

# Unexpected long-term changes in chromosome inversion frequencies in a Neotropical *Drosophila* species

Marcos R. D. Batista, Galina Ananina, Louis B. Klaczko\*

Departamento de Genética, Evolução e Bioagentes, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil

**ABSTRACT:** Several long-term studies on *Drosophila* chromosome inversion polymorphisms have shown that inversions can be a valuable tool to monitor rapid genetic shifts with climate change. However, so far, no study has assessed the effects of climate change in populations of Neotropical *Drosophila* species. After more than 2 decades, new samples were collected from the Parque Nacional do Itatiaia, Rio de Janeiro, to assess any changes in inversion frequencies and to detect possible global warming effects on the inversion polymorphism of the second chromosome of *D. mediopunctata*. Our results show unexpected simultaneous changes in inversion frequencies associated with climate change. Perhaps climatic variables other than temperature underlying the process caused such change, although potential genetic drift effects or demographic factors cannot be excluded. Further studies assessing population genetic structure may help clarify the changes observed.

**KEY WORDS:** Global warming · Chromosomal inversion · Brazilian Atlantic Forest · Temporal variation · *Drosophila mediopunctata*

Resale or republication not permitted without written consent of the publisher

## 1. INTRODUCTION

Climate change has affected global biota, producing rapid evolutionary responses (Parmesan 2006, Lavergne et al. 2010). Montane tropical biota are believed to be particularly vulnerable to global warming effects (Sheldon et al. 2011). Several long-term studies on *Drosophila* inversion polymorphisms have shown that changes in inversion frequencies can be a valuable tool to monitor rapid genetic shifts caused by climatic change (Stamenkovic-Radak et al. 2008, Balanyà et al. 2009, Levitan & Etges 2009, Rodriguez-Trelles & Rodriguez 2010; for review see: Hoffmann & Rieseberg 2008 and Rezende et al. 2010). None of these studies, however, addresses the potential effects of climate change on a *Drosophila* Neotropical species.

*Drosophila mediopunctata* belongs to the *tripunctata* group in the subgenus *Drosophila*. This is the

second largest *Drosophila* group from the Neotropical region, comprising 79 species (Yotoko et al. 2003, Hatadani et al. 2009, Bächli 2011).

This species has a wide distribution and has been reported in many regions in Brazil, such as the south where it is the most commonly collected species, and in other places of South and Central America, especially at high altitudes (Saavedra et al. 1995).

*Drosophila mediopunctata* is an almost exclusively forest dwelling species (rare or absent in Brazilian savannah [cerrado] and caatinga; see Tidon 2006, Mata et al. 2010). It has 5 pairs of acrocentric chromosomes and 1 pair of dot chromosomes that do not polytenize (Kastritsis 1966), and is highly polymorphic for chromosome inversions. The X chromosome has 3 inversions, one of which is related to the sex-ratio trait (Carvalho et al. 1989). Chromosome IV has 2 inversions, and chromosome II has 17 gene arrangements: 8 in the distal region (DA, DI, DP, DS, DV, etc.) and 9

in the proximal region (PA0, PB0, PC0, PC1, etc.). There is strong linkage disequilibrium between inversions in the distal and proximal regions of the second chromosome. Among the 72 possible combinations of distal region and proximal inversions found (Peixoto & Klaczko 1991, Ananina et al. 2002, Klaczko 2006), ~90% of the total is represented by 5 haplotypes (DA-PA0, DI-PB0, DS-PC0, DP-PC0 and DV-PC0).

A natural population of *Drosophila mediopunctata* from the Parque Nacional do Itatiaia, Rio de Janeiro State, was investigated in the late 1980s, where an altitudinal cline was detected for morphological traits and inversion frequencies (Bitner-Mathé et al. 1995, Bitner-Mathé & Klaczko 1999, Ananina et al. 2004). Moreover, the frequencies of inversions DA, DP and DS showed cyclical seasonal variation (Klaczko 2006). The frequency of DA (usually associated with proximal arrangement PA0) increased in cold dry months and decreased in warmer rainy months, while pooled frequencies of DP and DS (both associated with PC0) showed an opposite pattern. Consistently, this pattern was observed in an altitudinal cline in which the frequency of DA showed a significant positive correlation with altitude ( $r = 0.87$ ,  $p < 0.01$ ), while the pooled frequencies of DP and DS showed a highly significant yet negative correlation ( $r = -0.91$ ,  $p < 0.001$ ). Gene arrangement DI did not show any seasonal variation or correlation with either temperature or altitude. This pattern suggests that natural selection is maintaining the observed variation (Ananina et al. 2004, Klaczko 2006).

Hatadani & Klaczko (2008) showed that *Drosophila mediopunctata* wing size and shape were influenced by second chromosome karyotype, sex, and temperature under controlled laboratory conditions. They also found evidence of interaction between karyotype and temperature on wing shape, suggesting the existence of genotype-environment interaction on this character, and that this interaction may be correlated with temperature differences across seasons. Thus, the genetic content of the inversions may have accumulated different alleles adapted to different temperatures. Similarly, Andrade et al. (2009) showed that different chromosome karyotypes affected not only wing shape, but also male genital (aedeagus) morphology.

After >2 decades, new collections were carried out at the Parque Nacional do Itatiaia in order to characterize long-term changes in inversion frequencies and to assess the possible effects from climatic change, in particular the potential impact of global warming on the inversion polymorphism of the

second chromosome. We tested the hypothesis that, after 2 decades, inversions associated with hot-rainy weather (DS and DP) increased in frequency at the expense of DA (DA being the inversion correlated with cold-dry climate conditions).

## 2. MATERIALS AND METHODS

### 2.1. *Drosophila mediopunctata* samples

Between June 2007 and August 2010, 7 field trips were carried out in the Parque Nacional do Itatiaia, Rio de Janeiro, Brazil (22° 26' S, 44° 37' W). Our collection strategy was designed to test the following hypotheses: (1) if the current overall frequency of gene arrangements was similar to those previously described by Ananina et al. (2004), and if the overall correlations with environmental variables were still valid; we sampled different altitudinal points varying among a total of 9 points (from 590 to 1190 m above sea level, a.s.l., see Table A1 in Appendix 1) in each of the 7 collections in different seasons; (2) if gene arrangements DA, DS and DP were still significantly associated to altitude; we sampled fly populations from 7 different altitudes (from 590 to 1190 m a.s.l., Table A1) on one single occasion (cold-dry season 2009); and (3) if gene arrangements DA, DS and DP were still cycling seasonally; 2 areas (sites at altitudes of 750 and 950 m a.s.l.) were sampled on each of the 7 collecting dates to avoid confounding the effects of seasonal cycling and clinal variation of the inversion frequencies.

Flies were collected over fermented banana baits with entomological nets. They were then taken to the laboratory for analysis.

### 2.2. Cytological procedures and statistical analysis

Inversion frequency distributions were estimated using the 'egg sample' and 'male' methods (Arnold 1981, Ananina et al. 2004). Wild caught males were individually crossed with 3 virgin females from the ITC-229-ET homokaryotypic strain, maintained at 16.5°C and reared on trimeveledon culture medium (whole wheat flour, yeast, agar, sugar and powdered milk, see Carvalho et al. 1989). From each cross, up to 8 F1 larvae were karyotyped to infer the male parent karyotype, which was used to estimate inversion frequencies. The polytene salivary gland chromosomes of third instar larvae were prepared with 1N HCl, and subsequently stained with lacto-acetic-

orcein, as described by Ashburner (1989). For the 'egg sample' method, one F1 larvae from each wild caught female was analyzed and the observed karyotype used to estimate the inversion frequencies. For each collection, the estimates of the 2 methods were compared with a chi-square test (Table A2). Since no case of significant difference was found, the frequencies were pooled. We must note that when the number of individuals in one of the sexes was  $<5$ , we did not test the differences; however, no relevant difference could be seen by visual inspection (data not shown).

In 2009, during the fall collection, altitude was measured with a GPS navigation device (Table A1). Climatic variables were obtained from Resende Automatic Station; available data can be found at [www.agritempo.gov.br](http://www.agritempo.gov.br) (accessed May 10, 2011). For the analysis, the frequency of the arrangement DV was pooled with the frequencies of DS and DP, since they are all preferentially associated with PCO.

We compared the inversion frequencies with those previously published (Peixoto & Klaczko 1991, Ananina et al. 2004) using a chi-square test (Zar 2010). Pearson's correlation tests were performed to assess associations between inversion frequencies (after angular transformation, Zar 2010) and geographical as well as climatic variables including altitude, temperature and precipitation.

### 3. RESULTS

#### 3.1. Long-term variation of the climatic variables

Fig. 1 shows the long-term variation for the maximum, minimum and mean temperatures over the last 3 decades. Positive and significant correlations between years and mean as well as minimum temperatures were observed ( $T_{\text{mean}}$ :  $r = 0.46$ ,  $p = 0.0195$ ;  $T_{\text{min}}$ :  $r = 0.62$ ,  $p = 0.0009$ ). Fig. 2 shows a marginally significant trend of decreasing annual precipitation in the region ( $r = -0.399$ ,  $p = 0.048$ ). If we remove the outlier years 2006 and 2007 from the analysis, however, temporal changes in precipitation are no longer significant ( $r = 0.318$ ,  $p = 0.130$ ).

#### 3.2. Temporal variation of the inversion frequencies

Table A1 shows second chromosome inversion frequencies on different collecting dates at different altitudinal sites. Current average second chromosome

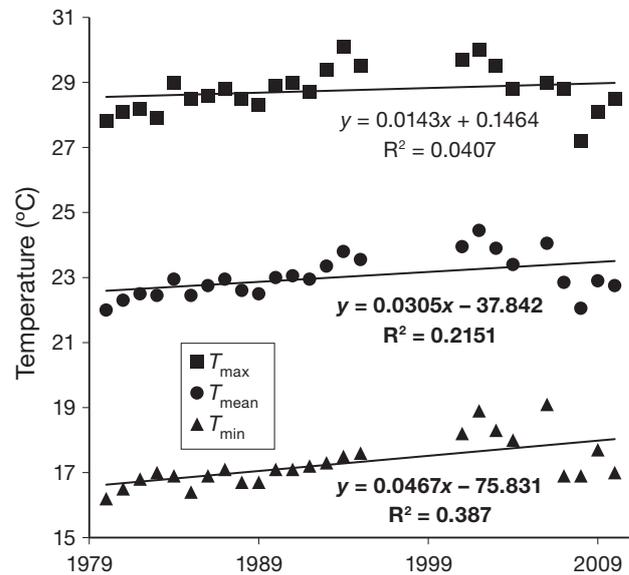


Fig. 1. Long-term temperature variations at Parque Nacional do Itatiaia, Rio de Janeiro. Annual means of average monthly maximum ( $T_{\text{max}}$ ) and minimum ( $T_{\text{min}}$ ) temperatures;  $T_{\text{mean}}$ : mean of  $T_{\text{max}}$  and  $T_{\text{min}}$ . Coefficients of determination ( $R^2$ ) and equations in **bold** are significant at 5% level

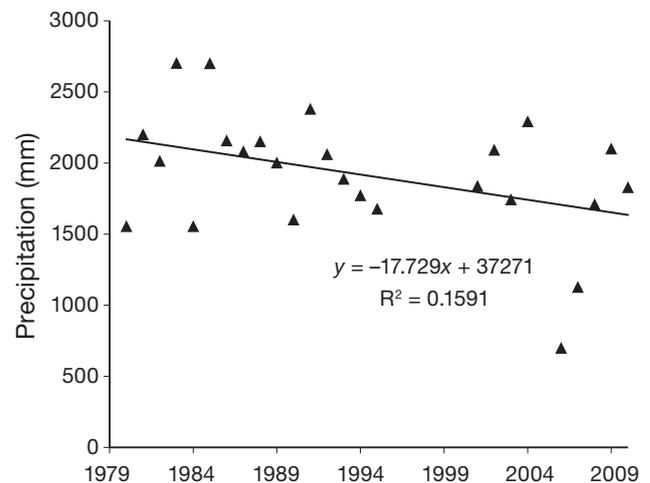


Fig. 2. Long-term variation of precipitation (annual sum of precipitation) at Parque Nacional do Itatiaia, Rio de Janeiro

inversion frequencies (Table 1) were significantly different from those in 1986 to 1988 ( $\chi^2 = 74.97$ ,  $p < 0.001$ ). Unexpectedly, increases of DA and especially of DI frequencies, as well as a decrease in the frequencies of DS, DP and DV, were significant (Fig. 3).

Furthermore, we compared the average frequency estimates for 2 different seasons: cold and dry (collections performed during fall and winter); hot and rainy (collections performed during spring and summer). To avoid confounding the effects of altitudinal clines

Table 1. *Drosophila mediopunctata*. Chromosome II inversion frequencies (%) of *D. mediopunctata* averaged over seasons (data only from 750 and 950 m altitude areas) and overall average for the 7 collections (overall frequency: data pooled from all areas sampled) at Parque Nacional do Itatiaia, Rio de Janeiro. 2N: number of chromosomes analyzed. OT: rare arrangements (DJ, DR, DT)

Inversions	Hot-rainy season (%)	Cold-dry season (%)	Overall frequency (%)
DA	46.3	57.0	54.8
DI	29.3	29.9	29.5
DS	13.0	4.1	7.5
DP	4.9	2.9	2.7
DV	4.9	4.5	4.0
OT	1.5	1.6	1.5
2N	324	314	1178

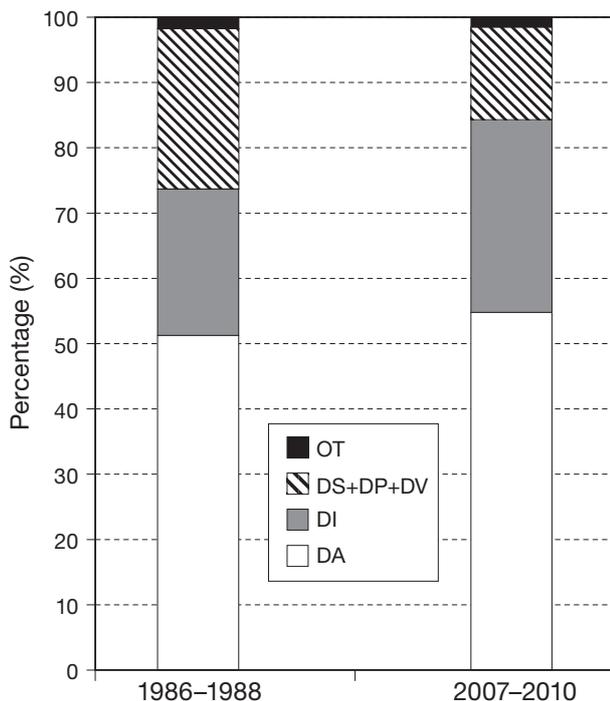


Fig. 3. *Drosophila mediopunctata*. Comparison between current (2007–2010) and previous (1986–1988) average frequencies of *D. mediopunctata* second chromosome inversions. OT: rare arrangements (DJ, DR, DT)

with seasonal effects, only data from population samples at 950 and 750 m were considered, since these populations were collected in every field trip. Fig. 4 shows that the frequencies of DA and the combined frequencies of DS + DP + DV continued to show seasonal variation as previously described in Ananina et al. (2004). Also, DI did not show any noticeable seasonal variation as previously described (Figs. 3 & A1).

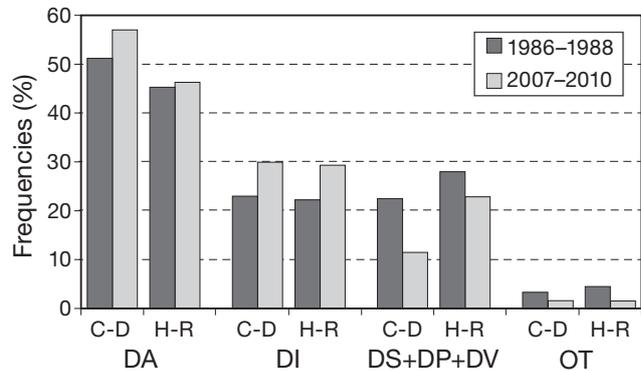


Fig. 4. *Drosophila mediopunctata*. Seasonal cycle of inversion frequencies (%) at Parque Nacional do Itatiaia, Rio de Janeiro. Only data from areas at 950 and 750 m were considered, since they covered the entire seasonal cycle. C-D: cold-dry season; H-R: hot-rainy season

### 3.3. Clines and correlations

To assess if previously described altitudinal clines were still present in these populations, we calculated correlations of inversion frequencies with altitudes from a single collecting date: fall 2009 (Fig. 5). The DA gene arrangement no longer showed a significant correlation with altitude ( $r = -0.12$ ,  $p = 0.78$ ), but DS continued to show a significant negative correlation ( $r = -0.71$ ,  $p < 0.05$ ) as previously found. Since DP is now found in low frequencies, we pooled the data for inversions DS, DP and DV, consistently finding a significant and negative correlation with altitude ( $r = -0.82$ ,  $p = 0.013$ ). Surprisingly, we also observed a positive and significant correlation between DI and altitude ( $r = 0.75$ ,  $p = 0.032$ ).

Correlations between inversion frequencies and temperature as well as precipitation data from the Resende climatic station (Table 2) were consistent with previous results (Ananina et al. 2004): DA was negatively correlated with temperature (but no longer significant:  $r = -0.55$ ,  $p = 0.20$ ), as was precipitation (close to but not significant:  $r = -0.74$ ,  $p = 0.055$ ). While DS was positively and significantly correlated with these variables (temperature:  $r = 0.756$ ,  $p = 0.049$ ; precipitation:  $r = 0.797$ ,  $p = 0.032$ ), no significant correlations were detected between DI and these climatic variables.

## 4. DISCUSSION

The observed overall climatic changes in the region of the Parque Nacional do Itatiaia were consistent with the effects of global warming as considered by the Intergovernmental Panel on Climate Change

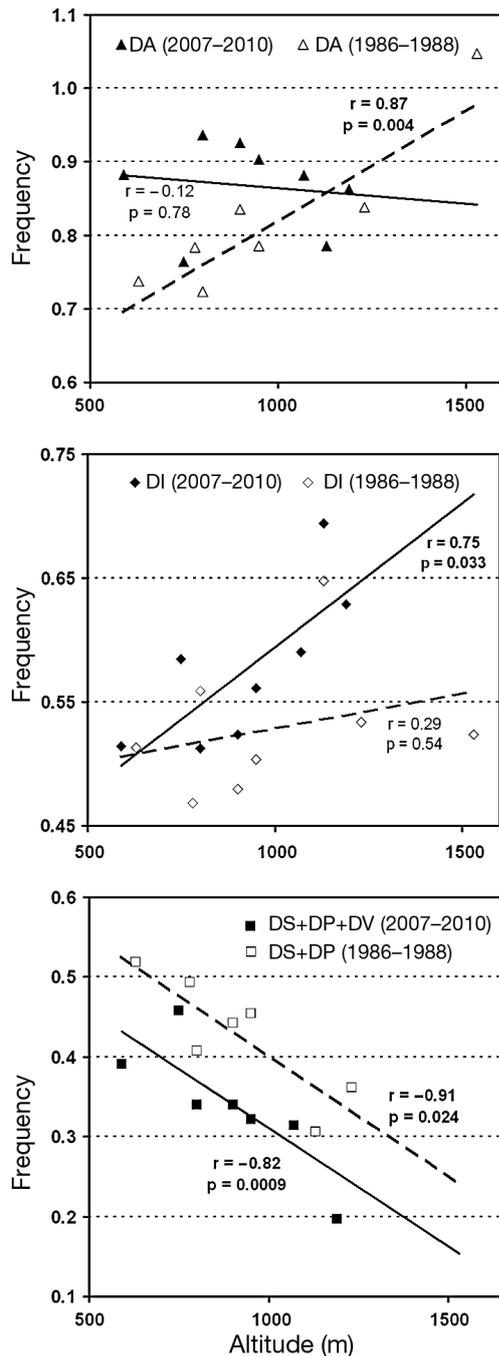


Fig. 5. *Drosophila mediopunctata*. Altitudinal variation in chromosomal inversion frequencies (proportions after angular transformation). Solid lines: fall 2009 collection; dashed lines: previous collections pooled (1986–1988); frequencies of DA (triangles), DI (diamonds), DS+DP+DV (squares). Pearson’s correlation coefficients ( $r$ ) and  $p$ -values in **bold** are significant at 5% level

(IPCC 2007). There was a clear trend in the increasing averages of minimum temperatures; although this was not apparent for maximum temperatures (Easterling et al. 1997).

Table 2. *Drosophila mediopunctata*. Pearson’s correlation ( $r$ ) between inversion frequencies and different climatic variables. \*Significant correlations. OT: rare arrangements (DJ, DR, DT)

Inversions	Temperature		Precipitation	
	$r$	$p$	$r$	$p$
DA	-0.548	0.202	-0.744	0.055
DI	-0.390	0.386	0.076	0.871
DS	0.756	0.049*	0.796	0.032*
DS + DP + DV	0.725	0.065	0.748	0.053
OT	0.355	0.434	-0.101	0.829

Long-term analyses of chromosomal inversion polymorphisms in different *Drosophila* species have shown changes in inversion frequencies associated with recent climate change, suggesting that widespread species may undergo adaptive shifts in response to global warming (Hoffmann & Rieseberg 2008). In Australian *D. melanogaster* populations, the In(3R)Payne inversion increased in frequency near the equator, forming a latitudinal cline (Knibb et al. 1981). After ~20 yr, Anderson et al. (2005) and Umina et al. (2005) showed frequency increase in this inversion in all Australian populations, suggesting that this was a direct response to global warming.

In *Drosophila robusta*, arrangements more frequent in northern regions for different North American populations decreased in frequency while ‘southern’ gene arrangements increased in frequency over the last 20 yr (Etges & Levitan 2008, Levitan & Etges 2009). Rodríguez-Trelles & Rodríguez (1998) showed O chromosomal arrangement frequency changes in populations of *D. subobscura* with temporal increases of southern arrangement frequencies. Further, Balanyà et al. (2006) showed that *D. subobscura* populations from 3 different continents evolved similar patterns in response to the impact of global warming. Stamenkovic-Radak et al. (2008) observed unexpected shifts in the chromosome polymorphism of *D. subobscura* where the appearance and stable maintenance of complex gene arrangements were attributed to reduced effective population sizes.

However, long-term temporal changes in inversion frequencies have not been observed in all species studied. North American populations of *Drosophila pseudoobscura* showed no consistent variation over 60 yr (Schaeffer 2008 and citations therein); although Schaeffer (2008) pointed out that in populations from California: ‘one can speculate that environmental changes were brought on by changes in agricultural

practice, in pesticide use, in air pollution, or global warming'. Similarly, Indian populations of *D. ananassae* have not shown any temporal variation in 20 yr of monitoring chromosomal inversion frequencies (Singh & Singh 2007).

In Itatiaia populations of *Drosophila mediopunctata*, the DI gene arrangement showed significant temporal changes in frequency. The frequency increased nearly 7% from the previous (1986–88 collections) 22.4%, as opposed to DS, DP and DV, which decreased ~10% from the previous 24.5% (Ananina et al. 2004). This is an unexpected result, as DS and DP gene arrangements previously showed a positive correlation with temperature, while the gene arrangement DI was not correlated to temperature (Table 2; Ananina et al. 2004).

Santos et al. (2005) showed that the chromosomal inversion polymorphism of *Drosophila subobscura* favored at high laboratory temperatures was not necessarily the same as the most common arrangements in populations of *D. subobscura* at warmer latitudes. This may be taken as evidence that the factors associated with genetic shifts might be more complex than a simple environmental variable and, perhaps, other underlying biotic and abiotic factors might be at work independently or interacting with each other, thus influencing inversion frequencies.

Ananina et al. (2004) also described altitudinal clines for DA, DS and DP, in which DA increased in frequency in higher altitudinal populations; however, DS and DP showed a contrary pattern. After 2 decades, DS, DP, DV maintained the same pattern with a highly significant negative correlation with altitude. The DI inversion, on the other hand, showed a significant positive altitudinal cline, while DA was not correlated with altitude (Fig. 5). These puzzling findings must be further investigated.

In contrast, seasonal variation of the gene arrangements was still present in our most recent collections. Inversion DA continues to show seasonal variation, increasing in frequency in the cold and dry season, while DS, DP and DV decreased in frequency. Repeated seasonal cycles of changes in inversions frequencies provide the clearest and least equivocal evidence of strong selection acting on inversion polymorphism in natural populations (Krimbas & Powell 1992, Powell 1997). Bradshaw & Holzapfel (2008) suggested that consequences of climate change will be longer warm seasons and shorter winters, causing phenological shifts in temperate zone organisms. Such shifts may have greater genetic consequences from factors other than merely temperature changes. Climate change in the neotropical region involves

warmer and drier winters and hotter summers with torrential rains influenced by other climatic phenomena such as 'El Niño' (Ab'Saber 2009). Nevertheless, these changes in climate seem to have had little effect on the seasonal cycles of *Drosophila mediopunctata* gene arrangement frequencies.

The observed changes in *Drosophila mediopunctata* chromosomal inversion frequencies over the last 20 yr may reflect responses to (1) climatic changes of abiotic factors, e.g. temperature, humidity, etc. (Chown et al. 2011); (2) changes in flora and fauna composition (Lavergne et al. 2010, Sheldon et al. 2011) as a result of an improvement of public policies toward park preservation; and climatic changes, such as global warming, affecting the distribution of species along the elevation gradient and, consequently, the community structure (driven by competition and predation); (3) changes in the genetic content of each inversion; (4) modifications of the demographic structure of the population with a possible effect of genetic drift (Stamenkovic-Radak et al. 2008, Joubert & Bijlsma 2010, Hoffmann & Sgrò 2011).

Our results show that the chromosome polymorphism of *Drosophila mediopunctata* changed in unexpected ways over the last 25 yr concomitant with climate change in the region of Parque Nacional do Itatiaia. Moreover, these observed changes in climate may have affected morphological traits in these populations; whether these changes were consistent with previous observations (Bitner-Mathé et al. 1995, Bitner-Mathé & Klaczko 1999) remains an unresolved issue. Further studies assessing the geographical variation and the genetic structure of the population using molecular markers may aid in clarifying the observed pattern.

*Acknowledgements.* We thank Dr. L. Nascimento, Coordenador de Pesquisa do Parque Nacional do Itatiaia for the authorizations and hospitality at the Park and J. Bernardino for his help with the field work. We appreciate the technical help of M. S. Couto and C. Couto. We are also grateful for assistance from A. B. Martins, D. Moraes, F. Rocha, I. M. Ventura, K. A. de Carvalho and R. Cavasini on different collecting occasions. We appreciate the dedicated work of F. M. Boschiero of the Espaço da Escrita from CGU-Unicamp for the careful revision of the English version. Special thanks to 2 anonymous reviewers who gave valuable and careful suggestions that improved the paper, as well as E. and R. Hogan for correcting the English version of the final manuscript. Finally, we thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo ao Ensino e Pesquisa (FAEP-Unicamp) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support.

## LITERATURE CITED

- Ab'Saber AN (2009) A propósito da periodicidade climatohidrológica que vem provocando grandes crises em Santa Catarina. *Estud Avançados* 23:297–306
- Ananina G, Souza WN, Peixoto AA, Klaczko LB (2002) Polytene chromosome map and inversion polymorphism in *Drosophila mediopunctata*. *Mem Inst Oswaldo Cruz* 97: 691–694
- Ananina G, Peixoto AA, Blanche-Mathé BC, Souza WN, da Silva LB, Valente VLS, Klaczko LB (2004) Chromosomal inversion polymorphism in *Drosophila mediopunctata*: seasonal, altitudinal and latitudinal variation. *Genet Mol Biol* 27:61–69
- Anderson AR, Hoffmann AA, McKechnie SW, Umina PA, 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
- Andrade CAC, Vieira RD, Ananina G, Klaczko LB (2009) Evolution of the male genitalia: morphological variation of the aedeagi in a natural population of *Drosophila mediopunctata*. *Genetica* 135:13–23
- Arnold SJ (1981) Statistics of natural populations. I. Estimating an allele probability in cryptic fathers with a fixed number of offspring. *Biometrics* 37:495–504
- Ashburner M (1989). *Drosophila*: a laboratory manual. CSHL Press, Cold Spring Harbor, NY
- Bächli (2011) Taxodros: database 2010-12, v1.03, available at [www.taxodros.uzh.ch](http://www.taxodros.uzh.ch) (accessed April 06, 2011)
- Balanyà J, Oller JM, Huey RB, Gilchrist GW, Serra L (2006) Global genetic change tracks global climatic warning in *Drosophila subobscura*. *Science* 313:1773–1775
- Balanyà J, Huey RB, Gilchrist GW, Serra L (2009) The chromosomal polymorphism of *Drosophila subobscura*: a microevolutionary weapon to monitor global change. *Heredity* 103:364–367
- Bitner-Mathé BC, Klaczko LB (1999) Size and shape heritability in natural populations of *Drosophila mediopunctata*: temporal and microgeographical variation. *Genetica* 105:35–42
- Bitner-Mathé BC, Peixoto AA, Klaczko LB (1995) Morphological variation in a natural population of *Drosophila mediopunctata*: altitudinal cline, temporal changes and influences of chromosome inversions. *Heredity* 75:54–61
- Bradshaw WE, Holzapfel CM (2008) Genetic response to rapid climate change: it's seasonal timing that matters. *Mol Ecol* 17:157–166
- Carvalho AB, Peixoto AA, Klaczko LB (1989) 'Sex-ratio' in *Drosophila mediopunctata*. *Heredity* 62:425–428
- Chown SL, Sorensen JG, Terblanche JS (2011) Water loss in insects: an environmental change perspective. *J Insect Physiol* 57:1070–1084
- Easterling DR, Horton B, Jones PD, Peterson TC and others (1997) Maximum and minimum temperature trends for the globe. *Science* 277:364–367
- Etges WJ, Levitan M (2008) Variable evolutionary response to regional climate change in a polymorphic species. *Biol J Linn Soc* 95:702–718
- Hatadani LM, Klaczko LB (2008) Shape and size variation on the wing of *Drosophila mediopunctata*: influence of chromosome inversions and genotype-environment interaction. *Genetica* 133:335–342
- Hatadani LM, McInerney JO, Medeiros HF, Junqueira ACM, Azeredo-Espin AM, Klaczko LB (2009) Molecular phylogeny of the *Drosophila tripunctata* and related groups (Diptera: Drosophilidae). *Mol Phylogenet Evol* 51:595–600
- Hoffmann AA, 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
- Hoffmann AA, Sgrò CM (2011) Climate change and evolutionary adaptation. *Nature* 470:479–485
- Intergovernmental Panel on Climate Change (IPCC) (2007). Climate change 2007: the physical science basis. In: Solomon S, Qin D, Manning M, Chen Z and others (eds) Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge
- Joubert D, Bijlsma R (2010) Interplay between habitat fragmentation and climate change: inbreeding affects the response to thermal stress in *Drosophila melanogaster*. *Clim Res* 43:57–70
- Kastritsis CD (1966). Cytological studies of some species of the *tripunctata* group of *Drosophila*. *Univ Texas Publ* 6615:413-474
- Klaczko LB (2006) Evolutionary genetics of *Drosophila mediopunctata*. *Genetica* 126:43–55
- Knibb WR, Oakeshott JG, Gibbs J (1981) Chromosome inversion polymorphisms in *Drosophila melanogaster*. I. Geographic clines and climatic associations in Australasian populations. *Genetics* 98:833–847
- Krimbas CB, Powell JR (1992). *Drosophila* inversion polymorphism. CRC Press, Boca Raton, FL
- Lavergne S, Mouquet N, Thuiller W, Ronce O (2010) Biodiversity and climate change: integrating evolutionary and ecological responses of species and communities. *Annu Rev Ecol Evol Syst* 41:321–350
- Levitan M, Etges WJ (2009) Rapid response to perturbation of chromosome frequencies in natural populations of *Drosophila robusta*. *Genetica* 137:1–8
- Mata RA, McGeoch MA, Tidon R (2010) Drosophilids (Insecta, Diptera) as tools for conservation biology. *Nat Conservação* 8:60–65
- Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annu Rev Ecol Evol Syst* 37: 637–669
- Peixoto AA, Klaczko LB (1991) Linkage disequilibrium analysis of chromosomal inversion polymorphism in *Drosophila*. *Genetics* 129:773–777
- Powell JR (1997). Progress and prospects in evolutionary biology: the *Drosophila* model. Oxford University Press, New York, NY
- Rezende EL, Balanyà J, Rodriguez-Trelles F, Rego C and others (2010) Climate change and chromosomal inversions in *Drosophila subobscura*. *Clim Res* 43:103–114
- Rodríguez-Trelles F, 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, Rodríguez MA (2010) Measuring evolutionary responses to global warming: cautionary lessons from *Drosophila*. *Insect Conserv Diversity* 3:44–50
- Saavedra CC, Callegari-Jacques SM, Napp M, Valente VLS (1995) A descriptive and analytical study of Neotropical drosophilid communities. *J Zoological Syst Evol Res* 33: 62–74
- Santos M, Cespedes W, Balanyà J, Trotta V, Calboli FCF, Fontdevila A, Serra L (2005) Temperature-related genetic changes in laboratory populations of *Drosophila subobscura*: evidence against simple climatic-based expla-

- nations for latitudinal clines. *Am Nat* 165:258–273
- Schaeffer SW (2008) Selection in heterogeneous environments maintains the gene arrangement polymorphism of *Drosophila pseudoobscura*. *Evolution* 62:3082–3099
- Sheldon KS, Yang S, Tewksbury JJ (2011) Climate change and community disassembly: impacts of warming on tropical and temperate montane community structure. *Ecol Lett* 14:1191–1200
- Singh P, Singh BN (2007) Population genetics of *Drosophila ananassae*: genetic differentiation among Indian natural populations at the level of inversion. *Genet Res* 89: 191–199
- Stamenkovic-Radak M, Rasic G, Savic T, Kalajdzic P, Kurbalija Z, Kenig B, Andjelkovic M (2008) Monitoring of the genetic structure of natural populations: change of the effective population size and inversion polymorphism in *Drosophila subobscura*. *Genetica* 133:57–63
- Tidon R (2006) Relationships between drosophilids (Diptera, Drosophilidae) and the environment on two contrasting tropical vegetations. *Biol J Linn Soc* 87:233–248
- Umina PA, Weeks AR, Kearney MR, McKechnie SW, Hoffmann AA (2005) A rapid shift in a classic clinal pattern in *Drosophila* reflecting climate change. *Science* 308: 691–693
- Yotoko KSC, Medeiros HF, Solferini VN, Klaczko LB (2003) A molecular study of the systematics of the *Drosophila tripunctata* group and the tripunctata radiation. *Mol Phylogenet Evol* 28:614–619
- Zar JH (2010) *Biostatistical analysis*, 5th edn. Prentice Hall, Upper saddle River, NJ

### Appendix 1

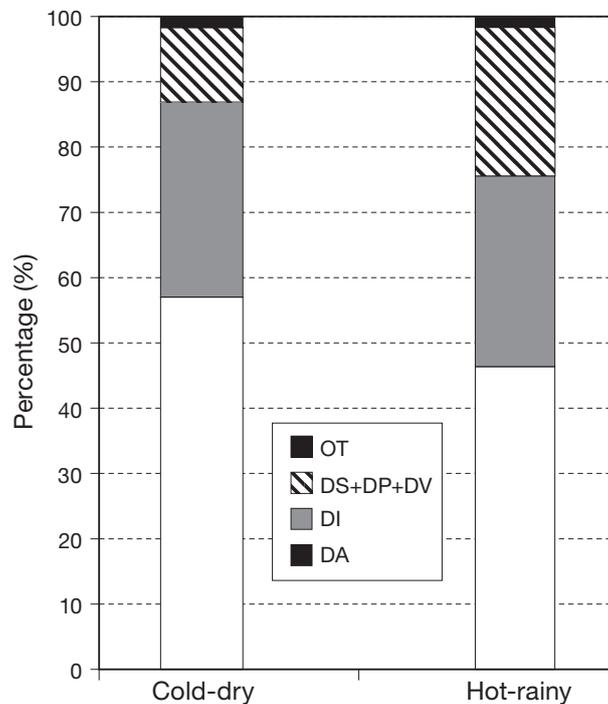


Fig. A1. *Drosophila mediopunctata*. Comparison between current inversion frequencies (2007–2010) contrasting cold-dry and hot-rainy seasons, considering only frequencies obtained at 2 altitudes: 750 and 950 m. OT: rare arrangements

Table A1. *Drosophila mediopunctata*. Second chromosome inversion frequencies (%) of *D. mediopunctata* from 7 collections at sites with different altitudes (m), at Parque Nacional do Itatiaia, Rio de Janeiro. 2N: number of chromosomes analyzed; C-D: cold-dry season; H-R: hot-rainy season. Inv: Inversions; OT: rare arrangements; 2N: number of chromosomes analyzed

Inv	1190	1130	1100	1070	950	900	800	750	590	Total
June 1–4, 2007; C-D										
DA			66.7		52.9			57.1		57.4
DI			33.3		41.2			35.7		37.2
DS			0		2.9			2.4		2.1
DP			0		0			0		0
DV			0		2.9			4.8		3.2
OT			0		0			0		0
2N			18		34			42		94
February 22–26, 2008; H-R										
DA			41.7		31.8			52.6		44.4
DI			41.7		40.9			26.3		33.3
DS			8.3		18.2			18.4		16.7
DP			8.3		4.5			0		2.8
DV			0		4.5			2.6		2.8
OT			0		0			0		0
2N			12		22			38		72
November 19–24, 2008; H-R										
DA			65.2		48.4			44		53.3
DI			29.3		25			29.8		28.3
DS			0		12.5			14.3		8.3
DP			0		6.3			7.1		4.2
DV			3.3		6.3			2.4		3.8
OT			2.2		1.6			2.4		2.1
2N			92		64			84		240
March 10–16, 2009; H-R										
DA			46.7		43.8			35.4		40.4
DI			30		25			33.3		30.9
DS			16.7		6.3			14.6		13.8
DP			0		6.3			8.3		5.3
DV			3.3		6.3			8.3		6.4
OT			3.3		12.5			0		3.2
2N			30		16			48		94
May 30–June 5, 2009; C-D										
DA	57.7	50		59.5	61.7	63.9	64.8	47.8	59.7	58.9
DI	34.6	40.9		31	28.3	25	24.1	30.4	24.2	28.4
DS	0	0		7.1	5	5.6	5.6	6.5	6.5	5.2
DP	3.8	0		2.4	1.7	0	1.9	4.3	3.2	2.3
DV	0	9.1		0	3.3	5.6	3.7	8.7	4.8	4.3
OT	3.8	0		0	0	0	0	2.2	1.6	0.9
2N	26	22		42	60	36	54	46	62	348
March 3–5, 2010; H-R										
DA	0	58.8		54.8	59.4	66.7		60		58.2
DI	0	23.5		26.2	34.4	16.7		20		26.1
DS	0	8.8		9.5	3.1	16.7		10		8.2
DP	0	0		0	0	0		0		0
DV	0	5.9		4.8	3.1	0		10		5.2
OT	0	2.9		4.8	0	0		0		2.2
2N		34		42	32	6		20		134
August 23–27, 2010; C-D										
DA				51.9	64			50	40	56.1
DI				38.9	19.8			37	30	29.6
DS				7.4	3.5			4.3	30	6.1
DP				1.9	5.8			2.2	0	3.6
DV				0	3.5			4.3	0	2.6
OT				0	3.5			2.2	0	2
2N				54	86			46	10	196

Table A2. *Drosophila mediopunctata*. Number of flies analyzed at different altitudes (Alt) on each collection occasion. N: number of female ( $\varphi$ ) and male ( $\sigma$ ) adults karyotyped; Seasons — C-D: cold-dry; H-R: hot-rainy. All  $\chi^2$  values are non-significant ( $p > 0.05$ ). Tests were carried out only for samples where the number of males and females was  $>5$

Collections	Alt (m)	N		df	$\chi^2$
		$\varphi$	$\sigma$		
June 1–4, 2007; C-D	1100	4	5		
	950	10	7	1	2.84
	750	4	30		
February 22–26, 2008; H-R	1100	4	4		
	950	11	0		
	750	16	3		
November 19–24, 2008; H-R	1100	30	32	1	0.16
	950	20	12	1	0.06
	750	20	22	2	4.53
March 10–16, 2009; H-R	1100	0	15		
	950	0	8		
	750	0	24		
May 30–June 5, 2009; C-D	1190	3	10		
	1130	5	6	1	2.89
	1070	6	15	1	1.79
	950	9	21	1	0.11
	900	9	9	1	2.84
	800	15	12	1	1.79
	750	11	12	1	0.11
	590	12	19	1	2.84
March 3–5, 2010; H-R	1130	10	7	1	0.77
	1070	7	9	1	0.20
	950	6	10	1	0.70
	900	10	3		
	750	5	5	1	3.33
August 23–27, 2010; C-D	1070	9	18	1	0.92
	950	20	23	1	2.37
	750	7	16	1	0.41
	590	5	6	1	1.56

Editorial responsibility: Nils Chr. Stenseth,  
Oslo, Norway

Submitted: July 11, 2011; Accepted: January 30, 2012  
Proofs received from author(s): May 25, 2012