



Chromosomal inversion polymorphism in *Drosophila mediopunctata*: seasonal, altitudinal, and latitudinal variation

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Abstract

The most polymorphic chromosome for inversions in *Drosophila mediopunctata* is the chromosome II, where 17 inversions have been found, eight of which occurring in the distal region and nine in the proximal region. We present an analysis of the chromosome II inversion polymorphism with respect to seasonal, altitudinal and latitudinal variation. In *D. mediopunctata* from the Parque Nacional do Itatiaia (southeastern Brazil), the frequencies of three of the distal inversions (namely *DA*, *DS*, and *DP*) vary seasonally. These inversions also show altitudinal clines in their frequencies. This microgeographic pattern was not observed on a macrogeographic scale. *D. mediopunctata* from Porto Alegre are less polymorphic for inversions than other populations, the most remarkable reduction occurring in the proximal region of chromosome II. There is a considerable difference between *D. mediopunctata* from Campinas and specimens from Serra do Japi, which are separated by only 50 km. In contrast, *D. mediopunctata* from Serra do Japi are much more similar to specimens from the Parque Nacional do Itatiaia, which is 200 km far.

Key words: cline, microgeographic variation, temperature, *tripunctata* group, Brazil.

Received: April 8, 2003; Accepted: July 28, 2003.

Introduction

Chromosomal inversion polymorphism in species of *Drosophila* is one of the best studied systems in population genetics. Inversions have been used to study phylogenies, geographical clines, temporal cycles, meiotic drive, and natural selection (for reviews see Sperlich and Pfriem, 1986; Krimbas and Powell, 1992). Since in a number of species this kind of polymorphism is under selection, it is common to assume *a priori* that this is true for any species and to conclude that this polymorphism is maintained by balancing selection (Dobzhansky, 1970). However, Krimbas and Loukas (1980), in their review of chromosomal inversion polymorphism in *D. subobscura*, insisted that one should try to demonstrate which type of selection,

if any, is actually occurring in each case. Thus, the question as to how chromosomal inversion polymorphisms are maintained remains unanswered, especially in very polymorphic species.

Drosophila mediopunctata, a species of the *tripunctata* group, is very common in many localities in South America, especially during the winter (Saavedra *et al.*, 1995). This species ranges from El Salvador to southern Brazil (Frota-Pessoa, 1954; Val *et al.*, 1981). Various aspects of its biology make *D. mediopunctata* an interesting model for studying the genetics of natural populations (for review and discussion, see Klaczko, 1995).

D. mediopunctata has $2n = 12$ chromosomes, consisting of five pairs of rods and a pair of dots (Kastritsis, 1966), and is highly polymorphic for inversions. Chromosomes X, II and IV show inversion polymorphisms. We found, in addition to the *Standard* arrangement, three inversions in chromosome X and one in chromosome IV (Carvalho *et al.*,

1989; Klaczko *et al.*, 1990; Peixoto and Klaczko, 1991). Chromosome II is the most polymorphic, with 17 inversions described. Understanding this complexity can be simplified by assigning the inversions to distal and proximal groups, based on the chromosome region in which they occur. The distal group includes eight inversions (*DA*, *DI*, *DS*, *DP*, *DV*, *DR*, *DL*, and *DJ*), while the proximal region contains nine (*PC0*, *PC1*, *PC2*, *PC3*, *PC4*, *PC5*, *PB0*, *PA0*, and *PA8*) (Peixoto and Klaczko, 1991; Ananina *et al.*, 2002).

In almost all combinations between proximal and distal inversions, there is no overlap between the two regions, although in principle recombination between them can occur. There is intense linkage disequilibrium between distal and proximal inversions, for example, *DA* is associated with *PA0*, as shown by the standardized coefficient of linkage disequilibrium (D') which presents a value of 0.98. Similarly, *DP* is associated with *PC0* ($D' = 0.97$), and *DS* with *PC0* ($D' = 0.95$) (Peixoto and Klaczko, 1991; Zapata, 2000).

In this paper, we examine the seasonal, altitudinal, and latitudinal variations in inversion frequencies in *D. mediopunctata*, in an attempt to understand how these polymorphisms are maintained.

Materials and Methods

Fly samples

Microgeographic variation in inversion frequencies was studied in the Parque Nacional do Itatiaia, on the slopes of Mantiqueira mountains, in the states of Rio de Janeiro and Minas Gerais, Brazil (22°25' S, 44°50' W). The fauna of this region was described by Barth (1957) and the flora by Brade (1956), who characterized it as a subtropical hygrosopic forest. *D. mediopunctata* were collected at various points, at altitudes from 700 m to 1600 m. The greatest distance between collection sites was 6 km. Approximately a dozen fermented banana baits spaced 5 to 15 meters apart were used per site.

Nine field trips were made to Itatiaia, but in only two areas (at 1020 m and 970 m) were specimens collected on all occasions. The collection dates were: fall 1986 (25-26 May), winter 1986 (29-30 September), summer 1987 (25-26 February), fall 1987 (30-31 May, 1-2 June), winter 1987 (28-30 August), spring 1987 (27-29 November), summer 1988 (11-13 March), fall 1988 (28-29 May), and spring 1988 (26-28 November).

Three other localities were chosen for macrogeographic analysis: Mata Santa Genebra (22°53' S, 47°04' W, Campinas, São Paulo), Serra do Japi (23°17' S, 47°00' W, Jundiaí, São Paulo), and Morro Sant'Ana (30°02' S, 51°13' W, Porto Alegre, Rio Grande do Sul). Ten field collections were made during the period 1991-2000, four of these in Campinas, three in Serra do Japi, and three in Porto Alegre.

Laboratory procedures

The inversion frequencies in the *D. mediopunctata* samples were estimated using the adult male sample and egg sample methods (Arnold, 1981; Klaczko, 1995). The adult sample method consisted of crossing each male collected with two or three virgin females from a homokaryotypic strain. We used strain *ITC-229ET*, which is homokaryotypic for the haplotype *DI-PB0* in chromosome II, and is routinely maintained in the UNICAMP and UFRJ laboratories. From each cross, the salivary gland chromosomes of up to eight F1 third instar larvae were analyzed cytologically to determine the male's genotype. The probability of a misidentification for a heterozygous male was negligible (less than 1%). In the egg sample method, we analyzed one F1 larva from each collected female.

Weather data used in the correlation analysis

The climatic variables analyzed included the maximum, minimum, and mean monthly temperatures and precipitation. The hydrometric data was supplied by the Brazilian Agency IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis). The hydrometric bulletin contained data for the period from September 1986 to December 1988. The average precipitation per day was calculated for each month of the period.

Unfortunately, no reliable records were available for temperatures within the park during the collecting period. Brade (1956) gives average data covering more than 15 years when the meteorological station in the park was in operation. A comparison of these data with recent temperature data for the town of Itatiaia (<http://br.weather.com/weather/climatology/BRXX0578>) shows that they are very similar. However, only the data from Brade (1956) were used in the correlation analysis, because they were considered to be more reliable.

Statistical analysis

The gene arrangements in the distal region are easily recognized in routine preparations. In contrast, proximal inversions are rather difficult to recognize in different homozygotes (Klaczko *et al.*, 1990). Since the linkage disequilibrium between the distal and proximal inversions is very strong in *D. mediopunctata* (see Peixoto and Klaczko, 1991), we used the distal inversions *DA*, *DI*, *DS*, *DP*, and *DV* as genetic markers for the haplotypes *DA-PA0*, *DI-PB0*, *DS-PC0*, *DP-PC0*, and *DV-PC0*, which together represent more than 85% of the total number of haplotypes. This procedure allowed us to pool the egg and male samples, since they were not significantly different, thus substantially increasing the sample size. We must emphasize that in no case (among all the samples analyzed) did we find a significant difference between the frequencies estimated using the adult male method and the egg sample method.

The correlation between the inversion frequencies and altitude, precipitation and temperature was examined after angular transformation of the chromosomal data.

Results

Altitudinal and seasonal changes in the inversion frequencies in the Parque Nacional do Itatiaia (microgeographic variation)

The frequencies of distal inversions in chromosome II, the number of chromosomes analyzed in each period and area of collection, and the weighted average frequencies between collections are reported in Table 1. There was a significant, positive correlation between the frequencies of *DA* (after angular transformation) and the altitude of the collection site ($r = 0.87$, $p < 0.01$) (Figure 1, Table 2). The opposite occurred in the case of *DS* ($r = -0.86$, $p < 0.01$) and *DP* ($r = -0.77$, $p < 0.05$) (not shown in the graph). When *DS* and *DP* were pooled, the negative correlation coefficient with altitude was greater ($r = -0.91$, $p < 0.01$) than for *DS* (Figure 1, Table 2). Such a grouping makes some biological sense, since the *DS* and *DP* inversions are almost completely associated with the proximal inversion *PC0* to form the haplotypes *DS-PC0* and *DP-PC0*. The *DI* inversion did not show a significant correlation with altitude ($r = 0.29$, $p > 0.05$) (Figure 1, Table 2). The correlation coefficients were calculated using the weighted average frequencies between collections, as shown at the bottom of Table 1.

The differences in the inversion frequencies between collections were highly significant ($\chi^2 = 74.2$, d.f. = 32, $p < 0.001$). The frequency of *DA* varied periodically (Figure 2), as it increased in cool months (end of fall and winter) and decreased in hot months (end of spring and summer), but we were unable to fit this to a sinusoidal curve. Nonetheless we could find a quadratic function with very good fit ($y = -0.0351x^2 + 0.1781x + 0.6037$; $r^2 = 0.89$; $p < 0.01$). There was a significant negative correlation with temperature ($r = -0.90$, $p < 0.001$, for the average temperature) and precipitation ($r = -0.75$, $p < 0.05$) (Table 2). The inversion *DS* showed an opposite pattern, since its frequencies increased in the hot months and fell in the cool months. The inversions *DI* (Figure 3) and *DP* did not vary cyclically, although, when *DP* was pooled with *DS*, a very clear pattern was observed (Figure 4), and a quadratic function could be successfully adjusted ($y = 0.0348x^2 - 0.1643x + 0.6184$; $r^2 = 0.62$; $p < 0.01$). The correlation coefficients for the inversion frequencies versus altitude and climatic variables are shown in Table 2. Figure 5 shows the distribution of *DA*, *DI* and *DS+DP* inversion frequencies in relation to the average temperature.

Macrogeographic variation

D. mediopunctata from Campinas, Serra do Japi, and Porto Alegre were also analyzed. The frequencies of distal inversions in chromosome II are shown in Table 3. In no

case were the differences between collections from the same locality significant, even when the collections were separated by over 10 years in Campinas and over nine years in Serra do Japi. The microgeographic pattern seen in the Parque Nacional do Itatiaia was not seen macrogeographically (Table 3). The frequencies of *DS*, *DP*, and *DV* were higher in Porto Alegre than at the other sites, whereas the frequency of *DA* was very low (Figure 6). *D. mediopunctata* from Campinas showed intermediate frequencies for all of these inversions. Thus, the frequencies of *DA* and *DI* decreased with latitude, while those of *DS* and *DP* increased (Figure 6).

The level of polymorphism decreased from the Parque Nacional do Itatiaia to Porto Alegre. Table 4 shows the expected frequencies of heterozygotes, calculated separately for the distal and proximal regions of chromosome II. This calculation was based on the data from males, for which we were sure of the identification of proximal region inversions. Heterozygosity decreased drastically in the proximal region of chromosome II in the Porto Alegre population, as well as the overall inversion number, which was smaller there than in the other populations studied. The overall inversion number in this population was also less than in the other populations studied. In contrast, the expected heterozygosity in the distal region remained almost unaltered.

Discussion

Our results provide strong evidence that the frequencies of chromosome II inversions *DA* and *DS* vary seasonally in *D. mediopunctata* from Itatiaia. Moreover, these two inversions and *DP* showed altitudinal clines in their fre-

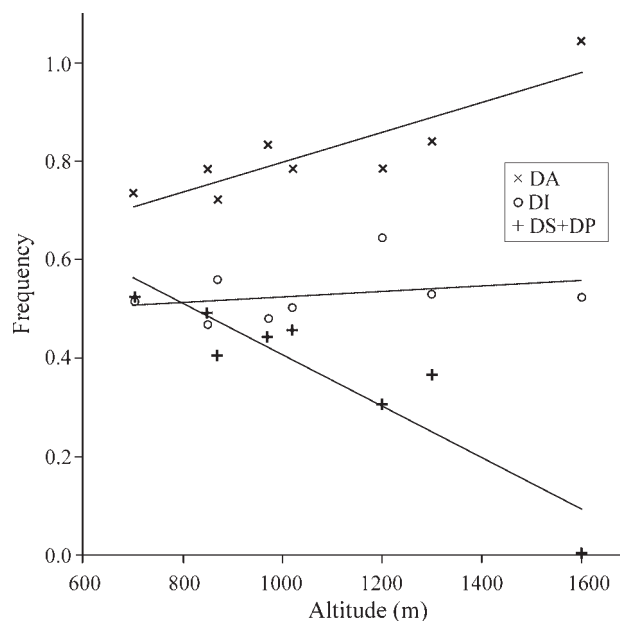


Figure 1—Distribution of *DA*, *DI*, and *DS+DP* inversion frequencies in chromosome II of *Drosophila mediopunctata* in relation to altitude (Parque Nacional do Itatiaia, Brazil).

Table 1 - Chromosome II inversion frequencies in *Drosophila mediopunctata* from several collections and altitudes in the Parque Nacional do Itatiaia (southeastern Brazil).

(A)

Inv	Altitude (m)								Av
	1600	1300	1200	1020	970	870	850	700	
Fall 1986 May (25-26)									
DA	-	0.500	-	0.541	0.533	-	-	-	0.528
DI	-	0.308	-	0.257	0.221	-	-	-	0.250
DS	-	0.058	-	0.095	0.107	-	-	-	0.093
DP	-	0.096	-	0.027	0.098	-	-	-	0.076
OT	-	0.038	-	0.081	0.041	-	-	-	0.052
N	-	52	-	74	122	-	-	-	248
Winter 1986 September (29-30)									
DA	-	0.750	-	0.477	0.580	-	-	-	0.535
DI	-	0.125	-	0.252	0.236	-	-	-	0.242
DS	-	0.125	-	0.113	0.088	-	-	-	0.100
DP	-	0	-	0.072	0.052	-	-	-	0.060
OT	-	0	-	0.086	0.044	-	-	-	0.063
N	-	8	-	222	250	-	-	-	480
Summer 1987 February (25-26)									
DA	0.750	0.571	0.500	0.425	0.398	-	-	-	0.438
DI	0.250	0.143	0.364	0.349	0.306	-	-	-	0.318
DS	0	0.143	0.091	0.132	0.176	-	-	-	0.143
DP	0	0.071	0.000	0.066	0.046	-	-	-	0.050
OT	0	0.071	0.045	0.028	0.074	-	-	-	0.050
N	8	14	22	106	108	-	-	-	258
Fall 1987 May (30-31), June (1-2)									
DA	-	0	-	0.541	0.592	0.438	-	0.456	0.537
DI	-	0.500	-	0.239	0.206	0.281	-	0.272	0.236
DS	-	0	-	0.077	0.101	0.063	-	0.140	0.096
DP	-	0	-	0.055	0.048	0.094	-	0.088	0.060
OT	-	0.500	-	0.088	0.053	0.125	-	0.044	0.071
N	-	2	-	272	228	32	-	114	648
Winter 1987 August (28-30)									
DA	-	0.611	-	0.559	0.598	-	0.525	0.455	0.530
DI	-	0.250	-	0.225	0.196	-	0.229	0.237	0.224
DS	-	0.028	-	0.118	0.103	-	0.142	0.143	0.125
DP	-	0.028	-	0.069	0.054	-	0.038	0.094	0.061
OT	-	0.083	-	0.029	0.049	-	0.067	0.071	0.060
N	-	36	-	102	184	-	240	224	786
Spring 1987 November (27-29)									
DA	-	-	-	0.424	0.549	-	0.410	0.471	0.461
DI	-	-	-	0.194	0.156	-	0.224	0.147	0.188
DS	-	-	-	0.171	0.153	-	0.216	0.265	0.185

Inv	Altitude (m)								Av
	1600	1300	1200	1020	970	870	850	700	
DP	-	-	-	0.129	0.076	-	0.052	0.118	0.091
OT	-	-	-	0.082	0.063	-	0.097	0	0.075
N	-	-	-	170	144	-	134	34	482
Summer 1988 March (11-13)									
DA	-	-	-	0.490	0.521	-	0.471	0.167	0.482
DI	-	-	-	0.186	0.188	-	0.186	0.333	0.190
DS	-	-	-	0.127	0.167	-	0.157	0.167	0.146
DP	-	-	-	0.108	0.083	-	0.100	0	0.097
OT	-	-	-	0.088	0.042	-	0.086	0.333	0.084
N	-	-	-	102	48	-	70	6	226
Fall 1988 May (28-29)									
DA	-	-	-	0.548	0.568	-	0.534	-	0.544
DI	-	-	-	0.197	0.205	-	0.172	-	0.188
DS	-	-	-	0.144	0.114	-	0.149	-	0.143
DP	-	-	-	0.067	0.045	-	0.075	-	0.068
OT	-	-	-	0.043	0.068	-	0.069	-	0.056
N	-	-	-	208	44	-	174	-	426
Spring 1988 November (26-28)									
DA	-	-	-	0.482	0.433	-	0.513	-	0.480
DI	-	-	-	0.232	0.183	-	0.179	-	0.209
DS	-	-	-	0.083	0.167	-	0.154	-	0.118
DP	-	-	-	0.095	0.133	-	0.103	-	0.104
OT	-	-	-	0.107	0.083	-	0.051	-	0.088
N	-	-	-	168	60	-	78	-	306

(B) Weighted average frequencies among collections

Inv	Altitude (m)								Av
	1600	1300	1200	1020	970	870	850	700	
Total									
DA	0.750	0.553	0.500	0.500	0.550	0.438	0.498	0.452	0.512
DI	0.250	0.259	0.364	0.233	0.213	0.281	0.204	0.241	0.224
DS	0	0.063	0.091	0.116	0.119	0.063	0.161	0.153	0.126
DP	0	0.062	0	0.077	0.064	0.094	0.063	0.093	0.071
OT	0	0.062	0.045	0.074	0.054	0.125	0.073	0.061	0.066
N	8	112	22	1424	1188	32	696	378	3860

N: number of chromosomes analyzed.

OT: other arrangements.

Av: Average; Inv: Inversions.

quencies. The pattern of clinal variation agreed with the seasonal variation, *i.e.*, the increase in frequency of inversion *DA* with altitude was similar to that in cool months and the decrease in frequency of inversion *DS* with altitude was similar to that in hot months. This pattern suggests that temperature or a related variable is the main factor responsible

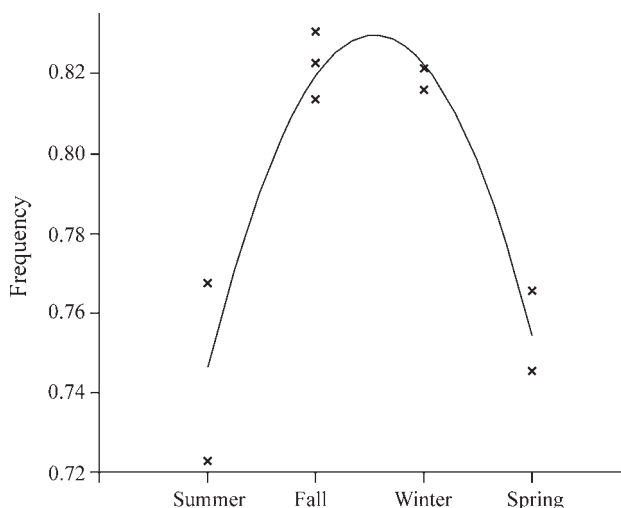
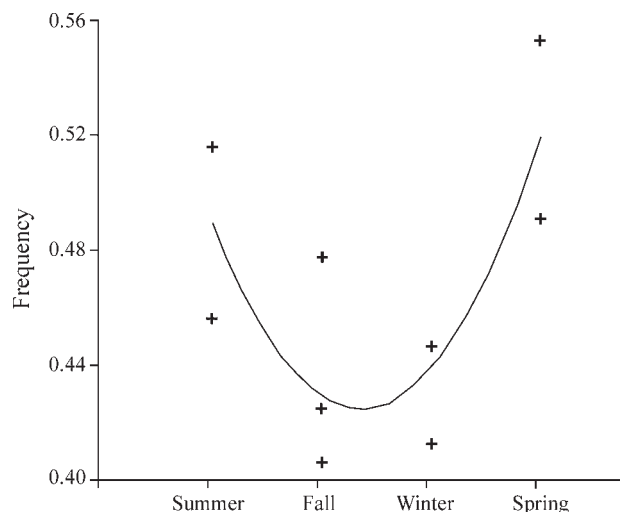
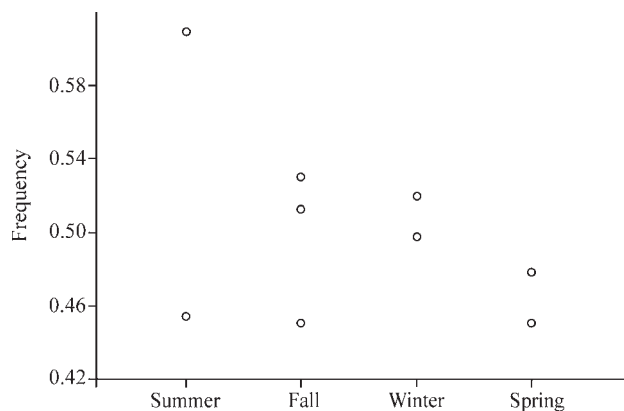
for this variation. The almost complete association of *DS* and *DP* with the proximal inversion *PC0* may explain the pattern shown in Figure 5, and why they vary similarly in relation to altitude (Figure 1).

Seasonal variations are *prima facie* evidence of natural selection (Levitan, 1973). This type of variation is not

Table 2—Pearson correlation coefficients (r) between inversion frequencies (after angular transformation) in chromosome II of *Drosophila mediopunctata* and altitude and other climatic data.

Inversion	Altitude	Precipitation	Temperature (°C)		
			Aver. max	Aver. min	Aver.
<i>DA</i>	0.8658**	-0.7494*	-0.8462**	-0.9522**	-0.9006***
<i>DI</i>	0.2907	0.2490	0.2978	0.1109	0.0424
<i>DS</i>	-0.8598**	0.3561	0.4884	0.5897	0.5929
<i>DP</i>	-0.7684*	0.2298	0.1480	0.4105	0.4481
<i>DS+DP</i>	-0.9133**	0.3900	0.4480	0.6503	0.6690*

*($p < 0.05$), **($p < 0.01$), ***($p < 0.001$).

**Figure 2**—Seasonal variation in the *DA* inversion frequency in chromosome II of *Drosophila mediopunctata* (Parque Nacional do Itatiaia, Brazil).**Figure 4**—Seasonal variation in the *DS* and *DP* (pooled) inversion frequency in chromosome II of *Drosophila mediopunctata* (Parque Nacional do Itatiaia, Brazil).**Figure 3**—*DI* inversion frequencies in chromosome II of *Drosophila mediopunctata* from various collections at the Parque Nacional do Itatiaia, according to the season.

uncommon in chromosomal inversion polymorphisms in species of *Drosophila*, e. g., *D. pseudoobscura* (see Dobzhansky, 1948), *D. melanica* (see Tonzetich and Ward, 1973), *D. subobscura* (see Fontdevila *et al.*, 1983), but does not occur in all species, e.g., *D. pavani* (see Brncic, 1973).

Microgeographic variation in inversion frequencies on mountain slopes is also not uncommon in species of *Drosophila* (Etges, 1984; Burla *et al.*, 1986) and is good evidence of selection.

Seasonal variation has at least two important consequences for chromosomal inversion polymorphism. First, it provides a mechanism that, under certain conditions, permits the maintenance of greater genetic variability in the population. Second, by its effect on chromosomal variability, such variation allows the coexistence of different inversions in the populations for a longer time.

The microgeographic pattern was not observed macrogeographically. The frequency of *DA* was unexpectedly low in the Porto Alegre population, whereas the frequencies of *DS* and *DP* were very high (Figure 6, Table 3). *D. mediopunctata* from Campinas showed intermediate frequencies. However, the limited number of populations examined makes it difficult to conclude that there is a latitudinal cline in the gene arrangement frequencies.

There is extensive data on clinal variations in *Drosophila* species, including chromosome inversions, allozyme loci, quantitative morphological traits, and physi-

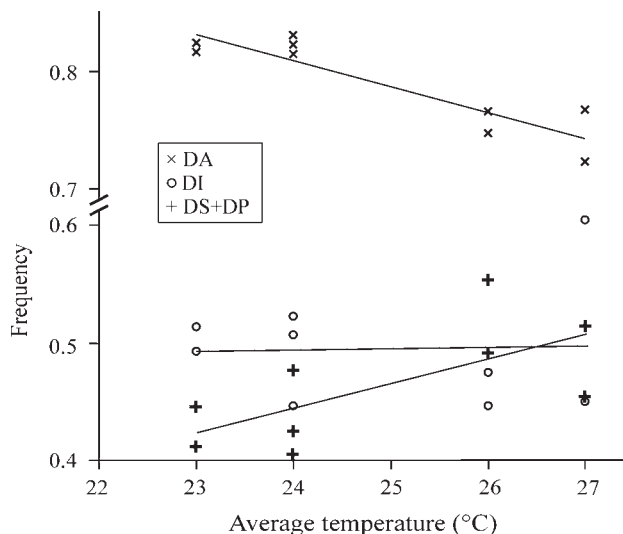


Figure 5-Distribution of *DA*, *DI*, and *DS+DP* inversion frequencies in chromosome II of *Drosophila mediopunctata* in relation to average temperature (Parque Nacional do Itatiaia, Brazil).

Table 3-Frequencies of distal inversions in chromosome II of *Drosophila mediopunctata* from various localities and collection dates.

Population and date	<i>DA</i>	<i>DI</i>	<i>DS</i>	<i>DP</i>	<i>DV</i>	<i>OT</i>	<i>N</i>
Campinas, Mata Santa Genebra							
Jun/1991	0.217	0.044	0.359	0.348	0.032	0	92
Sep/1996	0.228	0.080	0.377	0.204	0.098	0.012	162
Aug/2000	0.227	0.060	0.360	0.213	0.114	0.027	150
Sep/2000	0.213	0.017	0.374	0.257	0.113	0.026	230
Total	0.221	0.047	0.369	0.246	0.098	0.019	634
Serra do Japi							
Jun/1994	0.505	0.122	0.170	0.090	0.069	0.043	188
Apr/2002	0.649	0.079	0.088	0.105	0.079	0	114
May/2002	0.576	0.030	0.182	0.091	0.091	0.030	66
Total	0.562	0.092	0.147	0.095	0.076	0.027	368
P.Alegre, Morro Santa' Ana							
Apr/1999	0.083	0	0.417	0.292	0.208	0	24
Oct/1999	0.013	0	0.474	0.395	0.118	0	76
May/2000	0	0	0.425	0.433	0.142	0	120
Total	0.014	0	0.441	0.405	0.141	0	220

N: number of chromosomes analyzed.
OT: other arrangements.

ological and behavioral traits. Latitudinal clines are generally considered to be a consequence of an adaptive response to different climates. This conclusion is reinforced by the parallelism observed between continents harboring populations with different histories. It seems natural to expect that altitudinal clines should show some parallelism with latitudinal clines, since the same climatic variables have shaped both cline types. However, not all data fit these general ten-

Table 4- Expected heterozygosity for the chromosome in four Brazilian populations of *Drosophila mediopunctata*.

Populations	Heterozygosity	
	Distal region	Proximal region
Parque Nacional do Itatiaia	0.666	0.657
Serra do Japi	0.646	0.579
Campinas	0.732	0.414
Porto Alegre	0.615	0.024

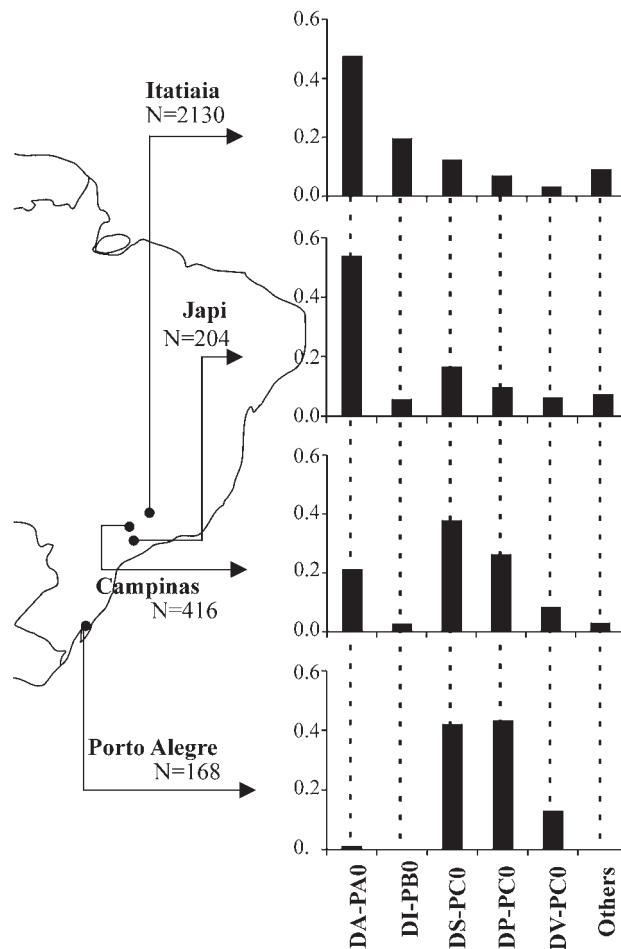


Figure 6-Distribution of haplotype frequencies in Brazilian populations of *Drosophila mediopunctata* from the Parque Nacional do Itatiaia, Serra do Japi, Campinas, and Porto Alegre. *N* = number of chromosomes analyzed.

dencies. For example, the cosmopolitan inversion *In(2L)t* of *D. melanogaster* shows worldwide latitudinal and seasonal fluctuations in frequencies, which are positively correlated with environmental temperature (Van Delden and Kamping, 1991; Kamping and Van Delden, 1999; Van't Land *et al.*, 2000). Laboratory experiments have shown a higher survival of karyotypes possessing *In(2L)t* in relation to high temperature (Kamping and Van Delden, 1999). In contrast, in African populations of *D. melanogaster*, no lat-

itudinal or altitudinal trends have been found for this inversion. Significant longitudinal clines have been detected for inversion frequencies, including *In(2L)t*, but these are difficult to correlate with a climatic or other ecological factor, and there is nothing to suggest any adaptive interpretation (Aulard *et al.*, 2002).

Another curious example is that of *D. subobscura*. This species is characterized by extensive rich inversion polymorphism that involves all five acrocentric chromosomes. Until 1980, the evidence that inversion polymorphism in *D. subobscura* was subject to selection was equivocal (Krimbas and Loukas, 1980). Later studies showed north-south geographical clines in gene arrangement frequencies in Europe (Menozzi and Krimbas, 1992). The analysis of the principal components of variance showed that the correlation coefficient between the values of the first principal component for inversions and those of climatic variables (temperature and humidity) was very high ($p < 0.0001$). Soon after the invasion of South America (Chile) and North America (western USA and Canada) by *D. subobscura* in the late 1970s, clines of gene arrangements were formed, as the species spread to the North and to the South (Prevosti *et al.*, 1988). The gradient of the clines increased with time and showed the same pattern as that found in Europe (but reversed in South America on account of the inversion of climatic conditions in the southern hemisphere) (Prevosti *et al.*, 1990). Seasonal changes also exist, but were overlooked in previous studies (Rodríguez-Trelles *et al.*, 1996; Sole *et al.*, 2002). All of these variations indicate the occurrence of natural selection for gene arrangements in natural populations of this species (Krimbas and Powell, 2000).

The existence of altitudinal variation in the gene arrangement frequencies in *D. subobscura* is controversial. Altitudinal cline was not found in Chilean populations of *D. subobscura* (Brncic and Budnik, 1987). In Switzerland, *Standard* gene arrangements of chromosomes *A*, *I*, *O*, and *U* showed a trend that was the opposite of what would be expected if the altitudinal gradients were parallel to the north-south one (Burla *et al.*, 1986). This finding resembles our results for *D. mediopunctata*.

In *D. pseudoobscura*, Dobzhansky (1943) observed that the altitudinal changes were almost opposite in pattern on a southern Californian mountain (San Jacinto) and the Sierras; gene arrangements more frequent at lower elevations in one mountain range were less frequent in the other. This was later explained assuming the population-specific coadapted nature of the inversions (Krimbas and Powell, 1992).

As shown here, the level of polymorphism decreased from Itatiaia to Porto Alegre. The total number of inversions was lowest in the Porto Alegre population, where only four haplotypes (*DS-PC0*, *DP-PC0*, *DV-PC0*, and *DA-PA0*) were found. The first three of these accounted for more than 98% of the total. Thus, the proximal region is al-

most monomorphic, and is represented by *PC0*, which is considered ancestral for the region (Peixoto and Klaczko, 1991). Since chromosome II can be divided into two regions according to the inversion groups, it is possible to calculate the expected Hardy-Weinberg heterozygosity for the proximal and distal regions separately. Table 4 shows the expected frequencies of heterozygotes for the proximal and distal regions of chromosome II. Despite the low total number of inversions in the Porto Alegre population, the expected frequency of heterozygotes for the distal region remained almost unaltered, whereas a drastic reduction in polymorphism occurred in the proximal region. The expected frequency of heterozygotes was 0.024 for the proximal region in this population (Table 4). As shown in Table 4, there was a tendency for heterozygosity to decrease from the Parque Nacional do Itatiaia to Porto Alegre, but this was limited to the proximal region. The Porto Alegre population is apparently distinct from the other populations studied, showing peripheral traits. The significance of this finding remains to be clarified.

A decrease in inversion heterozygosity towards the periphery of the species distribution is rather common in species of *Drosophila*. Soulé (1973) cited more than 10 species of *Drosophila* showing this pattern. For example, African populations of *D. melanogaster* considered to be ancestral are qualitatively more polymorphic than those in the rest of the world (Aulard *et al.*, 2002). There is some disagreement about the explanation for the greater inversion polymorphism seen in central populations. Da Cunha and Dobzhansky (1954) believed that inversions were differentially adapted to various conditions, so that central populations living in a more diverse environment would contain many inversions held in stable equilibrium by natural selection, whereas in marginal environments one arrangement would be most fit because the range of environments was narrow. Carson (1958) stated that peripheral populations were generally very small and suffered for inbreeding, and that natural selection favored "homoselection". Recombination is necessary to promote the formation of new allelic combinations. Whichever explanation is correct, there is little doubt as to the ubiquity of the pattern and the fact that some form of selection must be the causal agent.

The difference between the populations of Serra do Japi and Campinas is interesting, primarily because these areas are separated by only 50 km. Indeed, the population of Serra do Japi was much more similar to that of the Parque Nacional do Itatiaia, 200 km away (Figure 6). Salis *et al.* (1995) studied 26 forests in the State of São Paulo and showed that they could be divided into two floristic groups, with the Serra do Japi in one group and the Mata Santa Genebra (Campinas) in the other. Thus, the differences in the inversion frequencies may reflect adaptations to floristic variations. This hypothesis can be tested by col-

lecting specimens from other localities in the State of São Paulo.

Acknowledgments

The authors thank Hermes F. Medeiros for help in the field collections and for valuable discussion of manuscript, Aparecida Ramos Libânio, Clea Knauer da Silva, Glória Maria dos Santos, Laércio Santana, for technical support, and Stephen Hyslop for correcting the English. They also want to thank an anonymous referee for a thorough review of the manuscript. This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES), Fundação de Amparo à Pesquisa de São Paulo (FAPESP) and Fundo de Apoio ao Ensino e Pesquisa (FAEP-UNICAMP).

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Editor: João S. Morgante