Changes in chromosomal polymorphism and global warming: The case of Drosophila subobscura from Apatin (Serbia)

Goran Zivanovic¹ and Francesc Mestres²

¹Department of Genetics, Institute for Biological Research “Sinisa Stankovic”, University of Belgrade, Belgrade, Serbia.
²Departament de Genètica, Universitat de Barcelona, Barcelona, Spain.

Abstract

In this study, chromosomal inversion polymorphism data for a natural population of Drosophila subobscura from a swampy region near the town of Apatin (Serbia) were compared with data for the same population collected approximately 15 years earlier. The pattern of chromosomal inversion polymorphism changed over time. There were significant increases in the frequency of characteristic southern latitude (“warm” adapted) chromosomal arrangements and significant decreases in the frequency of characteristic northern latitude (“cold” adapted) chromosomal arrangements in the O and U chromosomes. The chromosomal arrangements O3+4 and O3+4+2 (derived from the O3+4 arrangement) showed significant increases in 2008 and 2009 with regard to the 1994 sample. There was also a significant increase (~50%) in the U1+2 arrangement, while U1+8+2 (a typical southern arrangement) was detected for the first time.

Since the Apatin swampy population of D. subobscura has existed for a long time in a stable habitat with high humidity that has not been changed by man, our results indicate that natural selection has produced chromosomal changes in response to the increase in temperature that has occurred in the Balkan Peninsula of central southeastern European.

Key words: chromosomal inversions, Drosophila subobscura, global warming, karyotypes.

Received: November 16, 2010; Accepted: March 28, 2011.

Introduction

The climate change of recent decades has led to heritable genetic changes in animal species as diverse as birds, squirrels and mosquitoes (Bradshaw and Holzapfel, 2006). Long-term changes in chromosomal inversion polymorphisms in Drosophila species suggest that the genetic constitution of populations is responding to climate change (Van Heerwaarden and Hoffmann, 2007). This suggestion is strongly supported by the fact that genes located within inversions are associated with a variety of traits including those involved in climate adaptation (Hoffmann and Rieseberg, 2008). This characteristic was observed in some species of Drosophila, such as D. melanogaster in Australia (Anderson et al., 2005; Umina et al., 2005), D. robusta in North America (Levitan and Etges, 2005) and D. subobscura in Europe (Rodríguez-Trelles and Rodríguez, 1998; Solé et al., 2002; Balanyà et al., 2004, 2006, 2009). This species is an excellent model organism for such studies because of its extensive polymorphism for chromosomal inversions: 67 inversions distributed in 93 chromosomal arrangements have been identified, with the frequencies of most of these varying clinally with latitude (Krimbas and Loukas, 1980; Prevosti et al., 1988; Menozzi and Krimbas, 1992; Krimbas, 1993).

Of the five acrocentric chromosomes in D. subobscura, the O chromosome (homologous to the 3R chromosomal arm of D. melanogaster and second chromosome of D. pseudoobscura) is the longest and most polymorphic, with about 40 natural chromosomal arrangements (Krimbas, 1993). Since the 1960s more than 150 natural populations have been studied throughout the Palearctic distribution of this species. However, few long-term analyses of changes in inversion polymorphism have been done in southeastern Europe or the Balkan Peninsula. For this reason, the main aim of this work was to study a population of D. subobscura from Apatin (Serbia). This population is
located in a swampy region on the left bank of the Danube river in the lowlands of the Pannonian plain. This area, which is midway along the Danube river about 1366-1433 km from the mouth of this river in the Black sea, is deliberately left to be flooded permanently or during spring across an area of at least 3500 ha. Consequently, the Apatin D. subobscura population has existed in a very humid habitat that has never been changed by man. This is probably the main reason for the large differences observed between the Apatin and other D. subobscura populations from the Balkan Peninsula (Zivanovic et al., 2002; Zivanovic and Marinkovic, 2003; Zivanovic, 2007; Zivanovic and Mestres, 2010a,b).

Specifically, we analyzed inversion polymorphisms in D. subobscura from Apatin 14 and 15 years after the initial studies, and used this information to assess variation in composition. Our intention was to compare samples from June 2008 and 2009 with the sample from June 1994 at exactly the same site, paying special attention to calendar and climatology data. The results may be especially interesting because during this period there were several heat waves, for example the first evidence of climate change in the region was recorded in summer 2003 (Schar and Jendritzky, 2004). The study should tell us whether long-term chromosomal inversion polymorphism changes, if they exist, are characteristic of all or only of specific chromosomes and their arrangements. Finally, the analysis of possible changes in frequency of some chromosomes in the O3+4 group (O3+4, O2+4, and so on) correlated to climate changes is of particular interest, because some genes involved in thermal adaptation are located in the O3+4 region (Quintana and Prevosti, 1991; Moltó et al., 1992; Laayouni et al., 2007).

Material and Methods

Drosophila subobscura flies were collected from a poplar (Populus alba) wood in a swampy region located on the left bank of the river Danube, near the town of Apatin (45°40’ N, 19°00’ E, approximately 200 km NW of Belgrade). This region is included in the Ramsar List, which lists wetlands of international importance, and is known as the Upper Danube wetland area. Meteorological data for the site were obtained from the Serbian Republic Hydro-meteorological Service. Samples were collected twice from exactly the same place in June 2008 and June 2009. To allow comparison with the June 1994 sample, the 2008 and 2009 samples were collected 2.5 days earlier per decade because spring/summer has advanced an average of 2.5 days per decade in Europe (Menzel et al., 2006). Unfortunately, the need to trap on precise days and Danube river flooding limited the sample size obtained. Only wild males (June 2008, June 2009) and the sons of wild females (June 2009) were used to assess chromosomal inversion polymorphism. The males were crossed individually with virgin females of the Kussnacht strain that were homokaryotypic for standard chromosomal arrangements in all five chromosomes. Polytene chromosomes were stained and squashed in aceto-orcein solution. At least eight larvae from the progeny of each cross were examined. The chromosomal map of Kunze-Mühl and Müller (1958) was used for cytological analysis of the chromosomal arrangements and the nomenclature for these arrangements followed that of Kunze-Mühl and Sperlich (1955). Departure of the observed frequencies of chromosomal arrangements and chromosomal karyotypes from expectations was tested using $\chi^2$ test contingency tables. The degree of chromosomal inversion polymorphism in the population analyzed was assessed using the index of free recombination (IFR) (Carson, 1955). Chromosomal inversion polymorphism patterns were obtained by computing Nei’s genetic distances (Nei, 1972) between Apatin 2008+2009 and Apatin 1994 and another five previously analyzed Balkan populations (Kamariste, June 1996; Djerdap, June 2001; Petnica, June 1995; Jastrebac, June 1993; Zanjic, June 1997) (Zivanovic et al., 2002; Zivanovic and Marinkovic, 2003; Zivanovic, 2007).

Results

The frequencies of chromosomal arrangements in the Apatin population from June 2008 and June 2009, together with those previously obtained for this population in June 1994 (Zivanovic et al., 2002), are shown in Table 1. A total of 18 chromosomal arrangements were found in the old and new samples. Some of the chromosomal arrangements found earlier (U1, E1+2, O6, O3+4, in 1994) were not observed in 2008 and 2009, whereas some chromosomal arrangements, such as U1+8, E1+2+9+12, O22 and O3+4 that were not found 15 years earlier were observed in the 2009 samples (Table 1). There were significant differences in the frequencies of the U chromosomal arrangements between the June 1994 and June 2009 samples ($\chi^2$ = 21.2, d.f. = 4, p < 0.001). Similarly, the 1994 and 2008+2009 samples also differed significantly in the frequencies of five U chromosomal arrangements ($\chi^2$ = 17.4, d.f. = 4, p < 0.01). There was a significant decrease in the frequency of the U1+8 arrangement in the two comparisons ($\chi^2$ = 9.5, d.f. = 1, p < 0.01 and $\chi^2$ = 6.9, d.f. = 1, p < 0.01, for the first and second comparison, respectively). Over the same period of time, U5+2 showed a significant increase in frequency ($\chi^2$ = 8.0, d.f. = 1, p < 0.01, for the first case and $\chi^2$ = 8.1, d.f. = 1, p < 0.01, for second). The U1+8+12 arrangement was detected for the first time in the 2008 and 2009 samples.

There were also significant differences among seven O-chromosomal arrangement frequencies between the 1994 and 2009 samples (first case) ($\chi^2$ = 25.1, d.f. = 6, p < 0.001). This significant difference was also observed in the second case (comparing the 1994 and 2008+2009 samples: $\chi^2$ = 23.8, d.f. = 6, p < 0.001). The O4 arrangement showed a significant decrease in frequency ($\chi^2$ = 4.2, d.f. = 1, p < 0.05 and $\chi^2$ = 5.1, d.f. = 1, p < 0.05, for the first
and second case, respectively), while the O$_{3+4}^+$ arrangement was observed for the first time in the 2009 sample. The O$_{3+4}$ arrangement increased in frequency, but there were no significant differences ($\chi^2 = 1.3$, d.f. = 1, p > 0.05 and $\chi^2 = 3.3$, d.f. = 1, p > 0.05, for the first and second case, respectively). However, O$_{3+4}$ and the other O$_{3+4}$ chromosomal arrangements derived from O$_{3+4}$ (O$_{3+4}^+$, O$_{3+4}^+22$) showed significant increases in frequency ($\chi^2 = 5.5$, d.f. = 1, p < 0.05 and $\chi^2 = 7.1$, d.f. = 1, p < 0.01 for the two cases, respectively). Finally, there was no significant difference in frequency for any chromosomal arrangement between the 2008 and 2009 samples.

The frequencies of the chromosomal karyotypes obtained for the Apatin population are shown in Table 2. The 1994 and 2008+2009 samples had 28 and 23 chromosomal karyotypes, respectively. Sixteen chromosomal karyotypes were found in both the old and new samples. Twelve chromosomal karyotypes previously observed were not found in the new samples, while seven chromosomal karyotypes present in the new samples were not detected in 1994. There were significant differences in the frequency of the U karyotypes ($\chi^2 = 19.6$, d.f. = 8, p < 0.05) between the 1994 and 2008+2009 samples. The U$_{w}/U_{u}$ karyotype showed a dramatic decrease (not seen in the 2008 and 2009 samples), whereas U$_{w}/U_{1+8}^+$ appeared for the first time in the 2009 sample. There were also significant differences in the frequencies of all 14 O-chromosome karyotypes for the two samples analyzed (1994 versus 2008+2009) ($\chi^2 = 29.2$, d.f. = 13, p < 0.01). O$_{w}/O_{a}$ showed a significant decrease in frequency ($\chi^2 = 7.3$, d.f. = 1, p < 0.01), while O$_{w}/O_{3+4}$ showed a significant increase ($\chi^2 = 7.8$, d.f. = 1, p < 0.01) and O$_{3+4}/O_{3+4}^+$ was detected for the first time in the 2009 sample. A similar result was found for all nine E chromosome karyotypes ($\chi^2 = 16.8$, d.f. = 8, p < 0.05). E$_{u}/E_{u}$ showed a significant decrease in frequency ($\chi^2 = 4.5$, 

### Table 1 - Frequencies of chromosomal arrangements for the Apatin natural population of *Drosophila subobscura*.

| Chromosomal arrangement | June 1994 | June 2008 | June 2009 | June 2008+2009 |
|-------------------------|-----------|-----------|-----------|----------------|
|                         | n (p (%)) | n (p (%)) | n (p (%)) | n (p (%))      |
| A$_{a}$                 | 25 (50.0) | 3 (60.0)  | 7 (46.7)  | 10 (50.0)      |
| A$_{1}$                 | 21 (42.0) | 2 (40.0)  | 7 (46.7)  | 9 (45.0)       |
| A$_{2}$                 | 4 (8.0)   | /         | 1 (6.6)   | 1 (5.0)        |
| Total                   | 50 (100)  | 5 (100)   | 15 (100)  | 20 (100)       |
| J$_{a}$                 | 39 (39.0) | 4 (40.0)  | 11 (36.7) | 15 (37.5)      |
| J$_{1}$                 | 61 (61.0) | 6 (60.0)  | 19 (63.3) | 25 (62.5)      |
| Total                   | 100 (100)| 10 (100)  | 30 (100)  | 40 (100)       |
| U$_{w}$                 | 52 (52.0) | 5 (50.0)  | 6 (20.0)  | 11 (27.5)      |
| U$_{1}$                 | 1 (1.0)   | /         | /         | /              |
| U$_{1}^+$               | 23 (23.0) | 4 (40.0)  | 15 (50.0) | 19 (47.5)      |
| U$_{1+2}$               | 24 (24.0) | 1 (10.0)  | 6 (20.0)  | 7 (17.5)       |
| Total                   | 100 (100)| 10 (100)  | 30 (100)  | 40 (100)       |
| E$_{w}$                 | 70 (70.0) | 7 (70.0)  | 18 (60.0) | 25 (62.5)      |
| E$_{1}$                 | 2 (2.0)   | /         | /         | /              |
| E$_{1+2}$               | 11 (11.0) | /         | /         | /              |
| E$_{1+2+9}$             | /         | /         | /         | /              |
| E$_{1+2+9+12}$          | /         | /         | /         | /              |
| E$_{u}$                 | 17 (17.0) | 3 (30.0)  | 4 (13.4)  | 7 (17.5)       |
| Total                   | 100 (100)| 10 (100)  | 30 (100)  | 40 (100)       |
| O$_{a}$                 | 61 (61.0) | 4 (40.0)  | 12 (40.0) | 16 (40.0)      |
| O$_{a}$                 | 6 (6.0)   | /         | /         | /              |
| O$_{2}$                 | /         | /         | /         | 1 (3.3)        |
| O$_{2}$                 | 23 (23.0) | 6 (60.0)  | 10 (33.3) | 16 (40.0)      |
| O$_{3+4}$               | 8 (8.0)   | /         | 2 (6.6)   | 2 (5.0)        |
| O$_{3+4}$               | 2 (2.0)   | /         | /         | /              |
| O$_{3+4}$               | /         | /         | /         | 5 (16.7)       |
| Total                   | 100 (100)| 10 (100)  | 30 (100)  | 40 (100)       |
d.f. = 1, p < .05), while for E8/E8 there was a significant increase ($\chi^2 = 5.1, d.f. = 1, p < 0.05$) and E8/E1+2+9+12 was observed for the first time in the 2009 sample.

The variations in chromosomal polymorphisms were compared with the meteorological data shown in Table 3. The combined average for the maximum, minimum and mean temperatures (26.4 °C, 14.4 °C and 20.6 °C, respectively) for 2008 and 2009 were higher than in 1994. A year-by-year analysis from 1994 onwards revealed several heat waves such as the well documented heatwave of 2003.

Table 2 - Frequencies of chromosomal karyotypes for the Apatin natural population of *Drosophila subobscura*.

| Chromosomal karyotype | June 1994 | June 2008 | June 2009 | June 2008+2009 |
|-----------------------|-----------|-----------|-----------|----------------|
|                       | n  | p (%)   | n  | p (%)   | n  | p (%)   | n  | p (%)   |
| Jst/Jst                | 9  | 18.0    | 1  | 20.0    | 1  | 6.7     | 2  | 10.0    |
| Jst/J1                 | 21 | 42.0    | 2  | 40.0    | 9  | 60.0    | 11 | 55.0    |
| Jst/J1                 | 20 | 40.0    | 2  | 40.0    | 5  | 33.3    | 7  | 35.0    |
| Total                  | 50 |          | 5  |          | 15 |          | 20 |         |
| Jst/J1+2               | 17 | 34.0    | /  | /       | /  | /       | /  | /       |
| Jst/J1+2               | 10 | 20.0    | 4  | 80.0    | 3  | 20.0    | 7  | 35.0    |
| Jst/J1+2               | 8  | 16.0    | 1  | 20.0    | 1  | 6.7     | 2  | 10.0    |
| Ust/Ust                | /  | /       | /  | /       | /  | /       | /  | /       |
| Ust/U1+2               | 6  | 12.0    | /  | /       | 3  | 20.0    | 3  | 15.0    |
| Ust/U1+2               | /  | /       | /  | /       | 1  | 6.7     | 1  | 5.0     |
| Total                  | 50 |          | 5  |          | 15 |          | 20 |         |
| E8/E8                 | 29 | 58.0    | 3  | 60.0    | 3  | 20.0    | 6  | 30.0    |
| E8/E1+2               | 1  | 2.0     | /  | /       | /  | /       | /  | /       |
| E8/E1+2               | 8  | 16.0    | /  | /       | 6  | 40.0    | 6  | 30.0    |
| E8/E1+2               | /  | /       | /  | /       | 2  | 13.3    | 2  | 10.0    |
| E8/E8                 | 3  | 6.0     | 1  | 20.0    | 4  | 26.7    | 5  | 25.0    |
| E8/E8                 | 2  | 4.0     | /  | /       | /  | /       | /  | /       |
| E8/E8                 | 1  | 2.0     | 1  | 20.0    | /  | /       | 1  | 5.0     |
| E8/E1+2               | 1  | 2.0     | /  | /       | /  | /       | /  | /       |
| E8/E1+2               | 5  | 10.0    | /  | /       | /  | /       | /  | /       |
| Total                  | 50 |          | 5  |          | 15 |          | 20 |         |
| O6/O3+4               | 22 | 44.0    | /  | /       | 2  | 13.3    | 2  | 10.0    |
| O6/O3+4               | 4  | 8.0     | /  | /       | /  | /       | /  | /       |
| O6/O3+4               | /  | /       | /  | /       | 1  | 6.7     | 1  | 5.0     |
| O6/O3+4               | 7  | 14.0    | 4  | 80.0    | 5  | 33.3    | 9  | 45.0    |
| O6/O3+4               | 4  | 8.0     | /  | /       | 1  | 6.7     | 1  | 5.0     |
| O6/O3+4               | 2  | 4.0     | /  | /       | /  | /       | /  | /       |
| O6/O3+4               | /  | /       | /  | /       | 1  | 6.7     | 1  | 5.0     |
| O6/O3+4               | 1  | 2.0     | /  | /       | /  | /       | /  | /       |
| O6/O3+4               | 1  | 2.0     | /  | /       | /  | /       | /  | /       |
| O6/O3+4               | 7  | 14.0    | 1  | 20.0    | 1  | 6.7     | 2  | 10.0    |
| O6/O3+4               | 1  | 2.0     | /  | /       | /  | /       | /  | /       |
| O6/O3+4               | 3  | 20.0    | 3  | 15.0    |
| O6/O3+4               | 1  | 2.0     | /  | /       | /  | /       | /  | /       |
| O6/O3+4               | 1  | 6.7     | 1  | 5.0     |
| Total                  | 50 |          | 5  |          | 15 |          | 20 |         |
The climate of the Apatin region is changing, with increased rainfall and temperatures. The Table 3 presents meteorological data for the Apatin region for the month of June from 1994 to 2009. The data suggest a gradual increase in temperature at the locality studied. However, desiccation does not seem to affect the Apatin habitat: although the rainfall pattern is irregular, a general decrease has not been observed. Indeed, data from 2006 onwards suggest that there has been a tendency for rainfall to increase, in agreement with global warming predictions (Houghton, 2005; Wentz et al., 2007).

The IFR values obtained for the Apatin population were: 75.60 ± 1.88 (June 2008) and 77.13 ± 2.11 (2009), with a mean of 76.75 ± 1.63. These values were only slightly lower than that for 1994 (84.62 ± 1.55). Table 4 shows Nei's genetic distances between the Apatin and other Balkan populations. This table also provides older genetic distances between Apatin (1994) and these Balkan populations (Zivanovic et al., 2002; Zivanovic, 2007). The new genetic distances were lower than those obtained previously.

**Discussion**

The world's climate is changing rapidly, with a global temperature increase of 0.6 °C in the past three decades, and 0.8 °C in the past century (Hansen et al., 2006; Van Heerwaarden and Hoffmann, 2007). Many animals and plants adapt rapidly to variations in environmental conditions (Endler, 1986). In particular, widespread generalist species such as *D. melanogaster* and *D. subobscura*, which have short generation times and rapidly form phenotypic and genotypic clines, are excellent sensitive indicators of such global warming (Van Heerwaarden and Hoffmann, 2007; Balanyà et al., 2009). The results of long-term studies indicate that there have been changes in the frequency of chromosomal arrangements in *D. subobscura*. In recent decades, a significant decrease in the frequency of standard chromosomes (“cold” adapted and abundant in northern latitudes) and an increase in some non-standard chromosomal arrangements (“warm” adapted and characteristic of southern latitudes) have been described in natural *D. subobscura* populations from southwestern and central Europe (de Frutos and Prevosti, 1984; Gosteli, 1990; Orengo and Prevosti, 1996; Rodriguez-Trelles et al., 1996; Solé et al., 2002; Balanyà et al., 2004, 2006, 2009) and in American colonizing populations (Balanyà et al., 2006, 2009). These changes have been attributed to the adaptation of chromosomal inversion polymorphism to a warmer climate in Europe and America.

The effect of global warming in the Apatin population is reflected as variation in chromosomal polymorphism. Specifically, there was a significant increase in the frequency of some chromosomal arrangements characteristic of southern latitudes (“warm” adapted) and a significant decrease in the frequency of some standard inversions (“cold” adapted) in the O and U chromosomes. Thus, O3+4 showed a significant increase when considered together with O3+4+x arrangements (~40% on average) in 2008 and 2009 compared to 1994. Similar results have been reported for other southwestern European populations (Orengo and Prevosti, 1996; Rodriguez-Trelles and Rodriguez, 1998). Interestingly, associations between heat-tolerance and the O3+4 arrangement have been described previously for *D. melanogaster*.
subobscura (Quintana and Prevosti, 1991). Furthermore, several genes that code for heat stress proteins (Moltó et al., 1992) and others involved in thermal adaptation (Laayouni et al., 2007) are located in this arrangement. In addition, the O_{3+4} and O_{3+4-x} frequencies in the old Apatin population (June 1994) were 53% and 59% lower, respectively, that in all other southern Balkan populations (Jastrebac, June 1993; Petnica, June 1995; Kamariste, June 1996; Zanjic, June 1997; Djdap, June 2001; Avala, June 2004) (Zivanovic et al., 2002; Zivanovic and Marinov, 2003; Zivanovic, 2007; Zivanovic and Mestres, 2010a,b). In contrast, the frequencies of the O_{3+4} and O_{3+4-x} arrangements in the new Apatin population (2008 + 2009 sample) were no much lower (by 19% and 28%, respectively) than those previously reported for the southern Balkan populations. Data for the O-chromosome suggest that the Apatin population is evolving towards a more “southern” population. Similarly, for the U chromosome, a significant increase in the U_{1+2} frequency (~50%) during the period studied, as well as the presence of the U_{1+2} arrangement which was not previously observed, indicate evolution towards a “southern” population.

Our results agree with those from southwestern and central European populations (Rodriguez-Trelles and Rodriguez, 1998; Solé et al., 2002; Balanyà et al., 2004, 2006, 2009). The relatively low IFR values obtained in this study (not previously observed in 1994) are characteristic of ecologically central European populations of D. subobscura (Krimbas, 1993). This result, and the smaller genetic distances between the new Apatin population (2008 + 2009) and all other Balkan populations (Table 4) compared to the distances previously found, provide further evidence that the Apatin population is becoming more “southern”. A similar decrease in genetic distances over time has been observed in Mediterranean, Atlantic and Central European populations of D. subobscura (Balanyà et al., 2004).

The absence of frequency changes for chromosomal polymorphism in the remaining chromosomes (A, J and E) suggests that our results were most probably not a consequence of gene flow from the south, where the frequencies of all the arrangements in these three chromosomes are completely different in all southern D. subobscura populations analyzed (Zivanovic et al., 2002; Zivanovic and Marinkovic, 2003; Zivanovic, 2007; Zivanovic and Mestres, 2010a,b). Together, these observations suggest that we have detected the effect of selection on chromosomal polymorphism composition (see also the discussion of this topic in Hoffman and Rieseberg, 2008). Some form of natural selection appears to be acting as a reaction to the increase in temperature associated with many heat waves that have markedly affected the Balkan Peninsula; random processes and genetic drift do not appear to be involved. The latter conclusion is supported by the high migration rate among D. subobscura populations from the Balkan Peninsula (Zivanovic et al., 2007) since migration among populations efficiently purges most effects of genetic drift.

Acknowledgments

This study was supported by grants from the Ministry of Science and Technological Development of the Republic of Serbia (no. 173025), Ministerio de Ciencia e Innovación (Spain) (no. BFU2009-07564) and “Generalitat de Catalunya” (Spain) (no. 2009 SGR 636). We thank Prof. C. Arenas (Dept. Estadistica, Universitat de Barcelona) for her statistical advice and Mr. C. Evans (Servei d’Assessorament Lingüístic, Universitat de Barcelona) for correcting the English.

References

Anderson AR, Hoffmann AA, McKechnie SW, Umina PA and Weeks AR (2005) The latitudinal cline in the In(3R) Payne inversion polymorphism has shifted in the last 20 years in Australian Drosophila melanogaster populations. Mol Ecol 14:851-858.

Balanyà J, Solé E, Oller JM, Sperlich D and Serra L (2004) Long-term changes in the chromosomal inversion polymorphism of Drosophila subobscura. II. European populations. J Zool Syst Evol Res 42:191-201.

Balanyà J, Oller JM, Huey RB, Gilchrist GW and Serra L (2006) Global genetic change tracks global climate warming in Drosophila subobscura. Science 313:1773-1775.

Balanyà J, Huey RB, Gilchrist GW and Serra L (2009) The chromosomal polymorphism of Drosophila subobscura: A micro evolutionary weapon to monitor global change. Heredity 103:364-367.

Bradshaw WE and Holzapfel CM (2006) Evolutionary response to rapid climate change. Science 312:1477-1478.

Carson HL (1955) The genetic characteristics of marginal populations of Drosophila. Cold Spring Harbor Symp Quant Biol 20:276-287.

de Frutos R and Prevosti A (1984) Temporal changes of chromosomal polymorphism in natural populations of Drosophila subobscura. Genetica 63:181-187.

Endler JA (1986) Natural Selection in the Wild. Princeton University Press, Princeton, 336 pp.

Gosteli M (1990) Chromosomal polymorphism in natural populations of Drosophila subobscura near Zurich, Switzerland: A contribution to long-term comparisons. Genetica 81:199-204.

Hansen J, Sato M, Ruedy R, Lo K, Lea DW and Medina-Elizade M (2006) Global temperature change. Proc Natl Acad Sci USA 103:14288-14293.

Hoffmann AA and Rieseberg LH (2008) Revisiting the impact of inversions in evolution: From population genetic markers to drivers of adaptive shifts and speciation? Annu Rev Ecol Evol Syst 39:21-42.

Houghton J (2005) Global warming. Rep Prog Phys 68:1343-1403.

Krimbas CB (1993) Drosophila subobscura: Biology, Genetics and Inversion Polymorphism. Verlag Dr Kovac, Hamburg, 395 pp.

Krimbas CB and Loukas M (1980) The inversion polymorphism of Drosophila subobscura. Evol Biol 12:163-234.
Kunze-Mühl E and Müller E (1958) Weitere Untersuchungen über die chromosomale Struktur und die natürlichen Strukturen von *Drosophila subobscura*. Chromosoma 9:559-570.

Kunze-Mühl E and Sperlich D (1955) Inversionen und chromosomale Strukturen bei *Drosophila subobscura*. Z. Indukt Abstamm Vererb Lehre 87:65-84.

Laayouni H, García-Franco F, Chávez-Sandoval BE, Trotta V, Beltran S, Corominas M and Santos M (2007) Thermal evolution of gene expression profiles in *Drosophila subobscura*. BMC Evol Biol 7:e42.

Levitan M and Etges WJ (2005) Climate change and recent genetic flux in populations of *Drosophila robusta*. BMC Evol Biol 5:e4.

Menozzi P and Krimbas CB (1992) The inversion polymorphism of *D. subobscura* revisited: Synthetic maps of gene arrangement frequencies and their interpretation. J Evol Biol 5:625-641.

Menzel A, Sparks TH, Estrella N, Koch E, Aasa A, Ahas R, Alm-Kubler K, Bissolli P, Braslavskia O, Briede A, et al. (2006) European phenological response to climate change matches the warming pattern. Global Change Biol 12:1969-1976.

Moltó MD, Pascual L, Martinez-Sebastian MJ and de Frutos R (1992) Genetic analysis of heat shock response in the three *Drosophila* species of the obscura group. Genome 35:870-880.

Nei M (1972) Genetic distance between populations. Am Nat 106:283-292.

Orengo DJ and Prevosti A (1996) Temporal changes in chromosomal polymorphism of *Drosophila subobscura* related to climatic changes. Evolution 50:1346-1350.

Prevosti A, Ribó G, Serra L, Aügade M, Balaña J, Monclus M and Mestres F (1988) Colonization of America by *Drosophila subobscura*: Experiment in natural populations that supports the adaptive role of chromosomal-inversion polymorphism. Proc Natl Acad Sci USA 85:5597-5600.

Quintana A and Prevosti A (1991) Genetic and environmental factors in the resistance of *Drosophila subobscura* adults to high temperature shock. III. Chromosomal-inversion and enzymatic polymorphism variation in lines selected for heat shock resistance. Genetica 84:165-170.

Rodríguez-Trelles F and Rodríguez MA (1998) Rapid microevolution and loss of chromosomal diversity in *Drosophila* in response to climate warming. Evol Ecol 12:829-838.

Rodríguez-Trelles F, Alvarez G and Zapata C (1996) Time-series analysis of seasonal changes of the O inversion polymorphism of *Drosophila subobscura*. Genetics 142:179-187.

Schar C and Jendritzky G (2004) Hot news from summer 2003. Nature 432:559-560.

Solè E, Balanyá J, Sperlich D and Serra L (2002) Long-term changes in the chromosomal inversion polymorphism of *Drosophila subobscura*. I. Mediterranean populations from southwestern Europe. Evolution 56:830-835.

Van Heerwaarden B and Hoffmann AA (2007) Global warming: Fly populations are responding rapidly to climate change. Curr Biol 17:R16-R18.

Wentz FJ, Ricciardulli L, Hilburn K and Mears C (2007) How much more rain will global warming bring? Science 317:233-235.

Zivanovic G (2007) Seasonal changes in chromosomal inversion polymorphism in a *Drosophila subobscura* natural population from a south-eastern European continental refugium of the last glaciation period. Russ J Genet 43:1344-1349.

Zivanovic G and Marinkovic D (2003) Viabilities of originally natural O-chromosomal inversion homo and heterokaryotypes in *Drosophila subobscura*. Hereditas 139:128-142.

Zivanovic G and Mestres F (2010a) Viabilities of *Drosophila subobscura* homo- and heterokaryotypes at optimal and stress temperatures. I. Analysis over several years. Hereditas 147:70-81.

Zivanovic G and Mestres F (2010b) Viabilities of *D. subobscura* homo- and heterokaryotypes at optimal and stress temperatures. II. Seasonal component analysis. Hereditas 147:82-89.

Zivanovic G, Andjelkovic M and Marinkovic D (2002) Chromosomal inversion polymorphism of *Drosophila subobscura* from south-eastern part of Europe. J Zool Syst Evol Res 40:201-204.

Zivanovic G, Arenas C and Mestres F (2007) The genetic structure of Balkan populations of *Drosophila subobscura*. Hereditas 144:120-128.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.