

## REVIEW

# The chromosomal polymorphism of *Drosophila subobscura*: a microevolutionary weapon to monitor global change

J Balanyà<sup>1</sup>, RB Huey<sup>2</sup>, GW Gilchrist<sup>3</sup> and L Serra<sup>1</sup>

<sup>1</sup>Grup de Biologia Evolutiva, Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, Barcelona, Catalonia, Spain;

<sup>2</sup>Department of Biology, University of Washington, Seattle, WA, USA and <sup>3</sup>Department of Biology, College of William & Mary, Williamsburg, VA, USA

The Palaearctic species *Drosophila subobscura* recently invaded the west coast of Chile and North America. This invasion helped to corroborate the adaptive value of the rich chromosomal polymorphism of the species, as the same clinal patterns than those observed in the original Palaearctic area were reproduced in the colonized areas in a relatively short period of time. The rapid response of this polymorphism to environmental conditions makes it a good candidate to measure the effect of the global rising of temperatures on the genetic

composition of populations. Indeed, the long-term variation of this polymorphism shows a general increase in the frequency of those inversions typical of low latitudes, with a corresponding decrease of those typical of populations closer to the poles. Although the mechanisms underlying these changes are not well understood, the system remains a valid tool to monitor the genetic impact of global warming on natural populations.

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*Drosophila subobscura* is a Palaearctic species that has been studied extensively by population and evolutionary geneticists for more than 70 years. In 1978 it was discovered in Puerto Montt, Chile; within a few years it extended over much of Chile and into Argentina and became the most common drosophilid in many places. In 1982, it appeared in the American northwest, shortly thereafter it was found extensively distributed from southern British Columbia, through Washington and Oregon, into central California. The karyotype of *D. subobscura* consists of five acrocentric chromosomes, named A, J, U, E and O, plus a small dot-like chromosome. Variations in sequence arrangement have been found in every one of the five large chromosomes: more than 80 different arrangements in total. There are differences between localities, but in every population most or all five chromosomes are polymorphic. The frequencies of the various chromosomal arrangements are remarkably similar in South America and in North America. The presence of the same 19 sequences, of 80 known sequences, and in similar frequencies, on both continents suggest that the two colonizations are not independent; the colonizers of one of the two continents

probably originated from individuals from the other (Ayala *et al.*, 1989).

In the Palaearctic region, Prevosti (1974) and others (reviewed in Krimbas and Loukas, 1980) determined that the frequencies of many of the chromosome arrangements of *D. subobscura* vary in clinal patterns that are correlated with latitude. The association between climatic variables and the *D. subobscura* chromosomal polymorphism has been interpreted as the result of adaptation, although some authors have argued that historical factors could explain the correlations without invoking adaptive factors (Krimbas and Loukas, 1980). The American populations, however, offer a rare opportunity to test the role of selection in maintaining the inversion-frequency clines: soon after the discovery of the species in America (3 years in the case of South American populations), the frequencies of the chromosome arrangements in nine South American and seven North American populations were analysed (Prevosti *et al.*, 1988). Correlations between chromosomal arrangement frequencies and latitude were calculated for these populations. Signs of these correlations are highly coincident with those found in the Old World. The correlation coefficients are statistically significant for six chromosome arrangements in South America and for six in North America (Ayala *et al.*, 1989). If we ignore the significance of individual correlations and consider only their sign, 12 out of 16 correlations have the same sign in all three continents. Not all these correlations are independent. If we exclude one per chromosome, 9 of 12 correlations are of the same sign in all three continents. The probability of this coincidence being

Correspondence: Dr J Balanyà, Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal, 645. Annex planta 2, 08028 Barcelona, Spain.  
E-mail: jbalanya@ub.edu

This paper is dedicated to A Prevosti on the occasion of his 90th anniversary.

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due to chance, as shown by a sign test, is  $P < 0.001$ . Thus, the initial evolution of chromosomal polymorphism on a continental scale was remarkably rapid and consistent.

Although the signs of correlations between frequency and latitude were similar on all three continents, the New World clines were nonetheless much shallower than those in the Old World. Would the New World clines become steeper over time and, thus, continue to converge on the Old World ones? To evaluate this possibility, additional samples were collected in North and South America. This effort has resulted in a genetic time series that now spans approximately two decades on both continents and offers insights into the strength and consistency of selection along latitudinal gradients (Balanyà *et al.*, 2003). Large samples were collected at six sites in South America in 1986 and 1999 and at seven sites in North America in 1992 and 1994. Of the 18 gene arrangements found on all three continents over the entire census period, between 14 and 17 (depending on the particular census), form clines of the same sign on all three continents. It is interesting to note that the clines in South and North America had not become steeper over time as expected, but remained distinctly shallower than those in the Old World. In fact, none of the 18 gene arrangements on either continent showed a significant increase in slope. We speculate that the shallower slope may be due to the more limited number of inversions, coupled with a general pattern of weak heterosis (Mestres *et al.*, 2001): all else being equal, more gene arrangements for a given chromosome allow more ways to be a heterozygote. Coupled with graded selection pressures along the cline plus some gene flow, the fewer inversions would mean more shallow slopes. Indeed, the slopes of the steepest clines (per each chromosome) in the New World are suggestively correlated with the number of gene arrangements (Pearson's  $r = 0.569$ ,  $t_{[8]} = 1.96$ ,  $P = 0.085$ ) for that chromosome.

The adaptive value of the chromosomal polymorphism of *D. subobscura* and the capacity to respond very rapidly and consistently to different environmental conditions makes it a valuable tool to monitor global change. Newer samples of inversion frequencies have been obtained for the colonizing populations since these first analyses: the time series now spans approximately two decades for both North America and South America (Balanyà *et al.*, 2003). The various New World samples have clinal slopes of the same sign as those in Europe, with minor fluctuations in the relative frequencies within a continent over time. This stable clinal pattern in the frequency of gene arrangements on all three continents allows us to examine shifts in overall frequencies over time.

The first long-term studies on the inversion polymorphism of *D. subobscura* showed a trend toward a frequency decrease in standard gene arrangements and a frequency increase in nonstandard gene arrangements (Frutos and Prevosti, 1984; Gosteli, 1990; Orengo and Prevosti, 1996; Rodríguez-Trelles and Rodríguez, 1998; Rodríguez-Trelles *et al.*, 1998). These studies, however, were limited to one chromosome or to one population. Solé *et al.* (2002) extended these long-term studies to seven Mediterranean populations, analyzing the frequencies of the chromosomal arrangements of all the acrocentric chromosomes of the species. These authors compared the chromosomal polymorphism of these populations with that of the same populations collected 26–35 years ago. The frequencies of gene

arrangements of all chromosomes had changed significantly during this period in a systematic way: an increase in the frequency of those arrangements typical of southern latitudes and a decrease for those more common in northern latitudes were observed in all populations. These changes could be due to climatic factors that are correlated with latitude, making the chromosomal composition of this species more 'equatorial' (Solé *et al.*, 2002). The same authors extended the analysis to three Atlantic and three Central European populations, obtaining similar results (Balanyà *et al.*, 2004). The genetic distances of the new populations to an average southern population of reference had decreased in comparison with those of the old populations. Again, these changes could be the result of climatic factors that are correlated with latitude; in particular, the assumption that global warming was responsible for all the changes observed appeared rather likely.

Given the evidence of shifts in chromosome-arrangement frequency in Europe, might a similar change be taking place in the American colonizing populations, as would be expected from global climate warming? Six South American and seven North American populations were resampled in 1999 and 2004, respectively, and their chromosomal polymorphism compared with those of the corresponding first samples. A similar analysis that the one made in the Old World populations was then carried out. Table 1 shows the number of positive and negative differences in each of the American subcontinents between the frequencies in the old and new samples for those arrangements showing significant positive or negative correlation coefficients with latitude in Europe (Menozzi and Krimbas, 1992). These results show the same trend as in the Old World: those arrangements whose frequency shows a positive correlation coefficient with latitude (potentially adapted to colder environments) tend to be found at lower frequency in the new samples, whereas those with negative correlation coefficient tend to increase their frequency. These long term changes detected in the chromosomal polymorphism of *D. subobscura* in three discontinuous areas match those expected to take place in response to global warming.

Balanyà *et al.* (2006) decided to test more directly whether climate and chromosomes were, in fact, shifting in parallel. They first used a principal component analysis to combine arrangement frequencies into a single Chromosomal Index (the first principal component), which explained 45.8% of the total variability. The values of this index decreased with latitude on all three continents, such that high positive values corresponded to a polymorphism associated with warmer sites. The index increased between initial and final samples in 24 of the 26 sites: thus, polymorphism frequencies became more equatorial (warm adapted) at most localities during the time interval considered.

Did climates in fact warm at these sites? To find out Balanyà *et al.* (2006) computed the first principal component for weather data, using the mean monthly minimum and maximum temperatures at the nearest weather station for each sample locality. The first principal component, explaining 79.8% of the variance, was the Temperature Index. It was negatively correlated with latitude and increased over the sample period in 22 of the 26 localities (12 out of 13 in Europe, 5 out of 6 in South America and 5 out of 7 in North America), indicating that temperature had, in fact, increased

**Table 1** Long-term changes of the frequencies of chromosomal arrangements in the American populations

New–old frequencies:	Group 1: Arrangements with positive correlation coefficient with latitude			Group 2: Arrangements with negative correlation coefficient with latitude		
	South America	North America	South and North America	South America	North America	South and North America
Number of differences with positive sign	15	12	27	32	36	68
Number of differences with negative sign	22	31	53	16	20	36
Sign test	NS	$P < 0.05$	$P < 0.05$	$P < 0.10$	$P < 0.10$	$P < 0.02$

Two groups are considered: one including all arrangements with positive correlation coefficients with latitude (more frequent towards the poles), and a second including all arrangements with negative correlation coefficients (more frequent towards the equator). For each chromosomal arrangement and for each population, the sign of the difference between the frequency of the arrangement in the new sample and in the old sample has been computed; thus, a positive sign indicates that the frequency of the arrangement has increased in that population during the period of time considered. The values given in the table correspond to the number of positive and negative signs scored in South American populations, North American populations and in both continents (pooled data). The significance of a sign test comparing the number of positive and negative differences within each group is also given. The  $P$ -values have been obtained using the sequential Bonferroni correction.

at most sites. Most interestingly, the changes in the Chromosome Index paralleled those of the Temperature Index in 22 of 26 cases (Rayleigh test,  $P < < 0.001$ ), consistent with the hypothesis that the changes observed in the inversion polymorphism of *D. subobscura* were driven by the changes in temperature (Balanyà *et al.*, 2006, Table 1 and Figure 2 therein).

Whether these systematic changes of the chromosomal polymorphism are a consequence of local natural selection or have been produced by migration from the lower latitudes (or both) remains an open question. The fact that the species has been recently detected further north than previously in Europe supports the migration hypothesis (Saura, 1994). Similarly, the recent appearance of some low-latitude arrangements at high-latitude sites seems to point to the same direction (Solé *et al.*, 2002; Balanyà *et al.*, 2004), although an effect of local adaptation cannot be excluded.

Similar rapid changes in the chromosomal polymorphism of other drosophila species in apparent response to climate warming have been reported. The inversion *In(3R)Payne* of *D. melanogaster* exhibits a North-South cline in eastern Australia, with its frequency increasing towards the equator (Knibb *et al.*, 1981). In samples separated 20 years, a general increase of the frequency of the inversion in all populations, with a corresponding shift in elevation of the clinal pattern of about 7° towards the equator, is detected (Anderson *et al.*, 2005; Umina *et al.*, 2005). Levitan and Etges (2005) and Etges and Levitan (2008) also documented a decline in the frequencies of some northerly gene arrangements and an increase in several southern gene arrangements in North American populations of *D. robusta* in correlation with increasing temperatures. In *D. pseudoobscura*, the seasonal cycling of some arrangements of the third chromosome as well as the altitudinal clines detected in different localities of the North American Pacific Coast constitute evidence that this polymorphism reacts to temperature, although the behaviour of the same chromosomal arrangement with respect to altitude or seasonal abundance may differ among populations (Dobzhansky, 1948). In agreement with this inconsistent behaviour of individual arrangements, North-South clines are not evident in this species (Powell, 1992). Thus, in a scenario of global warming, it

is difficult to predict which inversions are expected to change their frequency. As a matter of fact, Anderson *et al.* (1991) were not able to detect major changes in data from 48 populations spanning more than 40 years. The only changes emerging from these data were an increase in frequency of the arrangements Pikes Peak and Tree Line in the Pacific Coast area. These arrangements are more abundant in eastern populations who are subject to higher mean temperatures (Schaeffer, 2008).

Although it seems likely that temperature has an important role in the distribution of the chromosomal polymorphism in natural populations of *D. subobscura*, attempts to reproduce the clinal patterns in the laboratory by culturing the flies at different temperature regimes have failed (reviewed in Krimbas, 1993; Santos *et al.*, 2005). These results suggest that temperature alone might not be the selective agent. One alternative proposed by Bradshaw and Holzapfel (2008) argues that 'the increasing length of the growing season and the advance and delay of spring and winter, respectively, impose greater selection on animal populations than does the direct effect of temperature'. Nevertheless, these laboratory experiments cannot rule out a direct effect of temperature, as they were conducted at constant temperature in vials or small cages and do not mimic the regime found in nature (Huey and Rosenzweig, 2009).

In summary, the chromosomal polymorphism of *D. subobscura* is changing in step with climate in both its original and colonized distribution areas. Although the mechanism underlying these changes are not yet well understood, this system provides a powerful micro-evolutionary tool to monitor the genetic impact of climate warming on natural populations.

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