



Rapid evolution of wing size clines in *Drosophila subobscura*

George W. Gilchrist¹, Raymond B. Huey² & Lluís Serra³

¹Department of Biology Box 5805, Clarkson University, Potsdam, NY 13699-5805, USA (Phone: 315-268-2359; E-mail: gilchrgw@clarkson.edu); ²Department of Zoology Box 351800, University of Washington, Seattle, WA 98195-1800, USA; ³Departamento de Genètica, Universitat de Barcelona, 08071 Barcelona, Spain

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Abstract

Parallel latitudinal clines across species and continents provide dramatic evidence of the efficacy of natural selection, however little is known about the dynamics involved in cline formation. For example, several drosophilids and other ectotherms increase in body and wing size at higher latitudes. Here we compare evolution in an ancestral European and a recently introduced (North America) cline in wing size and shape in *Drosophila subobscura*. We show that clinal variation in wing size, spanning more than 15 degrees of latitude, has evolved in less than two decades. In females from Europe and North America, the clines are statistically indistinguishable however the cline for North American males is significantly shallower than that for European males. We document that while overall patterns of wing size are similar on two continents, the European cline is obtained largely through changing the proximal portion of the wing, whereas the North American cline is largely in the distal portion. We use data from sites collected in 1986/1988 (Pegueroles et al. 1995) and our 1997 collections to compare synchronic (divergence between contemporary populations that share a common ancestor) and allochronic (changes over time within a population) estimates of the rates of evolution. We find that, for these populations, allochronically estimated evolutionary rates within a single population are over 0.02 haldanes (2800 darwins), a value similar in magnitude to the synchronic estimates from the extremes of the cline. This paper represents an expanded analysis of data partially presented in Huey et al. (2000).

Introduction

Drosophila, like many other insects, exhibit predictable patterns of genetically based latitudinal variation in body size. Specifically, high-latitude populations have larger wing and body sizes than do low-latitude populations, even when populations are grown in a laboratory common garden. Regular, graded changes in organismal traits with geography are termed clines (Huxley, 1939; Haldane, 1948; Endler, 1977). Although clines can result from historical accident (Krimbas & Powell, 1992), the consistently positive correlations of size with latitude among many independently evolving species of *Drosophila* strongly suggests that clines are adaptive responses to geographic variation in natural selection (Endler, 1977;

Partridge & French, 1996). In *Drosophila melanogaster*, for example, parallel latitudinal clines in body size have been documented in North America (Coyne & Beecham, 1987; Capy Pla & David, 1993), western Europe and Africa (Capy Pla & David, 1993), eastern Europe and Asia (Imasheva, Bublil & Lazebny, 1994), South America (van't Land et al., 1995), and Australia (James, Azevedo, Partridge, 1995).

Although size clines probably result from geographic variation in natural selection, the dynamics of cline formation is virtually unknown. Specifically, how rapidly do latitudinal clines form? Can clines form in year, decades, or centuries? Are clinal patterns within a species similar across continents, reflecting similar selective regimes imposed by the physical environment, or does contingency dominate, reflecting

either differences in selection imposed by differences in the biotic community or differences in the genetic constitution of the founding populations. One way to study the dynamics of cline formation is to monitor the time course of evolution in recently introduced species that have colonized broad geographic areas (Johnston & Selander, 1964; Baker & Stebbins, 1965; Parsons, 1983): clinal evolution would be demonstrably rapid if the introduced populations soon evolved clines that are converging on those in the ancestral populations (Prevosti et al., 1988; Ayala, Serra & Prevosti, 1989; Brncic, 1994; Pegueroles et al., 1995). Very rapid and dramatic evolutionary responses to environmental perturbation are now well documented in studies of single populations (reviewed in Hendry & Kinnison, 1999; Kinnison & Hendry, 2001). Although such studies provide dramatic testimony to the efficacy of natural selection, they may tell us little about the long-term patterns of evolutionary change within populations or especially the geographic scale patterns among populations.

The fruit fly, *Drosophila subobscura*, provides a unique 'experiment in nature' that allows us to explore the dynamics of clinal evolution on a continent-wide scale. Historically, *D. subobscura* is a Palearctic species that is native over a broad geographic area from North Africa into southern Scandinavia; and it shows a pronounced latitudinal cline in wing size among ancestral populations (Prevosti, 1955; Misra & Reeve, 1964; Pfriem, 1983). It was accidentally introduced into both North and South America just over two decades ago. In February 1978, the species was discovered in Puerto Montt, Chile (Brncic et al., 1981). It spread rapidly and, by 1981, occupied a latitudinal range from La Serena (29° 55' S) to Coyhaique (45° 55' S). The flies were first discovered in North America in 1982 at Port Townsend, Washington (Beckenbach & Prevosti, 1986). Collections made in 1983 recovered the species from Davis, CA (38° 33' N) to Lillooet, BC (49° 15' N). Currently, the North American latitudinal range is from Ojai, CA (38° 29' N) to Port Hardy, BC (50° 42' N), with northern populations extending eastward across the Cascades into British Columbia (Noor, 1998; Huey et al., 2000). It is also expanding to the east and was recently found in the foothills of the Rockies near Provo, Utah in 1998 (Noor et al., 1998).

The first studies of possible rapid evolution of clines in the introduced populations focused on chromosomal inversions. Old World *D. subobscura* have a wealth (over 80) of inversions covering over 50%

of the genome (Krimbas & Loukas, 1980; Krimbas & Powell, 1992), and many exhibit north–south clinal variation in frequencies in Europe (Menozzi & Krimbas, 1992) that is correlated with climatic factors. The North and South American populations undoubtedly represent two replicate propagules from a single population (presumably via a ship transporting fruit): both share the same subset of 19 chromosome inversions, including a rare lethal (reviewed in Ayala, Serra & Prevosti, 1989; Prevosti et al., 1989). In collections from seven sites in Chile in 1981, only 3 years after the introduction, clines in inversion frequency had already begun to form (Prevosti et al., 1990). By 1986, 12 of the 19 inversions in both North and South America had formed frequency clines parallel to those in the ancestral European populations (Ayala, Serra & Prevosti, 1989; Prevosti et al., 1990). The repeated evolution of similar chromosome inversion clines on three continents suggests that similar patterns of natural selection are involved, not historical accident.

Although European populations show strong clinal variation in wing size, (Prevosti, 1955; Misra & Reeve, 1964; Pfriem, 1983), collections from North and South America about one decade after the introductions found no evidence of a latitudinal cline in wing size on either colonizing continent (Budnik, Cifuentes & Brncic, 1991; 1995). However, two decades after the introduction in North America (1997), a cline in wing size had evolved in North America (Huey et al., 2000). The selective mechanism favoring large size at higher latitudes for this species is unknown, however most general hypotheses focus on adaptive responses to temperature (Partridge & French, 1996).

In this paper, we examine the evolutionary dynamics of wing size clines in North American *Drosophila subobscura*. We compare latitudinal patterns of wing size and shape for introduced (North American) and for ancestral (European) populations. In addition, we compare the evolutionary dynamics of selection on wing size between six North American sites collected in both 1986 and 1997; these collections record a time series of evolution in action. Finally, we compute rates of divergence between populations at the ends of the cline and compare these with the longitudinal samples (a comparison of synchronic vs. allochronic designs, per Hendry & Kinnison, 1999). This paper is an extension of an initial report (Huey et al., 2000) that examined rapid evolution in wing length of these flies.

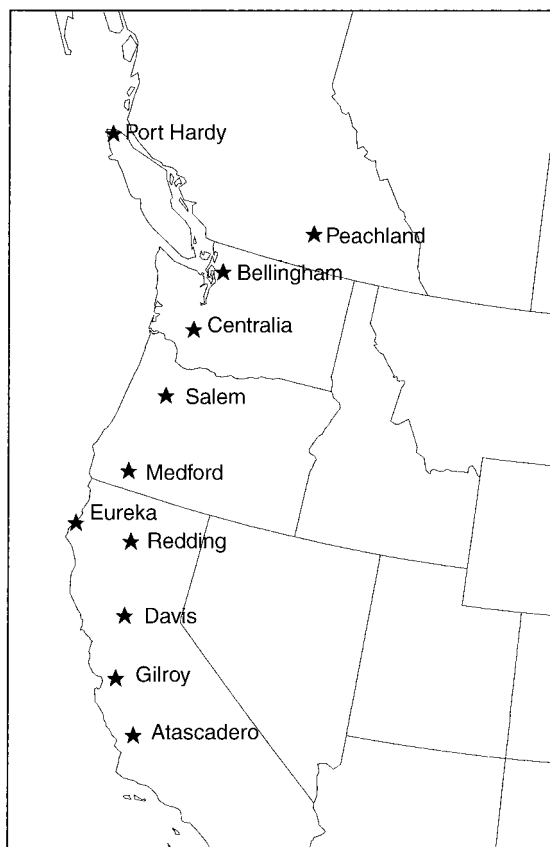


Figure 1. The North American sites collected in 1998.

Materials and methods

Drosophila subobscura were collected from 11 sites in North America (Figure 1) in April 1997 and from 10 sites in Europe in April 1998 (Table 1). Between 15 and 25 isofemale lines were established from each population and maintained on molasses-cornmeal medium in a Percival incubator at 20°C, 14L:10D for two generations. Ten males and 10 females were then collected from each isofemale line and combined in a sleeved population cage (25 cm × 14 cm × 12 cm). These stocks were mass cultured for five to six generations to ensure that any observed phenotypic differences among the lines reflected genetic differences (see Huey et al., 2000).

To obtain flies for the size measurements, we collected eggs and set up four vials per population (50 eggs per vial) and reared the flies to adulthood. Shortly after eclosion, adults were collected and the wings were mounted (approximately 20 individuals of each sex per population). Following Robertson and Reeve (1952) and Pegueroles et al. (1995), we measured

wing length in two segments along vein IV (Figure 2), $L1$ (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 (Figure 2). Wing dimensions are often used as an index of body size in *Drosophila* as it is typically correlated with other body dimensions (Robertson & Reeve, 1952; Misra & Reeve, 1964; Anderson, 1966; Sokoloff, 1966). All measurements were performed on the left wings using NIH Image on an Apple Macintosh video image analysis system.

Statistical analysis

Principal components analysis was performed on the size measurements to produce a set of orthogonal variables describing wing size and shape; we pooled all populations on both continents for this analysis. Each of the three wing dimensions was also analyzed separately. Hypotheses regarding the comparison between the North American and European populations were tested through a combination of regression analyses, tests for homogeneity of slope, and analyses of covariance.

We compared the rates of change in the six NA populations sampled in both 1986/1988 (Pegueroles et al., 1995) and in 1997 (Huey et al., 2000). In the Pegueroles et al. collections, Arlington (48.1°N), Centralia (46.7°N), Woodburn (45.2°N) and Medford (42.3°N) were sampled in 1986; and Eureka (40.8°N) and Davis (38.6°N) were sampled in 1988. These same sites were sampled in 1997, with the exceptions that we collected flies from Bellingham (48.7°N) and Salem (44.9°N) rather than Arlington and Woodburn, respectively. Flies in the two decadal collections were reared in different labs under different culture conditions, such that the mean size of the 1986/1988 flies was smaller than the mean size in 1997. We felt the most conservative approach would be to assume that the difference in continent-wide mean size between the two collections was due to rearing conditions, not to evolution. We removed this effect by standardizing the data in each year to the grand mean and standard deviation of all populations in all years. We computed the z -scores for each wing dimension ($L1$, $L2$, and W) separately for the 1986/1988 samples and the 1997 samples (pooling over sex and population).

Table 1. Locations, latitudes and wing dimensions of *Drosophila subobscura* collections

Location	Abr.	Lat.	N	Females (mean \pm SE)			Males (mean \pm SE)		
				L1 (mm)	L2 (mm)	W (mm)	L1 (mm)	L2 (mm)	W (mm)
Århus, DK	AA	56.1	20	1.37 \pm 0.013	1.17 \pm 0.010	1.13 \pm 0.012	1.24 \pm 0.014	1.08 \pm 0.010	1.05 \pm 0.008
Leiden, NL	LN	52.1	20	1.38 \pm 0.013	1.13 \pm 0.012	1.13 \pm 0.009	1.21 \pm 0.010	1.06 \pm 0.008	1.03 \pm 0.008
Lille, FR	LI	50.4	20	1.35 \pm 0.010	1.17 \pm 0.009	1.12 \pm 0.005	1.23 \pm 0.014	1.09 \pm 0.009	1.04 \pm 0.009
Gif-sur-Yvette, FR	GF	48.4	20	1.39 \pm 0.014	1.18 \pm 0.011	1.12 \pm 0.011	1.24 \pm 0.009	1.11 \pm 0.006	1.04 \pm 0.009
Dijon, FR	DI	47.2	20	1.37 \pm 0.011	1.16 \pm 0.006	1.14 \pm 0.007	1.19 \pm 0.014	1.07 \pm 0.012	1.02 \pm 0.011
Lyon, FR	LY	45.3	20	1.32 \pm 0.014	1.14 \pm 0.011	1.09 \pm 0.010	1.20 \pm 0.010	1.04 \pm 0.004	1.00 \pm 0.007
Montpellier, FR	MO	43.4	20	1.38 \pm 0.011	1.15 \pm 0.008	1.12 \pm 0.006	1.22 \pm 0.008	1.05 \pm 0.008	1.00 \pm 0.007
Barcelona, SP	BA	41.2	45	1.34 \pm 0.005	1.16 \pm 0.005	1.10 \pm 0.004	1.19 \pm 0.009	1.07 \pm 0.007	1.00 \pm 0.006
Valencia, SP	VA	39.3	20	1.28 \pm 0.019	1.16 \pm 0.007	1.09 \pm 0.011	1.16 \pm 0.009	1.08 \pm 0.006	1.00 \pm 0.007
Malaga, SP	MA	36.4	20	1.33 \pm 0.018	1.15 \pm 0.013	1.11 \pm 0.012	1.16 \pm 0.014	1.04 \pm 0.012	0.98 \pm 0.009
Port Hardy, BC	PH	50.7	18	1.37 \pm 0.011	1.17 \pm 0.010	1.13 \pm 0.007	1.21 \pm 0.011	1.05 \pm 0.007	1.03 \pm 0.007
Peachland, BC	PL	49.8	17	1.36 \pm 0.013	1.16 \pm 0.010	1.12 \pm 0.007	1.21 \pm 0.009	1.07 \pm 0.006	1.03 \pm 0.009
Bellingham, WA	BE	48.7	16	1.38 \pm 0.006	1.15 \pm 0.007	1.12 \pm 0.006	1.21 \pm 0.012	1.02 \pm 0.011	1.02 \pm 0.008
Centralia, WA	CE	46.7	16	1.35 \pm 0.011	1.15 \pm 0.010	1.12 \pm 0.009	1.21 \pm 0.008	1.06 \pm 0.008	1.03 \pm 0.008
Salem, OR	SA	44.9	12	1.37 \pm 0.015	1.12 \pm 0.014	1.11 \pm 0.010	1.23 \pm 0.010	1.02 \pm 0.012	1.02 \pm 0.007
Medford, OR	ME	42.3	16	1.33 \pm 0.011	1.12 \pm 0.009	1.11 \pm 0.009	1.23 \pm 0.011	1.02 \pm 0.007	1.03 \pm 0.008
Eureka, CA	EU	40.8	17	1.34 \pm 0.012	1.12 \pm 0.007	1.09 \pm 0.008	1.22 \pm 0.007	1.05 \pm 0.007	1.02 \pm 0.006
Redding, CA	RE	40.6	17	1.36 \pm 0.011	1.12 \pm 0.009	1.09 \pm 0.008	1.22 \pm 0.010	1.03 \pm 0.007	1.00 \pm 0.007
Davis, CA	DA	38.6	20	1.36 \pm 0.009	1.11 \pm 0.008	1.10 \pm 0.006	1.22 \pm 0.011	1.03 \pm 0.005	1.01 \pm 0.006
Gilroy, CA	GI	37.0	17	1.34 \pm 0.011	1.11 \pm 0.009	1.11 \pm 0.007	1.22 \pm 0.008	1.03 \pm 0.006	1.01 \pm 0.007
Atascadero, CA	AT	35.5	18	1.34 \pm 0.008	1.12 \pm 0.006	1.09 \pm 0.007	1.20 \pm 0.010	1.02 \pm 0.007	0.99 \pm 0.007

European flies were collected in spring, 1998; North American flies (see Figure 1 for map of locations) were collected in spring, 1997. North American sites shown in bold italics were collected in both 1986/1988 and 1997.

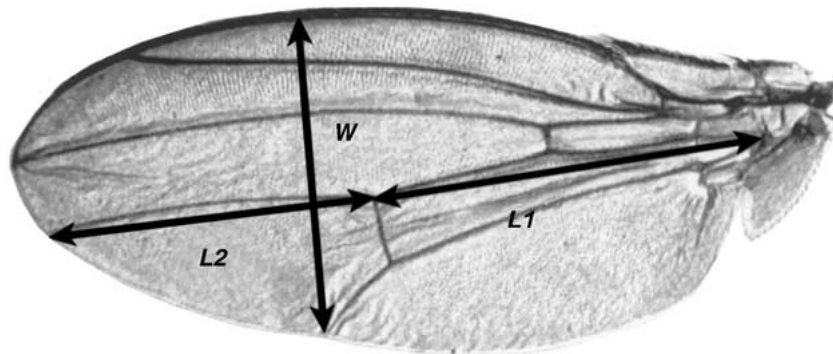


Figure 2. Wing dimensions measured. We measured the proximal (*L1*) and distal (*L2*) segments of vein IV, and the width (*W*) from the intersection of vein V with the trailing edge to the leading edge along a trajectory perpendicular to vein III.

We then back transformed to millimeters by multiplying the *z*-scores by the grand standard deviation and adding the grand mean (Note: this back transformation is necessary to compute evolutionary rates in darwins). 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 trans-

formed data (Hendry & Kinnison, 1999). Males and females were analyzed separately. All statistical procedures were performed using S-Plus 2000 (MathSoft; Seattle, WA).

Results

The mean and standard errors of each wing measure for the 1997 North American and 1998 European

Table 2. Principal components analysis of 1997 North American and 1998 European samples of *Drosophila subobscura*

	PC1	PC2	PC3
Standard deviation	0.118	0.040	0.023
Proportion of variance	0.868	0.100	0.032
Loadings			
<i>L1</i>	0.732	-0.562	-0.385
<i>L2</i>	0.461	0.825	-0.327
<i>W</i>	0.501		0.863

data sets are presented in Table 1. The principal components analysis for these samples is summarized in Table 2. Note that the first principal component, which describes overall size, accounts for 87% of the variance. The second principal component (which accounts for 10% of the variance) defines an inverse relationship between *L1*, the proximal portion of wing vein 4, and *L2*, the distal portion. The third principal component defines an inverse relationship between wing length and width; large negative values describe relatively long, narrow wings whereas large positive values describe relatively short, wide wings.

The clinal patterns of variation in size (*PCI*) for the 1997/1998 samples are shown in Figure 3. Ancestral (European) females and males both show a marked and linear increase in overall wing size with latitude (regression slopes: females: 0.0040 ± 0.0008 , $R^2 = 0.094$, $p < 0.001$; males: 0.0053 ± 0.0008 , $R^2 = 0.160$, $p < 0.001$) with no significant difference in slopes between the sexes (comparison of slopes: $F_{1,446} = 1.168$, $p = 0.28$). Introduced (North American) females and males also show a linear increase in wing size with latitude (regression slopes: females: 0.0038 ± 0.0007 , $R^2 = 0.125$, $p < 0.001$, males: 0.0016 ± 0.0007 , $R^2 = 0.025$, $p = 0.027$). North American female wing size, however, has a much steeper cline than that for males (comparison of slopes: $F_{1,376} = 4.65$, $p = 0.032$). Slopes of the clines for females don't differ significantly between North America and Europe (comparison of slopes: $F_{1,405} = 0.047$, $p = 0.83$). In contrast, the cline for North American males is decidedly shallower than that for European males (comparison of slopes: $F_{1,417} = 11.03$, $p < 0.001$). Similar patterns were observed when wing length (summing *L1* and *L2*), rather than *PCI*, was analyzed (Huey et al., 2000).

Because the second and third principle components explain relatively little of the clinal pattern of

variance, we will present the clinal patterns in the individual wing dimensions *L1*, *L2*, and *W* to assess changes in wing shape between the continents. European males and females show similar clinal patterns in the proximal portion of the wing (Figure 4, *L1*) (regression slopes: females: 0.0036 ± 0.0007 , $R^2 = 0.095$, $p < 0.001$, males: 0.004 ± 0.0006 , $R^2 = 0.150$, $p < 0.001$), however there is almost no clinal pattern in North America (regression slopes: females: 0.0015 ± 0.0006 , $R^2 = 0.030$, $p < 0.05$, males: -0.0002 ± 0.0006 , $R^2 = 0.0009$, $p = 0.684$). In contrast, the distal portion of the wing shows marked clinal variation in North American females (Figure 4, *L2*; regression slope: 0.0036 ± 0.0005 , $R^2 = 0.198$, $p < 0.001$). North American males (0.0019 ± 0.0005 , $R^2 = 0.071$, $p < 0.001$), European males (0.0014 ± 0.0005 , $R^2 = 0.030$, $p < 0.01$) and European females (0.0007 ± 0.0005 , $R^2 = 0.009$, $p = 0.150$) all exhibit much lower slopes that, by Tukey's *a posteriori* comparisons, do not differ significantly (Figure 4, *L2*). Finally, wing width (*W*) exhibits parallel clinal variation (Figure 4, *W*; by Tukey's *a posteriori* comparisons) in European females (0.0021 ± 0.0005 , $R^2 = 0.079$, $p < 0.001$ and in both sexes in North America (females: 0.0020 ± 0.0005 , $R^2 = 0.10$, $p < 0.001$; males: 0.0017 ± 0.0004 , $R^2 = 0.074$, $p < 0.001$). The wing width cline for European males is significantly steeper (0.0034 ± 0.0005 , $R^2 = 0.204$, $p < 0.001$). Thus, in Europe, most of the clinal variation occurs in *L1*, the proximal portion of the wing; whereas in North American females, most of the clinal variation occurs in *L2*, the distal portion (Figure 4). North American males have significant, but much shallower, patterns of clinal variation than European males.

The magnitude and direction of changes in each of the three wing measurements for each locality sampled in 1986/1988 and in 1997 sample are shown in Figure 5 (Hendry & Kinnison, 1999 describe this as the allochronic method). The panels on the left show the change in size for each sex in each population; the panels on the right show the rate of change, measured in haldanes, for each population. Evolutionary rates in darwins are presented in Table 3. In general, rates of evolution are highly and positively correlated between the sexes despite the relative lack of clinal variation in males (*L1*: $r = 0.92$, $p < 0.01$; *L2*: $r = 0.79$, $p < 0.05$; *W*: $r = 0.77$, $p < 0.05$). For females, Bellingham (Arlington in Pegueroles et al., 1995) and Medford showed significant evolutionary rates between 1986/1988 and 1997 for all three traits,

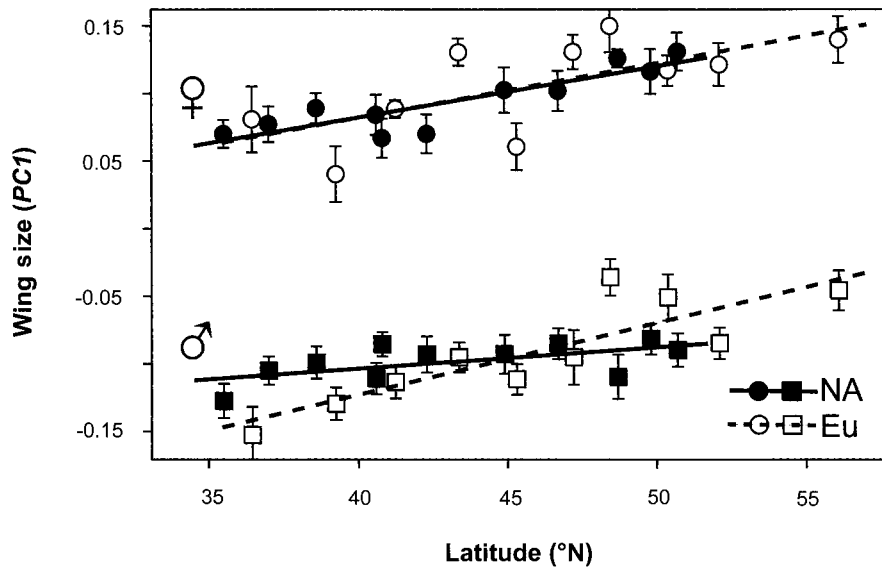


Figure 3. Clinal variation in overall wing size (*PC1*). In less than 20 years, a wing size cline for North American females has evolved that is statistically indistinguishable from that in Europe (upper lines). Males, however, are different (lower lines). Whereas in Europe, the slope for males and females is similar, in North America the slope of the male cline is very shallow. The error bars indicate ± 1 standard error.

whereas Medford was the only consistently significant site for males (Figure 5, Table 3). Note that the average wing size for Medford in the 1986 sample is exceptionally large; in the original analysis of these data, Pegueroles et al. (1995) remarked that the Medford population was a statistical outlier. Deleting the Medford measurements had no effect on the conclusions for the analyses conducted in this paper, so we elected to include the data. For comparison, we present the synchronic evolutionary rates of divergence in Table 4 (computed from bootstrapped estimates of the fitted values of the latitudinal extreme populations, assuming 20 years since the introduction and five generations per year).

Discussion

Clinal variation in wing size

Wing dimensions in North America evolve rapidly, forming a cline similar to that observed in the Old World. Although no clinal pattern was present in samples from 1986/1988 (Budnik, Cifuentes, Brncic, 1991; Pegueroles et al., 1995), a significant cline in wing size (represented by the first principal component) is present in 1997 that, for females, is statistically indistinguishable from the latitudinal size cline in Europe (Figure 3; see also Huey et al., 2000).

Two lines of evidence suggest that size clines, like the one that has evolved within the last decade in North America, represent an adaptation due to selection imposed by temperature. First, similar latitudinal clines in wing size are found in many species of *Drosophila* (*D. robusta*: Stalker & Carson, 1947; *D. simulans*: David & Bocquet, 1975; *D. obscura*: Pegueroles et al., 1995) and in multiple populations within *Drosophila melanogaster* (North America: Coyne & Beecham, 1987; Capy, Pla & David, 1993, western Europe and Africa: Capy, Pla & David, 1993; Gilchrist et al., 2000, eastern Europe and Asia: Imasheva Bubli & Lazebny, 1994, South America: van't Land et al., 1995; Gilchrist et al., 2000, and Australia: James, Azevedo & Partridge, 1995, but note Long & Singh, 1995 for an exception). Many other species of ectotherms exhibit a similar increase in body size with latitude (copepods: Lonsdale & Levinton, 1985; houseflies: Bryant, 1977; wood frogs: Riha & Berven, 1991). Similar clines are also associated with changes in altitude (Stalker & Carson, 1948; Berven, 1982) and body size tracks seasonal changes in temperature (Stalker & Carson, 1949; Tantawy, 1964; Kari & Huey, 2000).

Second, long term studies of laboratory evolution at constant temperatures consistently produces genetically larger individuals at low temperatures and genetically smaller individuals at high temperature (*D. melanogaster*: Cavicchi, Giorgi & Mochi, 1978;

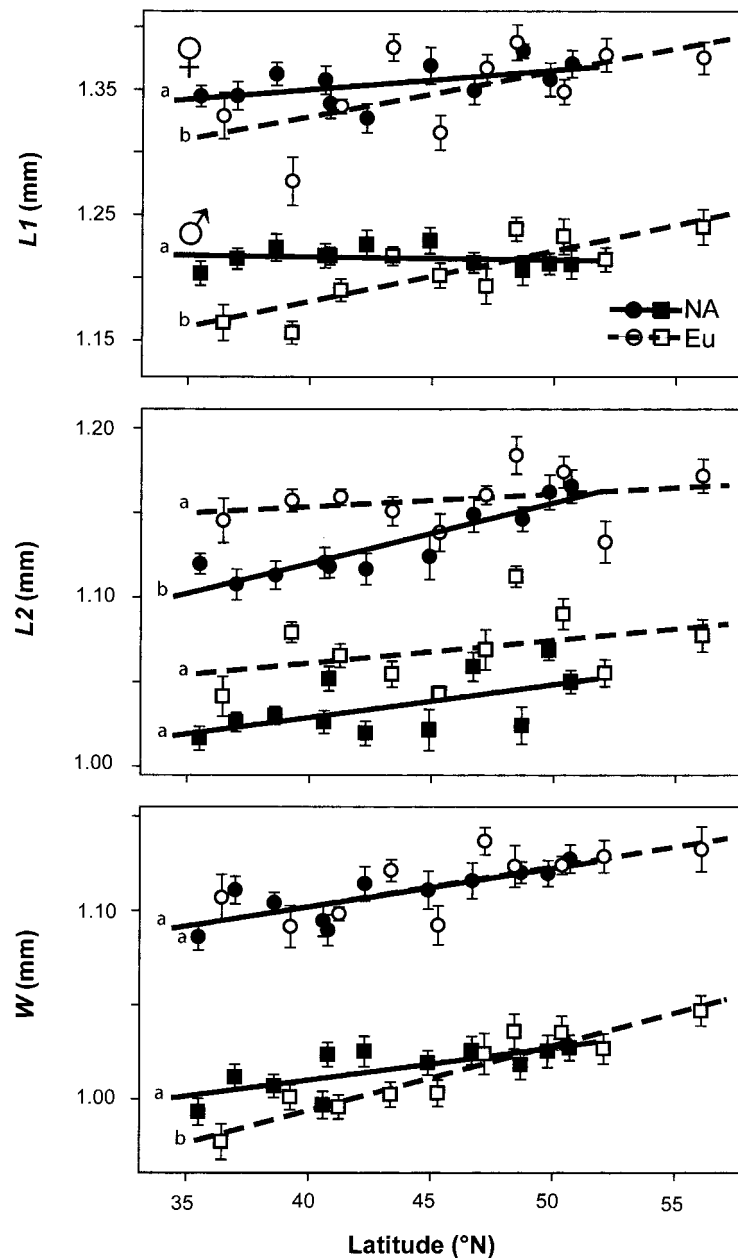


Figure 4. Clinal variation in different portions of the wing. Slopes were compared with Tukey *a posteriori* contrasts; lines having a statistically similar slope are indicated by a shared letter. Different portions of the wing in North American and Europe achieve the cline in wing size. In Europe, the cline is largely in the proximal portion of the wing ($L1$), whereas in North American females, the cline is largely in the distal portion of the wing ($L2$). European males have a steeper cline in wing width. The error bars indicate ± 1 standard error.

Cavicchi et al., 1985; Huey, Partridge & Fowler, 1991; Partridge et al., 1994; James & Partridge 1995; *D. pseudoobscura*: (Anderson, 1966, 1973). These experiments suggest that the observed latitudinal clines in wing size may reflect selection for larger body size via the cooler mean temperatures associated with higher latitudes. The adaptive significance of this pat-

tern, however, remains obscure (Partridge & French, 1996; van der Have & de Jong, 1996).

Males versus females

In contrast to the rapid parallel evolution of clinal variation in wing size in North American females,

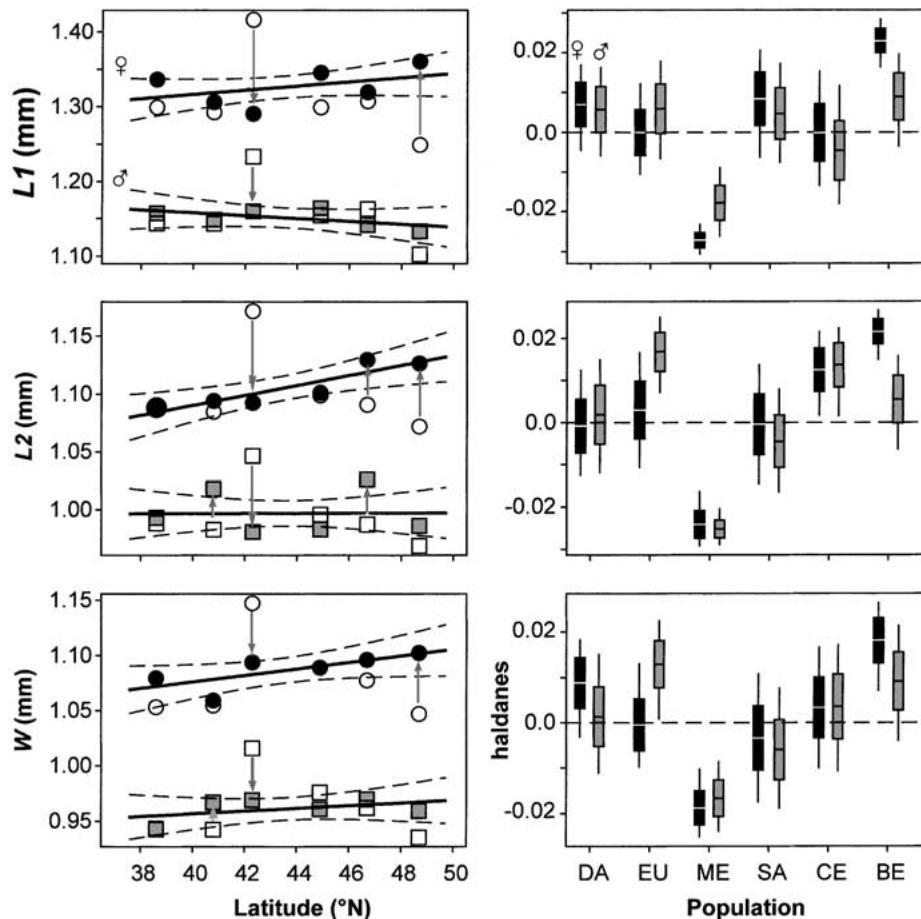


Figure 5. Allochronic rates of evolution. The panels on the left show the change in $L1$, $L2$, and W from 1986 or 1988 (open symbols) to 1997 (shaded symbols); those on the right show the allochronic estimate of evolution in haldanes. The regression and its 95% confidence limits are shown on the left for the 1997 samples. The arrows indicate the direction of significant evolutionary change as determined by bootstrap analyses. On the right are the bootstrapped means (center bar), standard deviations (box), and 95% confidence intervals of the allochronic estimates of evolutionary rates for males and females in each population.

the colonizing males are definitely lagging behind their European counterparts (Figure 3; Huey et al., 2000). *Drosophila* are sexually dimorphic, with males roughly 10% smaller than females in linear wing and body traits. In spite of the difference in intercepts of PCI (Figure 3), the slope of the cline in European *D. subobscura* males is statistically indistinguishable from that of females. North American males, however, exhibit a much weaker cline with a substantially shallower slope (Figure 3). Parallel clines for male and female body and wing dimensions are common in other drosophilids. In *Drosophila melanogaster*, the slopes of wing area versus latitude are the same for males and females in South America (Zwaan et al., 2000) and Australia (James, Azevedo & Partridge, 1995). Extensive measurements of *D. melanogaster* wing shape

in clines on three continents revealed highly conserved patterns of gender-related shape differences in spite of substantial variation among continents in the wing shape (Gilchrist et al., 2000). Misra and Reeve (1964) found no sex by latitude interaction for five morphological traits including wing length and width in European *D. subobscura*.

This parallelism across the sexes is expected as male and female body dimensions are highly genetically correlated. Cowley (1986) found that most wing characters in *Drosophila melanogaster* have a genetic correlation greater than 0.90. Reeve and Fairbairn (1996) found genetic correlations greater than 0.90 between male and female thorax width. Intersexual genetic correlations for body and wing size in *D. subobscura* have not been measured, however Pfriem

Table 3. Evolutionary rates between 1986/1988 and 1997 in darwins for three wing measures in North American flies

Loc.	Lat.	Sex	<i>L1</i>	<i>L2</i>	<i>W</i>
BE	48.7	F	2815 ± 625.9 (1562, 4061)	1550 ± 327.9 (915, 2150)	1585 ± 427.3 (802, 2414)
		M	818 ± 658.5 (-431, 2114)	541 ± 442.4 (-308, 1464)	734 ± 507.5 (-295, 1636)
CE	46.7	F	270 ± 722.5 (-1154, 1596)	1073 ± 484.7 (88, 2043)	508 ± 679.2 (-832, 1871)
		M	-584 ± 663.7 (-1918, 734)	1135 ± 490 (181, 2098)	249 ± 609 (-875, 1452)
SA	44.9	F	1189 ± 637.1 (-97, 2430)	47 ± 567.6 (-1173, 1089)	28 ± 526 (-957, 1075)
		M	250 ± 584.7 (-881, 1368)	-334 ± 609.6 (-1464, 850)	-441 ± 481.1 (-1354, 546)
ME	42.3	F	-3166 ± 470.8 (-4082, -2300)	-2226 ± 418.8 (-3035, -1399)	-1476 ± 471.8 (-2376, -506)
		M	-1924 ± 481.4 (-2891, -1029)	-1898 ± 307.7 (-2506, -1305)	-1377 ± 363.4 (-2107, -665)
EU	40.8	F	356 ± 414.5 (-491, 1095)	258 ± 369.7 (-482, 957)	156 ± 361.3 (-589, 841)
		M	142 ± 310.1 (-442, 753)	1027 ± 301.3 (469, 1626)	718 ± 318.8 (88, 1337)
DA	38.6	F	945 ± 476.6 (9, 1885)	39 ± 423.9 (-809, 892)	739 ± 349.1 (43, 1407)
		M	335 ± 509.9 (-685, 1363)	131 ± 339.8 (-530, 803)	18 ± 397.3 (-763, 785)

For each sex, the bootstrapped mean ± standard deviation are shown on the first line; below in parentheses are the lower and upper 95% confidence intervals. Values significantly different from zero are shown in boldface.

Table 4. Rates of evolutionary divergence (synchronic) estimated by bootstrapping the entire North American data set and computed divergence in haldanes and darwins from the fitted values of the extreme populations (Port Hardy and Atascadero)

	Haldanes			Darwins		
	<i>L1</i>	<i>L2</i>	<i>W</i>	<i>L1</i>	<i>L2</i>	<i>W</i>
Females	0.0047 ± 0.0023 (0.0005, 0.0092)	0.0138 ± 0.0022 (0.0099, 0.0184)	0.0086 ± 0.0016 (0.0055, 0.0115)	729 ± 372.1 (69.7, 1477.5)	2408 ± 395.4 (1608.4, 3190.5)	1413 ± 311.4 (822.5, 1997.1)
Males	0.0018 ± 0.0013 (0.0001, 0.0050)	0.0101 ± 0.0017 (0.0067, 0.0134)	0.0082 ± 0.0017 (0.0048, 0.0116)	325 ± 242.8 (16.3, 906.9)	1627 ± 310.2 (1025.1, 2239.0)	1363 ± 344.1 (720.3, 2034.5)

The mean ± standard error is shown, with the lower and upper 95% confidence intervals given below in parentheses. We assume that 20 years has elapsed since the introduction, with five generations per year for computing haldanes.

(1983) found a parallel increase in size when males and females were heterozygous (rather than homozygous) for inversions on the O chromosome. Pfriem also demonstrated that this pattern was similar across latitudes in Europe.

The sex difference in clinal pattern in North America is surprising, given the probable high intersexual genetic correlation. If females are under strong dir-

ectional selection for larger size at higher latitudes, then one would expect a correlated response to selection in the males (Fisher, 1958; Lande, 1980). Artificial selection studies on sexual dimorphism in *Drosophila* confirm that selection on one sex elicits a parallel change in the unselected sex. (Frankham, 1968; Alicchio & Palenzona, 1971; Reeve & Fairbairn, 1996).

We propose two possible mechanisms that could account for the slow rate of clinal evolution in the invading males. First, the colonizing populations may have passed through a genetic bottleneck, resulting in a change in the intersexual genetic correlation matrix. The founding populations in North and South America carried a sample of 19 out of approximately 80 chromosomal arrangements found in the Old World. Prevosti et al. (1989) estimate a minimum number of colonizers at 10–15 individuals. Allozyme surveys (Prevosti et al., 1989) show a deficiency of rare alleles in the New World, compared to the Old World, indicating a mild founder effect. On the other hand, the similarities of the gene pools in North and South America, coupled with the known rapid expansion of the populations shortly after their introduction suggests that strong founder effects are unlikely.

A second scenario is a change in the selective environment to one favoring small male wing size across all latitudes, perhaps mediated by female mate choice. Old World *D. subobscura* females prefer mates from their own geographic populations, with the degree of preference negatively correlated with distance separating the populations (Constanti et al., 1986). Because the original colonists probably came from the Mediterranean region, New World females would prefer southern European males, which have a smaller wing size. If North American females prefer some aspect of courtship performed by small winged males, then stabilizing sexual selection could potentially maintain uniform male wing size from all latitudes across the cline, assuming the genes controlling wing size and mating preferences are not tightly linked. This hypothesis predicts that similar patterns should be seen in the South American populations currently being analyzed.

Contingency in wing evolution

Although the overall size patterns are similar between the ancestral and introduced populations, at least for females, contingency plays a role in determining which part of the wing varies with latitude (see also Figure 2(B) in Huey et al., 2000). In Europe, most of the clinal variation lies in the proximal portion of the wing (Figure 4, L1), whereas in North America females, the distal portion (Figure 4, L2) forms most of the cline. It is extremely unlikely that this difference, which corresponds to a small change in the position of the posterior cross vein, has any adaptive significance. The posterior and distal portion of the wing may

be under fewer functional constraints than the basal and anterior regions (Dickinson, Lehmann & Sane, 1999) and would therefore be more likely to evolve through genetic drift. Gilchrist et al. (2000) found considerable variation across continents in the position of the posterior cross vein in *Drosophila melanogaster*, consistent with the hypothesis of relaxed functional constraints on this character.

Wing width

Wing width also increases in size at higher latitudes on both continents, with European males showing a significantly steeper cline than other populations. Stalker (1980) found that *D. melanogaster* flying at lower ambient temperatures had relatively larger wings for a given body weight. Because of physiological constraints, wing beat frequencies of ectotherms are lower at cool temperatures (Reed, Williams & Chadwick, 1942). Stalker argued that larger wings allowed the generation of relatively greater lift at low wing beat frequencies, hence would be selectively advantageous under cool ambient conditions. What is not clear is why there is a difference between the sexes. Gilchrist et al. (2000) observed that male wings were relatively wider and shorter than female wings across continents in *D. melanogaster* clines, however they did not remark on changes in relative width within the clines. Perhaps some aspect of courtship by *Drosophila* males favors short winged individuals; increasing width would be a means of meeting the functional requirement of larger wings at high latitudes.

Rates of evolution

The recent papers by Hendry and Kinnison (1999, 2001) reviewing rates of evolution provides ample evidence that evolutionary rates within populations are often very rapid. Kinnison and Hendry point out that two methods have been used to estimate rates. The allochronic method requires a time series of measurements within a population, an approach exemplified by Peter and Rosemary Grant's classic study of body and beak sizes in Darwin's finches in the Galapagos islands over the last 25 years (Grant & Grant, 1995) and Reznick and colleagues' (1990, 1997) experimental introductions of guppies to different stream habitats in Trinidad. Synchronic methods measure divergence between two or more populations sharing a common ancestry; Carroll's study of divergence in beak length

of soapberry bugs (Carroll, Dingle & Klassen, 1997) exemplifies this approach.

The *Drosophila subobscura* introduction presents a unique opportunity for comparing these two methods of estimating evolutionary rates. Because several sites were sampled in both 1986/1988 and 1997, and because we know the approximate date of introduction, we can apply both synchronic and allochronic methods to the three measured wing traits in the invading North American populations (Figure 5, Tables 3 and 4). In Figure 5 (allochronic method, measured in haldanes), most of the evolution has apparently occurred at higher latitudes, with increases in all measures of wing size, especially at Bellingham. Males generally show a slower rate of evolution than females, as would be expected given the relatively weak pattern of clinal variation. The major exception to these trends is Medford, where both males and females sampled in 1986 were unusually large in all dimensions. In the original analysis of these data, Pegueroles et al. (1995) noted that Medford was an outlier. Because omitting Medford from the data analyses made no significant difference in our results at other sites, we have elected to include the data.

The pattern of more evolution in the north, shown in Figure 5 and Table 3, is consistent with the hypothesized Mediterranean origin of the colonists. The founders of the North American population should have had relatively small wings; selection would thus favor an increase in size in the high latitude populations. Note that these analyses are conservative; the 1986/1988 flies were much smaller than the 1997 flies. We attribute this discrepancy to differences in rearing conditions and transformed the data in both samples to the grand mean and standard deviation (see Methods and materials for details). By the argument above, however, it is likely that the 1986/1988 samples were, on average, genetically smaller than the 1997 flies. Although rearing at higher densities in the 1986/1988 studies may have obscured clinal variation, we find that clinal patterns in more recent collections are detectable at rearing densities comparable to those used in the earlier study (Gilchrist & Huey, unpublished data).

The mean values for allochronic evolutionary change in the most rapidly evolving populations are within a factor of two to the estimates obtained by the synchronic method (Table 4). Thus, at least in this system, synchronic estimates of evolutionary rates that average out factors such as local reversals and stasis are similar in magnitude to allochronic estim-

ates obtained over many (approximately 50 in this case) generations within a single population. Hendry and Kinnison (1999) point out that this might rarely be the case because diverging lineages are potentially exposed to quite different selective regimes. The similarity here is because most of the significant evolution is occurring at only the high latitudinal populations; the low latitude populations have evolved relatively little with regard to the measured traits since the 1988 collections (Figure 5, Table 3). (Note: the synchronic rates of evolution in wing length in haldanes reported in Huey et al. (2000) were in error. A correction was published in Science 2000, volume 288: 809. For wing length ($L1+L2$), the correct values were 0.011 haldanes for females and 0.004 haldanes for males. No major conclusions in that study were affected by the errors.)

The rates of evolution observed in *D. subobscura* are an order of magnitude below the fastest rates tabulated in Hendry and Kinnison's (1999) review. The fastest rates, however, represent the response of a single population to an extraordinary event such as a drought (Boag & Grant, 1981) or an encounter with a new predator (Reznick et al., 1997), which may represent transient and possibly reversible changes (Gibbs & Grant, 1987). What we see in *D. subobscura* is the evolution of a continent-wide pattern in wing size in response to the same sort of 'normal' environmental conditions that have favored clines in other species and on other continents. Rapid evolution of wing size clines may be the rule rather than the exception in *Drosophila*; the Australian and South American clines in *D. melanogaster* wing size may be less than 100-year-old (Bock & Parsons, 1981). This implies that, for whatever adaptive reason, selection for a positive latitudinal size cline in flies and other ectotherms is strong and similar on many continents. It should be noted that the *D. subobscura* cline is maintained in the face of what appears to be relatively high gene flow. The known spread of the species in South America from its point of introduction at Puerto Montt in 1978, to its occupation of over 15° of latitude by 1981 suggests that these flies are extremely vagile. No significant differences in microsatellite allele frequencies are found between populations from Seattle, WA and Mount St. Helena (northern Napa Valley), CA (Noor, Pascual & Smith, 2000), suggesting a potential for high gene flow in spite of stable and predictable clines in chromosomal arrangements (Ayala, Serra & Prevosti, 1989; Serra, unpublished data).

Conclusions

Species introductions that are followed by a rapid invasion of a broad geographic range represent an extraordinary opportunity for evolutionary biologists to study rates and direction of evolution (Baker & Stebbins, 1965; Parsons, 1983). *Drosophila subobscura*, a European fruit fly introduced almost simultaneously in North and South America, provides a unique opportunity to assess the rate and similarity of evolutionary processes in replicated populations on a broad geographic scale. We have shown in this paper and in Huey et al. (2000) that North American females have evolved a cline in wing size statistically indistinguishable from the European cline in less than two decades.

Contingency plays a role: the cline in North American males is much less steep than the cline in European males and different parts of the wing seem to have evolved on the different continents. Evolutionary rates are higher in the high latitude populations, suggesting that most of the evolution in North America has involved an increase in size from small-bodied, Mediterranean colonists. The similarity of clines within species across continents and among drosophilid species suggests that the evolution of these functionally important traits is predictable, however the particular combination of traits that combine to yield that function may be highly contingent. We are currently analyzing data from Chilean flies collected in 1999 and testing hypotheses about the role of selection on flight ability, mate choice and genetic covariances in the formation of these clines.

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