LATITUDINAL VARIATION FOR TWO ENZYME LOCI AND AN INVERSION POLYMORPHISM IN *DROSOPHILA MELANOGASTER* FROM CENTRAL AND SOUTH AMERICA

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Abstract.—Many organisms show latitudinal variation for various genetically determined traits. Such clines may involve neutral variation and originate from historical events or their maintenance may be explained by selection. For *Drosophila melanogaster,* latitudinal variation for allozymes, inversions, and quantitative traits has been found on several continents. We sampled *D. melanogaster* populations in Panama and along a transect of 40 latitudinal degrees on the west coast of South America. Negative correlations with latitude were found for *Adh^S* and α*Gpdh^F* allele frequencies and for the frequency of the cosmopolitan inversion *In(2L)t* in *Adh^S* α*Gpdh^F* chromosomes. A positive correlation existed between wing length and latitude. Significant correlations were found between these traits and climatic variables like temperature and rainfall. The observed clines show considerable resemblance to those found on other continents. Gametic disequilibrium between *Adh^S* and α*Gpdh^F* occurred predominantly at higher latitudes and was caused by the presence of *In(2L)t.* The reasons for the clinal distributions are discussed and it is argued that selection is the most likely explanation. However, the exact nature of the selective force and the interactions of allozymes with each other and with *In(2L)t* are complex and not fully understood. In tropical regions *In(2L)t*-containing genotypes have higher fitness than *ST/ST* and *Adh* and α*Gpdh* hitchhike with the inversion, but there is also evidence for balancing selection at the *Adh* locus.

Key words.—Alcohol dehydrogenase, α glycerophosphate dehydrogenase, *Drosophila melanogaster,* gametic disequilibrium, inversions, latitudinal clines, polymorphism.

Many species show an ecogeographical distribution with several traits varying with longitude or latitude. Such clines can be attributed either to adaptation to different environments, historical events, or to random processes like genetic drift, gene flow, or isolation by distance (Endler 1977). Extensive research has been carried out with respect to the existence of latitudinal clines for *Drosophila melanogaster.* The cline for allele frequencies of the alcohol dehydrogenase locus (*Adh*) is perhaps one of the best-studied examples, and this cline (with an increasing frequency of the *Adh^S* allele toward the equator) has been observed on several continents, with an emphasis on North America and Australasia (Berger 1971; Johnson and Schaffer 1973; Vigue and Johnson 1973; Voelker et al. 1977; Oakeshott et al. 1982; Capy et al. 1986; Anderson et al. 1987; Berry and Kreitman 1993; Bubli and Imasheva 1997). A similar clinal pattern has been found in populations in Europe and Africa (Capy et al. 1986; David et al. 1989; Bénassi and Veuille 1995; Veuille et al. 1998).

The general view is that the worldwide cline for *Adh* is the result of selection (Oakeshott et al. 1982; Anderson et al. 1987; Berry and Kreitman 1993), with temperature or temperature-related factors being the main selective agents (Malpica and Vassallo 1980; Van Delden 1982; McKenzie et al. 1994). The ADH^S enzyme has a higher in vitro stability at higher temperatures (Vigue and Johnson 1973). However, flies homozygous for *Adh^S* showed a higher in vivo mortality

at high (i.e., 35°C) temperatures (Van Delden and Kamping 1980). In addition, the *Adh^FF* genotype is better adapted to the presence of alcohol in the medium, which is presumed to be available in larger quantities in fruits in temperate regions (Van Delden et al. 1978; Van Delden 1982 and references therein).

However, it is not yet clear whether selection acts directly on the *Adh* gene or whether this cline simply reflects selection at linked loci or chromosomal regions (see Berry and Kreitman 1993). Two important candidates for this type of selection are the α-glycerophosphate dehydrogenase (σ*Gpdh*) gene and the cosmopolitan inversion *In(2L)t.* The α*Gpdh* locus is related functionally to the *Adh* locus through the NADH: NAD⁺ ratio (Cavener 1983). *In(2L)t* includes the α*Gpdh* locus, one of its breakpoints is located close to the *Adh* locus, and is nearly always found in combination with *Adh^S* and α*Gpdh^F* (Voelker et al. 1978). Clinal patterns have been observed for *In(2L)t* frequency, varying from 0% to 5% in temperate populations to 70% toward the equator (Mettler et al. 1977; Inoue and Watanabe 1980; Yamaguchi et al. 1980; Knibb et al. 1981; Knibb 1982, 1983; Inoue et al. 1984; Anderson et al. 1987; Das and Singh 1991; Singh and Das 1992; Inoue and Igarashi 1994; Veuille et al. 1998). For α*Gpdh,* a clinal pattern was also observed: the frequency of the *F* allele increases towards the equator (Johnson and Schaffer 1973; Oakeshott et al. 1982, 1984; Knibb 1983; Gibson et al. 1991; Parkash and Shamina 1994).

As for *Adh,* the latitudinal cline for α*Gpdh* is thought to be related to temperature. The α*Gpdh^F* allele is more resistant to higher temperatures (Alahiotis et al. 1977; Voelker et al. 1978; Barnes et al. 1989; Van Delden and Kamping 1989) and, at lower temperatures, α*Gpdh^FF* flies have a lower rate

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of development than αGpdh^{SS} flies (Barnes et al. 1989). In addition, experimental evidence indicates that the αGpdh^{SS} genotype shows a larger flight output at low temperature (Barnes and Laurie-Ahlberg 1986). However, there are a number of studies that failed to demonstrate significant αGpdh effects on metabolic rate, high temperature resistance, or ethanol tolerance (for references, see Oudman et al. 1992).

Laboratory experiments showed that independently of the *Adh* and α*Gpdh* loci homozygotes for *In(2L)t* have a selective advantage at high temperatures. Moreover, these homozygotes are smaller and have a longer development, whereas the heterozygotes for the inversion were found to have a higher viability under high-temperature conditions (Stalker 1980; Knibb 1982; Van Delden and Kamping 1989, 1991; Singh and Das 1992). In addition, seasonal variation in *In(2L)t* frequency has been observed, with higher frequencies during or at the end of the warmer season (Langley et al. 1977; Stalker 1980; Zacharopoulou and Pelecanos 1980; Aguadé and Serra 1987; Kim and Sung 1988; Sanchez-Refusta et al. 1990; Kamping and Van Delden 1999).

Many studies have found a direct correlation between wing length (which is a measure for body size) and flight activity, as well as inversion and allozyme polymorphism (Jones 1974; Pieragostini et al. 1979; Stalker 1980; Pfriem 1983; Serra and Oller 1984; Barnes and Laurie-Ahlberg 1986; Hasson et al. 1992; Bitner-Mathé et al. 1995). As with inversion and allozyme frequencies, clear latitudinal clines have been revealed for body size and, particularly, wing length. Smaller wings are found in equatorial regions (Capy et al. 1993; Imasheva et al. 1994; James et al. 1995), but this cline is not always monotonic (Long and Singh 1995). Previous papers have reported conflicting conclusions about the degree of linkage disequilibrium among *Adh,* α*Gpdh,* and *In(2L)t* (Voelker et al. 1978; Malpica and Vassallo 1980; Yamaguchi et al. 1980; Knibb 1983; Inoue et al. 1984; Oakeshott et al. 1984; Alonso-Moraga and Muñoz-Serrano 1986; Anderson et al. 1987; Van Delden and Kamping 1989). These contrasting results may reflect differences among continents in the degree of linkage disequilibrium or even differences in research methodology, which demonstrates the complexity of the association between *Adh,* α*Gpdh,* and *In(2L)t.* For this reason, and because Central and South America have only scarcely been sampled with respect to the existence of a cline for *Adh,* α*Gpdh,* and *In(2L)t,* we sampled wild populations of *D. melanogaster* in Panama and along the west coast of South America. We have estimated the frequency of *Adh* and α*Gpdh* alleles, scored *In(2L)t* genotype, determined gametic disequilibrium between *Adh* and α*Gpdh,* and measured wing length. Here, we report on the associations among these polymorphisms and discuss their adaptive significance and latitudinal distribution.

MATERIALS AND METHODS

Populations

Between June and November 1991, flies were collected at five locations in Panama. During February and March 1995, flies were collected at one location in Ecuador and nine locations in Chile (Fig. 1). The 15 sampling sites cover approximately 40 latitudinal and 12 longitudinal degrees (Table
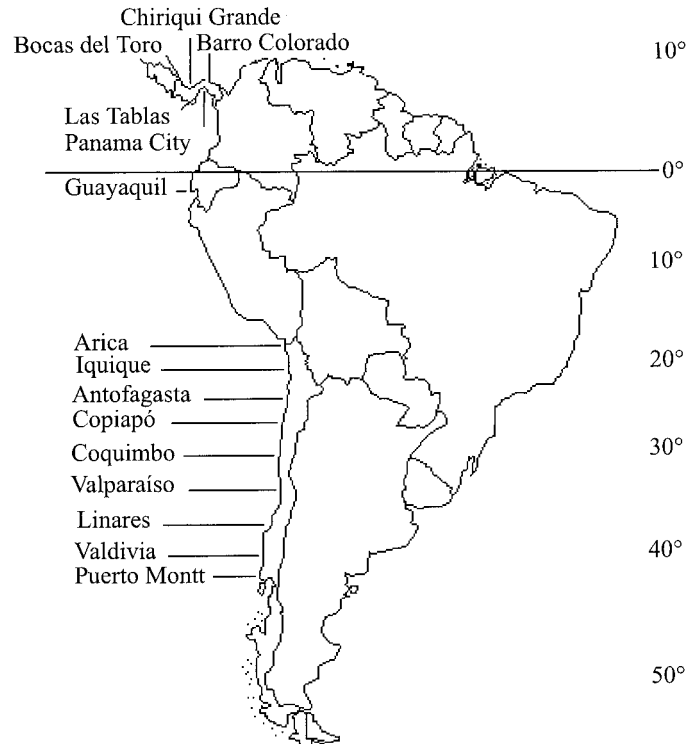

FIG. 1.   Map of the 15 sampling sites.

1). Flies were caught by sweeping a net over boxes containing rotten fruit in fruit markets. If necessary, additional traps with a mixture of fermenting bananas and yeast were used. Several authors have found an association between altitude and *Adh* allele frequencies and morphological characters in *Drosophila* (Grossman et al. 1970; Pipkin et al. 1976; Louis et al. 1982; Bitner-Mathé et al. 1995). Accordingly, all our samplings, except Linares (140 m) and Copiapó (350 m), were done at locations with an altitude below 100 m. No effect of altitude on allele or inversion frequency was observed in this study.

Allozyme Electrophoresis

Wild females collected in the sampling sites were placed individually in a separate vial containing instant *Drosophila* medium and allowed to produce eggs. Horizontal polyacrylamide gel electrophoresis was performed on the wild caught flies as described by Van Delden and Kamping (1989) to determine the joint genotype for *Adh* and α*Gpdh* (cytological locations 35B3 and 26A, respectively). The offspring were used for further tests.

Wing Length Measurement

Left wings of wild males were embedded in a drop of Euparal on a microscopic slide and measured under a light microscope (10 × 8) with an ocular micrometer. The absolute length of the anterior crossvein to the wingtip was taken as a measure for wing length (Prout 1958). For Panama, male wing length data were derived from female wing lengths by using a transformation factor of 0.875 (conforming to data

TABLE 1. Location and climatic data of the sampled populations. For further information on climatic data see the Materials and Methods section.

Population	Latitude (S/N)	Longitude (W)	T_{year} (°C)	R_{year} (mm)	S_{year} (h)	H_{year} (%)
Guayaquil, Ecuador (GU)	2°13′S	79°54′	25.3	950	1614	77
Las Tablas, Panama (LT)	7°45′N	80°15′	26.5	1200	n.a.	84*
Chiriqui Grande, Panama (CG)	8°56′N	82°07′	26.5	3750	n.a.	90*
Panama City, Panama (ST)	9°00′N	79°30′	25.6	2328	2337	86
Barro Colorado Island, Panama (BC)	9°08′N	79°50′	25.5	2250	n.a.	87*
Bocas del Toro, Panama (BT)	9°19′N	82°15′	26.5	3300	n.a.	82*
Arica, Chile (AR)	18°28′S	70°19′	18.7	0.5	2258	74
Iquique, Chile (IQ)	20°13′S	70°10′	17.9	0.6	2816	74
Antofagasta, Chile (AN)	23°38′S	70°24′	16.4	1.7	2966	77
Copiapó, Chile (CO)	27°20′S	70°21′	15.2	12	n.a.	74
Coquimbo, Chile (CQ)	29°56′S	71°24′	13.6	79	2185	83
Valparaíso, Chile (VA)	33°05′S	71°40′	14.0	373	2120	82
Linares, Chile (LI)	35°48′S	71°36′	12.7	967	2463	76
Valdivia, Chile (VD)	39°48′S	73°14′	11.0	1871	1876	83
Puerto Montt, Chile (PM)	41°30′S	72°50′	10.1	1803	1593	85

n.a., data not available.
* Data from 1991 only (see text).

of Robertson and Reeve 1952; Prout 1958; Zwaan et al. 1992; David et al. 1994).

Detection of In(2L)t

The frequency of *In(2L)t* (cytological limits: 22D2–E1, 34A8–9) was determined by performing single-pair matings between wildtype males and virgin females from a laboratory stock fixed for the second chromosome markers *dumpy* (*dp*: II, 13.0) and *black* (*b*: II, 48.5). Tests were done in the third generation after collection. Ninety pair-matings were done for each Panamanian sample site and 50 for the Chilean and Ecuadorian sites. Eight F_1 females from each cross were individually backcrossed to *dp b* males. The presence of flies with only the *dp* or only the *b* phenotype in the F_2 generation indicated the occurrence of recombination of the second chromosome and, consequently, absence of *In(2L)t* (Van Delden and Kamping 1989). The average number of F_2 individuals scored per cross was 50. This procedure enabled us to determine the frequency of heterozygous and homozygous flies for *In(2L)t*.

Climatic Data

In Chile, Ecuador, and Panama City, climatic data were recorded by weather stations adjacent to the collection sites and were kindly provided to us by Direccion Meteorologica de Chile, Santiago de Chile, Chile, and the Koninklijk Nederlands Meteorologisch Instituut, De Bilt, the Netherlands. Climatic data from the Panama locations were obtained from the Smithsonian Tropical Research Station. Climatic data included T_{year} (average annual temperature), T_{min} (average monthly minimum temperature), T_{max} (average monthly maximum temperature), R_{year} (average of total yearly rainfall), S_{year} (average of total yearly sun-hours), and H_{year} (average of annual relative humidity). All figures used are long-term (30-year) averages, except when indicated differently (Table 1). Because of the strong correlation of T_{year} with both T_{max} and T_{min} ($r = 0.99$ and 0.96, respectively), only T_{year} was used in the analyses.

Estimation of Gametic Disequilibrium

Joint gametic frequencies for *Adh* and $\alpha Gpdh$ could not be determined directly from the observed dilocus genotypes because it was not possible to distinguish between the coupling and repulsion heterozygotes. Therefore, the maximum-likelihood method for codominant loci was used to estimate gametic frequencies from the observed genotypes (Hill 1974). Assuming that D is normally distributed, the variance of D when $D = 0$ can be approximated by $V(D) = p_1 p_2 q_1 q_2 / n$, where p_1 and p_2 are the allele frequencies at the first locus and q_1 and q_2 are the allele frequencies at the second locus, and n is the sample size. The hypothesis of $D = 0$ can be rejected at the 0.05 significance level if $|D| > 1.96(V[D])$ (Hedrick 1983).

Changes in allele frequencies affect maximum potential gametic disequilibrium (D_{max}). Therefore, absolute values of gametic disequilibrium may give rise to misleading conclusions; for this reason, a relative measure (D/D_{max}) is preferable (Hedrick 1983, 1987). The latter value ranges from zero to one, and is independent of allele frequencies (Hedrick 1987). However, under circumstances where both D and D_{max} are small, the D/D_{max} ratio may take unrealistically high value and samples either with only two or three of four possible gamete types will give a D/D_{max} value of one.

Statistical Analysis

To normalize the distribution, wing length data were natural-log transformed. Frequencies of Adh^S, $\alpha Gpdh^F$, *In(2L)t*, population heterozygosities, and annual relative humidity data were angularly transformed. Total yearly rainfall was square-root transformed. Dilocus heterozygosities were obtained by averaging the heterozygosities for *Adh* and $\alpha Gpdh$ for each population.

RESULTS

Latitudinal Variation in Adh and αGpdh Allele Frequencies

Adh^S frequencies varied from 0.99 to 0.07 and $\alpha Gpdh^F$ frequencies between 0.97 and 0.43 (Table 2; Fig. 2). Both

TABLE 2. Number of collected flies (*n*), *Adh* and α*Gpdh* allele frequencies in the collected samples, and *In(2L)t* frequencies in the third generation. Significant deviations from Hardy-Weinberg equilibrium and significant gametic disequilibria are indicated by asterisks. See text for the calculation of *D* and D_{max} (gametic disequilibrium) and *H* (heterozygosity) and Table 1 for population abbreviations.

| Population | *n* | Allele frequency | | Frequency *In(2L)t* | *D* | D/D_{max} | *H* |
		Adh^S	α$Gpdh^F$				
GU	130	0.99	0.62	0.18	0.001	0.12	0.26
LT	81	0.91	0.90	0.18	−0.007	0.08	0.17
CG	31	0.81	0.92	0.30	−0.025	0.38	0.18
ST	122	0.85	0.86	0.13*	−0.005	0.04	0.25
BC	345	0.96	0.93	0.18*	0.002	0.73	0.11
BT	17	0.85	0.97	0.44	0.004	1.00	0.18
AR	49	0.25*	0.66	0.46	−0.051	0.62	0.30
IQ	28	0.34	0.63	0.40	−0.074	0.58	0.39
AN	72	0.26	0.66	0.43*	−0.075*	0.86	0.42
CO	103	0.17	0.47	0.17	−0.068*	0.76	0.41
CQ	22	0.16	0.45	0.27	−0.087	1.00	0.39
VA	334	0.14***	0.69	0.18	−0.041*	0.94	0.31
LI	194	0.07	0.43	0.10*	−0.029*	0.70	0.32
VD	192	0.11	0.47	0.04	−0.040*	0.68	0.33
PM	43	0.13	0.64	0.15	−0.046	1.00	0.37

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Adh^S and α$Gpdh^F$ frequencies were negatively correlated with latitude when all 15 samples from Panama and South America were considered (Table 3). A negative correlation between latitude and Adh^S frequency was also found for the ten samples from South America ($r = -0.89$, $P < 0.001$) and for the nine samples from Chile ($r = -0.86$; $P < 0.01$). No significant correlation between latitude and α$Gpdh^F$ frequency was present in the latter two cases. No correlations between latitude and Adh^S and α$Gpdh^F$ were found for the five samples from Panama. Given that latitude correlates with T_{year} ($r = -0.97$, $P < 0.001$), its correlation with allele frequencies (Table 3) can be attributed to the well-known correlation of allele frequencies with temperature. Using the equation given by Anderson et al. (1987), we calculated the frequencies for Adh^S and α$Gpdh^F$ in *Standard* (i.e., no *In[2L]t* carrying) chromosomes. Table 3 shows that, although most *r*-values decrease marginally, all significant correlations between allele frequencies and latitude or climatic variables remain significant. In other words, the latitudinal clines of *Adh* and α*Gpdh* are largely independent of the presence of *In(2L)t*.
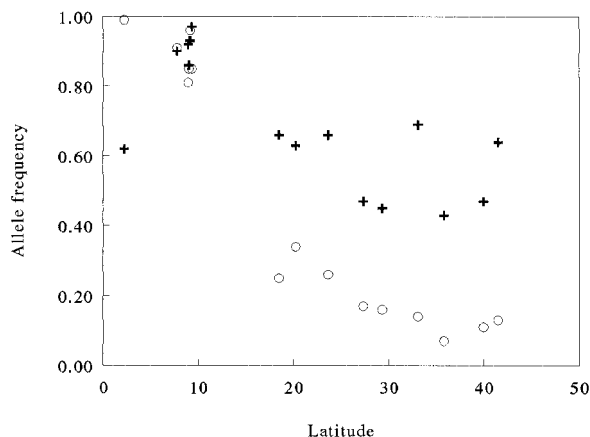

FIG. 2. Relation between Adh^S (○) and σ$Gpdh^F$ (+) frequencies and latitude.

Latitudinal Variation in *In(2L)t* Frequencies and Wing Length

Estimated *In(2L)t* frequencies ranged from 0.46 to 0.04 (Table 2). Four populations showed a smaller number of heterozygotes than would be expected if the populations were in Hardy-Weinberg equilibrium.

The average percentage of *dp* or *b* recombinants among F_2 progeny in which *In(2L)t* was absent varied between 31.1% and 33.2%. These percentages are less than expected (35.5%). This is probably due to presence in the samples of chromosomes with reduced recombination caused by small inversions (Van Delden and Kamping 1989). *In(2L)t* appeared always to be associated with the Adh^S/α$Gpdh^F$ allele combination.

For the total dataset, no latitudinal correlation was found for the frequency of *In(2L)t* (Table 3, Fig. 4), if frequency was calculated as percentage of chromosomes carrying *In(2L)t* among all chromosomes tested. However, all chromosomes carrying *In(2L)t* had an Adh^S α$Gpdh^F$ genotype. When the frequency of inversion-carrying chromosomes within all Adh^S α$Gpdh^F$ chromosomes was calculated, a highly significant positive correlation with latitude was found (Table 3), with the frequency of Adh^S α$Gpdh^F$ chromosomes carrying *In(2L)t* approaching 100% at 20° latitude (Fig. 5). When only the nine Chilean samples were taken in consideration, a negative correlation of latitude and *In(2L)t* frequency ($r = -0.90$, $P < 0.001$) was found.

Wing length varied from 1.08 mm to 1.45 mm (Fig. 3) and was positively correlated with latitude (Table 3).

Gametic Disequilibrium

It can be seen from Figure 4 that, although there is a significant decline in *In(2L)t* frequency between 20° and 40° latitude ($r = -0.90$, $P < 0.001$ for this latitudinal range only), the leveling of this decline is primarily due to the rarity of Adh^S α$Gpdh^F$ chromosomes in this latitudinal range. Figure 4 also shows the strong latitudinal cline of Adh^S α$Gpdh^F$ chromosomes ($r = -0.93$, $P < 0.001$ for the correlation of

TABLE 3. Correlation coefficients (*r*) of *Adh^S*, α*Gpdh^F*, and *In(2L)t* frequencies and wing length on latitude and four climatic variables. For *Adh^S* and α*Gpdh^F*, frequencies for all chromosomes (population) and frequencies corrected for the presence of *In(2L)t* (*ST* chromosomes) are given. A distinction is also made for *In(2L)t* frequencies: The proportion of all chromosomes carrying *In(2L)t* (population) and the proportion of *Adh^S*α*Gpdh^F* chromosomes with *In(2L)t* (*Adh^S*α*Gpdh^F* chromosomes) are presented. Sample sizes are shown in parentheses.

Trait	Latitude	T_{year}	R_{year}	S_{year}	H_{year}
Adh^S (population)	−0.93***	0.95***	0.53*	−0.19	0.40
	(15)	(15)	(15)	(10)	(15)
Adh^S (*ST* chromosomes)	−0.88***	0.91***	0.61*	−0.34	0.42
	(15)	(15)	(15)	(10)	(15)
α*Gpdh^F* (population)	−0.72**	0.83***	0.62*	0.11	0.59*
	(15)	(15)	(15)	(10)	(15)
α*Gpdh^F* (*ST* chromosomes)	−0.64**	0.76**	0.59*	0.01	0.45
	(15)	(15)	(15)	(10)	(15)
In(2L)t (population)	−0.36	0.29	−0.36	0.58	−0.29
	(15)	(15)	(15)	(10)	(15)
In(2L)t (*Adh^S*α*Gpdh^F* chromosomes)	0.81***	−0.87***	−0.59*	0.19	−0.42
	(15)	(15)	(15)	(10)	(15)
Wing length	0.97***	−0.98***	−0.43	−0.04	−0.29
	(15)	(15)	(15)	(10)	(15)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Adh^S α*Gpdh^F* chromosome frequency and latitude), where in tropical populations the *Adh^S* α*Gpdh^F* chromosomes reach 0.90–0.95. This is clearly due to gametic disequilibrium between *Adh^S* and α*Gpdh^F* (see below).

Both allozyme loci were tested for deviations from Hardy-Weinberg equilibrium (Table 2). For α*Gpdh*, no deviations were detected. *Adh*, however, did show significant deviations for two populations (Arica and Valparaíso), caused by shortage of heterozygotes.

To test for independence of segregation between *Adh* and α*Gpdh*, *D*-values for gametic disequilibrium were calculated and the resulting values were tested against the hypothesis that $D = 0$. The results are summarized in Table 2. It is clear that *D* tends to become increasingly negative when the distance of the population from the equator becomes larger ($r = −0.61$, $P < 0.05$ for the correlation between *D* and latitude), and this points to a relative excess of chromosomes with the *Adh^S* α*Gpdh^F* and the *Adh^F* α*Gpdh^S* combinations at higher latitudes. This holds also for D/D_{max}, a measure for

the degree of gametic disequilibrium that is more independent of allele frequencies than *D* (correlation with latitude: $r = 0.65$, $P < 0.01$).

Heterozygosity

Heterozygosity (*H*) was estimated as a measure for genetic diversity. Contrary to the results presented by Oakeshott et al. (1984) and Singh et al. (1982) (who screened populations from several continents for 10 and 26 loci, respectively) our results showed a positive correlation between heterozygosity and latitude ($r = 0.69$, $P < 0.01$). Oakeshott et al. (1984) found no significant relationship between *H* and latitude, while Singh et al. (1982) found a negative correlation. One of the reasons for this discrepancy may be the fact that our data are based on two loci only: *Adh* and α*Gpdh*, which are both probably under some (direct or indirect) selection pressure. In addition, Table 2 shows that the correlation with latitude is mainly caused by the markedly lower *H*-values for the Panamanian populations. Heterozygosity based on *In(2L)t* karyotypes did not produce any indication of a correlation with latitude ($r = 0.36$, ns).

DISCUSSION

We found latitudinal clines in Central and South America for *Adh*, α*Gpdh*, wing length and, to a certain extent, for *In(2L)t* as well. All clines have a general correspondence to those reported for other continents (e.g., Voelker et al. 1977; Knibb 1982; Oakeshott et al. 1982; Anderson et al. 1987; David et al. 1989; Capy et al. 1993).

A point for discussion is the degree to which the latitudinal clines are actually the result of natural selection. *Drosophila melanogaster* is supposed to have originated in East Africa, spread out to Eurasia, and colonized Australia and the Americas only recently (Lemeunier et al. 1986; David and Capy 1988; Hale and Singh 1991; Capy et al. 1993). However, the history of South American populations remains unclear. As a result of the commensal relationship between *D. melano-*
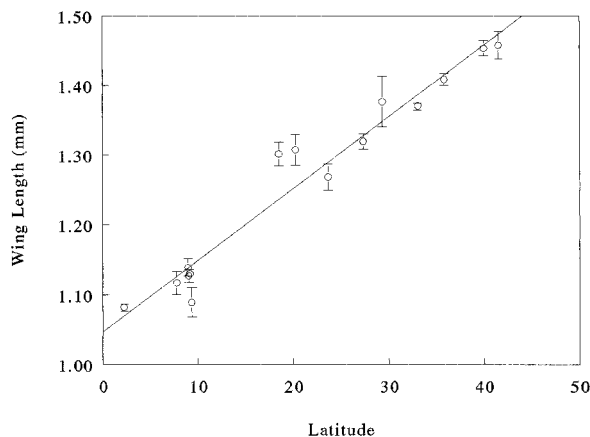

FIG. 3. Relation between male wing length (mm) and latitude. Vertical bars indicate standard error of the mean. Linear regression: $r^2 = 0.96$, $F = 303.66$, $P < 0.0001$.

gaster and humans, it is possible that the Chilean populations have descended from European populations, whereas the Panamanian and Ecuadorian flies could have originated from a mix of descendants from African and European flies (see Veuille et al. 1998). The clines presented in this paper could then be the result of migration and gene flow between adjacent source populations (Krimbas and Loukas 1980; Coyne et al. 1987). This could lead to a gradient of allozyme and inversion frequencies or morphological characters between the tropical African populations around the equator to the temperate European populations in Chile.

There are several arguments against this hypothesis. First, extensive research on inversion polymorphism in recently colonizing populations of *Drosophila subobscura* in South and North America has provided strong support for the adaptive value of inversion polymorphisms and for the speed at which these latitudinal clines can be formed (Prevosti et al. 1988, but for conflicting results on quantitative traits, see Pegueroles et al. 1995). Second, latitudinal correlations are now known to exist for five continents. These are strong arguments for the hypothesis that the allele frequencies of *Adh* and α*Gpdh* and the frequency of *In(2L)t* in wild populations of *D. melanogaster* are under natural selection. Finally, extensive laboratory research on *Adh*, α*Gpdh* and *In(2L)t* has provided experimental support for selection.

With respect to the maintenance of the *Adh* polymorphism, evidence is accumulating that balancing selection is involved. Strong indications come from analyses of DNA polymorphisms in and around the *Adh* region. Berry and Kreitman (1993) investigated populations in a north-south cline along the east coast of North America. They found a distinct cline of the *S/F* polymorphism and the $\nabla 1$ insertion/deletion polymorphism (located in the 5′ adult intron), but not for numerous silent DNA polymorphisms (see also Kreitman and Aguadé 1986; Simmons et al. 1989). Berry and Kreitman concluded that despite high levels of homogenizing gene flow, the *S/F* and $\nabla 1$ polymorphisms were under clinal selection. Kreitman and Hudson (1991) found an excess of silent nucleotide variation around the *F/S* site indicating balancing selection. This fits with the results of Bijlsma-Meeles and Bijlsma (1988), who estimated fitnesses of *Adh* genotypes under laboratory conditions without the presence of ethanol. They found overdominance for female fecundity and lower male virility for *Adh^{SS}* compared to *Adh^{FF}* and *Adh^{FS}*. These results agree with the finding of converging allele frequencies over generations in experimental populations started with different initial frequencies (Van Delden et al. 1978; Bijlsma-Meeles and Bijlsma 1988), thus pointing to the existence of an equilibrium allele frequency.

We observed that the correlation between latitude and *Adh^S* frequency is not linear, but shows a steeper part between 10° and 18° (Fig. 2). David et al. (1989) described a ''Mediterranean instability'' in populations sampled between 30° and 42° latitude, which results in a nonlinear relation between latitude and *Adh* allele frequencies when these frequencies are plotted over a broader range of latitudinal degrees. This seems not to be the case for the Mediterranean-like region in Chile, which is located between 32° and 37°. Also, the location and the climate of an instability region varies between studies (30° to 42° in the Mediterranean, David et al.
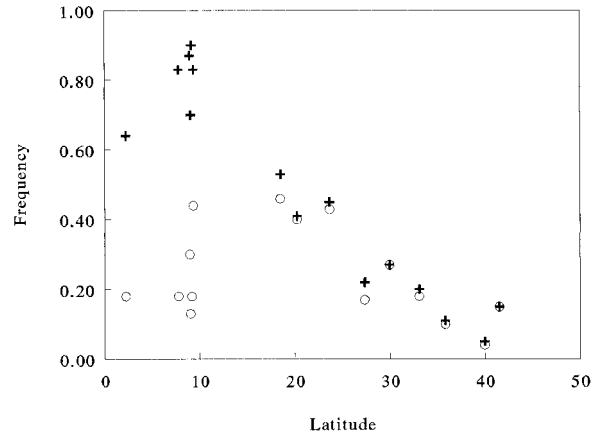

FIG. 4.   Relation between frequencies of *In(2L)t* (○) and *Adh^S*/α*Gpdh^F* chromosomes (+) with latitude.

1989; 20° to 28° in India, Parkash and Shamina 1994; 10° to 18°, this study), indicating that it is not the typical Mediterranean climate (dry, hot summers and mild rainy winters) that, as claimed by David et al. (1989), is the direct cause of the high variation in *Adh* allele frequencies.

However, the exact nature of the selection forces on all the polymorphisms considered here is still not clear. Our results show that *Adh* and α*Gpdh* alleles become more strongly linked the further the population is from the equator and that the significant negative *D*-values and the large D/D_{max} values are caused by the fact that virtually all *Adh^S* alleles in these southern populations are found in combination with α*Gpdh^F* alleles. When the frequency of the *Adh^S*/α*Gpdh^F* gamete is calculated on the basis of the respective one-locus allele frequencies, it becomes clear that in all populations from Copiapó southward is approximately two times higher than expected. It is very likely that this is due to the presence of *In(2L)t*. Given this, we can conclude: (1) that there is a strong association between *Adh^S* and α*Gpdh^F* at temperate latitudes that is caused by *In(2L)t*; and (2) that even at these higher latitudinal degrees, there exists some selective force that maintains *In(2L)t* and therefore the *Adh^S* α*Gpdh^F* combination as well at a relative high frequency. Such strong gametic disequilibrium due to association with *In(2L)t* has also been observed in laboratory populations kept at high temperature (Van Delden and Kamping 1989, 1991) and a seminatural population in a tropical greenhouse (Kamping and Van Delden 1999).

A negative latitudinal cline for *In(2L)t* was found for the Chilean populations (Fig. 4). The correlation between latitude and inversion frequency is not significant if the tropical populations are also taken into account. The fact that the *In(2L)t* frequencies from our tropical populations are high, but do not reach values higher than 46%, may be due to overdominance. Veuille et al. (1998) have also reported high *In(2L)t* frequencies in tropical African populations: from 0.23 in Malawi to 0.73 in the Ivory Coast. Van Delden and Kamping (1989, 1991, 1997) found that *In(2L)t* homozygotes developed more slowly and had a lower weight than the other two karyotypes, with the result that the *In(2L)t* frequency decreased in cultures reared at temperatures of 20°C and 25°C. At high temperatures (29°C or 33°C), however, *In(2L)t*-car-
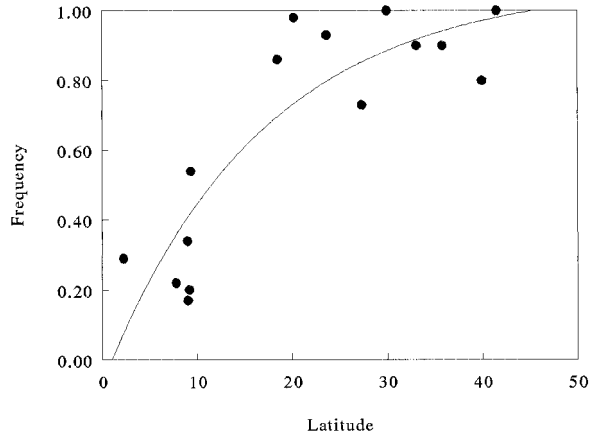
FIG. 5. Relation between *In(2L)t* frequency within *Adh^S^/αGpdh^F^* chromosomes and latitude. The solid line represents an empirically fitted exponential curve: $y = 1.08 - 1.14e^{(-x/16.78)}$ ($r^2 = 0.76$, $F = 19.42$, $P = 0.0002$).

rying genotypes were more heat resistant than *ST* homozygotes and overdominance was observed. Consequently the inversion frequencies were kept stable at equilibrium values. In addition, lower fitness of individuals carrying an *In(2L)t* chromosome was found on ethanol-supplemented food. Heterosis of *In(2L)t* was also described for other populations (Watanabe and Watanabe 1973; Stalker 1976). The combination of overdominance with local differences in ecoclimatic conditions (i.e., amount of ethanol in food and mean temperatures) may give rise to variable tropical *In(2L)t* equilibrium frequencies. However, four populations showed significant deviations from Hardy-Weinberg equilibria (Table 2), resulting from a shortage of *In(2L)t* heterokaryotypes, so we cannot confirm the presence of overdominance for this inversion in the Central and South American populations.

Adh, αGpdh, and inversion polymorphisms have all been related to body size and wing length (Pieragostini et al. 1979; Serra and Oller 1984; Barnes and Laurie-Ahlberg 1986; Oudman et al. 1991; Van Delden and Kamping 1991; Hasson et al. 1992). In our study, wing length was found to be strongly correlated with latitude and temperature. Many studies have reported a latitudinal cline for wing length in wild populations of *D. melanogaster* on other continents as well (e.g., Imasheva et al. 1994; James et al. 1995; Long and Singh 1995). A high rearing temperature causes shorter development time and smaller adult body size. However, the latitudinal cline for wing length still persists in our stocks when reared in the laboratory under standard conditions, which indicates a genetic influence on wing length (Van 't Land et al. 1999). Stalker (1980) found that flies adapted to high temperatures have a relative large wing-load index, i.e., relatively small wings and a rapid wing beat. This may relate to *Adh*, αGpdh, or *In(2L)t*. There exists experimental evidence that flies with either an αGpdh^FF^ or an *Adh^SS^* genotype are smaller and have shorter wings, and that αGpdh^FF^ flies have a higher flight output at high temperatures (Pieragostini et al. 1979; Serra and Oller 1984; Barnes and Laurie-Ahlberg 1986; Oudman et al. 1991). Our findings of higher frequencies for *Adh^S^* and αGpdh^F^ and shorter wings in the tropics are consistent with these results. However, the mechanism

responsible for the connection between the polymorphisms at these loci and a polygenic trait like wing length remains a matter of further investigation (see Van 't Land et al. 1999).

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