RESEARCH NOTE

Medium-term changes in *Drosophila subobscura* chromosomal inversion polymorphism: a possible relation with global warming?

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Introduction

Drosophila subobscura is a species with a rich chromosomal polymorphism for inversions. Evidence demonstrates that it is adaptive. In the present research, we studied whether it is possible to detect changes in the inversion chromosomal polymorphism of *D. subobscura* in a medium-term period of time. The Serbian population of Avala was selected and its inversion composition in 2004 and 2011 (a seven year period) was compared. Significant variation was found in the U chromosome. This result was due to a significant increase of $U_{\underline{1+2}}$ (warm) and a decrease of U_{st} (cold) and $U_{\underline{1+2+6}}$. Further, minimum, maximum and mean temperatures increased (although not significantly). Thus, U chromosome seems to be able to react in a medium-term to temperature changes in the way expected by the global warming.

Karyotype of *D. subobscura* comprises five acrocentric (X = A, E, J, O, U) and one dot chromosomes, with E and O being the most polymorphic for inversions (Krimbas 1992, 1993). Although historic factors cannot be ruled out, it is generally accepted that this polymorphism is adaptive (for a revision, see Zivanovic *et al.* 2012; Pegueroles *et al.* 2013). It is well known that it varies seasonally (Fontdevila *et al.* 1983; Rodríguez-Trelles *et al.* 1996), and the same has been observed in Serbian populations (Zivanovic 2007; Zivanovic and Mestres 2010b). According to global warming expectations, long-term changes have been observed in this species (Orengo and Prevosti 1996; Rodriguez-Trelles and Rodriguez 1998; Solé *et al.* 2002; Balanya *et al.* 2004, 2006, 2009). We have also reported this effect in several studies carried out in the Balkans (Zivanovic and Mestres 2010a, 2011; Zivanovic *et al.* 2012).

The aim of the present research was to assess whether in a medium-term period is it possible to detect changes in the inversion chromosomal polymorphism of *D. subobscura*. In a parallel way, variations in the following temperature measures, maximum, minimum and mean, have also been analysed. We studied Avala (Serbia) population of *D. subobscura* in 2004 and had the opportunity to collect flies again in 2011, exactly in the same location.

Materials and methods

D. subobscura flies were collected from Avala mountain (Serbia) population (44°48′N 20°30′E), located at 18 km south of Belgrade. The physical and biological characteristics of the trapping place are described in Zivanovic and Mestres (2010b). Collections were obtained in 2004 (from 2nd to 9th of June) and in 2011 (from 30th of May to 5th of June), strictly in the same place. The second sample was obtained many days earlier because spring/summer has advanced ∼2.5 days per decade in Europe (Menzel *et al.* 2006). Maximum, minimum and mean temperatures, and rainfall data were obtained from Republic Hydrometeorological Service (Serbia).

Wild males and one son of each wild female were individually crossed with virgin females of the Küsnacht strain, which is homokaryotypic for standard chromosomal arrangements in all five chromosomes (Zollinger 1950). Third instar larvae from the F_1 were dissected and polytene chromosomes were stained and squashed in aceto–orcein solution. For each cross, at least eight larvae were analysed. All crosses were carried out in individual vials with 25 mL standard corn-meal-sugar-agar-yeast medium maintained at 18°C, 60% relative humidity and 12 h light / 12 h dark cycle.

Fisher's exact test was used (statistically significant P value <0.05) to compare the chromosomal composition between 2004 and 2011. This test has been used since it is

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more precise than chi-squared test when the expected frequencies are small. The corresponding *P* values were obtained using the bootstrap procedure (100,000 runs). As described in Zivanovic *et al.* (2012), for analysing the temperature changes along years, a time series analyses was carried out. For all these computations, R package was used (http://CRAN.R-project.org).

Results

Inversion chromosomal polymorphisms from 2004 and 2011 samples are presented in table 1. There were no significant differences for the A chromosome ($P = 0.838$), J ($P = 1$), E ($P = 0.569$) and O ($P = 0.874$). However, there were significant differences for the U chromosome ($P = 0.008$). Using the classification of Menozzi and Krimbas (1992) in 'cold' and 'warm' arrangements, U_{st} (cold) showed a slight decrease ($P = 0.417$), but $U_{\underline{1+2}}$ (warm) showed a significant increase ($P = 0.003$), and the difference was significant even after Bonferroni correction. Finally, $U_{\underline{1+2+6}}$ decreased ($P = 0.118$) and $U_{\underline{1+8+2}}$ (warm) showed a negligible increase ($P = 0.785$). In general, the pattern of changes for the U chromosome agrees with the global warming expectations. As previously mentioned, changes over time were not significant for other chromosomes, but a qualitative observation allows us to conclude that no variation was present for the A and J chromosomes. Thus, comparing the variation in frequency of 'cold' inversions (A_{st} and A_1) with that of 'warm' inversion (A_2), no changes were observed between 2004 and 2011. In the J chromosome, the only inversions observed (J_{st} classified as 'cold' and J_1 considered 'warm') did not show any changes over the period of time analysed. Considering E chromosome, E_{st} ('cold') showed a slight decrease ($P = 0.848$), but the 'warm' $E_{\underline{1+2+9}}$ also decreased ($P = 0.627$). The 'warm' adapted $E_{\underline{1+2+9+12}}$ (not present in 2004) increased ($P = 0.299$). Finally for the O chromosome, O_{st} ('cold') frequency slightly increased ($P = 1$), and among the 'warm' adapted, $O_{\underline{3+4}}$ decreased ($P = 0.755$), whereas $O_{\underline{3+4+1}}$ ($P = 1$) and $O_{\underline{3+4+8}}$ ($P = 0.527$) increased. Following Rego *et al.* (2010), we have tested whether there were differences between 'cold' and 'warm' groups of inversions in this seven year period. Analysing all chromosomes together or individually, in both conditions no significant variation was seen ($P = 0.520$, when analysing all chromosomes) and individually: A ($P = 1$), J ($P = 1$), E ($P = 1$), O ($P = 1$) and U ($P = 0.082$).

Table 1. Frequencies of chromosomal inversions from the Avala mountain population of *D. subobscura*, both in 2004 and 2011.

Chr inversion	2004 *n*	2004 %	2011 *n*	2011 %
A_{st} (cold)	14	45.2	20	40.0
A_1 (cold)	9	29.0	18	36.0
A_2 (warm)	8	25.8	12	24.0
Total	31		50	
J_{st} (cold)	13	21.0	22	22.0
J_1 (warm)	49	79.0	78	78.0
Total	62		100	
U_{st} (cold)	8	12.9	8	8.0
$U_{\underline{1+2}}$ (warm)	20	32.2	57	57.0
$U_{\underline{1+2+6}}$	29	46.8	25	25.0
$U_{\underline{1+8+2}}$ (warm)	5	8.1	10	10.0
Total	62		100	
E_{st} (cold)	15	24.2	22	22.0
$E_{\underline{1+2}}$	2	3.2	2	2.0
$E_{\underline{1+2+9}}$ (warm)	31	50.0	45	45.0
$E_{\underline{1+2+9+12}}$ (warm)	–	–	4	4.0
E_8	14	22.6	27	27.0
Total	62		100	
O_{st} (cold)	12	17.1	18	18.0
O_6	2	2.9	–	–
$O_{\underline{3+4}}$ (warm)	33	47.2	44	44.0
$O_{\underline{3+4+1}}$ (warm)	7	10.0	11	11.0
$O_{\underline{3+4+2}}$	–	–	2	2.0
$O_{\underline{3+4+5}}$	3	4.3	2	2.0
$O_{\underline{3+4+6}}$	1	1.4	2	2.0
$O_{\underline{3+4+7}}$	1	1.4	1	1.0
$O_{\underline{3+4+8}}$ (warm)	3	4.3	7	7.0
$O_{\underline{3+4+17}}$	–	–	1	1.0
$O_{\underline{3+4+22}}$	8	11.4	12	12.0
Total	70		100	

Inversions and arrangements are classified as 'cold-adapted' (cold) and 'warm-adapted' (warm) according to Menozzi and Krimbas (1992); *n*, number of chromosomes; –, absent.

Table 2. Meteorological data for the Avala mountain population for the month of June from 2004 to 2011.

Year	$T_{max.}$ (°C)	$T_{min.}$ (°C)	T_{mean} (°C)	Rainfall (mm)
2004	25.3	15.1	19.9	113.3
2005	24.5	14.8	19.6	107.1
2006	24.2	15.6	19.8	127.1
2007	28.4	17.7	23.0	59.6
2008	27.4	17.5	22.2	84.6
2009	25.1	15.6	20.1	150.6
2010	25.0	16.2	20.5	172.1
2011	26.7	16.5	21.3	43.2

$T_{max.}$, maximum temperatures; $T_{min.}$, minimum temperature.

Data regarding temperature and rainfall variations between 2004 and 2011 are shown in table 2. During this period, maximum temperature increased (25.3 to 26.7°C) and the same pattern was recorded for minimum (15.1 to 16.5°C) and mean (19.9 to 21.3°C) temperatures. Although these measures of temperature showed an increasing trend, none of the changes were significant: maximum ($t = 0.693$, $P = 0.514$), minimum ($t = 1.256$, $P = 0.256$) and mean ($t = 0.876$, $P = 0.415$). Rainfall followed an erratic profile as expected under global warming conditions.

Discussion

In long-term studies, it has been observed that *D. subobscura* inversions change in frequency according to global warming expectations in three continents (Balanyà *et al.* 2006). For the medium-term study, our main result is that a significant change in frequency was observed for one of the chromosomes of *D. subobscura* karyotype. The significant variation for the U chromosome was found in a medium-term period (seven years) and according to global warming expectations, because in this period, temperatures tend to increase. Likely, it was due to the significant increase of $U_{\underline{1+2}}$ (warm) arrangement, whereas $U_{\underline{1+2+6}}$ clearly decreases. This later arrangement is considered to be not associated with temperature adaptation, but is characteristic of the Balkan region (Krimbas 1992, 1993). Additionally, another 'cold' adapted arrangement (U_{st}) decreased and a 'warm' adapted one ($U_{\underline{1+8+2}}$) increased, both in small proportion. Studying long-term changes, other authors have found significant variations for the U chromosome. Thus, De Frutos and Prevosti (1984), in a 21 years study and analysing different sites of the same locality (Tibidabo hill, near Barcelona) found significant changes for the U, A, E and O chromosomes. In the same place and continuing the previous research, Orengo and Prevosti (1996) found significant variations for the $U_{\underline{1+2}}$ and $U_{\underline{1+8+2}}$ arrangements. In another long-term study, Solé *et al.* (2002) found a significant effect for the U chromosome in Montpellier (south of France) and Calvià (Majorca Island). In another long-term analysis using Atlantic and central Europe populations, significant differences in frequency for the U and O chromosomes were also detected (Balanyà *et al.* 2004). Finally in Balkans, Zivanovic and Mestres (2011) we found a significant variation for the U chromosome in Apatin (Serbia) population during 1994–2009. In this case, results were similar to those presented in the present research: U_{st} (cold) significantly decreased, $U_{\underline{1+2}}$ (warm) significantly increased and $U_{\underline{1+8+2}}$ (also considered 'warm') was found for the first time in this population. Similar results were found at Petnica (Serbia) for the period 1995–2010 (Zivanovic *et al.* 2012): U_{st} (cold) slightly decreased, $U_{\underline{1+2+6}}$ dramatically decreased, and $U_{\underline{1+2}}$ and $U_{\underline{1+8+2}}$ (both considered 'warm') clearly increased.

We speculate that U chromosome responds to medium and long-term changes because it presents a reduced number of arrangements in most studied populations. Further, the majority of these arrangements are associated with temperature. Maybe they do not react directly to temperature, but to an environmental factor linked to it. However, not all arrangements are adaptive to temperature. As mentioned previously, $U_{\underline{1+2+6}}$ is considered to be not related with thermal adaptation, but is rather frequent in the Balkan region and Asia Minor. In particular, environmental conditions of this region, it probably presents an adaptive advantage to their carriers. In the Balkans, it has also been observed to decrease in frequency over time in different studies (Zivanovic and Mestres 2011; Zivanovic *et al.* 2012), likely due to some kind of environmental changes. Thus, it seems that natural conditions are probably changing along the years in these populations and *D. subobscura* is adapting to them.

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