



Climate change and chromosomal inversions in *Drosophila subobscura*

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ABSTRACT: In natural populations, the large changes in chromosomal structure occurring through chromosomal inversions show pronounced variations in frequency that often correspond to temporal and spatial climatic trends, which suggests that they may be employed to monitor the impact of global warming. Here we review and update the evidence on the association between chromosomal inversions and climate in *D. subobscura*, which provides one of the best studied models in this context. Chromosomal inversion frequencies of *D. subobscura* populations vary predictably with latitude, and this association has evolved independently in Europe and South and North America. They also exhibit clear seasonal trends that are consistent with temperature fluctuations. More importantly, latitudinal clines in chromosomal inversion frequencies seem to be responding to the global rise in mean temperatures in all continents. We analyze the relevance of these results in the light of climate change, and discuss how a better understanding of the mechanisms underlying these patterns may contribute to our knowledge on the impacts of global warming in biological systems.

KEY WORDS: Chromosomal inversions · Latitudinal clines · Thermal adaptation · Global warming

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1. INTRODUCTION

Understanding the impact of human-driven climate change in biological systems is one of the most important challenges that researchers face during the 21st century, with important repercussions in the medium and long term (IPCC 2007). It is currently clear that species are responding in various ways to global warming (Peñuelas & Filella 2001, Peñuelas et al. 2002, Walther et al. 2002, Root et al. 2003, Parmesan 2006, 2007 and references therein), yet predicting how species and communities will respond to changes in environmental conditions on a global scale remains considerably difficult. This is partly because these changes vary geographically as well as

due to the inherently complex relationship organisms have with their surrounding biotic and abiotic environment.

Recent studies have shown that predictions of organisms' responses based solely on climatic variables can be dramatically different to those that take into account additional knowledge of biology. For example, although the impact of global warming is more pronounced at high latitudes, comparative studies on the ranges of thermotolerance in ectotherms suggest that tropical species may be more vulnerable to increases in mean environmental temperatures because they already encounter temperatures close to their physiological limits (Deutsch et al. 2008, Tewksbury et al. 2008, but see Hoffmann 2010) or lack the appropriate

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genetic variation in key traits (Kellermann et al. 2009). This discrepancy illustrates how consideration of biological data may drastically alter the outcome of predictions on the impacts of climate change, and emphasizes the necessity of more holistic approaches to monitoring and investigating the effects of global warming on biological systems.

Long-term monitoring surveys capitalize on bioindicators or biomonitors for which comprehensive historical records are available. In this sense, phenology (i.e. the study of changes in plant and animal life-stage events, driven by environmental change) is becoming a major component of climate change research, largely because long-term phenological changes are relatively easy to identify (see e.g. Sparks et al. 2009). Even though current DNA technologies make it possible to obtain information from archived samples (see Nielsen & Hansen 2008), time series of genetic data remain almost nonexistent, with a few exceptions provided by a small number of *Drosophila* species that have been regularly scored for chromosomal inversions. Here we present a brief overview (including some unpublished results) of what is known on the rich natural variation for these genetic markers in *D. subobscura*, and discuss the potential relevance of this model to understand: (1) how changes in climatic conditions might be affecting the genetic composition of species; (2) which environmental factors might be more relevant for predictive purposes; and (3) how species may respond to these environmental challenges.

1.1. Chromosomal inversion polymorphisms

Chromosomal inversions were first detected from an alteration in the offspring proportions derived from some genetic crosses (Sturtevant 1917). The use of cytological techniques introduced in 1935 uncovered an almost bewildering amount of inversion variation (polymorphism) in natural populations of *Drosophila pseudoobscura* and related species, which allowed the construction of phylogenetic relationships (Dobzhansky 1937). Extensive surveys in southern California from 1939 to 1946 showed that chromosomal inversion frequencies of *D. pseudoobscura* presented marked seasonal cycles (Dobzhansky 1947), leading to a fundamental turning point in evolutionary genetics because these patterns suggested that these polymorphisms could be adaptive.

Succinctly, an inversion is a change in chromosomal structure in which a fragment of the chromosome involving several genes rotates 180°, resulting in a region where the positions of these genes are inverted relative to the original sequence (Fig. 1). Cytological methods for detecting inversions have been predominantly employed in Diptera (flies, mosquitoes, etc.) because these insects present specialized cells that undergo repeated rounds of DNA replication without cell division, forming giant chromosomes (polytenic chromosomes) that could thus be studied at a greatly enhanced resolution. These chromosomes have characteristic banding patterns which can be used to identify structural changes, and often form an inversion loop in individuals containing 2 different gene arrangements on the same chromosome: one with and the other without the inverted fragment (heterokaryotypes; see Fig. 1). Another important consequence of inversions in an evolutionary context is that they suppress recombination in heterokaryotypes (i.e. suppress the reshuffling of genes between chromosomes with inverted and non-inverted regions). As a result, genetic differences accumulate in genes associated with the inversions, which may play a significant role in population divergence and speciation (reviewed in Hoffmann & Rieseberg 2008).

Following Dobzhansky's pioneering works, many researchers studied chromosomal inversion polymorphisms, gathering an extensive historical record of the distribution of different inversions in *Drosophila* species worldwide. It was realized very soon that inversion frequencies show conspicuous latitudinal clines in many

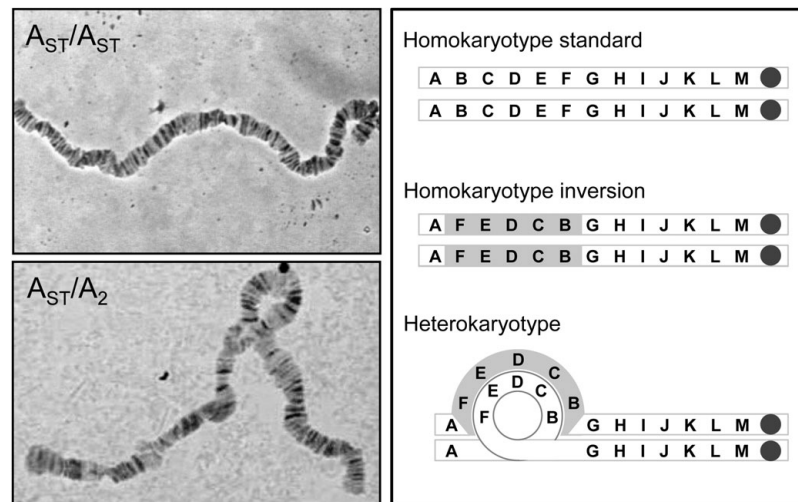


Fig. 1. Chromosomal inversions can be detected in *Drosophila* by analyzing the banding patterns or the inversion loops of polytene chromosomes. The pictures show individuals that are homokaryotypic (top) and heterokaryotypic (bottom) for gene arrangements on the A chromosome (light micrograph). A schematic diagram showing how inversions result in chromosomal loops in heterokaryotypes is shown on the right

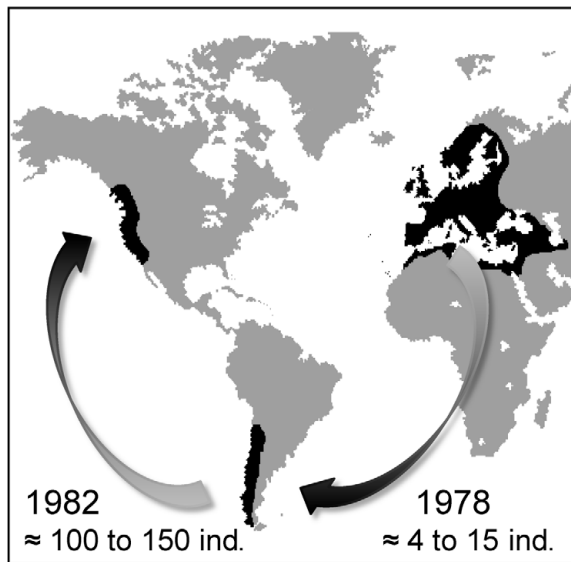


Fig. 2. *Drosophila subobscura*. Global distribution, and invasion of the Americas, depicting the estimated population size during the colonization events

species (see Krimbas & Powell 1992). Together with the cyclical changes already reported by Dobzhansky in *D. pseudoobscura*, the idea that climatic factors favor different inversions at different latitudes and/or in different seasons is nowadays widely accepted.

2. THE INVASIVE SPECIES *DROSOPHILA SUBOBSCURA*: A CASE STUDY

2.1. Historical records of clinal patterns

Drosophila subobscura is a native Palearctic species with a wide geographic distribution ranging from Scandinavia to Northern Africa (Fig. 2). This species has become particularly suitable for studies of experimental evolution on the adaptive role of inversions because of its well-studied chromosomal inversion polymorphism, which encompasses more than 90 gene arrangements present in its 5 large acrocentric chromosomes. Naturally occurring inversions in *D. subobscura* are nowadays distinguished as e.g. A_1 , J_1 or O_5 , where the letter stands for the corresponding chromosome and the subscripted number for the inverted segment on that chromosome relative to a so-called standard orientation).

The geographical distribution of gene arrangements in European populations of *Drosophila subobscura* has been studied extensively (see Krimbas 1992 for a review), and can be briefly summarized as follows. (1) The frequency of different gene arrangements varies dra-

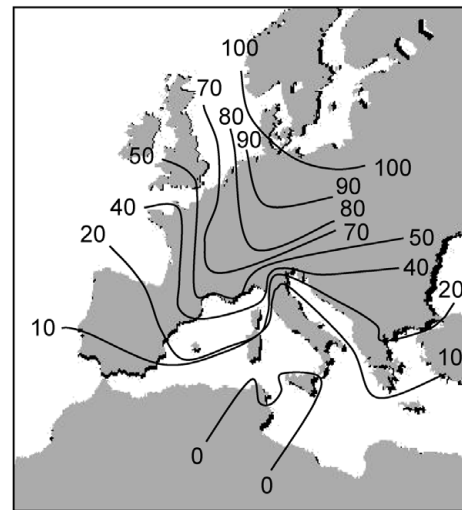


Fig. 3. *Drosophila subobscura*. Latitudinal cline for the gene arrangement O_{ST} observed in Europe. Lines represent isoclines with similar frequencies of O_{ST} (from 0 to 100%). Note that O_{ST} is absent in southern populations and fixed in northern populations, hence it is considered a 'cold-climate' arrangement

matically between different populations, and several arrangements that are frequent in some populations may be absent in others. (2) There is a general correspondence between the genetic and geographical distance between populations, hence the genetic constitution of different populations becomes increasingly different as their geographic distance increases. The effects of geographic barriers are also evident in this context, and genetic differentiation between populations separated by sea straits and high mountains is generally higher than expected in the absence of geographic barriers (Prevosti et al. 1975). (3) Whereas the genetic constitution of northern populations is relatively homogeneous and includes few inversions, southern populations present a considerably larger number of inversions that form complex gene arrangements, including many that are apparently unique to specific locations. (4) The relative frequencies of several gene arrangements present marked geographical clines running in the northeast–southwest direction (Fig. 3).

Taken together, geographic patterns suggest that historical processes, migration and local adaptation have been involved in shaping the distribution of gene arrangements across Europe and northwest Asia. The most widely accepted explanation for these patterns proposes that separate populations of *Drosophila subobscura* remained isolated in southern regions of Europe during the last glaciation, eventually becoming genetically differentiated (from a number of European case studies it appears that the Balkans, the Iberian Peninsula, Italy and Turkey contained

refugia and were a source for post-glacial population expansions of many species; Hewitt 2000, 2001). Subsequently, *D. subobscura* from different refugia recolonized the northern regions of Europe (Krimbas 1992). This scenario explains the geographic structure and increased number of chromosomal arrangements in southern populations, and partly accounts for the more homogeneous pattern observed in northern Europe. The latitudinal clines in several chromosomal arrangements provide circumstantial evidence that selective processes might have also been involved, and whether historical processes alone can explain the lower diversity in northern populations remains a matter of debate. Evidence that these clines are partly adaptive has emerged from research on the rapid invasion of the New World by *D. subobscura* (Fig. 2, Section 2.3).

2.2. Local seasonal variation

Seasonal changes in genetic markers can be either erratic or cyclical. As mentioned previously, the observation of seasonal changes—repeated over the years—in the frequencies of gene arrangements represented the first piece of evidence in support of the adaptive value of chromosomal inversion polymorphisms (Dobzhansky 1970, Lewontin 1974, Endler 1986). Seasonal cycling of chromosomal inversions has been recorded in a number of *Drosophila* species, including *D. pseudoobscura* (Dobzhansky 1947), *D. persimilis* (Dobzhansky 1956), *D. funebris* (Dubinin & Tiniakov 1945), *D. flavopilosa* (Brcnc 1972), *D. robusta* (Levitan 1973, Etges 1984), *D. melanica* (Tonzetich & Ward 1973), *D. melanogaster* (Stalker 1980, Sánchez-Refusta et al. 1990) and *D. mediopunctata* (Ananina et al. 2004), highlighting the generality of this pattern across species with contrasting geographic distributions.

In the case of *Drosophila subobscura*, its rich inversion polymorphism was initially supposed to be invariant, or to vary erratically with respect to the annual climatic cycle. This conclusion, however, was based on results from few studies in a limited number of populations. This situation may have favored the concealment of seasonal cycling of inversions if they cycle seasonally in some populations but not in others, such as has been reported for *D. pseudoobscura* (Dobzhansky 1962, Dobzhansky & Ayala 1973), *D. robusta* (Carson 1958, Dobzhansky 1962) and *D. persimilis* (Dobzhansky 1956, Dobzhansky & Ayala 1973). Seasonal cycling could

also have been neglected because the sampling periodicity and/or schedule may have missed the inversions' seasonal cycle (e.g. Krimbas 1967, who collected samples in spring and autumn), and in general because of the short length of the early monitoring studies, generally spanning 1 yr (e.g. De Frutos & Prevosti 1984), and in no case longer than 2 consecutive years.

The early view that the chromosomal inversion polymorphisms of *Drosophila subobscura* do not cycle with the seasons was subsequently challenged by a long-term seasonal study (spanning 2 sampling periods within 16 yr), using time series methods, which focused on the temporal variation of the O chromosomal polymorphisms at Mount Pedroso, northwest of the Iberian Peninsula (Rodríguez-Trelles et al. 1996). The study disclosed a complex picture: the most frequent gene arrangements exhibited pronounced seasonal cycles superimposed on long-term trends (Fig. 4). Although the intensity and direction of the temporal patterns differed from one arrangement to the next, there was a general agreement between seasonal, long-term and latitudinal trends. So-called cold-climate or cold-adapted gene arrangements (i.e. those gene arrangements that show a negative correlation coefficient with maximum temperatures, or a positive correlation coefficient with latitude, such as O_{ST} ; Menozzi & Krimbas 1992) increased in frequency in winter and decreased in summer. In addition, these arrangements also showed a decrease in frequency in the long-term (Fig. 4) that coincided with a mean increase in environmental temperatures within the 16 yr survey period (Rodríguez-Trelles & Rodríguez 1998).

How widespread are the seasonal cycles observed at Mount Pedroso? Seasonal changes of inversions con-

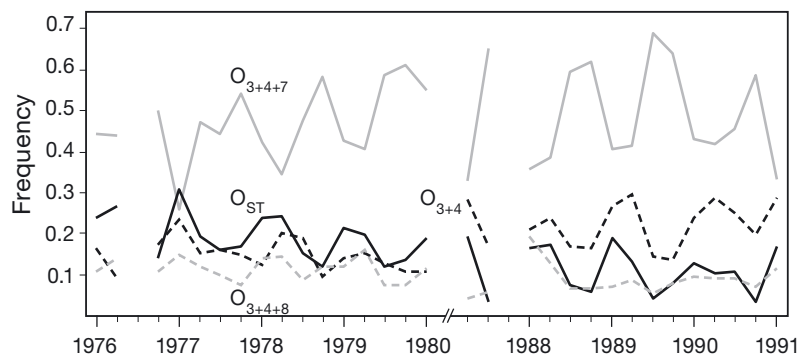


Fig. 4. *Drosophila subobscura*. Seasonal fluctuations of gene arrangement frequencies in the population of Mount Pedroso, Spain. Different arrangements in the O chromosome show seasonal cycles and long-term trends that coincide with temperature fluctuations and global warming: cold-climate arrangements such as O_{ST} decrease in frequency during summer and in the long-term, whereas the opposite is true for warm-climate arrangements such as O_{3+4} (redrawn from Rodríguez-Trelles et al. 1996, Rodríguez-Trelles & Rodríguez 1998)

sistent with those found in this population were also observed at other locations (e.g. Burla & Götz 1965, Zivanovic 2007), although these studies lacked the resolution to differentiate erratic from cyclic variation and resolve whether gene arrangement frequencies were shifting in response to climate change in those locations. In summary, the most extensive temporal survey performed in a single population and a single chromosome of *Drosophila subobscura* does suggest that seasonal changes in gene arrangement frequencies can be an important source of temporal variation, yet the generality of this pattern remains poorly studied.

2.3. Invasion of the Americas

The invasion of the Americas by *Drosophila subobscura* about 30 yr ago provided a unique opportunity to study this colonization event from its inception, and somewhat revitalized the use of this species in evolutionary studies. The first individuals of *D. subobscura* in South America were detected in Puerto Montt, southern Chile, in February 1978 (Brncic & Budnik 1980). By then, researchers had been collecting *Drosophila* species in Chile on a yearly basis for almost 2 decades, which strongly suggests that colonization was indeed detected at its beginnings. Within a year, *D. subobscura* had been collected near Santiago, located 1000 km north from Puerto Montt (Brncic et al. 1981, Prevosti et al. 1988). This strongly suggests that the species has huge dispersal ability. Also remarkable was the discovery in 1982 of *D. subobscura* in North America near Port Townsend, WA (Beckenbach & Prevosti 1986), because those 2 colonization events could now be considered a large-scale natural experiment with 2 replicates (i.e. 'a grand experiment in evolution'; Ayala et al. 1989).

Although it was initially unclear whether the arrival in North America represented a second independent invasion, South and North American flies are now known to share identical genetic markers (gene arrangements, lethal genes, microsatellite loci, etc.) likely derived from a source population in southwestern Europe (Mestres et al. 2005, Pascual et al. 2007 and references therein). Recent analyses using microsatellite loci provide compelling evidence that the spread of *Drosophila subobscura* in the Americas occurred following 2 colonizing events: from Europe to South America, and from South to North America (Fig. 2). The initial propagule size was small (<15 ind.), but a substantially larger number of effective founders (around 100 to 150) colonized North America (Pascual et al. 2007). The genetic diversity of New World populations was greatly reduced in comparison with native European populations as a consequence of the initial

genetic bottleneck, hence it was initially unclear whether *D. subobscura* would be able to adapt to the new conditions (e.g. inbreeding can have important effects on thermal tolerance and fitness in *D. melanogaster*; Joubert & Bijlsma 2010, this Special).

The regular tracking of the inversion polymorphism and body dimensions since the colonization of the Americas has provided clear evidence that this species can evolve very fast. For instance, just 3 yr after the South American invasion, Prevosti et al. (1985) collected flies from 7 Chilean sites spanning 12° of latitude and found incipient clines for gene arrangements that were convergent with the long-standing clines in native Palearctic populations. Further collections following the second invasion from 8 North American sites spanning 13° of latitude revealed clines that were again congruent with those in Europe and South America (Prevosti et al. 1988). Such a degree of concordance on all 3 continents (summarized in Balanyà et al. 2003) provided unambiguous evidence that selection on the chromosomal inversion polymorphism of *Drosophila subobscura* must be strong, and that environmental factors associated with latitude probably play an important role in this context.

Some contrasting patterns between Old and New World populations are, however, likely due to the initial bottleneck. About 2 decades after the American invasion, Huey et al. (2000) collected flies from 11 localities in North America and 10 localities in continental Europe. Their aim was to determine whether body size clines had eventually evolved in the New World since large-scale patterns of body size variation, with genetically larger individuals encountered at higher latitudes, are common in *Drosophila* (see Blanckenhorn & Demont 2004 and references therein), including native Old World populations of *D. subobscura* that offered an evolutionary baseline as these flies have evolved for millennia. Convergence of clinal variation in wing size (a proxy for body size) between Old and New World populations was detected (see also Gilchrist et al. 2001, 2004), but an interesting observation was that such convergence was achieved by latitudinal changes in the relative length of different parts of the wing. Contingent, unpredictable evolution was suggested as an explanation for the different details involved in the otherwise parallel wing size clines between continents (Huey et al. 2000, Gilchrist et al. 2001). However, recent results suggest that the association between inversions and particular genes influencing wing traits have changed as a consequence of the bottleneck, and that variable evolutionary patterns might be predictable as long as the genetic makeup of founder populations is taken into account (Fragata et al. 2010; see Section 4.2).

3. GLOBAL WARMING

3.1. Tracking the change in chromosomal inversions

The repeated evolution of latitudinal clines following colonization has allowed researchers to make large-scale comparisons to forecast unprecedented changes in ecological systems due to global warming. Historical surveys suggest that warm-climate gene arrangements are increasing in frequency in European populations of *Drosophila subobscura* (Orengo & Prevosti 1996, Rodríguez-Trelles et al. 1996; Fig. 5), possibly due to the ongoing climate warming (Rodríguez-Trelles & Rodríguez 1998, Rodríguez-Trelles et al. 1998, Solé et al. 2002, Balanyà et al. 2004). The same trend has been reported in populations of South and North America, indicating warm-climate inversions are increasing in frequency at higher latitudes in all continents (Balanyà et al. 2006). Analyses pooling data from European, South American and North American sites showed that shifts in chromosomal arrangement frequencies reflect quite accurately changes in ambient temperature: in 22 of the 26 sampled sites, the shifts occur in the direction predicted from observed temperature trends (Balanyà et al. 2006).

This tendency is not limited to *Drosophila subobscura*. Surveys in populations of the cosmopolitan species *D. melanogaster* from eastern Australia show a similar pattern (Umina et al. 2005). Although the magnitude of the response varies depending on the gene or chromosomal arrangement targeted, Umina et al. (2005) reported a latitudinal shift of nearly 800 km within roughly 20 yr for one particular inversion. In

addition, studies performed in populations of *D. robusta* from eastern North America also suggested latitudinal shifts possibly associated with changing temperatures (Levitan & Etges 2005, Etges et al. 2006, Etges & Levitan 2008). Chromosomal arrangements in *D. robusta* populations have changed within 15 to 20 yr, though responses in some populations were not always consistent with expectations based on environmental trends (Etges & Levitan 2008). Long-term changes in some chromosomal arrangements have also been reported in North American populations of *D. pseudoobscura* (Anderson et al. 1991) and in Japanese populations of *D. melanogaster* (Inoue et al. 1984). However, in these cases it is not clear how the observed patterns correlate with climatic trends.

Although much of the evidence is fragmented and comes from a few studies, the apparent generality of some patterns emphasizes the necessity of increased monitoring efforts. As briefly outlined in the previous paragraphs, the latitudinal shifts in inversion frequencies in *Drosophila* populations seems to be a widespread phenomenon that encompasses a great variety of chromosomal arrangements, relatively distant species of drosophilids (e.g. whereas the *robusta* group is currently classified within the subgenus *Drosophila*, the *melanogaster* and *obscura* groups are classified within the *Sophophora* subgenus, and both subgenera diverged about 40 million years ago; Markow & O'Grady 2006), and a broad geographical range that spans North and South America, Europe, Asia and Oceania. The evidence pointing towards an association between these patterns and climate change is

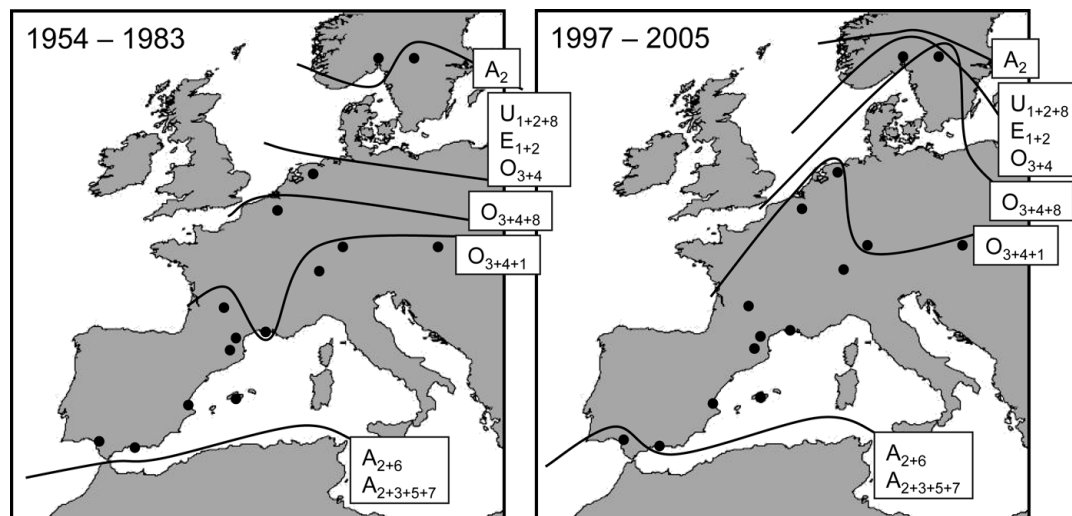


Fig. 5. *Drosophila subobscura*. The shift observed in the northern limit of warm-climate gene arrangements after comparing recent surveys with historical records. Isoclines were drawn separating populations (circles) with detected chromosomal arrangements from those for which they were apparently absent. The generality of the pattern suggests that southern populations are shifting towards higher latitudes, though the possibility that rare gene arrangements were not detected in historical samples cannot be completely discarded

robust but still limited to a few species, and additional work is therefore necessary in this context.

3.2. What do these shifts indicate?

Results described above indicate that variation in chromosomal polymorphisms and other non-neutral genetic markers may eventually be employed to monitor the impact of climate change on the genetic constitution of populations at different locations. Nonetheless, little is known on the causal mechanisms behind such a pattern. A potentially serious problem consists of what is the most appropriate period to update historical records. Specifically, in species with short generation times relative to season length, the seasonal climatic cycle can induce constantly recurring seasonal changes in climate-related traits superimposed on long-term trends (Section 2.2). To prevent confounding both patterns, collections may be updated using the same calendar dates of old samples. However, this relies on the premise that the seasonal cycles have remained stationary over the monitoring period in the populations being compared (cf. Rodríguez-Trelles & Rodríguez 2007, 2010, Balanyà et al. 2007). However, another possibility is that the latitudinal clines and observed shifts in gene arrangement frequencies have evolved in response to seasonality. Bradshaw & Holzapfel (2008) suggested that changes in the length of the growing season might be more important than the direct effects of temperature (this may be the case particularly in regions at higher latitudes with more marked seasonal variation; see Fig. 1 in Chown et al. 2004). Recent reports of shifts in the timing of events such as reproduction or migration, which are presumably associated with climate change, partly support this alternative (Peñuelas & Filella 2001, Parmesan & Yohe 2003, Root et al. 2003). Interestingly, gene arrangement frequencies of the population at Mount Pedroso exhibit both seasonal and long-term trends (Fig. 4), emphasizing that these alternatives are not mutually exclusive (Rodríguez-Trelles & Rodríguez 2010).

Latitudinal clines of polymorphic inversions in *Drosophila subobscura* seem to emerge from the interaction between dispersal and local adaptation. This idea is reinforced by the fact that the dispersal ability of this species is known to be very high (i.e. the expansion from Puerto Montt to Santiago within 1 yr points to a dispersal rate of roughly 2.7 km d⁻¹; see Section 2.3). Furthermore, no genetic differentiation between European populations has been detected using microsatellite loci, which suggests that gene flow due to migration is sufficiently high to homogenize the genetic constitution of populations at putatively neutral markers (Pascual et al. 2001). Gene flow prevents popula-

tions from optimally adapting to their environments (Ronce & Kirkpatrick 2001), but there is no clear amount of migration or gene flow at which point populations can become effectively isolated with respect to their evolutionary dynamics (Rice 2004). The rapidly evolved inversion clines in the Americas, however, suggests that local adaptation is taking place in spite of high gene flow (Kirkpatrick & Barton 2006). Therefore, chromosomal arrangements in *D. subobscura* likely play an important role in preventing the genetic exchange between locally adapted alleles from other populations or genetic backgrounds. The clinal shifts in the frequency of chromosomal inversions correlated with current climate change might then reflect: (1) locally ongoing adaptation to global warming trends, and/or (2) invasion from more equatorial populations carrying warm-climate gene arrangements that nowadays encounter better conditions to flourish at higher latitudes.

We took advantage of one of the most remarkable features of *Drosophila subobscura* (i.e. its extremely rich inversion polymorphism encountered in Palearctic populations; see Section 2.1) and asked the question: Can we now detect chromosomal arrangements that were formerly restricted to southern populations at higher latitudes? If this were the case, it would provide compelling evidence that migration is taking place, because overwhelming evidence favors the view that chromosomal inversions in *Drosophila* are monophyletic (Powell 1997). In fact, the geographical distributions of some gene arrangements now appear at relatively high frequencies in some northern locations (Fig. 5), where they were undetected in historical surveys (Solé et al. 2002, Balanyà et al. 2004, J. Balanyà et al. unpubl. data). A remarkable example is the gene arrangement O₃₊₄₊₈, the most frequent genetic configuration found in North Africa (Prevosti 1974), but whose frequency is now 22.6% in Groningen, Netherlands, and 2.7% in Sunne, Sweden. A potential caveat with these data is that the gene arrangements could have been already present in historical times at the same locations, but were not detected because they had very low frequencies. However, this pattern was observed repeatedly in several gene arrangements in different chromosomes (Fig. 5), which suggests that the overall trend probably does not reflect a sampling artifact.

To summarize, gene arrangement frequencies in *Drosophila subobscura* are shifting in response to climate change in Europe and South and North America, but it remains unclear why. Although this does not exclude the use of chromosomal polymorphisms as biological monitors of climate change, a better understanding of the processes involved is certainly necessary. Determining which factors are more relevant in

shaping the response of *D. subobscura* to global warming is crucial to predicting how other biological systems may be affected by—and may eventually respond to—climate change.

4. TESTING HYPOTHESES DERIVED FROM NATURAL PATTERNS

4.1. Phenotypic latitudinal clines

Several complementary approaches may be employed to elucidate which factors ultimately influence the evolution of latitudinal clines. Perhaps the most intuitive approach involves determining which phenotypic traits that are directly associated with Darwinian fitness (such as fecundity, developmental rates, heat or cold tolerance, etc.) show significant latitudinal clines. This is particularly challenging because measurements of this nature involve very large sample sizes and controlling for environmental effects that might obscure real genetic trends. In addition, in the case of *Drosophila subobscura*, the genetic bottleneck during the colonization of the New World (Section 2.3) has resulted in somewhat different genetic compositions in American and European populations, which is another confounding factor that might obscure comparisons across continents.

Few studies have addressed whether latitudinal gradients exist at the phenotypic level in *Drosophila subobscura* (for phenotypic clines in other *Drosophila* species, see e.g. Hoffmann et al. 2003, Hoffmann & Weeks 2007, Arthur et al. 2008, Turner et al. 2008 and references therein). A recent example is the analysis of latitudinal patterns in desiccation- and starvation-resistance (Gilchrist et al. 2008). One would intuitively expect that these phenotypic traits may be important determinants of survival in the field. Their analysis involved 9 populations from Europe, 9 from North America and 10 from South America, that were distributed across a gradient of more than 15° of latitude on each continent. Although some clinal patterns were detected, results were not conclusive: starvation tolerance did not vary with latitude on any continent, and the latitudinal cline in desiccation resistance in South America showed the opposite trend (i.e. an increase in resistance with latitude) than in Europe and North America.

Conversely, convergent trends were observed across Europe and North and South America in the evolution of larger body sizes at higher latitudes (Section 2.3). However, latitudinal variation in quantitative traits potentially unrelated to climatic variables may occur as a correlated response to selection on other targets. This is particularly relevant because inversions involve

many genes (Section 1.1); hence different chromosomal gene arrangements from natural populations can differ in their mean value for one or more phenotypic traits. For instance, Orengo & Prevosti (2002) uncovered a relationship between chromosomal polymorphisms and wing size in a European population of *D. subobscura* (see also Fragata et al. 2010). In accordance with the clinal patterns, they found bigger sizes to be preferentially associated with gene arrangements whose frequencies increase with increasing latitude. Correlated clinal patterns in inversion frequencies and body size might therefore have a similar underlying cause, although it is unclear how temperature mediates selection of body size in small ectotherms (Blanckenhorn & Demont 2004). In summary, determining if observed clinal patterns result from direct selection of body size or as a correlated response requires a mechanistic understanding of how variation in size translates into differences in Darwinian fitness, which is not possible with a strictly correlational approach. Researchers must then rely on different approaches to understand how these patterns emerge.

4.2. Experimental evolution

In an attempt to determine if differences in temperature could account for the observed latitudinal patterns in chromosomal inversion frequencies, lines derived from a South American population of *Drosophila subobscura* from Puerto Montt (the epicenter of the American invasion) were maintained in the laboratory for several generations at 3 constant temperatures: 13°C (cold), 18°C (around the optimum temperature; see Krimbas 1993, Rego et al. 2010) and 22°C (warm) (Santos et al. 2004, 2005). If temperature was the main factor driving the evolution of latitudinal clines of gene arrangements, one would expect chromosomal frequencies of laboratory lines subjected to colder temperatures to resemble those from natural populations at increasing latitudes.

Succinctly, the evolutionary responses detected in the laboratory could not account for most trends observed in nature (see also discussion in Chown et al. 2010, this Special). Several gene arrangements quickly responded to the contrasting thermal regimes, but responses often contradicted expectations based on the clinal patterns. Phenotypic responses were also discrepant: selected lines did not differ in wing size in spite of the conspicuous convergent clines observed across continents, casting doubts on the adaptive value of size variation across different thermal environments (Section 4.1). Nonetheless, it remains debatable whether the selection regimes in the laboratory, submitting lines to constant temperatures, accurately

reflect the selective pressures in nature. In spite of some inherent limitations (see Huey & Rosenzweig 2009 for a general discussion on the topic, and Rego et al. 2010 on this particular experiment), experimental evolution under more well-established conditions can provide valuable information on the mechanisms underlying correlational patterns observed in nature.

4.3. Association between inversions and phenotypic traits

Another complementary approach involves determining how chromosomal gene arrangements correlate with variation at the phenotypic level. This approach can shed light on the genetic basis of observed phenotypic variation with an unprecedented level of resolution, and requires measuring the phenotypic trait of interest in individual flies (preferentially after removing environmental sources of variation) and then determining their gene arrangements.

For instance, a recent study has shown that flies carrying different gene arrangements differ in their thermal preferences and tolerances to high temperatures (Rego et al. 2010). Individual flies from a population collected near Lisbon, Portugal, and maintained for several generations in the laboratory were first allowed to select their preferred ambient temperatures in a thermal gradient, and then were submitted to increasing temperatures in a water bath until they became unconscious. After analyzing the gene arrangements of each individual, it became clear that flies carrying warm-climate arrangements chose and tolerated higher temperatures than their cold-climate counterparts, which agrees with the expectation from natural patterns. Interestingly, thermal preference and thermal tolerance were genetically independent, being primarily associated with inversions on different chromosomes. Comparisons between temporal surveys in the field and results from Rego et al. (2010) suggest that thermal tolerance is more important in the evolution and dynamics of latitudinal clines than thermal preference.

This approach has also been employed to study the discrepant morphological clines observed in Europe and the Americas. As discussed above, latitudinal clines in wing length were generally convergent but involved changes in different sections of the wing on each continent. In other words, 'the evolution of geographic variation in wing length has been predictable, but the means by which the cline is achieved is contingent' (Huey et al. 2000, p. 308). To test if this was the case, Fragata et al. (2010) estimated how wing length and shape (computed as a ratio of proximal wing length to total wing length; Huey et al. 2000) were associated

with gene arrangements in a European (Lisbon) and a South American (Puerto Montt) population, and found that the genetic basis underlying wing morphological variation was dramatically different (for comparisons on the genetic basis behind wing shape and size across *Drosophila* species, see Trotta et al. 2010, this Special).

It became apparent that the bottleneck during the colonization of the Americas affected the association between some gene arrangements and wing shape, raising the possibility that morphological responses were not related to the evolution of wing size clines. This hypothesis was tested by reanalyzing the data reported in Pegueroles et al. (1995), who sampled *Drosophila subobscura* flies from 6 North American populations in 1986 (i.e. 4 yr after the first colonizers arrived). Whereas no latitudinal cline was detected in wing size at this early stage of colonization, wing shape clines were already present in both males and females (Santos et al. 2004) and matched the patterns reported for populations collected 11 yr later (Fig. 1B in Huey et al. 2000, Gilchrist et al. 2001). Therefore, although the genetic composition of the population that invaded America was contingent on the bottleneck effect, the wing shape cline that evolved subsequently in North America was not, highlighting that contingent, unpredictable evolution might be less widespread than some evolutionary biologists assume (cf. Gould 1989, Conway-Morris 1998).

5. CONCLUDING REMARKS

Recent studies in natural populations of *Drosophila* have shown that species can respond considerably quickly to changes in environmental conditions, suggesting that genetic responses might provide relevant information on the impacts of climate change (Rodríguez-Trelles & Rodríguez 1998, Umina et al. 2005, Balanyà et al. 2006, 2009). Although observed patterns such as the latitudinal shifts in gene frequencies highlight their importance as biological monitors, a better understanding of the processes underlying these patterns is necessary. In this context, experiments in the field and/or in mesocosms may provide complementary approaches to the ones outlined in Section 4. Recent field release studies have been successfully employed to test the adaptive role of acclimatory responses in *D. melanogaster* (Loeschcke & Hoffmann 2007, Kristensen et al. 2008) and the putative role of local adaptation as a determinant of chromosomal frequencies in *D. robusta* (Levitán & Etges 2009), and similar experiments might shed light on the mechanisms underlying the geographical and temporal changes in the chromosomal polymorphism of *D. subobscura*.

A better understanding of the processes that underlie the evolution of latitudinal clines of inversion frequencies may allow researchers to determine which phenotypic traits are being influenced by environmental conditions and to extrapolate results obtained in *Drosophila* to other systems. Extrapolations should be performed with caution, however, because species differ in a variety of ways that will ultimately determine how they respond to environmental challenges (Rose et al. 2005). One way to partially circumvent this problem is to develop genetic and/or phenotypic markers in a wide range of model species belonging to different groups. These model species should share 2 basic attributes: distribution ranges must be large to minimize the confounding effects of local processes, and generation times must be short to ensure that evolutionary responses are observed. The list of potentially suitable models in this context includes not only other *Drosophila* species (a genus with over 1500 species distributed across the globe; Markow & O'Grady 2006), but also rodents (e.g. genera *Mus* and *Peromyscus*), plants such as the cosmopolitan weed *Arabidopsis* and small invertebrates from aquatic systems such as *Daphnia* and *Littorina*. Ultimately, these biological monitors may prove useful to quantify the impact of climate change in an ecosystem, assess which taxa are more vulnerable and determine which climatic variables are more relevant in a given scenario.

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