

EVOLUTIONARY PACE OF CHROMOSOMAL POLYMORPHISM IN COLONIZING POPULATIONS OF *DROSOPHILA SUBOBSCURA*: AN EVOLUTIONARY TIME SERIES

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Abstract.—Biologists have long debated the speed, uniformity, and predictability of evolutionary change. However, evaluating such patterns on a geographic scale requires time-series data on replicate sets of natural populations. *Drosophila subobscura* has proven an ideal model system for such studies. This fly is broadly distributed in the Old World, but was introduced into both North and South America just over two decades ago and then spread rapidly. Rapid, uniform, and predictable evolution would be demonstrated if the invading flies evolved latitudinal clines that progressively converged on those of the native populations. Evolutionary geneticists quickly capitalized on this opportunity to monitor evolutionary dynamics. Just a few years after the introduction, they surveyed chromosomal inversion frequencies in both North and South America. On both continents they detected incipient latitudinal clines in chromosome inversion frequencies that almost always had the same sign with latitude as in the Old World. Thus the initial evolution of chromosomal polymorphisms on a continental scale was remarkably rapid and consistent. Here we report newer samples of inversion frequencies for the colonizing populations: the time series now spans almost one decade for North America and almost two decades for South America. Almost all inversions in the New World continue to show the same sign of frequency with latitude as in the Old World. Nevertheless, inversion clines have not consistently increased in steepness over time; nor have they consistently continued to converge on the Old World baseline. However, five arrangements in South America show directional, continentwide shifts in frequency. Overall, the initial consistency of clinal evolutionary trajectories seen in the first surveys seems not to have been maintained.

Key words.—Chromosome polymorphism, clinal variation, *Drosophila subobscura*, introduced species, rate of evolution.

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Whether evolutionary trajectories are rapid or slow (Darwin 1859; Simpson 1944; Hendry and Kinnison 1999), gradual or punctuational (Simpson 1944; Gould and Eldredge 1977), and predictable or contingent (Gould 1989; Travisano et al. 1995; Losos et al. 1998) has been long and hotly debated by evolutionary biologists. The exact trajectories of trait evolution can be readily evaluated in replicated laboratory populations by monitoring phenotypes over time (Cohan 1984; Travisano et al. 1995). However, evaluating microevolutionary trajectories of replicated sets of natural populations on a geographic scale is rarely feasible. One favorable circumstance involves species that have recently invaded multiple areas: rapid, uniform, and predictable microevolution on a geographic scale would be demonstrated if the invading populations rapidly and independently evolved geographic clines that uniformly converged on baseline clines in the ancestral populations (Johnston and Selander 1964; Baker and Stebbins 1965; Parsons 1983; Endler 1986; Pimm 1988). Alternatively, a role for drift or contingency would be implicated if the invading populations evolved idiosyncratic clines (Endler 1977).

The fly *Drosophila subobscura* provides remarkable opportunities for evaluating microevolutionary trajectories (Ayala et al. 1989). These flies are native to the Old World, where they are broadly distributed from North Africa to Scandinavia (Krimbas 1993). They have five pairs of acrocentric chromosomes that are all polymorphic for inversions. The

frequencies of most chromosomal arrangements vary clinally with latitude (Prevosti 1964; Prevosti et al. 1988; Menozzi and Krimbas 1992). Moreover, time-series data for some Palearctic localities indicate recent increases in frequencies of inversions typical of low latitudes (Orengo and Prevosti 1996; Rodríguez-Trelles and Rodríguez 1998; Rodríguez-Trelles et al. 1998; Solé et al. 2002), suggesting that contemporary climate warming is influencing spatial and temporal patterns of inversion frequencies.

Drosophila subobscura were first discovered in South America (Brncic and Budnik 1980) and North America (Beckenbach and Prevosti 1986) 20 to 25 years ago. Because both the South and North American populations share the same inversions and other genetic markers, they are probably drawn from a single colonizing stock (Ayala et al. 1989; Mestres et al. 1992). Genetic data suggests the number of colonists was between 10 and 150 (Mestres et al. 1990; Pascual et al. 2001), such that the invading flies experienced a genetic bottleneck (Brncic et al. 1981; Prevosti et al. 1985; Rozas and Aguadé 1991; Balanya et al. 1994; Pascual et al. 2001). Indeed, only 23% of all inversions known in the Old World occur in New World populations (Brncic et al. 1981; Prevosti et al. 1988, 1989), though these are by far the most common inversions in the Old World (below). These flies explosively colonized broad latitudinal ranges (>15°) on both continents and became subject to climatic gradients similar to those facing the ancestral Old World populations (Brncic et al. 1981; Ayala et al. 1989; Prevosti et al. 1989). Consequently, the native Old World populations provide an evo-

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lutionary baseline (but see Discussion) for latitudinal patterns of variation in inversion frequency, and the New World populations serve as replicate natural experiments (Prevosti et al. 1985; Ayala et al. 1989; Brncic 1995).

Evolutionary geneticists immediately recognized and capitalized on this “grand experiment in evolution” (Ayala et al. 1989, p. 246). Within just a few years of the introductions, they surveyed geographic patterns of chromosome inversion frequencies in invading populations on both continents (1981 in South America, 1985–1986 in North America) and discovered astonishingly rapid evolution on a geographic scale (Brncic et al. 1981; Prevosti et al. 1985, 1988). Even in those initial surveys, chromosome inversions had already evolved latitudinal clines in frequency that remarkably were almost always in the same direction as in the native Old World populations. A second sample from South America in 1986 suggested that these clines were still evolving and were continuing to converge on the Old World baseline (Prevosti et al. 1990).

We have now made additional samples for South America (1999) and for North America (1991, 1994). By combining our new data with those from earlier surveys, we generate a replicated time series that enables us to explore the evolutionary dynamics of a genetic trait. We now explore these temporal series to determine whether inversion clines on both continents are continuing to evolve and specifically to converge on the Old World baseline. Such long-term time series are rare (especially for introduced species) but are nonetheless crucial for studying the evolutionary dynamics of traits (Anderson et al. 1991; Coyne et al. 1992; Orengo and Prevosti 1996; Rodríguez-Trelles et al. 1996, 1998; Losos et al. 1998; Bell 2001; Carroll et al. 2001; Solé et al. 2002).

MATERIALS AND METHODS

Geographic Samples

For South America, geographic data of inversion frequencies are drawn from seven populations sampled in 1981 (Prevosti et al. 1985) and again in 1986 (Prevosti et al. 1990). We now add new data from 1999 for six of these populations (Santiago, Chillán, Laja, Valdivia, Puerto Montt, and Coyhaique). These South American samples cover almost two decades and thus bracket virtually the entire colonization period.

For North America, published data are available for seven populations sampled in 1985 and 1986 (Prevosti et al. 1987). We report additional data for seven populations (Gilroy, Davis, Eureka, Medford, Salem, Centralia, and Bellingham) from 1991 and for eight populations (Gilroy, Davis, Eureka, Medford, Salem, Centralia, Bellingham, and Port Hardy) in 1994. One additional sample (Centralia in 1995) is included in Electronic Appendix A (see Electronic Appendices A and B, currently available from the *Evolution* Editorial Office at evolution@asu.edu), but was not considered in the analysis of the clines. North American data cover almost a decade, positioned in the middle of the colonization period.

To quantify baseline clines for the Old World populations, we analyzed data (courtesy of C. B. Krimbas; see Menozzi and Krimbas 1992) for 79 sites that were within the known latitudes bounded by the New World populations and that

were lower than 564 m (the maximum altitude of any New World sample site. This Old World dataset is larger than that used in previous comparisons; Prevosti et al. 1988, 1990.). Before estimating the frequency of each inversion at each site, we pooled all arrangements on a given chromosome that have never been detected in the New World samples. Thus, the European slopes include the effects of inversions not represented in the New World. However, excluding all the non-New World arrangements from the dataset does not change the conclusions that follow. The O_7 arrangement has been excluded from the analysis of the New World populations because this arrangement probably originated there via a rare recombination between O_{3+4+7} and O_{st} arrangements (see Results).

In the new as well as old samples, wild-caught males (or their progeny in isofemale lines) were mated individually to virgin females of the *chcu* strain, which is homokaryotypic for the arrangements A_{st} , J_{st} , U_{st} , E_{st} , and O_{3+4} . One third-instar larva from each cross was then examined for its polytene chromosomes to ascertain the arrangements of one set of chromosomes for each wild fly or line. The number of chromosomes sampled at each site in each collection year had a mean \pm SD of 121 ± 55 (range 18 to 301; values presented in Electronic Appendices A and B).

The symbol for each chromosome consists of a letter designating the chromosome and a subscripted number. Gene arrangements (multiple inversions on a chromosome) have multiple numbers (each separated by a plus sign) identifying the specific inversions involved in that arrangement (Krimbas 1993).

Previous studies (Prevosti et al. 1985, 1988, 1990; Menozzi and Krimbas 1992) of latitudinal clines in *D. subobscura* have concentrated on the magnitude of the correlation coefficient between frequency and latitude (or climate variables). However, here we analyze the slope of the regression of frequency on latitude, as this statistic allows a broader range of statistical comparisons. For each arrangement, we computed the slope of a regression of frequency (angular transformed, weighted by number of chromosomes sampled at each locality) on latitude. Frequencies of different arrangements on the same chromosome are not independent (Ayala et al. 1989), so we developed two permutation tests to evaluate statistical significance of latitudinal clines. Separate analyses were conducted for each sampling date in South America and in North America.

Tests for Similarity of Sign and Slope, and of Convergence

We developed a permutation test to evaluate whether signs of slopes of inversion frequency on latitude are disproportionately similar to those for the Old World populations. For each of the five chromosomes, we randomly assigned latitudes (without replacement) to the gene-arrangement frequency data to create a permuted list. We then computed the slopes of each gene arrangement on the permuted latitudes for all chromosomes and recorded the number of slopes in the permuted dataset that matched the sign of the slope for the same gene arrangement in Europe. By repeating this computation 1000 times, we generated a null distribution and

determined the probability of obtaining the observed value by chance alone for that continent and year.

Next, we developed a test to evaluate whether slopes (for each New World continent and year) of the regressions of frequency on latitude were similar in magnitude to those for the Old World flies. The procedure was similar to that described above; however, instead of counting the number of slopes of identical sign, we computed the mean squared deviations between the New World and European slopes of the gene arrangements within each chromosome and then summed those means across the five chromosomes. The null distribution of the summed mean-squared deviations was obtained from 1000 resampling runs to determine the probability of obtaining the observed value by chance.

To test whether slopes of inversion clines are converging on those of Old World populations, we ran an ordered-factor (by year) analysis of variance (Sokal and Rohlf 1995) on the squared deviation of jackknifed pseudoreplicates of the South or North American slopes from the European baseline slopes. Convergence is indicated if the linear contrast for year is significant and negative (thus indicating that the slopes are converging over time on the European slopes).

RESULTS

The frequencies of the chromosomal arrangements at each site in North and South America over time are compiled in Electronic Appendices A and B, respectively. Data for some surveys (North America in 1985 and 1986, South America 1981 and 1986) have been published previously (Prevosti et al. 1988, 1990) but are included for completeness. Data for 79 sites in Europe are not summarized (but see Menozzi and Krimbas 1992).

The colonization of the New World was associated with a major bottleneck, as revealed by data available on chromosomal polymorphism (Prevosti et al. 1988), lethal genes (Mestres et al. 1990), allozyme polymorphism (Balanya et al. 1994) and microsatellite loci (Pascual et al. 2001); the inversions present in the New World represent only a small sample of inversions known in the Old World (18 of approximately 80; see Menozzi and Krimbas 1992). Nevertheless, the inversions that became established in the New World are by far the most commonly encountered ones in the Old World. For example, the New World inversions on the A chromosome (A_{st} or A_2) are found in 79.0% of all Old World A chromosomes ever sampled (data summarized from Menozzi and Krimbas 1992). The same pattern holds for the other chromosomes ($J = 95.4\%$, $E = 87.9\%$, $O = 90.5\%$, $U = 85.3\%$).

Three new (i.e., unknown in Palearctic populations) inversions have been detected in the New World. Inversions E_{21} and O_{26} were discovered in North America (Port Hardy 1994 and Centralia 1995, respectively) in only one chromosome each (note: O_{26} is included in the arrangement $O_{3+4+2+26}$; Electronic Appendix A). Inversion E_{17} was discovered in low frequency in South America in 1981 (Santiago and Chillán) but has not been detected thereafter (Electronic Appendix B).

Two chromosomal arrangements likely evolved in the New World. Arrangements O_7 and U_1 , which are also found pe-

riodically in the Old World, are probably the result of recombination in the New World between an O_{3+4+7}/O_{st} heterozygote and between an U_{1+2}/U_{st} heterozygote, respectively.

Almost all of the remaining arrangements are present in all the populations and in each year sampled. Thus most of the geographic patterning (described below) is the result of changes in frequency rather than from postcolonization bottlenecks within continents. (Of course, such bottlenecks might be undetected if gene flow is high.) The inversions not detected in all the populations include E_{1+2} (absent in Viña del Mar, Bariloche, Castro, and Coyhaique in South America) and $E_{1+2+9+12}$ and O_{3+4+7} (both absent in Port Hardy, BC). These inversions are uncommon even when present (Electronic Appendices A and B), and so their apparent absence at some sites may represent sampling error, although a secondary bottleneck might have occurred when Vancouver Island (Port Hardy) was colonized.

Analyses of Clines

We analyze historical shifts in latitudinal clines in chromosomal arrangements in three ways. First, we compute the signs and magnitudes of regression slopes of inversion frequency on latitude for all Old and New World samples and then determine whether clines in inversions in the New World are similar in sign to those in the Old World. Second, we examine whether the slopes of the regressions of inversion frequency on latitude have changed over time in North and in South America. Thus we analyze changes of slope over time without reference to the Old World patterns. Third, we determine whether the New World slopes are continuing to converge on the Old World slopes as suggested by Prevosti et al. (1990).

European baseline clines

Slopes of frequency versus latitude have not been calculated in previous studies but are shown graphically for the European populations in Figure 1. Signs of correlations between inversion frequency and latitude for the Old World populations (see Materials and Methods; data not shown) are identical with those in previous surveys (Prevosti 1964; Prevosti et al. 1988; Menozzi and Krimbas 1992).

Signs of clines with latitude

Latitudinal clines evolved almost instantly in the invading South American populations. Even at first survey (1981), 17 of 18 arrangements—far more than expected by chance (permutation test, $P = 0.001$)—showed clines that had the same sign with latitude as in the Old World (see Fig. 1). This pattern has remained relatively stable in subsequent surveys: the number of arrangements that have the same sign with latitude as in the Old World has decreased slightly since 1981 (16 of 18 in 1986, $P = 0.001$); 15 of 18 in 1999, $P = 0.001$, but this decline is not significant (R X C test, $P > 0.50$).

Latitudinal clines also began to evolve almost instantly in North America. By the first sample (1985–1986), 14 of 18 arrangements showed clines that had the same sign with latitude as in the Old World ($P = 0.001$). As in South America,

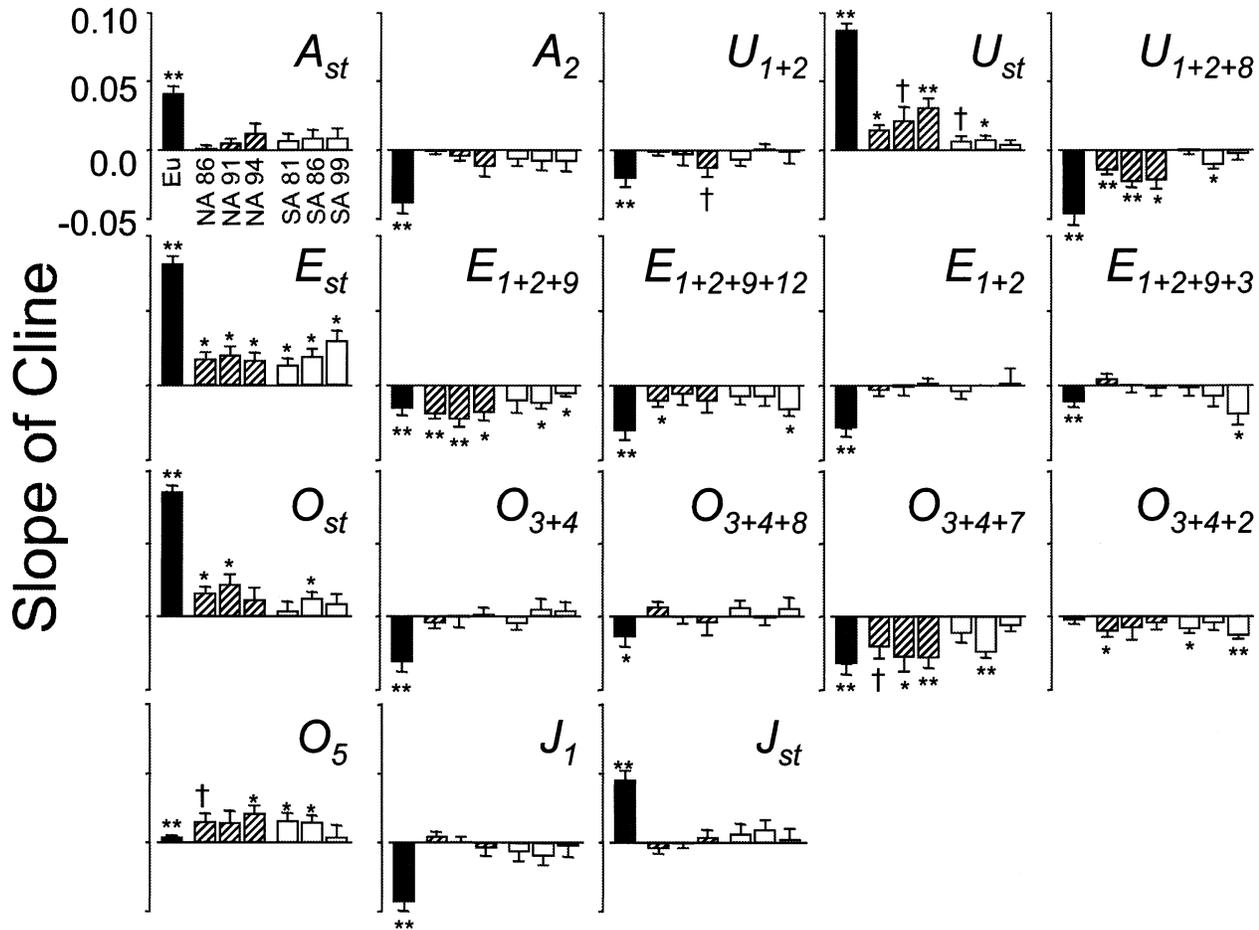


FIG. 1. Slopes of frequencies versus latitude (± 1 SE) for chromosome inversions of *Drosophila subobscura* in Europe (EU), in South America (SA), and in North America (NA). Slopes are generally the same sign in America as in Europe, but are invariably less steep and are not continuing to converge on the European slopes. Chromosomes are designated by capital letters (A, U, E, O, J), and inversions by associated subscripts. Significance levels for latitudinal clines are computed via permutation tests (see Materials and Methods): † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$.

this pattern has remained stable thereafter (18 of 18 in 1991, $P = 0.001$; 16 of 18 in 1994, $P = 0.001$).

Test for heterogeneity of New World slopes over time

To determine whether the slopes of the inversion clines have changed subsequent to the first census in the New World (see Fig. 1), we ran analyses of covariance on frequency of each inversion versus latitude for the various samples in the time series (three each in North and South America). If clines have changed in steepness, the interaction between time and latitude should be significant. In fact, no inversion in either North or South America showed a significant interaction (all $P > 0.1$), indicating that slopes were parallel. Next we examined whether the intercept (for frequency) had shifted over time, which would indicate a continentwide shift in inversion frequency. Five inversions in South America showed significant shifts in intercept even after a sequential Bonferroni correction (U_{st} : $P = 0.002$, U_{1+2+8} : $P \ll 0.001$, and O_{3+4} : $P \ll 0.001$, E_{1+2+9} : $P < 0.005$, $E_{1+2+9+12}$: $P < 0.005$). Interestingly, all five exhibit a significant linear change in overall frequency (i.e., a consistent shift in direction): O_{3+4} , U_{1+2+8} , and $E_{1+2+9+12}$ have increased in frequency, whereas U_{st} and

E_{1+2+9} have decreased (single degree of freedom contrasts on time as an ordered factor). In contrast, no inversion in North America showed a significant, continent wide shift in frequency. Thus, a shift in slope of clines is not detectable either in North or South America, but a few arrangements in South America appear to be changing consistently in frequency across all latitudes.

Test for similarity of slopes with the Old World baseline

The latitudinal clines in South America are significantly closer in slope to those of the European baseline than would be expected by chance alone in all three years sampled (Table 1). North American clines show a similar pattern (Table 1).

Even though clines for inversions in the initial sample (1981) for South America almost always had the same sign with latitude as in the Old World (above), the slopes of those clines were invariably much less steep than in the Old World (Fig. 1; all $P < 0.001$), suggesting that clines then were just forming. Are those clines becoming increasingly steep over time and thus continuing to evolve toward the baseline clines? The heterogeneity of slopes tests (above) suggest no general change in slope but do not specifically address convergence.

TABLE 1. Slopes of clines in the New World are closer in magnitude to those in the Old World than would be expected by chance alone. The observed mean-squared deviations across chromosomes are smaller than the null expected deviations of the bootstrapped South American (SA) and North American (NA) slopes from the European baseline for each sample date. Slopes for New World flies are much more similar to those of Old World flies than expected by chance (P).

Continent	Date	Observed Deviation	Expected Deviation \pm SD	P
SA	1981	0.00068	0.00099 \pm 0.000091	0.001
SA	1986	0.00057	0.00102 \pm 0.000128	0.001
SA	1999	0.00063	0.00102 \pm 0.000119	0.001
NA	1986	0.00069	0.00099 \pm 0.000091	0.001
NA	1991	0.00058	0.00102 \pm 0.000128	0.001
NA	1994	0.00052	0.00102 \pm 0.000119	0.001

Although patterns for a few inversions are suggestive of convergence (E_{st} ; Fig. 1), none of the 18 inversions showed significant convergence. Thus there is no evidence that inversion clines in South America are detectably continuing to converge on the Old World baseline (Table 2). To some extent, this may reflect insufficient power. Nevertheless, only 10 of 18 inversions in South America showed a negative sign for the linear contrast for the deviation of slope from the Old World as a function of year (Table 2; see Materials and Methods), suggesting that almost half of all arrangements fail to show any evidence of continued convergence on Old World patterns.

The patterns are similar in North America. Slopes of clines in the initial survey (1985–1986) were generally less steep in North America than in the Old World (Fig. 1). None of the 18 arrangements showed significant convergence on the Old World slopes in subsequent surveys (Table 2). Moreover, only 8 of 18 arrangements (Table 2) had a negative linear contrast for year (see *Tests for Similarity of Sign and Slope, and of Convergence*, above), suggesting that most arrangements in North America are not showing any evidence of convergence.

Whether the cline for a given arrangement is showing convergence or divergence with respect to the Old World is strikingly inconsistent between North and South America. Indeed, only four of the 18 arrangements show the same sign for the linear contrast for year on both continents (Table 2). Subsequent to the initial survey, the evolutionary trajectories of a given inversion are thus essentially independent on the two continents.

DISCUSSION

The invasion and subsequent rapid spread of *D. subobscura* into both North and South America provide a remarkable opportunity to examine the speed, uniformity, and predictability of evolutionary change on a geographic scale (Prevosti et al. 1988; Ayala et al. 1989; Brncic 1995). Evolutionary geneticists quickly capitalized on this as an opportunity to measure the speed and predictability of evolution (Brncic et al. 1981; Prevosti et al. 1985, 1988; Ayala et al. 1989). Knowing that the frequencies of chromosome inversions varied dramatically with latitude in the ancestral Old World populations, they quickly surveyed the inversion frequencies in

latitudinally separated populations in both North and South America. Their first surveys (1981 in South America, 1985–1986 in North America) were made just a few years after the introduction and expansion of the populations. Nevertheless, they discovered that inversion clines were already being established and almost always had the same sign with latitude as in the Old World, thus suggesting very rapid evolution toward the Old World baseline (Brncic et al. 1981; Prevosti et al. 1988; Ayala et al. 1989). A second sample for South America (1986) was interpreted as showing that inversion frequencies were continuing to converge on the Old World pattern and had not yet reached equilibrium (Prevosti et al. 1990). Thus, the results from these early surveys were clear: evolution in this system was rapid, continuing, and predictable.

Not all inversions in those initial surveys showed patterns that mirrored Old World ones. The conspicuous exception was the O_5 inversion. In the Palearctic region, this inversion is always rare and erratically distributed, and its frequency does not vary with latitude (Krimbas and Loukas 1980; Krimbas 1992, 1993; Zivanovic and Mestres 2000). In both North and South America, however, this inversion quickly developed significant latitudinal clines (Fig. 1).

Our new samples substantially extend this time series of evolution in action. For South America, they span almost the entire time since the colonization. A combined analysis of old and new samples shows that none of the gene arrangements in either South or North America has a slope that has become steeper or is continuing to converge on those of the Old World flies (Fig. 1). The only conspicuous change is that five arrangements in South America do show significant, continentwide shifts in overall frequency (below). Thus, the overall temporal pattern is one of rapid convergent evolution immediately after the invasion of essentially all chromosomal arrangements toward the Old World baseline (as evidenced by the similarity of signs of correlations in the first surveys), but of little or no convergence thereafter.

An earlier report based on the first two datasets from South America (1981, 1986) suggested that inversion clines were continuing to evolve closer to the Old World patterns (Prevosti et al. 1990), whereas our analyses suggest otherwise. The discrepancy may reflect different statistical approaches: the earlier study focused qualitatively on temporal shifts in the magnitude of the correlation coefficient between frequency and latitude, whereas we test quantitatively for convergence in slopes of regressions.

Interestingly, other traits show different patterns of clinal evolution from those seen for inversions. For example, wing length increases clinally with latitude in the baseline, Old World populations (Prevosti 1955; Misra and Reeve 1964; Pfreim 1983; Pegueroles et al. 1995; Huey et al. 2000; Gilchrist et al. 2001). No wing-size cline was evident in North or in South America one decade after the invasion (Budnik et al. 1991; Pegueroles et al. 1995), but a significant cline was evident after two decades in North American populations (Huey et al. 2000; Gilchrist et al. 2001; note that South American patterns are currently under study). Thus the evolution of wing-size clines appeared undetectable at first, and became apparent only later—just the opposite pattern seen with inversions. Some other traits show no latitudinal clines at all,

TABLE 2. Slopes of clines in the New World are not converging significantly on those of the Old World. The squared deviations of the jackknifed South American and North American chromosomal arrangement slopes from the European baseline slopes are given for each sample. Patterns between years were analyzed by analysis of variance, with year as an ordered factor. The *F* test examines the significance (*P*) of the linear contrast. Convergence would be indicated by a significant linear contrast and by a negative sign of the linear coefficient (see Materials and Methods). None of the gene arrangements shows significant convergence on the Old World slopes. Values for the A_2 and J_1 arrangements are the same as those for A_{st} and J_{st} , respectively and thus are not shown. Sign indicates positive or negative slope with latitude.

Arrangement	South America						North America							
	1981			1986			1991			1994				
	Squared deviation (mean ± SE (×10 ³))	Mean square (×10 ⁶)	Sign	Squared deviation (mean ± SE (×10 ³))	Mean square (×10 ⁶)	Sign	Squared deviation (mean ± SE (×10 ³))	Mean square (×10 ⁶)	Sign	Squared deviation (mean ± SE (×10 ³))	Mean square (×10 ⁶)	Sign		
A_{st}	2.22 ± 1.342	1.99 ± 1.144	2.43 ± 1.483	2.41	0.138	0.715	+	2.73 ± 6.962	2.41 ± 6.780	2.12 ± 6.780	2.12 ± 2.159	14.17	0.644	0.432
J_{st}	1.94 ± 1.598	1.65 ± 1.167	3.32 ± 3.865	81.59	1.501	0.236	+	2.50 ± 4.680	2.45 ± 1.726	2.02 ± 1.726	2.02 ± 1.551	7.93	0.403	0.533
U_{1+2}	0.60 ± 1.519	0.84 ± 1.493	1.06 ± 1.171	7.18	1.306	0.267	+	0.70 ± 0.492	1.10 ± 1.321	0.38 ± 0.958	0.38 ± 0.958	2.22	0.297	0.592
U_{st}	6.83 ± 1.691	6.65 ± 1.137	7.44 ± 3.950	16.58	0.897	0.356	+	5.60 ± 1.619	5.27 ± 3.697	3.68 ± 1.847	3.68 ± 1.847	125.98	1.971	0.177
U_{1+2+8}	2.82 ± 1.580	1.67 ± 4.489	2.43 ± 1.277	1.97	0.198	0.662	+	1.37 ± 4.466	0.81 ± 2.255	1.52 ± 1.157	1.52 ± 1.157	0.19	0.033	0.857
E_{st}	5.03 ± 1.898	4.67 ± 3.536	3.06 ± 1.882	147.60	2.307	0.145	-	4.60 ± 2.761	4.41 ± 1.937	4.66 ± 2.347	4.66 ± 2.347	0.04	0.001	0.979
E_{1+2+9}	0.60 ± 4.924	0.15 ± 6.230	0.23 ± 8.138	3.56	0.933	0.346	-	0.22 ± 0.335	0.13 ± 8.157	0.56 ± 1.887	0.56 ± 1.887	3.70	0.773	0.390
$E_{1+2+9+12}$	1.09 ± 5.739	1.46 ± 1.427	0.65 ± 4.254	9.89	1.112	0.305	-	0.97 ± 8.809	1.77 ± 3.015	1.55 ± 1.578	1.55 ± 1.578	14.58	0.365	0.553
E_{1+2}	0.82 ± 1.340	0.82 ± 5.833	3.13 ± 3.242	212.48	5.749	0.027	+	0.65 ± 6.334	1.09 ± 1.246	0.96 ± 3.658	0.96 ± 3.658	4.24	0.619	0.441
$E_{1+2+9+3}$	0.53 ± 0.513	0.53 ± 0.889	0.16 ± 5.180	5.32	1.442	0.245	+	0.64 ± 9.527	0.55 ± 0.625	0.73 ± 4.802	0.73 ± 4.802	0.17	0.038	0.848
O_{st}	6.86 ± 2.893	5.64 ± 2.633	5.83 ± 1.725	28.52	0.439	0.516	-	4.64 ± 9.620	4.45 ± 2.834	5.60 ± 2.148	5.60 ± 2.148	27.57	0.641	0.433
O_{3+4}	1.14 ± 1.473	2.28 ± 2.388	2.53 ± 3.282	59.30	1.070	0.314	+	1.05 ± 7.639	1.66 ± 1.758	1.53 ± 1.166	1.53 ± 1.166	9.76	0.608	0.445
O_{3+4+8}	0.67 ± 1.374	0.19 ± 1.207	0.40 ± 3.382	1.46	0.173	0.682	-	0.32 ± 8.222	0.43 ± 2.605	0.95 ± 1.689	0.95 ± 1.689	13.47	1.150	0.297
O_{3+4+7}	0.72 ± 7.851	0.18 ± 7.206	0.49 ± 3.239	0.92	0.276	0.605	-	0.72 ± 1.122	1.28 ± 1.693	0.41 ± 0.481	0.41 ± 0.481	1.92	0.138	0.714
O_{3+4+2}	0.13 ± 3.118	0.32 ± 5.686	0.07 ± 0.119	0.34	0.214	0.649	-	0.04 ± 1.054	0.77 ± 1.727	0.26 ± 4.285	0.26 ± 4.285	3.19	0.328	0.573
O_5	0.98 ± 1.653	0.42 ± 9.910	0.66 ± 1.209	2.25	0.134	0.719	-	0.65 ± 4.582	1.04 ± 1.327	0.54 ± 1.755	0.54 ± 1.755	0.13	0.021	0.887

even after two decades (photoperiodicity: P. Lankinen, pers. Comm.; sperm length: R. Snook, pers. Comm.; desiccation tolerance: G. W. Gilchrist, L. Dietrich, and D. DeSourdy, unpubl. data). Such differences among traits in the tempo of evolution are perhaps not surprising: traits undoubtedly differ in their genetic architecture and in the strength of selection on them and on correlated traits.

Continentwide Shifts in Frequency of South American Arrangements

One conspicuous trend is that five chromosomal arrangements are undergoing highly significant, continentwide shifts in frequency in South America: U_{st} and E_{1+2+9} are decreasing in frequency, whereas O_{3+4} , U_{1+2+8} , and $E_{1+2+9+12}$ are increasing in frequency (see Electronic Appendix B). We see two possible (nonexclusive) explanations as to why these arrangements might show significant shifts.

First, the most likely source of the New World flies was the western Mediterranean, because the inversions present in that region (and their frequencies) most closely match those seen in the initial (1981) sample from South America (Prevosti et al. 1987; Ayala et al. 1989; G. W. Gilchrist, unpubl. data). Note that nine arrangements in the 1981 sample differ significantly in frequency from those of four sites in eastern Spain (Barcelona, Ibiza, Riba-roja, and Valencia; *t*-tests with sequential Bonferroni corrections, data not shown). Four of those divergent inversions are the ones that show significant postcolonization change, and interestingly all four are reverting in frequency back toward the Spanish level. Therefore, perhaps the marked deviation in “founding” frequencies for these inversions was a consequence of a perturbing founder effect, and the subsequent shift in frequency reflects selection driving the frequency back toward that seen in Spain. However, the other five divergent inversions do not show a significant reversion toward the Spanish level.

Second, a climate-based explanation can be suggested. In the Old World, U_{st} is most common at high latitudes, whereas the other four inversions are most common at low latitudes (Menozzi and Krimbas 1992; Fig. 1). Over the past few decades in Spain, the frequency of some of these inversions has shifted apparently in response to observed climate warming: the “high-latitude” arrangement U_{st} has decreased in frequency as temperatures have warmed (Orengo and Prevosti 1996), whereas O_{3+4} , has increased in frequency (but see Orengo and Prevosti 1996; Rodríguez-Trelles and Rodríguez 1998). Thus the frequencies of these inversions seem to be climate sensitive. Therefore, perhaps the initial excess frequency of U_{st} in South America (relative to Spain) was maladaptive in a warm or warming climate, whereas the initial low (relative) frequency of O_{3+4} was similarly maladaptive in a warm or warming climate.

Why Has Clinal Evolution Stalled?

Two related but unresolved question emerge from our analyses. Why have the slopes of the inversion clines seemingly not evolved significantly subsequent to the first census? Why have the slopes not continued to converge on the European ones, even though the slopes of the inversion clines are still

shallow relative to those of the ancestral populations (Fig. 1)? Several answers are possible.

Perhaps our inability to detect significant change is due to insufficient power. This could easily be the case in North America, because the samples span less than a decade—not much time for detectable evolution to occur. Certainly, the number of sites per continent is limited (especially in South America), and the number of chromosomes scored per chromosome per site per sample averaged 136.2 for South America and 105.6 for North America (see Electronic Appendix A and B). Not surprisingly, the confidence intervals for any sample of inversion frequencies are necessarily large (as are SE of jackknifed deviations of slopes from the Old World ones; see Table 2). Despite limited power, we were able to detect highly significant shifts in continentwide inversion frequencies for five arrangements in South America. Moreover, because only about half of all inversions show even a hint of convergence on the Old World clines (Table 2), apparent stalling of clinal evolution does not appear to be merely an artifact of limited power.

Perhaps the expectation that clines in the New World should ever converge on those of the Old World (Ayala et al. 1989) is itself unreasonable. Lack of convergence could occur if the latitudinal patterns of selection differ markedly on the continents. Certainly, the continents differ somewhat in selective environments, but all share a common trend of hot and dry climates at low latitudes, and cool and wet climates at higher latitudes. (In fact, regressions of climate scores [PCA] on latitude are strikingly parallel for all three continents; G. Gilchrist, unpubl. Data.) Greater gene flow in the New World (relative to the Old World) could also buffer clinal evolution even if selection patterns were the same. Finally, new mutations in inversions might have altered their selective value in the new environment; or new mutations outside inversions might have altered epistatic interactions with inversions. These options remain to be examined.

Another possibility is that the lack of continued convergence may be a byproduct of a founder effect that occurred during the colonization (Balanya et al. 1994). Fewer than 150 individuals were believed involved in the initial colonization (Mestres et al. 1990; Pascual et al. 2001), and the resultant bottleneck reduced the number of gene arrangements (from about 80 in the Old World to 18), thereby reducing the available genetic variation (though not sufficiently so to inhibit the establishment and rapid expansion of *D. subobscura*). Moreover, some inversions were likely represented only once in the initial sample of colonizers, as suggested by the strong linkage disequilibrium between inversions and several genetic markers (Rozas and Aguadé 1991; Mestres et al. 1995). Overall, then, the genetic background in the New World flies is impoverished relative to that in the Old World. Therefore, the observed limited convergence might be a consequence either of limited genetic variation in the New World or of sensitivity of evolutionary trajectories to the particular genetic background present during the initial colonization (Barton and Charlesworth 1984).

Heterosis could also play a role (Krimbas 1992; Mestres et al. 2001) in constraining clinal evolution. The increased autozygosity following the bottleneck (Pascual et al. 2001) might buffer selection that favors an increased frequency of

particular inversion or arrangement along a geographic gradient. In other words, heterosis is more likely to restrain the evolution of steep clines whenever the diversity of chromosomal polymorphisms is low. Significant heterosis for two heterokaryotypes has indeed been detected in the American populations (Mestres et al. 2001). One case corresponds to the O_5 inversion. This gene arrangement is completely associated with a lethal gene in the American populations (Mestres et al. 1990; Menozzi and Krimbas 1992) and shows significant latitudinal clines there (Prevosti et al. 1988; Fig. 1). It persists apparently because heterokaryotypes carrying this inversion have a selective advantage; and its clinal distribution may reflect geographic variation in the magnitude of selection against individuals carrying other chromosomal combinations (Mestres et al. 2001).

If heterosis is generally significant in New World *D. subobscura*, little further change in inversion clines might be expected (Endler 1977), unless either new inversions or new mutations within existing arrangements amplify chromosomal polymorphism in the New World. New inversions could, of course, appear by mutation; and apparently new inversions have been detected in the New World (E_{17} , Brncic et al. 1982; $E_{1+2+9+18}$, Pegueroles et al. 1988; E_{21} and $O_{3+4+2+26}$, this study). However, all are rare, and at least two may have already been lost (E_{17} and $E_{1+2+9+18}$ have not been found since the mid-1980s). Nevertheless, if inversions do re-evolve in the New World (although this idea has largely been discounted, see Powell 1997), new coadapted gene complexes could arise and change clinal pattern distributions.

New inversions could also be added if additional samples of *D. subobscura* colonize from the Old World. Remarkably, additional colonizations seem not to have occurred. All of the Palearctic inversions found in the most recent New World samples were also found in the earliest samples (Tables 1, 2). Had other Palearctic flies invaded the Americas subsequent to the original colonization, additional Palearctic inversions should have been detected in recent samples. After all, the pool of inversions—many of which are often common—is large in Old World flies (Krimbas and Loukas 1980; Krimbas 1992, 1993).

Other genetic mechanisms can potentially amplify standing genetic variation. In fact, crossing over (O_7 and U_1 , see Results) and gene conversion (Rozas and Aguadé 1991) have already yielded new gene combinations. Moreover, patterns of selection due to climate changes (Rodríguez-Trelles and Rodríguez 1998; Solé et al. 2002) may modify the distribution pattern of the clines.

In conclusion, a replicated time series of evolution in action—now spanning almost two decades for South America—demonstrates that the evolution of geographic clines in inversion frequencies was initially rapid and predictable: weak inversion clines were detectable almost instantly and were in the same direction with latitude as in the ancestral clines. However, the evolution that has occurred subsequently seems more idiosyncratic. Clines have not become steeper nor have they continued to converge on those of the Old World. Genetic considerations relating to heterosis and founder effects suggest that little additional evolution is to be expected in the invading populations unless new coadapted gene com-

plexes arise: this prediction than can be tested with future samples.

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