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A TIME SERIES OF EVOLUTION IN ACTION: A LATITUDINAL CLINE IN WING SIZE IN SOUTH AMERICAN *DROSOPHILA SUBOBSCURA*

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Abstract.—*Drosophila subobscura* is geographically widespread in the Old World. Around the late 1970s, it was accidentally introduced into both South and North America, where it spread rapidly over broad latitudinal ranges. This invading species offers opportunities to study the speed and predictability of trait evolution on a geographic scale. One trait of special interest is body size, which shows a strong and positive latitudinal cline in many *Drosophila* species, including Old World *D. subobscura*. Surveys made about a decade after the invasion found no evidence of a size cline in either North or South America. However, a survey made in North America about two decades after the invasion showed that a conspicuous size cline had evolved and (for females) was coincident with that for Old World flies. We have now conducted parallel studies on 10 populations (13° of latitude) of flies, collected in Chile in spring 1999. After rearing flies in the laboratory for several generations, we measured wing sizes and compared geographic patterns (versus latitude or temperature) for flies on all three continents. South American females have now evolved a significant latitudinal size cline that is similar in slope to that of Old World and of North American flies. Rates of evolution (haldanes) for females are among the highest ever measured for quantitative traits. In contrast, the size cline is positive but not significant for South or North American males. At any given latitude, South American flies of both sexes are relatively large; this in part reflects the relatively cool climate of coastal Chile. Interestingly, the sections of the wing that generate the size cline for females differ among all three continents. Thus, although the evolution of overall wing size is predictable on a geographic scale (at least for females), the evolution of size of particular wing components is decidedly not.

Key words.—Cline, contingency, haldanes, latitude, rapid evolution, size.

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Drosophila subobscura is native to a broad latitudinal range in the Old World (north Africa to southern Scandinavia), where it shows marked latitudinal clines in body size and in many genetic markers (Prevosti 1955; Misra and Reeve 1964; Prevosti et al. 1985; Prevosti et al. 1988; Pegueroles et al. 1995; Huey et al. 2000; Gilchrist et al. 2001; Calboli et al. 2003). Similar body and wing-size clines are commonly found in many native species of *Drosophila* (Stalker and Carson 1947; Sokoloff 1965; David and Bocquet 1975; Pegueroles et al. 1995) and in introduced species such as *D. melanogaster* (Coyne and Beecham 1987; Capy et al. 1993; Imasheva et al. 1994; James et al. 1995; van't Land et al. 1995). These clines have probably evolved at least partly in response to latitudinal gradients of temperature (Partridge and French 1996). Nevertheless, the adaptive significance of latitudinal size clines in ectotherms remains elusive (James et al. 1995; Partridge and French 1996; van der Have and de Jong 1996; van Voorhies 1996; Azevedo et al. 1998; Reeve et al. 2000).

How fast evolution can proceed within localized populations is a fundamental question that has received renewed interest in recent years (Hendry and Kinnison 1999; Kinnison and Hendry 2001). Even so, few studies have attempted to address a related question, namely, how fast can clinal evolution occur on a geographic scale? The repeated documentation of latitudinal size clines in introduced species such as *D. melanogaster* suggests that a cline can evolve within hundreds of years (David and Capy 1988; Imasheva et al. 1994; Gilchrist and Partridge 1999), but determining whether geographic clines can evolve even faster requires monitoring species that have been introduced far more recently than has *D. melanogaster*.

One invading species that is suitable for estimating maximal rates of clinal evolution is *Drosophila subobscura*. Just over two decades ago, this fly was accidentally introduced into the New World in both North and South America. Genetic evidence documents unequivocally that the founding stock for both introductions was the same (Prevosti et al. 1988; Ayala et al. 1989; Prevosti et al. 1989; Mestres et al. 1992; Balanyà et al. 1994, 2003; Mestres et al. 1994). The invading flies rapidly colonized broad latitudinal ranges on both continents and soon occupied areas with climatic patterns roughly similar to those experienced by the native Old World flies (Fig. 1).

Species that have invaded new continents can also be used to evaluate a second fundamental question: Is the evolution of clines repeatable (Losos et al. 1998)? Similar clinal patterns with latitude for a given species on multiple continents suggests that natural selection, imposed by common characteristics of the environment that vary with latitude, dominates evolutionary trajectories on a geographic scale (Endler 1977).

Here we evaluate size clines in *D. subobscura* on three continents and examine the rates and predictability of evo-

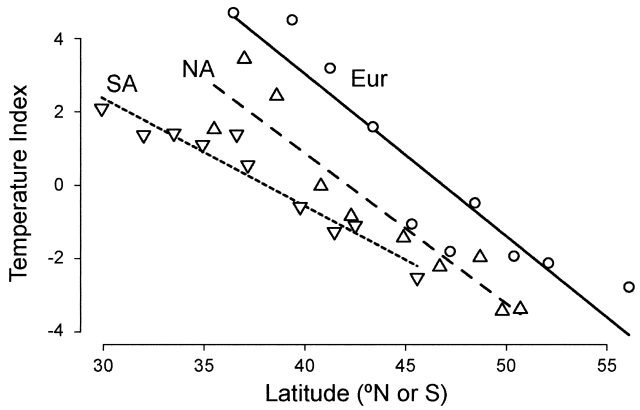

FIG. 1. Temperature index (first principal component of mean seasonal T_{min} and T_{max} for each site) as a function of latitude for Europe (Eur), North America (NA), and South America (SA).

lution on a geographic scale. Rapid and predictable evolution would be evident if, for example, both the North and South American populations have evolved latitudinal clines that have converged on the ancestral Old World clines (Endler 1977).

Rapid and repeatable evolution at the genetic level has already been well documented in *D. subobscura*. Indeed, evolutionary geneticists quickly recognized this opportunity to study evolution in action and seized upon this ''grand experiment in evolution'' (Ayala et al. 1989). In studies made less than a decade after the invasion, researchers discovered that both the North and South American populations had evolved latitudinal clines in chromosomal inversion frequencies that were generally in the same direction (though not nearly as steep) as in the native Old World populations (Brncic et al. 1981; Prevosti et al. 1985, 1987, 1988, 1989, 1990; Balanyà et al. 2003). Thus, the evolution of clines in inversion frequencies was remarkably rapid and predictable, at least in general direction. Nevertheless, studies about one decade after the introduction found no evidence that a cline in wing size had yet evolved in either North or South America (Budnik et al. 1991; Pegueroles et al. 1995).

Some two decades after the introduction, we decided to re-examine the North American populations to evaluate whether a wing size cline had finally evolved (Huey et al. 2000; Gilchrist et al. 2001). We collected latitudinal series for North America in 1997 and for Europe in 1998 and found that the North American females had indeed evolved a significant latitudinal cline in wing length that was remarkably parallel to that for the native Old World females. The cline for males was less steep than that for Old World males. Interestingly, different sections of the wing were involved in generating the North American versus Old World clines. In a more expansive report (Gilchrist et al. 2001), we found that these patterns applied to overall wing dimensions, not just to wing length. Our prior studies with the North American flies thus demonstrated a remarkably convergent evolution of a size cline for females that is one of the fastest examples of quantitative trait evolution ever recorded in nature (Hendry and Kinnison 1999; Gilchrist et al. 2001; Kinnison and Hendry 2001). These studies also demonstrated that the evolution of a latitudinal cline in overall wing size is repeatable on a continental scale, although the particular morphological adjustment by which it was achieved varies among continents.

Here we examine patterns of clinal variation in the South American populations of *D. subobscura*. Have these flies now evolved a latitudinal cline in wing size in the decade since the prior survey (Budnik et al. 1991; Pegueroles et al. 1995)? If so, did the particular morphological adjustment underlying that size cline follow the pattern for North American, that for Europe, or even a different pattern? We also examined whether size clines on all three continents are correlated with climatic measurements, not just with latitude. If wing size evolution were driven primarily by climatic factors such as temperature, then we would expect coincident relationships between size and climate on all three continents.

To answer these questions, we traveled to Chile in the austral spring of 1999. We collected flies from 10 populations over a broad latitudinal range (~13°, Table 1), established laboratory stocks of each, and subsequently measured wing sizes. We find that females in the Chilean populations have now also evolved a latitudinal size cline that parallels those found in the native European populations and the introduced North American ones (see preliminary report in Calboli et al. 2003). At any given latitude, however, the Chilean flies are relatively large, which seems to be related in part to the relatively cooler climates (based seasonal summaries of minimum and maximum daily temperature) along coastal South America. Our studies document that evolution of wing size can be remarkably fast and predictable, even on a continental scale. Nevertheless, the evolution of size of particular wing components is decidedly not predictable.

MATERIALS AND METHODS

We collected *D. subobscura* (between 20 and 25 isofemale lines) from 10 low-altitude sites in Chile (Table 1) during November 1999. Isofemale lines were reared for two generations in the laboratory at 20°C (14L:10D). Groups of 10 males and 10 females from each isofemale line were combined in a population cage (25 cm X 14 cm X 12 cm) for each population. The flies were mass reared (at low larval density) for seven to nine generations.

To obtain flies for the size measurements, we collected eggs and set up four vials per population (50 eggs per vial, 10 ml medium per vial) and reared the flies to adulthood at 20°C. Shortly after eclosion, adults were collected and the wings were mounted (five to 10 individuals of each sex per vial per population for a minimum of 20 males and 20 females). Left wings were measured with Sigma Scan software (SPSS, Chicago, IL). Following Robertson and Reeve (1952) and Pegueroles et al. (1995), we measured wing length in two segments along vein IV (diagrammed in Gilchrist et al. 2001): *L1* (from the base of the fourth longitudinal vein to the posterior cross vein) and *L2* (from the posterior cross vein to the distal extreme of the fourth longitudinal vein). We also measured wing width, *W*, as the distance from the distal extreme of the fifth longitudinal vein on the trailing edge of the wing to the leading edge in a line perpendicular to the vein III. (Note that wing dimensions, which are often used as an index of body size in *Drosophila*, are typically correlated with other body dimensions; Robertson and Reeve

TABLE 1. Source sites for flies used in this study and weather stations used for constructing the temperature index. Loc, locality index; Lat, latitude; Long, longitude; Alt, altitude. Start, the date the weather records started; End, the date of the last weather data; Rec, the number of daily records used in the analysis.

Population	Loc	Lat (N)	Long	Alt (meters)	Weather station	Country	Lat (N)	Long	Alt (meters)	Start	End	Rec
Aarhus	AA	56°9′	10°13′E	0	Gniben	Denmark	56°0′	11°17′E	4	11/79	12/91	3242
Leiden	LN	52°9′	4°30′E	−1	Valkenburg	Netherlands	52°11′	4°25′E	1	10/77	12/91	5067
Lille	LI	50°38′	3°4′E	24	Lille	France	50°34′	3°6′E	47	10/77	12/91	5147
Gif-sur-Yvette	GF	48°44′	2°8′E	127	Grouin De Cancale	France	48°43′	1°51′W	40	10/77	12/91	5082
Dijon	DI	47°21′	5°1′E	235	Dijon	France	47°16′	5°5′E	222	10/77	12/91	5151
Lyon	LY	45°31′	4°50′E	260	Lyon/Satolas	France	45°44′	5°5′E	248	10/77	12/91	5129
Montpellier	MO	43°38′	3°53′E	18	Montpellier	France	43°35′	3°58′E	5	10/77	12/91	5151
Barcelona	BA	41°25′	2°11′E	0	Barcelona	Spain	41°24′	2°11′E	12	10/77	9/89	3938
Valencia	VA	39°26′	0°22′W	7	Valencia	Spain	39°29′	0°23′W	13	10/77	12/91	4758
Malaga	MA	36°45′	4°25′W	0	Malaga/El Rompedizo	Spain	36°40′	4°29′W	8	10/77	12/91	5181
Port Hardy	PH	50°42′	127°25′W	24	Port Hardy	Canada	50°41′	127°22′W	22	10/77	12/91	5179
Peachland	PL	49°46′	119°44′W	342	Penticton	Canada	49°28′	119°36′W	344	10/77	12/91	5177
Bellingham	BE	48°44′	122°28′W	30	Bellingham	USA	48°48′	122°32′W	48	10/77	12/91	5079
Centralia	CE	46°40′	122°58′W	58	Olympia	USA	46°58′	122°54′W	61	10/77	12/91	4039
Salem	SA	44°55′	123°1′W	47	Salem	USA	44°55′	123°1′W	61	10/77	12/91	5177
Medford	ME	42°20′	122°51′W	117	Medford	USA	42°22′	122°52′W	405	10/77	12/91	5177
Eureka	EU	40°48′	124°10′W	13	Eureka	USA	40°48′	124°10′W	18	10/77	12/91	5171
Davis	DA	38°33′	121°44′W	15	Fairfield/Travis AFB	USA	38°16′	121°56′W	19	10/77	12/91	4076
Gilroy	GI	37°1′	121°35′W	61	San Jose/Muni	USA	37°22′	121°55′W	17	6/81	12/91	3828
Atascadero	AT	35°29′	120°41′W	268	Paso Robles	USA	35°40′	120°38′W	255	10/77	12/91	5075
La Serena	LS	−29°55′	71°15′W	28	Forada	Chile	−29°55′	71°14′W	137	10/77	12/91	5179
Illapel	IP	−32°0′	71°10′W	388	Quintero	Chile	−32°47′	71°31′W	3	10/77	12/91	2440
Santiago	SG	−33°30′	70°40′W	521	Santiago/Pedahuel	Chile	−33°23′	70°53′W	480	10/77	12/91	5179
Curico	CU	−34°55′	71°14′W	214	Curico	Chile	−34°58′	71°13′W	225	10/77	12/91	5169
Chillan	CH	−36°37′	72°7′W	129	Chillan/O'Higgins	Chile	−36°35′	72°2′W	148	10/77	12/91	734
Laja	LJ	−37°10′	72°42′W	49	Concepcion	Chile	−36°46′	73°3′W	12	10/77	12/91	5169
Valdivia	VD	−39°46′	73°14′W	4	Valdivia	Chile	−39°39′	73°5′W	53	10/77	12/91	5159
Puerto Montt	PM	−41°28′	72°56′W	0	Puerto Montt/El Tepual	Chile	−41°26′	73°5′W	85	10/77	12/91	5171
Castro	CA	−42°30′	73°46′W	0	Ancud	Chile	−41°54′	73°48′W	11	10/77	4/90	491
Coyhaique	CQ	−45°35′	72°4′W	302	Coyhaique	Chile	−45°35′	72°7′W	310	10/77	12/91	732

1952; Misra and Reeve 1964; Anderson 1966; Sokoloff 1966.)

European and North American flies had been collected and reared in an identical manner (for additional data and details see Gilchrist et al. 2001).

Climate Data

Climate data were obtained from NOAA's Global Daily Summary for 1982 to 1991 (NOAA 1994). More recent data is available but only for some sites. Because general climate patterns have not changed dramatically, we opted for using this smaller, more consistent dataset. When data were unavailable for a particular collecting locality, the closest locality of similar altitude was used (see Table 1). We computed the mean minimum (T_{min}), mean maximum (T_{max}), and total precipitation for each season at each site. We determined that the precipitation statistics had little impact in preliminary analyses, so we included only temperatures in the subsequent analyses. The four seasonal T_{min} values and the four seasonal T_{max} values were transformed into a temperature index (*TI*) by computing the first principal component (see below).

Data Analyses

We log-transformed the three wing measurements to normalize the variance and then computed principal components over both sexes and all populations on the three continents to obtain estimates of overall size. Because *D. subobscura* is sexually dimorphic, we analyzed females and males separately.

Analyses of clinal patterns have used two different approaches. Some include data from multiple animals at each site (e.g., Coyne and Beecham 1987; Imasheva et al. 1994; Huey et al. 2000), whereas others analyze only population means (Gilchrist and Partridge 1999; Calboli et al. 2003). The former increases power, but may violate the assumption of independence of the residuals as flies within sites are likely more closely related than are flies among sites. Accordingly, we choose to be conservative and have analyzed only population means.

We used a linear model to analyze latitudinal variation in wing size, with latitude as a covariate nested inside of continent; this procedure enabled us to obtain separate estimates of slope and intercept for each continent. We tested the null hypothesis of parallel slopes by using a standard ANOVA comparison of slopes. When the slopes were significantly heterogeneous, we then used orthogonal contrasts with a single degree of freedom to test two a priori hypotheses: (1) European slopes did not differ significantly from the New World slopes; and (2) North American slopes did not differ from the South American ones. When we could not reject the null hypothesis (slopes are not significantly heterogeneous), we tested for overall size differences among the continents by assuming a common slope and applying an ANCOVA. If this analysis indicated significant heterogeneity, we then used contrasts with a single degree of freedom to test two a priori hypotheses: (1) the body sizes are similar in Europe and the New World; and (2) the body sizes are similar in North and South America. Parallel analyses were carried out for regressions of size on the *TI*. The ANCOVA statistics and contrasts are presented in the Appendix. Finally, similar analyses of the three measured wing dimensions were carried out by regressing each variable on latitude and on the temperature indices.

Rates of evolution were computed by both allochronic and synchronic methods (Hendry and Kinnison 1999) and bootstrapped to obtain estimates of variation and confidence intervals. We compared the rates of change in the six NA and five SA populations sampled in both 1986/1988 (Pegueroles et al. 1995) and in 1997/1999. These collections are not ideal for comparison as the flies were reared in different laboratories under different culture conditions. Moreover, the 1986 values represent the vial means of 10 males and 10 females per vial, rather than measurements of individuals. Because the overall size of the 1986/1988 flies was much smaller than that of the 1997/1999 samples, we felt that the most conservative approach would be to attribute this to a difference in rearing conditions. Thus, for each decade's data, we computed the *z*-scores for each wing dimension (*L1*, *L2*, and *W*) for that decade (pooling over sex, population, and continent). The *z*-scores were then back-transformed to millimeters by multiplying the values by the standard deviation then adding the mean of the combined 1986/1988 and 1997/1999 data. We estimated the mean and confidence limits for the rate of change in each wing dimension in each population, computing both haldanes (assuming five generations per year in all populations) and darwins using a bootstrap analysis ($B = 1000$) of the transformed data (Hendry and Kinnison 1999). More computational details are presented in Gilchrist et al. (2001). All of the statistical analyses were carried out in R (Ihaka and Gentleman 1996).

RESULTS

Patterns of Climate with Latitude

The first principal component (PC1) of the seasonal temperature data (the *TI*, see Materials and Methods) explained approximately 70% of the variation and consisted of positive loadings (0.281–0.406) for all temperatures. Thus, large positive scores for PC1 indicate relatively warm locales. The second principal component (PC2), which explained 20% of the variation, described the difference between spring and summer T_{max} (0.467–0.564) and the rest of the temperature data (−0.459–0.188). The second and higher temperature principal components were not correlated with any of the measures of wing size, so they were excluded from further consideration.

The *TI*, varies inversely with latitude in a similar manner on all three continents (Fig. 1, comparison of slopes test: $F_{2,25} = 2.230$, $P = 0.1277$). However, at any given latitude, the South American sites are cooler (lower *TI*) than are comparable North American or European latitudes (Fig. 1). ANCOVA revealed a significant difference in intercept among the three continents ($F_{2,27} = 18.90$, $P < 0.001$). Tukey's post hoc comparisons detect no significant difference between the intercepts for Europe and North America, but a highly significant difference between those two continents and South America. (Note, however, that each NA site has a lower *TI* than European sites at a similar latitude, no doubt reflecting the impact of the Gulf Stream on western sites in Europe.

The lack of a significant difference between Europe and the west coast of North America is probably a power issue.)

Overall Wing Patterns: Principal Components

The PC1 describes overall wing size (loadings 0.544–0.630), and PC2 describes a trade-off between *L1* (0.755) and *L2* plus *W* (−0.275 to −0.595). The third principal component (PC3) explains only 3% of the variance and was omitted from further analyses.

Clinal Variation in Size: Latitude

Wing size (PC1) is plotted as a function of latitude in Figure 2, and the slopes of the regressions are shown in Table 2. In an overall test among all continents, female wing size increases significantly with latitude ($F_{1,24} = 6.16$, $P = 0.020$).

Next we compare clines for females among continents. Slopes were not significantly different (comparison of slopes: $F_{2,24} = 0.534$, $P = 0.59$), so we computed intercepts for each continent and sex, assuming a common slope (Appendix). For PC1, intercepts differ significantly among continents (ANCOVA: $F_{2,26} = 17.78$, $P < 0.001$). We used a priori contrasts to test the null hypotheses that the intercept of European females was similar to those from the New World populations and that North and South America shared a similar intercept. Both hypotheses were rejected. At a given latitude, New World females are marginally larger overall than Old World females ($F_{1,26} = 3.48$, $P = 0.073$), and South American females are larger than North American females (ANCOVA: $F_{1,26} = 32.09$, $P < 0.001$).

Males show somewhat different patterns. Although PC1 scores for males increase with latitude on all continents, the cline is significant only in Europe (Table 2). The slopes are not significantly different between Old and New Worlds or between North and South America, reflecting the relatively low power that comes from using the population means (see Materials and Methods). Male *D. subobscura* exhibit relatively large variation in wing size among populations within each continent, especially so in South America.

Clinal Variation in Size: Temperature Index

The above analyses show that wing size increases with latitude, suggesting that latitudinal changes in thermal regimes may be responsible. Overall wing size (PC1) varies clinally and inversely with the *TI* both for females ($F_{1,24} = 14.14$, $P < 0.001$) and for males ($F_{1,24} = 5.07$, $P = 0.034$). Females exhibit significant clinal variation on all three continents (Fig. 2), with no significant heterogeneity among the slopes ($F_{2,24} = 0.87$, $P = 0.43$). European males show marginally significant clinal variation (Fig. 2), with no significant heterogeneity of slopes ($F_{2,24} = 0.30$, $P = 0.74$).

After fitting a common slope for all continents within each sex, we find that *y*-intercepts are heterogeneous (Appendix; ANCOVA: females: $F_{2,26} = 7.30$, $P < 0.01$; males: $F_{2,26} = 9.99$, $P < 0.001$). European flies are not significantly different from the New World flies (females: $F_{1,26} = 0.042$, $P = 0.84$; males: $F_{1,26} = 1.99$, $P = 0.17$), but the South American flies are significantly larger overall than those from North America (females: $F_{1,26} = 14.56$, $P < 0.001$; males: $F_{1,26} = 17.99$, $P < 0.001$).

Continentwide Trends in Size

The wing size of the SA flies (particularly of males) is larger overall than that of flies in North America or Europe (Figs. 2,3). ANCOVA results cited above show that females from South America are larger (Appendix; more positive PC1) than those from North America, and that New World females are larger than European. Although male slopes are not significantly different from zero overall, South American males clearly are larger than those from North America and Europe. We hypothesize that the South American pattern could simply reflect continental differences in the relation between latitude and climate. Recall that South America is colder than North America or Europe (Fig. 1) at a given latitude and that flies from colder environments are generally larger. Thus, if the size differences among populations simply represent a response to temperature, then the residuals from a regression of PC1 on temperature should show no heterogeneity among continents. In Figure 4, we show boxplots of the residuals for each continent and sex, pooled across latitudes. For females, no clinal variation remained in the residuals of PC1 after removing the effect of temperature (latitude: $F_{1,26} = 2.41$, $P = 0.133$) but significant heterogeneity was still present among the continents ($F_{2,26} = 7.75$, $P < 0.005$). Tukey's HSD tests showed no significant difference between Europe and either of the New World populations, however South American females were still significantly larger than North American females. For males, the residuals were also not significantly correlated with latitude ($F_{1,26} = 0.91$, $P = 0.349$); however, significant heterogeneity remained among the continents ($F_{2,26} = 10.16$, $P < 0.001$). Tukey's HSD revealed no significant difference between Europe and North America; however, the South America males were still significantly larger than those on the other continents.

Clinal Variation in Shape: Latitude

Large positive values in PC2 indicate a wing that has a relatively long proximal portion (*L1*), a relatively short distal proportion (*L2*), and is relatively narrow, whereas large negative values indicate the opposite. The continents show no consistent clinal relationship between PC2 and latitude for either sex (females: $F_{1,24} = 0.669$, $P = 0.422$; males: $F_{1,24} = 0.261$, $P = 0.614$); however, interactions between continent and latitude are significant or nearly so (females: $F_{2,24} = 3.012$, $P = 0.068$; males: $F_{2,24} = 3.522$, $P = 0.046$), indicating heterogeneity among slopes. PC2 increases significantly with latitude for European flies of both sexes (Table 2), but not for North American or South American flies. Note, however, that the sign of the slope in North America is in the opposite direction to that in Europe and South America.

Examination of the original wing dimensions reveals why the clinal pattern in PC2 varies among continents. Recall that *L1* measures the proximal portion of vein IV; both sexes show strong clinal variation in *L1* with regard to latitude (Fig. 2, Table 2; females: $F_{1,24} = 16.38$, $P < 0.001$; males: $F_{1,24} = 11.987$, $P < 0.01$). European and South American flies of

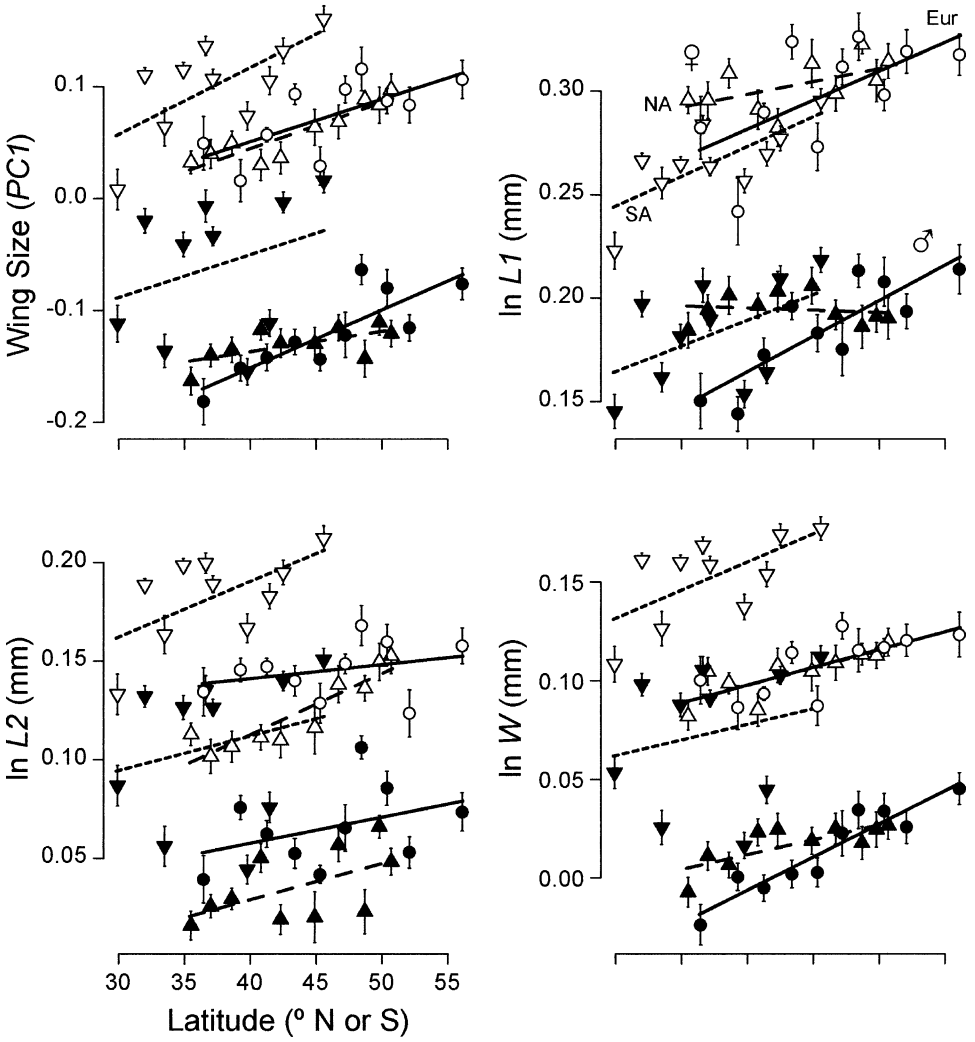

FIG. 2. Latitudinal patterns in wing size. Females are indicated by open marks, males by solid marks. Error bars indicate ±1 standard error. Slopes (given in Table 2) are computed on the population means. PC1 is the first principal component describing overall wing size. *L1* is the proximal portion of wing vein IV. *L2* is the distal portion of wing vein IV. *W* is the width of the wing.

both sexes show significant positive increases in *L1* with latitude, whereas those for North American flies have shallower slopes that are not significantly different from zero. Nonetheless, the comparison of slopes test could not reject the null hypothesis of parallel slopes for females ($F_{2,24}$ = 0.86, P = 0.435).

The distal portion of vein IV (*L2*) shows significant clinal variation for females ($F_{1,24}$ = 16.288, $P < 0.001$), but not males ($F_{1,24}$ = 2.889, P = 0.102). Clinal patterns are significant and parallel for North American and South American females, but not significant for Europe (Fig. 2, Table 2). Nonetheless, the null hypothesis of parallel slopes cannot be rejected ($F_{2,26}$ = 2.39, P = 0.113), probably because of insufficient power to detect differences in slope. None of the continents exhibits significant clinal variation in *L2* among males.

Finally, wing width (*W*, Table 2) shows a strong latitudinal cline for both sexes (females: $F_{1,24}$ = 20.92, $P < 0.001$; males: $F_{1,24}$ = 8.79, $P < 0.01$) with no significant heterogeneity of slopes. Nevertheless, South American flies have significantly wider wings than flies from Europe or North America (Appendix).

Clinal Variation in Shape: Temperature

PC2 shows no consistent clinal pattern with the *TI* for either sex (Table 2; females: $F_{1,24}$ = 0.77, P = 0.389; males: $F_{1,24}$ = 0.44, P = 0.514); however, continent × *TI* interactions are significant (females: $F_{2,24}$ = 3.83, P = 0.036, males: $F_{2,24}$ = 3.87, P = 0.035). European females exhibit a weak but significant negative cline (Table 2); European and North American males have marginally significant negative and positive clines respectively. Neither sex in South America shows any clinal pattern with regard to PC2.

The proximal portion of the wing (*L1*), shows significant clinal variation with the *TI* for both sexes (Fig. 3, Table 2; females: $F_{1,24}$ = 10.65, $P < 0.005$); males: $F_{1,24}$ = 8.27, $P < 0.01$), with what appear to be steeper slopes in Europe and South America than in North America for both sexes. For females, however, the slopes of *L1* on *TI* for Europe, North

TABLE 2. Slopes and comparisons of slopes for regression of wing traits on latitude or temperature index. The values shown are the slope ±1 standard error. The significance of slope (≠0) is indicated by: † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The letters (italicized) indicate significant a priori contrasts testing for heterogeneity of slopes. The letters *a* and *b* indicate a significant contrast with a single degree of freedom between European and New World clines. No contrast was significant between North American and South American slopes.

	Latitude		Temperature index	
	Females	Males	Females	Males
PC1				
Europe	0.004 ± 0.0014*	0.005 ± 0.0021*	−0.007 ± 0.0033*	−0.010 ± 0.0047†
North America	0.004 ± 0.0015*	0.002 ± 0.0024	−0.008 ± 0.0040†	−0.004 ± 0.0057
South America	0.006 ± 0.0017**	0.004 ± 0.0026	−0.017 ± 0.0063*	−0.009 ± 0.0090
R^2	0.633	0.535	0.530	0.489
PC2				
Europe	0.002 ± 0.0008* *a*	0.001 ± 0.0008† *a*	−0.004 ± 0.0017* *a*	−0.003 ± 0.0017† *a*
North America	−0.001 ± 0.0009 *b*	−0.002 ± 0.0009† *b*	0.003 ± 0.0020 *b*	0.004 ± 0.0021† *b*
South America	0.000 ± 0.0010 *b*	0.001 ± 0.0010 *b*	−0.001 ± 0.0032 *b*	−0.003 ± 0.0033 *b*
R^2	0.901	0.835	0.906	0.839
ln L1				
Europe	0.003 ± 0.0009**	0.003 ± 0.0009*** *a*	−0.006 ± 0.0020**	−0.007 ± 0.0020** *a*
North America	0.001 ± 0.0010	0.000 ± 0.0010 *b*	−0.002 ± 0.0024	0.001 ± 0.0025 *b*
South America	0.003 ± 0.0011*	0.002 ± 0.0011* *b*	−0.008 ± 0.0038*	−0.006 ± 0.0039 *b*
R^2	0.671	0.483	0.625	0.406
ln L2				
Europe	0.001 ± 0.0008	0.001 ± 0.0015	−0.001 ± 0.0018 *a*	−0.002 ± 0.0032
North America	0.003 ± 0.0009**	0.002 ± 0.0017	−0.007 ± 0.0022** *b*	−0.004 ± 0.0039
South America	0.003 ± 0.0010**	0.002 ± 0.0018	−0.008 ± 0.0035* *b*	−0.004 ± 0.0061
R^2	0.814	0.616	0.786	0.600
ln W				
Europe	0.002 ± 0.0007*	0.003 ± 0.0012*	−0.004 ± 0.0017*	−0.006 ± 0.0027*
North America	0.002 ± 0.0008*	0.002 ± 0.0013	−0.003 ± 0.0020	−0.003 ± 0.0032
South America	0.003 ± 0.0009**	0.002 ± 0.0015	−0.008 ± 0.0032*	−0.003 ± 0.0051
R^2	0.817	0.704	0.776	0.677

America, and South America cannot be distinguished ($F_{2,24} = 1.19$, $P = 0.32$), so we fitted a common slope and applied analysis of covariance. The *y*-intercepts were heterogeneous among continents (Appendix; $F_{2,26} = 12.10$, $P < 0.01$), and differed significantly between Europe and the New World ($F_{1,26} = 6.39$, $P < 0.05$) and between North America and South America ($F_{1,26} = 17.80$. $P < 0.001$). *L1* in the South American females was smaller over all temperatures than in North America or Europe. For males, we found a marginal heterogeneity of slopes ($F_{2,26} = 2.801$, $P = 0.081$); European males have a marginally stepper slope in *L1* on *TI* than New World males ($F_{1,26} = 3.35$, $P = 0.08$).

L2 (the more distal portion of the wing) shows significant clines on *TI* for females (Fig. 3, Table 2; $F_{1,24} = 9.32$, $P < 0.01$), but not males ($F_{1,24} = 1.64$, $P = 0.212$). For females, the slopes were marginally heterogeneous among the continents ($F_{2,26} = 2.87$, $P = 0.076$), with a significant contrast between Europe and the New World ($F_{1,26} = 5.72$, $P > 0.05$). Both South America and North America show a relatively steep negative slope, with *L2* increasing at lower temperatures, whereas European females exhibit virtually no clinal pattern. For males, no significant clinal pattern in *L2* was detected for any continent.

Wing width (*W*) exhibits parallel clinal variation with temperature on all three continents (Fig. 3, Table 2; females: $F_{1,24} = 11.83$, $P < 0.005$, males: $F_{1,24} = 6.65$, $P < 0.05$, no significant continent × *TI* interactions). In both sexes, *y*-intercepts are heterogeneous (Appendix; ANCOVA, females: $F_{2,26} = 37.45$, $P < 0.001$; males: $F_{2,26} = 23.61$, $P < 0.001$), with New World flies having significantly wider wings than European flies (females: $F_{1,26} = 10.48$, $P < 0.005$; males: $F_{1,26} = 11.54$, $P < 0.005$) and South American flies having wider wings than North American flies (females: $F_{1,26} = 64.41$, $P < 0.001$; males: $F_{1,26} = 35.68$, $P < 0.001$).

Rates of Evolution

We computed allochronic (Hendry and Kinnison 1999) rates of evolution in haldanes (Fig. 5) and synchronic rates in both haldanes and darwins (Table 3) for both North and South American flies. Estimation of allochronic rates requires an actual time series, whereas synchronic rates of evolution are computed from divergent populations sampled at a single time. The synchronic rates (Table 3) are similar in magnitude to the allochronic. On both continents, the highest allochronic rates of evolution are at the highest latitude populations, with one exception. In North America, high rates of evolution were observed between 1986 and 1997 in Medford, Oregon, a mid-latitude site; however, the 1986 flies were exceptionally large, suggesting a possible error in the data.

DISCUSSION

Two decades after the introduction of *D. subobscura* to North and South America, significant wing size clines for

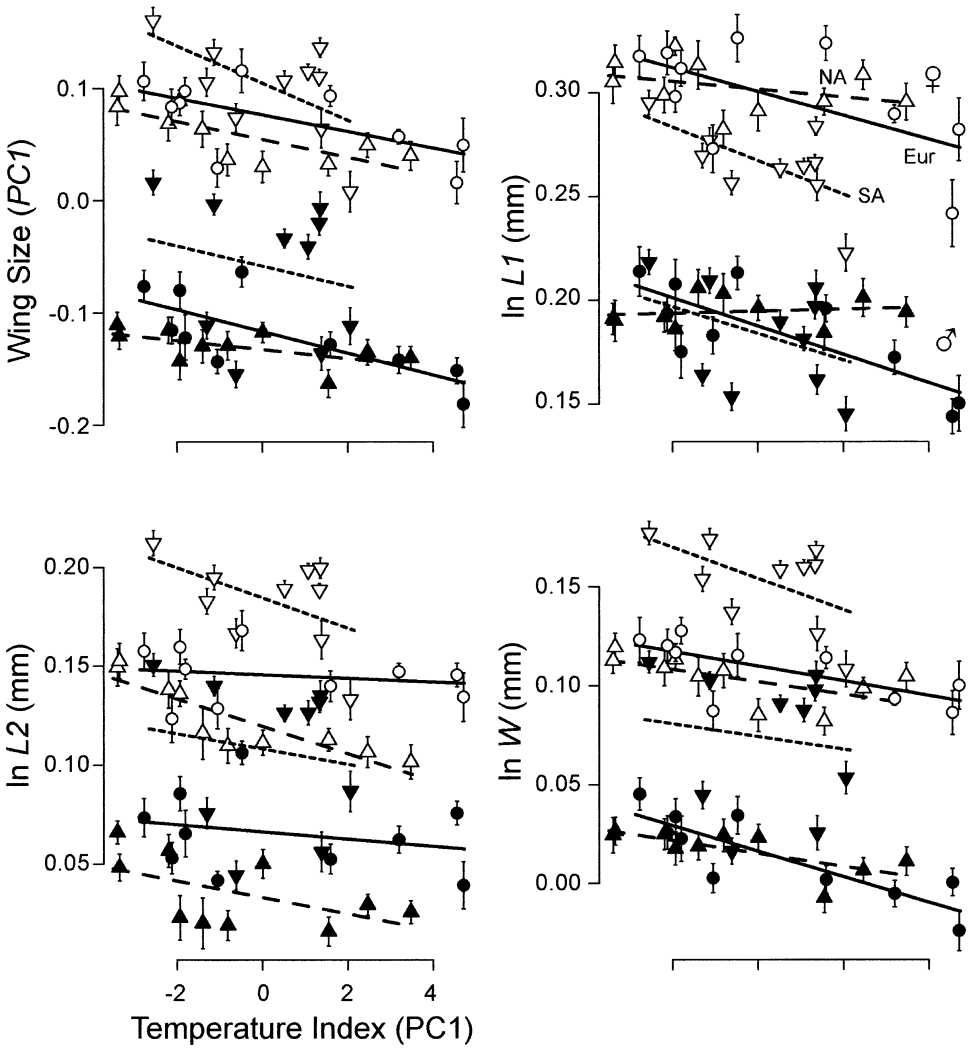

FIG. 3. Clinal patterns of wing size on the temperature index. Females are indicated by open marks, males by solid marks. Error bars indicate ±1 standard error. Slopes (given in Table 2) are computed on the population means. PC1 is the first principal component describing overall wing size. *L1* is the proximal portion of wing vein IV. *L2* is the distal portion of wing vein IV. *W* is the width of the wing.

females are evident on both continents (see also Huey et al. 2000; Gilchrist et al. 2001; Calboli et al. 2003). For females in the New World, the clines are now strikingly parallel to the ancestral cline in Europe, and in fact slopes in overall wing size with latitude are statistically indistinguishable among continents (Fig. 2, Table 2).

Clinal patterns for males are different in the New World. In Europe, males and females show parallel wing size clines (Huey et al. 2000; Gilchrist et al. 2001; Calboli et al. 2003), but colonizing males on both New World continents show less steep and weaker clines than do females. Given the high genetic correlation in body size between the sexes in other drosophilids (Cowley et al. 1986; Reeve and Fairbairn 1996), the difference in clines between males and females is surprising. Either the genetic correlation between wing sizes of males and females is relatively weak in the invading populations, or some selective factor is acting differentially on the sexes in the New World.

Although the general increase in wing size with latitude, especially among females, is similar on all three continents, the portion of the wing responsible for that increase differs among continents. In Europe, differences in the length of the proximal portion of the wing (*L1*) are primarily responsible for the cline, whereas in North America, differences in distal portion of the wing (*L2*) are responsible (Table 2). In South America, both parts of the wing contribute to the cline. Clinal variation in wing width is positive and similar for both males and females among all the continents, however South American flies of both sexes have much wider wings than do either the North American or European populations.

The ubiquity of size clines in drosophilids (*D. pseudoobscura*: Sokoloff 1965; *D. robusta*: Stalker and Carson 1947; *D. simulans*: David and Bocquet 1975; *D. obscura*: Pegueroles et al. 1995; *D. melanogaster*: Coyne and Beecham 1987; Capy et al. 1993; Imasheva et al. 1994; James et al. 1995; van't Land et al. 1995; Gilchrist et al. 2000; *Zaprionus in-*

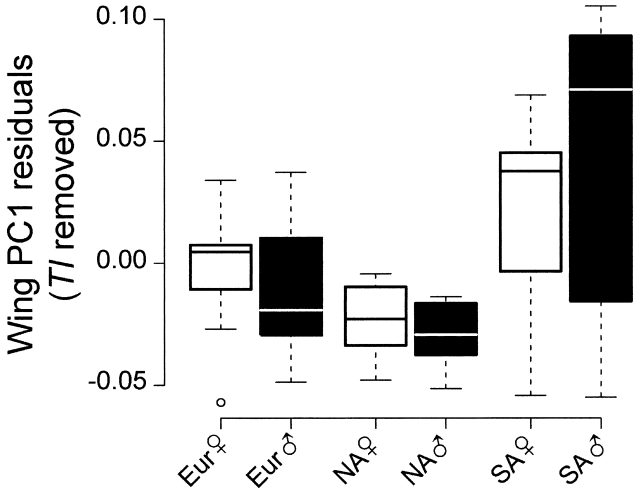

FIG. 4. Box plots of the residual wing size, after removing the effects of the temperature index. The bar indicates the mean, the box the 25% and 75% quantiles, and the bars the 10% and 90% quantiles. South American (SA) flies are significantly larger than North American (NA) or European (Eur) flies. Females are indicated by open boxes, males by shaded.

dianus Karan et al. 2000), and other species of ectotherms (copepods: Lonsdale and Levinton 1985; houseflies: Bryant 1977; wood frogs: Riha and Berven 1991) suggests that clines in which size increases with latitude have an adaptive basis that is related to temperature. However, the selective bases of this pattern remain elusive (Partridge and French 1996). Bergmann's rule, wherein animals gain a body temperature excess by reduced surface-to-volume ratios in cold climates, does not benefit animals of less than a few milligrams in body mass (Willmer and Unwin 1981; Stevenson 1985).

Although the slopes of female wing size versus latitude are similar for all continents, the South American cline is shifted approximately 6° toward the equator. (Thus at a given latitude, South American flies are much larger than those from North America or Europe.) Because coastal South America has a colder climate than North America or Europe (because of its smaller landmass at high latitudes and its exposure to the cold Humboldt Current), the larger size of South American flies (Fig. 4) might be related to climate. We tested this hypothesis by statistically removing the effect of temperature from PC1, our index of overall wing size, and asking whether significant differences remained among the continents. The residuals obtained from the regression were not significantly correlated with latitude for any continent in

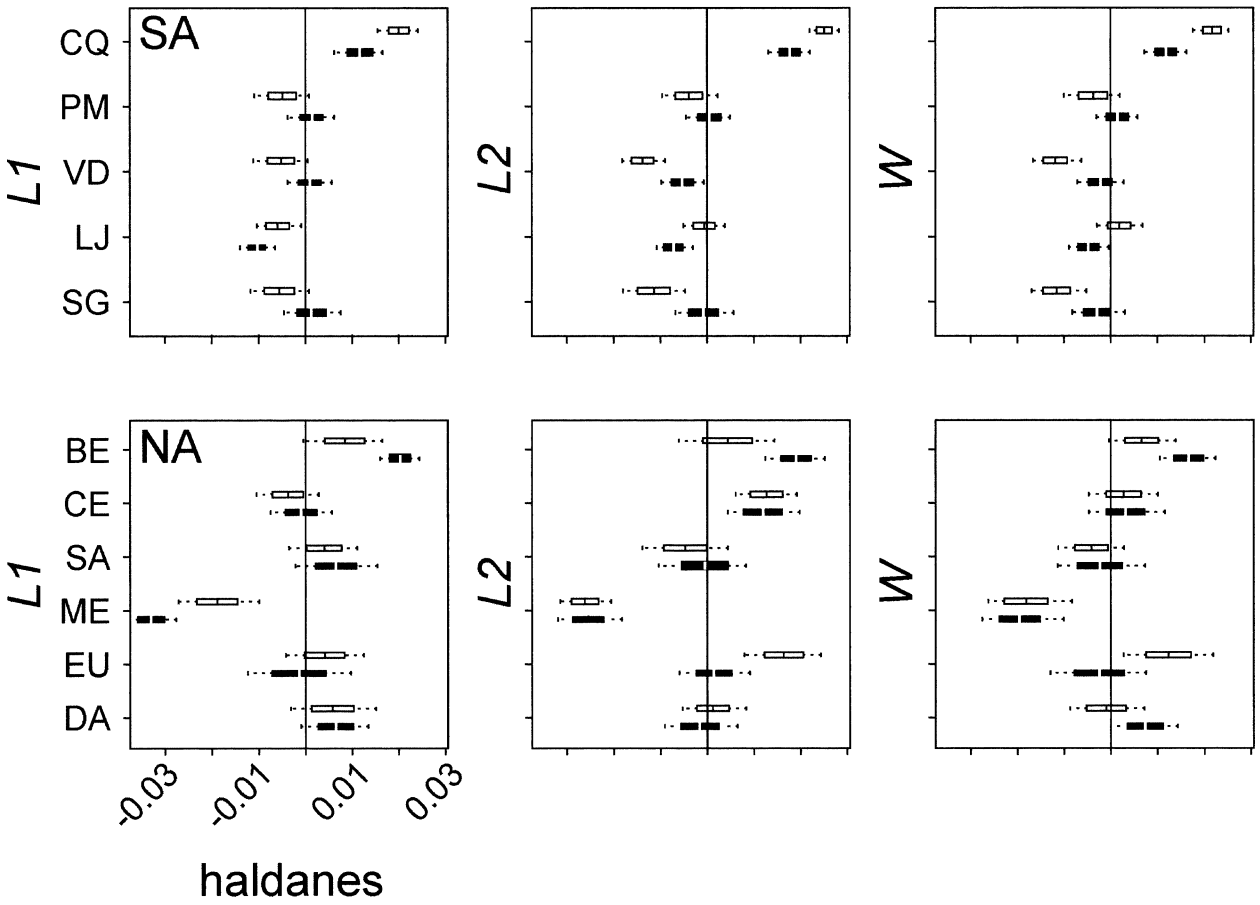

FIG. 5. Allochronic rates of evolution from 1986 to 1999 in South America (SA, top) and from 1986 to 1997 in North America (NA, bottom). Females are indicated by open boxes, males by shaded. The data graphed are jackknifed estimates of the mean, the standard error (box) and 95% confidence intervals (bars) for the sites in SA and NA for which there were data available for both sampling periods. *L1* is the proximal portion of wing vein IV. *L2* is the distal portion of wing vein IV. *W* is the width of the wing.

TABLE 3. Synchronic rates of divergence in North and South America. The statistics are based on the predicted (by regression) mean at the high and low latitudinal extremes on each continent (Atascadero and Port Hardy in North America [NA], La Serena and Coyhaique in South America [SA]). The statistic was bootstrapped by randomly sampling with replacement within each locality 1000 times and computing the best-fit regression line.

		L1		*L2*		*W*	
		Mean ± SD	95% CI	Mean ± SD	95% CI	Mean ± SD	95% CI
haldanes							
Female	NA	0.0047 ± 0.00226	0.0005, 0.0092	0.0138 ± 0.00219	0.0099, 0.0184	0.0086 ± 0.00155	0.0055, 0.0115
	SA	0.0075 ± 0.00112	0.0053, 0.0097	0.0063 ± 0.00111	0.0040, 0.0082	0.0063 ± 0.00110	0.0041, 0.0083
Male	NA	0.0018 ± 0.00135	0.0001, 0.0050	0.0101 ± 0.00173	0.0067, 0.0134	0.0082 ± 0.00174	0.0048, 0.0116
	SA	0.0080 ± 0.00119	0.0055, 0.0103	0.0048 ± 0.00135	0.0021, 0.0074	0.0052 ± 0.00137	0.0023, 0.0078
darwins							
Female	NA	729 ± 372.1	69.7, 1477.5	2408 ± 395.4	1608.4, 3190.5	1413 ± 311.4	822.5, 1997.1
	SA	2104 ± 370.4	1409.0, 2841.3	1983 ± 407.4	1161.0, 2734.2	1745 ± 374.9	1021.6, 2478.6
Male	NA	325 ± 242.8	16.3, 906.9	1627 ± 310.2	1025.1, 2239.0	1363 ± 344.1	720.3, 2034.5
	SA	2098 ± 391.0	1331.0, 2851.3	1326 ± 426.1	517.1, 2134.1	1229 ± 369.4	484.8, 2003.7

either sex; however, South American flies were still significantly larger than the North American colonists for both sexes. Thus, the larger size of South American flies is not entirely due to a colder climate. Of course, the difference in size might merely be an artifact of flies from the three continents having been collected and measured in three different years. This explanation is unlikely, however; all flies were maintained on medium from the same recipe and in the same incubators. Moreover, we have measured European stocks in different years under the same rearing conditions and see no significant differences in wing size (G. W. Gilchrist and R. B. Huey, unpubl. data).

An alternative and intriguing possibility is that the increase in size in South America (beyond that explained by continental differences in temperature) is due to competitive release. *Drosophila subobscura* is the only *obscura*-group drosophilid in Chile. Decreased interspecific competition in *Drosophila* may result in an evolutionary increase in body size (Alatalo and Gustafsson 1988; Schluter 2000), in a manner similar to increase in size observed at low conspecific density (e.g., Santos et al. 1992, 1994; Partridge and Fowler 1993; but see Santos et al. 1997). Although Chilean *D. subobscura* larvae likely compete with native species (such as *D. pavani* and *Scaptomyza denticauda* as well as abundant cosmopolitans such as *D. simulans*), the peak abundance of *D. subobscura* occurs early in the year before that of the most common drosophilids; and so the overall impact of these other species on *D. subobscura* appears small (Budnik and Brncic 1983; Brncic et al. 1985; Benado and Brncic 1994; Brncic 1994). In contrast, North American *D. subobscura* entered a community with five native *obscura*-group species (Beckenbach and Prevosti 1986; Ayala et al. 1989) that overlap substantially in seasonal activity patterns (Pascual et al. 1993). Laboratory experiments suggest that *D. subobscura* may be an inferior competitor to *D. pseudoobscura* (Pascual et al. 1998) although superior to *D. azteca* (Pascual et al. 2000). Nevertheless, *D. subobscura* has become the numerically dominant drosophilid at mid to higher latitudes (Pascual et al. 1993) and has largely displaced most of the other *obscura*-group flies in Washington and Oregon (A. Beckenbach, pers. comm.), at least in urban areas.

Genetic data suggest that the original colonists were probably descended from flies from the Mediterranean coast of Europe (Prevosti et al. 1987; Prevosti et al. 1988; Ayala et al. 1989; Prevosti et al. 1989; Mestres and Serra 1995). If so, then the original colonists should have had a relatively small body size; and thus most of the evolution responsible for the cline should have taken place at high latitude. This appears to be the case in South America, with the highest positive rates of evolution in all wing regions at Coyhaique, the highest latitude site (Fig. 5). The pattern for North America is less clear, with relatively rapid positive evolution for all traits in Bellingham, Washington, particularly for males, but also large positive changes in Eureka, California, females. The only other site with comparable evolutionary rates is Medford, Oregon. As noted above, however, size data for this site in 1986 are anomalous and probably in error.

The synchronic rates (Table 3) reveal significantly higher rates of evolution of *L1* in South America than North America for both sexes, but higher rates for *L2* in North America than in South America. This reflects the continental differences in the contribution of the various regions of the wing to the overall clinal pattern of wing size: most of the clinal variation in South America is due to *L1*, whereas most of the variation in North America is due to *L2*.

Synchronic rates of evolution between the geographic extremes (Table 3) are of comparable magnitude to allochronic rates (Fig. 5). This result, however, may not be typical. Synchronic studies, which estimate rates based on divergence among different contemporary populations, are far more common than are allochronic studies, which follow individual populations over time. The interpretation of synchronic estimates, however, is relatively difficult as they conflate the independent evolutionary history of two populations into a single estimate (Kinnison and Hendry 2001). Indeed, the heterogeneity among allochronic rates in our different populations (Fig. 5) suggests that one could obtain a wide range of synchronic estimates, depending entirely on which pair of populations was chosen for study. Allochronic rates, however, are not without their problems. Uncontrolled phenotypic plasticity, seasonal variation, or mere changes in methodology could result in large differences in measurements of a single trait taken several generations apart. Accordingly, we standardized all measurements to the overall mean in size,

but this results in a minimal rate of evolution. Nevertheless, the rates observed in *D. subobscura* are among the fastest ever recorded (Hendry and Kinnison 1999) and represent the only example where data on replicated allochronic rates of microevolution are available (Kinnison and Hendry 2001).

Concluding Remarks

The evolution of wing size clines in *D. subobscura* in only two decades or so demonstrates morphological evolution acting largely in parallel on three continents. The similarity of the clines, particularly for females, is striking testimony to the power of natural selection in rapidly shaping these clines. The exact adaptive significance of the increased wing size at higher latitudes, however, remains unanswered. The shift of the cline approximately 6° closer to the equator in South America, in a pattern that mirrors the shift in temperature, strongly implicates the thermal environment (and not photoperiod) as an important source of selection. The fact that a parallel increase in wing size at higher latitudes is obtained on each continent, although achieved by different developmental and cellular means, suggests that overall size itself may be under selection. The greater overall size of the South American flies, which appear to have reduced interspecific competition relative to Europe or North America, is consistent with the hypothesis of character divergence due to competitive release.

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Appendix

ANCOVA tests for heterogeneity of Y intercepts, with all continents fit to a common slope. The values given are the Y intercept ±one standard error. The letters in the next column indicate significant single degree of freedom a priori contrasts. The letters *a* and *b* indicate a significant difference in intercept between the Old World and the New World. The letters *c* and *d* indicate a significant difference between North and South America. Cells with *not* ‖ indicate that there was significant heterogeneity of slopes, so the ANCOVA would be meaningless.

	Latitude		Temperature index	
	Females	Males	Females	Males
PC1				
Europe	−0.133 ± 0.0406	−0.291 ± 0.0624	0.077 ± 0.0091	−0.117 ± 0.0126
N. America	−0.136 ± 0.0385 *c*	−0.292 ± 0.0592 *c*	0.054 ± 0.0091 *c*	−0.135 ± 0.0127 *c*
S. America	−0.066 ± 0.0333 *d*	−0.199 ± 0.0512 *d*	0.103 ± 0.0090 *d*	−0.059 ± 0.0126 *d*
PC2				
Europe	*not* ‖	*not* ‖	*not* ‖	*not* ‖
N. America	*not* ‖	*not* ‖	*not* ‖	*not* ‖
S. America	*not* ‖	*not* ‖	*not* ‖	*not* ‖
ln L1				
Europe	0.192 ± 0.0266	*not* ‖	0.300 ± 0.0056 *a*	*not* ‖
N. America	0.202 ± 0.0252 *c*	*not* ‖	0.300 ± 0.0056 *bc*	*not* ‖
S. America	0.179 ± 0.0218 *d*	*not* ‖	0.267 ± 0.0056 *bd*	*not* ‖
ln L2				
Europe	0.051 ± 0.0252	−0.008 ± 0.0426	*not* ‖	0.067 ± 0.0085
N. America	0.034 ± 0.0239 *c*	−0.034 ± 0.0404 *c*	*not* ‖	0.034 ± 0.0085 *c*
S. America	0.106 ± 0.0206 *d*	0.047 ± 0.0350 *d*	*not* ‖	0.108 ± 0.0085 *d*
ln W				
Europe	0.013 ± 0.0209 *a*	−0.091 ± 0.0355 *a*	0.110 ± 0.0046 *a*	0.016 ± 0.0071 *a*
N. America	0.014 ± 0.0198 *bc*	−0.082 ± 0.0336 *bc*	0.101 ± 0.0046 *bc*	0.014 ± 0.0072 *bc*
S. America	0.075 ± 0.0171 *bd*	−0.011 ± 0.0291 *bd*	0.153 ± 0.0045 *bd*	0.075 ± 0.0071 *bd*