956) and Mesa et al. (1982) stated that there were at least three Robertsonian translocations in various polymorphisms in different populations of this species, but they were not described nor otherwise studied. Rocha et al. (2004) described the standard karyotype

TABLE 2. *Leptysma argentina*. Multiple regression of rainfall vs. T_{min} (independent variables) and fusion 3/6 frequency (independent variable). β – multiple index of regression; SE – standard error; B – individual index of regression; $t(6)$ – level of the parameter of distribution t of Student with 6 degrees of freedom.

	β	SE	B	SE	$t(6)$	P -level
T_{min}	-0.953851	0.168041	-0.312488	0.055051	-5.67631	0.001288
Rainfall	0.352801	0.168041	0.017486	0.008329	2.09950	0.080534

TABLE 3. Populations sampled, latitude and longitude, fusion frequency, minimum temperature and rainfall in *Cornops aquaticum*.

Population	Latitude	Longitude	Fusion 1/6	Fusion 2/5	Fusion 3/4	Tmin (in °C)	Rainfall (in mm)
Corrientes	27°28'S	58°83'W	0	0	0	15.70	1389
Santa Fe	31°40'S	60°46'W	0.020	0.042	0	12.11	981
Rosario	32°57'S	60°40'W	0.026	0.026	0	11.78	993
San Pedro	33°40'S	59°41'W	0.250	0.250	0.250	11.44	1167
Zárate	34°02'S	59°12'W	0.521	0.542	0.438	11.06	1044
Tigre	34°26'S	58°36'W	0.884	0.846	0.884	10.54	1101

of this species in a monomorphic population (without fusions) near Pernambuco, Brazil, and Colombo (2007, 2008, 2009) identified the rearrangements as three Robertsonian translocations occurring between acrocentrics 1 and 6 (fusion 1/6), 2 and 5 (fusion 2/5) and 3 and 4 (fusion 3/4). All three fusion frequencies increase along the course of the Paraná River en route to the River Plate; thereby running roughly southwards, thus creating a latitudinal cline (Colombo, 2008). In fact, in the northernmost population (Corrientes) there are no Robertsonian translocations, whereas in the southernmost one (Tigre) frequencies of these rearrangements are high (in the order of 0.85). In the present study, the relationship between fusion frequencies and environmental variables was assessed. If all six populations are included into the double regression analysis, both environmental variables taken into consideration (Tmin and rainfall) show non-significant regressions with fusion 1/6, fusion 2/5 and fusion 3/4 frequencies. But if the population from Corrientes is excluded (justified since this population is situated too far north) and the focus lies into the area of polymorphism in the lower course of the Paraná River (Santa Fe, Rosario, San Pedro, Zárate and Tigre), a clear and significant regression emerges between Tmin and fusion 1/6, 2/5 and 3/4 frequencies (Table 4).

Trimerotropis pallidipennis

The male karyotype of *T. pallidipennis* comprises 23 chromosomes (22 + X0). Pairs 1–3 are long (L), pairs 4–8 are medium-sized (M), and pairs 9–11 are small (S). All three long chromosomes are submetacentric (SM), the X chromosome is metacentric (M), whilst the remainder are basically acrocentric (Vaio et al., 1979). Chromosomes 4, 6, 7, and 8 are polymorphic for seven pericentric inversions that change the basic acrocentric morphology (A)

into many submetacentric (SM1, SM2, SM3, SM4), metacentric (M), and inverted acrocentric (AI) forms (Confalonieri, 1994). The correlation of inversion frequencies with environmental variables (above all Tmin and rainfall) has been presented elsewhere (Confalonieri & Colombo, 1989; Confalonieri, 1994; Colombo & Confalonieri, 1996). However, the findings are mentioned in this section because they are discussed below in the Discussion. The inversion frequencies in selected populations are given in Fig. 3.

DISCUSSION

Correlation of a trait with environmental factors is a common means of detecting natural selection in wild populations (Endler, 1977, 1986, see below for further discussion). If the same correlation is seen in independent groups of populations over a wide area, selection is then one possible explanation. In previous contributions, 17 natural populations of *T. pallidipennis* polymorphic for 3–7 pericentric inversions in 4 medium-sized pairs – showed altitudinal and latitudinal clines for nine inversion karyotypes over a wide area in Argentina. Joint analysis had also shown correlations with altitude, latitude and longitude (Confalonieri & Colombo, 1989; Confalonieri, 1994). Multiple regression analyses among inversion frequencies and environmental variables not only confirmed the consistency of altitudinal gradients for all sequences but also identified minimum temperature (Tmin), among several other environmental variables, as the main climatic variable responsible for this pattern, to such extent that a population with an extreme Tmin value was monomorphic. In addition, some karyotypes correlated with rainfall (Colombo & Confalonieri, 1996). The results indicate that at extremely low Tmin (in the Andes or in

TABLE 4. *Cornops aquaticum*. Multiple regression of rainfall vs. minimum temperature (independent variables) and fusions 1/6, 2/5 and 3/4 frequencies (independent variables). β – multiple index of regression; SE – standard error; B – individual index of regression; $t(6)$ – level of the parameter of distribution t of Student with 6 degrees of freedom.

	β	SE	B	SE	$t(2)$	P-level
a) Fusion 1/6:						
Tmin	-1.02605	0.168620	-0.613486	0.100820	-6.08497	0.025960
Rainfall	-0.08577	0.168620	-0.004861	0.009557	-0.50865	0.661556
b) Fusion 2/5:						
Tmin	-1.02962	0.180555	-0.589028	0.103293	-5.70252	0.029402
Rainfall	-0.09881	0.180555	-0.005358	0.009791	-0.54724	0.639119
c) Fusion 3/4:						
Tmin	-0.987736	0.200648	-0.592646	0.120390	-4.92273	0.038875
Rainfall	-0.028318	0.200648	-0.001611	0.011412	-0.14113	0.900697

southern Patagonia) inversion frequency is zero, whereas at the other extreme of T_{min} distribution inversions are fixed (frequency equal to one). No hint of a central-marginal pattern was found here.

L. argentina is a South American species which contains populations that are polymorphic for a centric fusion between pairs 3 and 6 (fusion 3/6). This rearrangement reveals consistent effects on chiasma frequency and/or distribution (Colombo 1990, 1993a), whilst morphometrical analyses established that fusion 3/6 is positively correlated with increased body size (Colombo, 1989). Here again, as in the case of the pericentric inversions of *T. pallidipennis*, recombination is reduced in heterozygotes, due to a decrease in chiasma frequency in the trivalent alongside with the displacement of chiasmata to distal positions (Colombo, 1990), and also because of the automatic reduction in number of chromosome combinations in gametes due to the joining of two linkage groups into one. This twofold reduction of recombination, unlike inversions, is maintained in fusion homozygotes. The negative correlation of the rearrangement with minimum temperature, as found in the present study, again proves to be the best indicator of fusion frequency in the area of chromosome polymorphism.

As with the case of *T. pallidipennis*, the area of lowest recombination was the southernmost extreme of the species' distribution, showing no hint of the central-marginal pattern; rather, as in the previous case, it yields an environmental cline.

Cornops aquaticum occurs in the New World from latitude 23° N (South of Mexico) to latitude 35° S (Uruguay and central Argentina). It is an obligate tropical and subtropical species (Adis & Junk, 2003; Adis et al., 2004, 2008), so it is remarkable that in the southernmost margin of its distribution (the lower course of the Paraná river) a parallel polymorphism of three polymorphic centric fusions occurs (Colombo, 2008) that also provokes, as is usually the case in Robertsonian rearrangements (Colombo, 2012), a strong intra-chromosomal drop of chiasma frequency both in fusion heterozygotes and homozygotes, along with a shift of chiasmata to distal positions (Colombo, 2007). It is clear that the drop in recombination arises in the margin, and not in the center, of the species' distribution (which would correspond to the Amazonian basin). Interestingly enough, in Central Brazil there are no centric fusions (Rocha et al., 2004). In the present study, as in *L. argentina*, fusion frequency is inversely correlated with temperature; it may again be a case of an environmental cline. Microsatellite (DNA) marker studies in the area displaying the rearrangements revealed that these selectively neutral markers show no environmental cline, so here it is clear that centric fusion distribution may respond to selective pressures due to the environment (Romero et al., 2011). No central-marginal pattern is evident.

In all these cases, the ultimate evidence of adaptiveness would be the finding of the non-neutral loci responsible for temperature adaptations and its linkage with the rearrangements; until then, adaptiveness is a good working

hypothesis. As mentioned by Endler (1986), correlation with environmental variables is the first indicator of natural selection operating in the wild, other evidence being departures from null models (i.e. Hardy Weinberg or gametic equilibrium) or differences between age classes. In the case of *L. argentina*, all of these indications were found (Colombo, 1993b; Colombo et al., 2004), only that the “smoking gun” of temperature as a cause for karyotype frequency distribution has not yet been detected. A stronger hint is given in the case of *T. pallidipennis*, since the cline is not only latitudinal but also altitudinal (Confalonieri & Colombo, 1989; Confalonieri, 1994; Colombo & Confalonieri, 1996).

With no definite link between clines and environment, it must be reckoned that other factors may be responsible for the clinal variation. In fact, two chromosome races with hybridisation and only weak selection against heterozygotes would create a chromosome cline (McAllister, 2002; McAllister et al., 2008). However, both *L. argentina* and *C. aquaticum*'s trivalents of structural heterozygotes were studied for their orientation in metaphase I and the result was more than 97% of “disjunctional orientation”, i.e. the only configuration that can create balanced gametes (Colombo, 2009). Furthermore, in such cases there may be reorientation of the trivalent, thus raising the percentage of viable gametes (Bidau & Mirol, 1988); effectively, all of the metaphase II plates of *C. aquaticum* were euploid, thus suggesting that negative selection on heterozygotes may be extremely slight or nonexistent. In the case of the paracentric inversions of *T. pallidipennis*, it is known that the heterozygotes for these rearrangements may be affected by deficiencies and duplications when there is homologous pairing, thus creating inviable gametes (White, 1973); however, pairing in inversion heterozygotes among grasshoppers is always non-homologous and straight (Diez & Santos, 1993), arguing that selection against heterozygotes does not occur. Besides, the patchy distribution of *T. pallidipennis*' standard arrangements following altitudinal clines further reduces this suggestion (Confalonieri & Colombo 1989; Confalonieri, 1994).

A further objection raised against clinal variation as evidence of adaptive selection is that the rapid dispersion of chromosomally-unstable species may create clinal patterns, as in the case of the wood white butterfly, *Leptidea sinapis* L. (Lukhtanov et al., 2011). However, this is a very special case because it smacks of chromosomal speciation, given that lepidopterans have holocentric chromosomes (Lukhtanov & Kuznetsova, 2009). In fact, as recently demonstrated, fusions/fissions of holocentric chromosomes restrict gene flow, whilst their effects are cumulative (Hipp et al., 2010). This is clearly not the case in grasshoppers, with their large monocentric chromosomes; additionally, a precondition of chromosome evolution is negative heterosis of the rearrangements (King, 1993); as previously mentioned, the rearrangements described here have no discernable negative heterosis (see above).

D. pratensis, like the other Acridid grasshopper species here discussed, is a New World (specifically South-American) species which bears a complex system of Robertsonian translocations with seven different centric fusions which form different chromosome races. In the central area (the Pampas) there is a maximum of chromosome variation, including a highly interesting hybrid zone with the formation of multivalents (see Bidau et al., 2003, for a review). The Robertsonian translocations of *D. pratensis* cause, as usual, a reduced recombination rate due to the proximal and interstitial chiasma frequency reduction in the chromosomes involved in the rearrangements; besides, the loss of independent segregation in anaphase I also impinges on overall genetic recombination (Bidau & Mirol, 1988; Bidau, 1990, 1993; Bidau et al., 1991). Chromosomal studies of many populations from Argentina (Bidau & Martí, 1995; Martí & Bidau, 1998) was performed on both sexes alike and no difference in chiasma patterns nor of geographic differentiation was noticed.

The geographical distribution of the rearrangements was studied in *D. pratensis*, and the authors proposed a central-marginal pattern (Bidau & Martí, 2002, 2005); in fact, the central populations from the Pampas, where this species is more abundant, have a higher frequency of centric fusions than those from northern populations (sited in the Andes Range) and in Patagonia, where there are marginal or relict populations of this species (Bidau & Martí, 2002). Indeed, some of the marginal populations are monomorphic without fusions. These, according to the authors, are the hallmark of a central-marginal distribution of the Robertsonian translocations (Bidau & Martí, 2005).

The case of *D. pratensis* seems different to those of the aforementioned species. For a start, there are very few populations with fixed centric fusions, i.e. populations where the standard acrocentrics are not found (except for a few in the highly anomalous region of the hybrid zone, Bidau, 1991; Tosto & Bidau, 1991) and most of the central populations are highly polymorphic, as predicted by the central-marginal model. However, the studies overlook one feature: they do not examine the correlation between fusion frequency and environmental variables. When temperature is taken into account (judging from the published fusion frequencies and latitude and altitude, Bidau & Martí, 2002), a clear correlation emerges between minimum temperature and average number of fusions per population. Admittedly, the authors have a point since, from the scatterplot, it is easily seen that the populations from the central temperatures still stray apart from the line and into higher fusion frequencies. I am not claiming that the authors are wrong in proposing a central-marginal pattern in this case. Even so, caution is here necessary and all variables should be considered. Clearly, correlation with environmental variables has not previously been taken into account.

Admitting that the case of *D. pratensis* is one revealing a clear central-marginal pattern, it is obvious that the polymorphisms of the other species described are far

removed from this situation. Instead, they clearly correlate with environmental variables, most of all with minimum temperature. This suggests that the central-marginal model is not automatically applicable to all chromosome polymorphisms, but that in some cases environmental clines are the reason of the geographic distribution of chromosome polymorphisms in natural populations.

Note added in proof: The species *Trimerotropis pallidipennis* is currently undergoing revision. The variety that lives in arid and semiarid regions of Argentina will be given species rank (M.M. Cigliano, pers. comm.). Therefore our study covers almost the whole area of distribution of this new species.

ACKNOWLEDGEMENTS. The author wishes to express gratitude to two anonymous reviewers and H.D. Loxdale whose advice significantly improved the quality of the manuscript.

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Received January 17, 2012; revised and accepted March 20, 2012