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INFERENCES FROM A RAPIDLY MOVING HYBRID ZONE

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Abstract.—*Anartia fatima* and *Anartia amathea* (Lepidoptera: Nymphalidae) are sister taxa whose ranges abut in a narrow hybrid zone in eastern Panama. At the center of the zone, hybrids are abundant, although deviations from Hardy-Weinberg and linkage disequilibria are strong, due in part to assortative mating. We measured differences across the zone in four wing color-pattern characters, three allozyme loci, and mitochondrial haplotype. Wing pattern, allozyme, and mitochondrial clines were coincident (i.e., had the same positions) and concordant (i.e., all markers had similar cline shapes, about 28 km wide). Repeated samples demonstrated that the hybrid zone has been moving eastwards at an average rate of 2.5 km/year over the past 20 years, accompanied by an equivalent movement of the mtDNA cline. No introgression of mtDNA haplotypes were found in the ''wake'' of the moving cline, as might be expected for a neutral marker. The concordance of morphological and mtDNA clines between 1994 and 2000, in spite of hybrid zone movement, suggests strong epistasis between the mitochondrial genome and nuclear loci. Cline movement is achieved mainly by pure *fatima* immigrating into *amathea* populations; hybrids had little effect, and were presumably outcompeted by fitter pure *fatima* genotypes. This movement can be explained if random dispersal of 7–19 km.gen$^{-1/2}$ is coupled with a competitive advantage to *A. fatima* genomes of 2–5%. Hybrid zone motion is equivalent to Phase III of Wright's shifting balance. Hybrid zone movement has rarely been considered likely in the past, but our results show that it may be more important in biogeography and evolution than generally realized.

Key words.—*Anartia,* concordant clines, cytonuclear disequilibrium, linkage disequilibrium, moving hybrid zones, shifting balance, speciation.

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A particularly interesting feature of hybrid zones is that they can move (Barton and Hewitt 1981).

Hybrid zones are regions where genetically distinct populations meet and produce hybrids (Barton and Hewitt 1985, 1989; Harrison 1993). Clines at different loci and characters involved in hybrid zones are often highly ''coincident'' (the centers of the clines are in the same position) and concordant (they have similar width and shape). A cline can be defined as a more or less continuous geographic change in the frequency of alternative forms of a single gene, chromosome, or character. Concordance and coincidence of clines are presumed to result partly because of their origin as a result of secondary contact between populations that diverged in geographic isolation, and partly because strong gametic disequilibria set up across the entire genome cause populations in the center of the hybrid zone act as a genetic barrier (Barton and Hewitt 1985).

However, if the selection pressures acting on a variety of characters and loci across a hybrid zone are different, each may be expected to introgress through the hybrid zone to different extents resulting in the multiple clines being in some cases neither coincident nor concordant. It is often assumed that mtDNA should show the greatest introgression because it is thought by many to be neutral, and thus may flow relatively easily through the hybrid zone. There are some excellent examples where clines at mtDNA haplotypes are strongly displaced from the location of hybrid zones at nuclear genes affecting fitness (Ferris et al. 1983; Marchant et al. 1988; Thulin et al. 1997; Rohwer et al. 2001), and these cases are often interpreted as due to past hybrid zone movement. However, the evidence for neutrality of mtDNA is largely circumstantial, and due to its lack of recombination, the entire mtDNA genome would be involved in a selective sweep if even a single differentiated codon were under selection. Introgression of mitochondrial haplotypes across some hybrid zones may be explained equally parsimoniously as evidence of a global selective advantage for the introgressing mitochondrial genome.

The position and width of a narrow cline are maintained by a balance between selection and migration. Some clines are maintained by ''exogenous'' selection due to the environment, for example a climatic boundary (Haldane 1948; Endler 1977; Moore and Price 1993). In this case, differential selection for or against particular genotypes across an ecotone will cause the clines to settle on the boundary between environments. The clines will not move unless the environment changes. Other clines may be maintained by ''endogenous''

selection, for example selection against heterozygotes, epistatic selection, or frequency-dependent selection against rare genotypes. For these types of selection, the position of a cline in the environment is arbitrary; any asymmetry of selection or migration across the cline will cause it to move (Bazykin 1969; Barton 1979; Mallet and Barton 1989; Johnson et al. 1990; Gavrilets 1998).

Many hybrid zones are under a mixture of endogenous and exogenous selection. For example, in hybrid zones of the toad *Bombina* there is strong selection against hybrids, but there are also many morphological and physiological differences across the zones which influence desiccation resistance, and which correlate with changes in size and permanence of breeding ponds (Szymura and Barton 1986, 1991). Such clines will be rooted to the environmental boundary because of linkage disequilibrium and pleiotropy between traits influenced by both exogenous and endogenous selection. In other cases moving clines can be trapped in regions of low density, which act as sinks for migration (Barton 1979; Barton and Hewitt 1981). For such reasons, cline movement in hybrid zones has usually been discounted as an important force in evolution.

Yet the possibility that clines and hybrid zones move remains alluring and largely untested. Hybrid zones have often been used as evidence for secondary contact between faunas and floras that evolved in geographic isolation as many as 10^4–10^7 years ago (e.g., Mayr 1963; Remington 1968; Whitmore and Prance 1987). However, if hybrid zones commonly move, the clustering of hybrid zones in biogeographic ''suture zones'' could have more to do with current ecotones or dispersal barriers than an indication of events during the remote past.

The movement of clines is also of key importance in the debate about adaptation via a ''shifting balance'' mechanism. In Wright's shifting balance hypothesis (Wright 1932, 1977), a new form arises locally via genetic drift or temporary fluctuations in the direction of selection (Phase I), and then becomes fixed locally via natural selection (Phase II). Finally, if the new form is superior to (or its ''adaptive peak'' is higher than) the older form, a net efflux of fitter individuals may allow the novel adaptive peak to spread to other populations (Phase III). The importance of the shifting balance has often been discounted on the grounds of little empirical evidence for Phase III, and for all three phases acting in the same system (Coyne et al. 2000). Because moving clines export adaptive or more stable genetic equilibria to new populations, they are concrete examples of a process involved in Phase III.

To date, few studies have reported evidence for hybrid zone movement (see Discussion). In some cases, the movement of hybrid zones may be due to unnatural introductions or other human interference (e.g., Goodman et al. 1999). The general lack of cline movement may, as already suggested, be due to their becoming trapped in population density troughs and on environmental gradients (Barton and Hewitt 1985). However, clines are generally expected to move somewhat slowly compared to the typical research grant cycle. In addition, a prevailing belief that hybrid zones demarcate stable ancient biogeographic boundaries may have discouraged investigations into their movement.

In this paper, we document movement of a hybrid zone between *Anartia* butterflies in Panama over a period of more than 20 years, and quantify introgression and its asymmetry at a variety of molecular and morphological markers. We use this data to infer patterns and strength of selection across the hybrid zone.

The Genus Anartia

Anartia (Lepidoptera: Nymphalidae) are among the commonest Neotropical butterflies. The genus consists of five species. *Anartia jatrophae* is widespread throughout the Neotropics and is sympatric with the other four species. *Anartia chrysopelea* and *A. lytrea* are restricted to some islands in the Greater Antilles, whereas *A. fatima* and *A. amathea* are confined to the mainland and the Lesser Antilles. *Anartia fatima* is distributed north and west from Panama to the U.S. border with Mexico, whereas *A. amathea* ranges from eastern Panama through the entire South American mainland as far south as northern Argentina (Silberglied et al 1979). The ranges of *A. fatima* and *A. amathea* meet only in the Darién Province in eastern Panama, where naturally occurring hybrids are common (Brown 1976; Silberglied et al. 1979; Silberglied 1984). It has been suggested that this hybrid zone exists as a result of secondary contact between two species that evolved in allopatry, following the rise of the Isthmus of Panama during the late Pliocene, three million year ago (Silberglied et al. 1979; Knowlton and Weigt 1998).

The biology of *Anartia* has been well studied (Brown 1976; Emmel and Leck 1970; Silberglied et al. 1979). Here, we summarize the main points. *Anartia* are restricted to humid, grassy habitats, and have benefited from human activities such as forest clearing and agriculture. Food plants are small herbs growing in open areas. *Blechum* species (Acanthaceae) are the most widely used larval host plants for *A. fatima* and *A. amathea* (Scott 1986; Smith et al. 1994). Adults feed at a number of flowers common in open areas, especially *Lantana camara*, *Hyptis mutabilis*, and *Sida* sp. (Fosdick 1973). Adult life is short, averaging one to two weeks, but females are highly fecund, laying several hundred eggs over the course of a few days. The adults are palatable to birds, and are widely used in experiments on butterfly mimicry and predator learning (Brower et al. 1963; Chai 1986).

Silberglied (1984) and Davies et al. (1997) demonstrated strong assortative mating between Panamanian *A. fatima* and *A. amathea*. Survival of F_1 hybrids is normal, but larval survival is strongly reduced in F_2 hybrids. Haldane's Rule effects are evident in that F_1-hybrid females (the heterogametic sex) have somewhat reduced fertility; F_1-hybrid females from the cross *A. amathea* (female) $\times$ *A. fatima* (male) also have a reduced tendency to mate (Davies et al. 1997). As mtDNA is maternally inherited, this second asymmetrical Haldane effect might be expected to cause asymmetrical mitochondrial introgression across the hybrid zone, leading to *A. fatima* haplotypes being commoner in *A. amathea* than vice versa.

Materials and Methods

Butterfly Collections and Study Sites

The *A. fatima*/*amathea* hybrid zone has been sampled at least four times over the past 23 years. Collections have been

TABLE 1. Details of collection sites.

Site no.	Distance from Panama City	GPS readings		Nearest town
		Latitude	Longitude	
1	147	08°58′N	78°29′W	Ipetí
2	221	08°26′N	77°54′W	Metetí
3	237	08°21′N	77°50′W	Sanson
4	247	08°17′N	77°49′W	Canglón
5	268	08°13′N	77°44′W	Yaviza
6	190			
7	214			
8	228			
9	239			
10	251			
11	261			
12	268			Yaviza
13	109			between Lago Bayano and Ipetí
14	159			Cañazas
15	187			El Tirado
16	229			Metetí/Sanson
17	246			Canglón
18	186			12 km northwest of Santa Fé

made at locations along the Pan-American highway that runs along the length of Panama, and represents a transect across the *Anartia* hybrid zone (Table 1, Fig. 1). The information from 1977 and 1980 was obtained from unpublished field notes (R. E. Silberglied, A. Aiello, R. Olberg); collection dates were 2–4 June 1977 and 26–27 May 1980. The data consisted of wing pattern scores (see below). Tissue samples were not available, nor were the wings themselves. The 1994 specimens were collected by ND on 20–21 December 1993 and 21 February 1994. Further specimens were collected by KD and MB on 4–7 May 2000, 2–4 June 2000, and 27–28 August 2000. Collections were not made beyond the end of the road at Yaviza (273 km from Panama City), as the terrain from here to the Colombian border is currently dangerous due to guerrilla activity. The 1994 individuals were stored at −70°C, while the 2000 individuals were stored in DMSO at 4°C, after their wings were removed. Individuals collected in 1994 and 2000 were scored for wing pattern, and their mtDNA haplotype was determined (except for the August 2000 collection) using the procedures described below. The 1994 individuals were also scored at diagnostic allozyme loci.

Wing pattern scores

All the individuals in the 1994 and 2000 collections were scored for four wing pattern characters clearly divergent be-

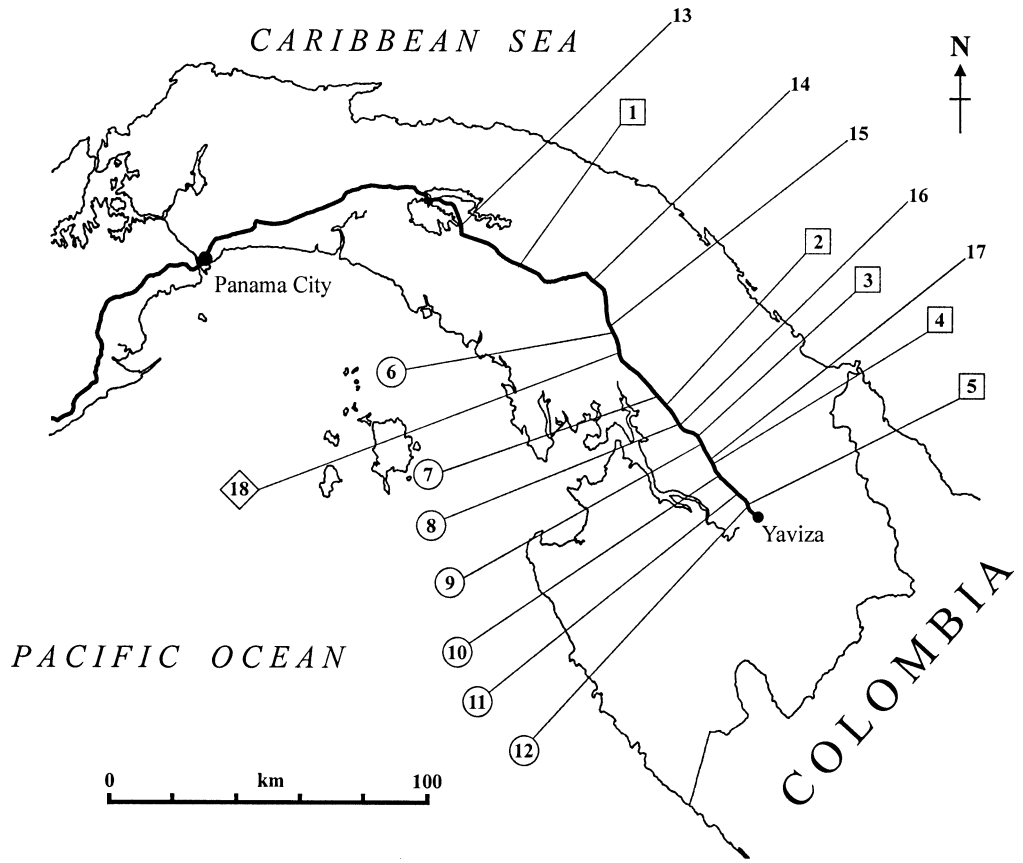

FIG. 1. Map of eastern Panama showing the locations of collecting sites along the Pan-American Highway. Numbers in squares (1–5) are sites for 2000 collections; in circles (6–12) are 1994 collection sites; plain numbers (13–17) are 1980 sites; and 18 is the 1977 site. These correspond to site numbers used in Table 1.

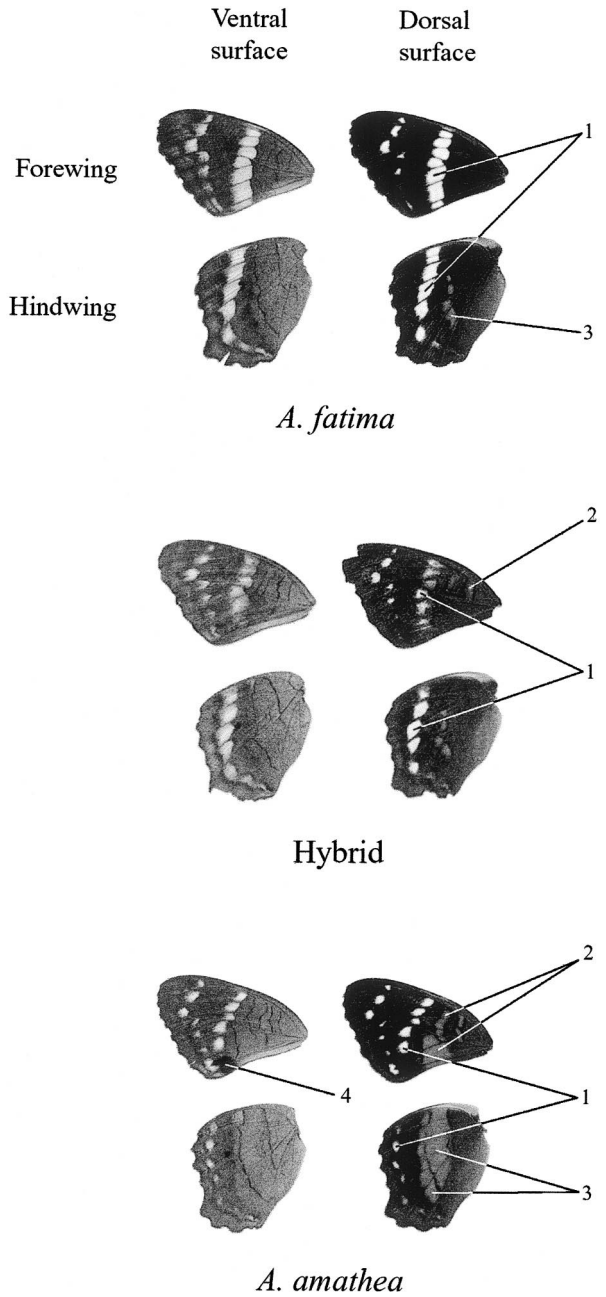

FIG. 2. Wing-color patterns in *Anartia amathea* (A.a), *A. fatima* (A.f), and a hybrid, showing the four characters used in the wing index. Character 1, pale band solidity (A.a-broken; A.f-solid). Character 2, red on dorsal surface of forewing (A.a-present; A.f-absent). Character 3, red on dorsal surface of hindwing (A.a-lots, medial band; A.f-little, distal spots). Character 4, black spot on under side of forewing (A.a-present; A.f-absent). The dark, gray, and pale colors of the dorsal surfaces are dark brown, red, and cream colored, respectively, in real life. The hybrid shows intermediate expression for characters 1 and 2 on the dorsal forewing.

tween the two species, but at the same time having intermediate levels of expression in hybrids. These characters are described and illustrated in Figure 2. Each character was given a score between 0 and 1. A pure *A. amathea* character scored 0, pure *A. fatima* scored 1, and intermediate characters received scores of 0.25, 0.5, or 0.75 depending on the level of its expression. The overall wing index used for each individual was an average of the scores of the four characters.

In the absence of wings (1977 and 1980 collections), the wing index developed here could not be used directly. However, proxy wing scores were given based on the information provided. In the field notes, individuals were placed in one of three categories: *A. fatima*, *A. amathea*, and hybrid. These were given wing index scores of 1, 0 and 0.5, respectively. In some cases where additional information was provided in the notes in the form of ''backcross to *fatima*'' or ''backcross to *amathea*,'' scores of 0.75 and 0.25 were assigned respectively.

Allozyme electrophoresis

The abdomen of each individual from the 1994 collection was homogenized on ice in 70 μl of grinding buffer (Mallet et al. 1993; Emelianov et al. 1995) and centrifuged to remove debris. Electrophoresis was performed on Gelman cellulose acetate plates using two buffer systems: phosphate (0.36% NaH_2PO_4, 0.22% Na_2HPO_4, pH 6.3) and Tris-gly (0.3% Trizma, 1.4% glycine, pH 8.6). Plates were run with samples of both species in order to ensure accurate scoring of alleles between runs. We used standard staining procedures for the enzyme loci *Got*-1, *Got*-2, *Hbdh*, *Pk*, *Gpi*, *Pgm*, *Fum*, *Ak*, *Eno*, α*Gpd*, *Acon*, *3Pgd*, *Idh*, *Me*, *Gpt*, *Pep* (*pro-pro*), and *Mpi* (Mallet et al. 1993, Emelianov et al. 1995). The frequencies of 69 alleles at these 17 loci were scored in six individuals from each of the five species of *Anartia*, representing eight populations. These frequencies were used to produce genetic distance measures and phenograms. Three loci had strong frequency differences between *A. fatima* and *A. amathea* (*Got*-2, *Hbdh*, and *Pk*). No linkage mapping has been performed on *Anartia*, so we do not know the linkage relations of these or any other loci, except that none appeared to be sex-linked. Table 2 gives details of allele frequencies and scores applied to each allele for use in a hybrid index. These scores were averaged to generate an overall allozyme hybrid index for each individual.

Mitochondrial DNA haplotyping

DNA was extracted from the thorax tissue of individuals using the phenol-chloroform method described by Brower (*http://www.ent.orst.edu/browera/insect.htm*). To gauge the amount of sequence variation both within and between *A. fatima* and *A. amathea*, and to determine the most suitable restriction enzymes to use for distinguishing the two, an ~800-bp region of the mitochondrial DNA spanning some of the cytochrome oxidase subunit I (COI) gene (position 2211 to 3009 relative to the *Drosophila yakuba* mitochondrial sequence) was sequenced in 40 individuals. These individuals (23 *A. fatima* and 17 *A. amathea*) consisted of representatives from all the 1994 and 2000 collection sites, as well as two *A. amathea* each from Trinidad and Grenada. The sequences have been deposited in GenBank (accession numbers AY010919–AY010958). PCR amplification was carried out using the published primers Jerry (5′-CAACATTTATTTTGATTTTTTGG-3′) and Pat (TCCAATGCACTAATCTGCCATATTA-3′) (Simon et al 1994). The PCR products were subject to electrophoresis through a 1% low-melting

TABLE 2. Allozyme allele frequencies across the hybrid zone—1994 data. *N* is the number of individuals scored for the allozyme loci at each site.

Locus	Mobility	Index score	Allele frequency at each site					
			214	228	239	251	261	268*
N			20	19	20	20	20	17
Glutamate oxaloacetate transaminase (*Got-2*)								
	−0.53	0	0.025	0.053	0.150	0.725	0.775	0.941
	−0.71	1	0.900	0.632	0.750	0.175	0.175	0.059
	−1.00	1	0.075	0.316	0.100	0.100	0.050	—
β-hydroxy-butyrate dehydrogenase (*Hbdh*)								
	10.00	0	0.025	—	0.025	0.125	0.050	0.233
	1.00	0	—	—	0.125	0.450	0.600	0.600
	−8.00	1	0.800	0.974	0.725	0.425	0.325	0.167
	−16.00	1	0.175	0.026	0.125	—	0.025	—
Pyruvate kinase (*Pk*)								
	1.00	0	0.025	0.132	0.100	0.725	0.775	0.912
	0.72	1	0.975	0.868	0.900	0.275	0.225	0.088

* Only 15 individuals were successfully scored for *Hbdh* at this site.

temperature agarose gel, and the DNA stained using ethidium bromide. The relevant band was cut, and the gel digested for a minimum of three hours.

These gelased PCR products were used as templates in d-Rhodamine based dideoxy-chain termination reactions. An internal primer, Dick (5′-CCAACAGGAATTAAAATTTT-TAGATGATTAGC-3′) (Simon et al 1994), was used in addition to the two flanking primers, to obtain a high level of overlap of the sequences. The reaction products were purified by centrifuging through sephadex columns and dried down. The rehydrated products were loaded into an ABI 377 automatic sequencer, and sequences were read using Sequencing Analysis 3.0 (ABI). Further editing and alignment was done with Sequencher 3.1.

Analysis of the sequences revealed that in the sequenced region, 21 base pair substitutions differentiated the *A. fatima* haplotype from the *A. amathea* haplotype. Two of these sites lay within the recognition sequences of the restriction enzymes *SacI* and *MspI*. *SacI* cut the *A. fatima* haplotype (at position 2335 relative to *D. yakuba*), and *MspI* cut the *A. amathea* haplotype (at position 2415 relative to *D. yakuba*). These differences allowed the diagnosis of individual haplotypes using a restriction fragment length polymorphism (RFLP) approach. The PCR product from each individual was separately digested overnight with each of the two enzymes (0.5 units μl^{-1}) at 37°C. Subsequently digestion products were separated out by electrophoresis on 2% agarose, and detected by staining with ethidium bromide.

Population Genetic Analysis

Allozyme allele frequencies for the five *Anartia* species were used to construct Cavalli-Sforza chord and Nei's genetic distances (Caville-Sforza and Edwards 1967; Nei 1972). The distance matrices were then used to construct phenograms using PHYLIP 3.5c (Felsenstein 1993).

Maximum-likelihood deviations from Hardy-Weinberg and two-locus equilibrium (based on the method of Hill 1974) were calculated and compared with the likelihood of no deviation in likelihood ratio tests. Deviations from Hardy-Weinberg in the allozyme data were assessed using the inbreeding coefficient F_{IS} (Wright 1965). To standardize deviations from two-locus equilibrium, we employed the pairwise gametic correlation coefficient:

$$R_{AB} = \frac{D_{AB}}{\sqrt{p_A p_B (1 - p_A)(1 - p_B)}}$$

(where p_A, p_B are the frequencies of alleles *A* and *B* at the two loci, P_{AB} is the frequency of haplotype *AB*, and $D_{AB} = P_{AB} - p_A p_B$ is the usual pairwise gametic disequilibrium coefficient (Hill 1974), which is here used for nuclear-nuclear ''linkage'' disequilibria as well as for cytonuclear disequilibria between nuclear loci and mitochondrial haplotype (see also Asmussen et al. 1989). Because double heterozygotes cannot be classified into coupling and repulsion genotypes, the interallozyme disequilibria estimated are equivalent to the composite measure Δ_{AB} of Weir (1990). We estimated correlation coefficients between each of the three allozyme loci and the mtDNA haplotype at each population sampled in 1994.

To compare disequilibria among allozymes and mitochondrial haplotypes with disequilibria at loci underlying quantitative traits (wing pattern) for the 1994 samples, we used the disequilibrium measure of *D** suggested by Nürnberger et al. (1996), as follows:

$$D^* = \frac{2\ \mathrm{cov}(z, z')}{(1 + F_{IS})\Delta z \Delta z'}$$

(where Δz and $\Delta z'$ are the differences between means of the quantitative characters z and z' across the hybrid zone). Each allozyme or haplotype locus was treated as if it had only two types of alleles—*A. fatima* (with a score of 1) or *A. amathea* (scored 0). The lumping of allozyme alleles into *A. fatima* and *A. amathea* types followed the index score shown for each allele in Table 2. For allozyme and mtDNA alleles, the value of Δz is 1, since the allelic differences were almost completely fixed. Our hybrid index for wing-color pattern is in this case a quantitative trait with Δz again standardized to 1. Values of F_{IS} were averaged across allozymes for 1994

data, but could not be calculated for 2000 data, where no allozyme data was available. However, the values of F_{IS} in this part of the hybrid zone in 1994 were low (~0.1) and anyway insignificant, so the results are little affected by setting $F_{IS} = 0$, which was accordingly done.

We present values of D^*, which, like D, has a maximum of 0.25 when the underlying allele frequencies are approximately 0.5. We also present values of the product-moment correlation, r, together with significance values for that measure. Because the variance for a trait depends on the number of loci, these values of r are not strictly comparable to the disequilibrium measures D^* or R. Nonetheless, the high values of r show clearly that the covariance between traits is near the maximum possible in populations with high D^* and R, so that r has some heuristic value.

Cline Analysis

We fitted maximum-likelihood clines to wing data from 1980, 1994, and 2000, the mtDNA data from 1994 and 2000, and to allozyme data from 1994 using the ''Fit 1D Cline'' routine in ANALYSE 1.30 PPC (Barton and Baird 1996). Using a Metropolis algorithm, ANALYSE can fit a tanh curve to cline data using four variables—p_{max}, p_{min} (the maximum and minimum gene frequency values in the tail ends of a cline), cline width, and cline center. However, when clines were fit for the purposes of this study, p_{min} and p_{max} were fixed and not allowed to vary. Though this might seem to take away much of the advantage of a using likelihood analysis method, the small number of collection sites mean that the software would otherwise try to fit four variables to six points or less (the number of sites used in this analysis), producing results that are not biologically sensible. For wing and mtDNA clines, p_{max} was fixed at the average index score at the 190 km site, this being the site furthest into the *A. fatima* side in 1994. This made $p_{max} = 1$ for the mtDNA, and > 0.98 for most of the other clines. For the allozyme cline, the average for the 214-km site was used for p_{max}, since individuals from the 190-km site were not scored for allozymes. The p_{min}-values were obtained in a slightly different manner. The 268-km collection was the site deepest into the *A. amathea* side, and in 1994 consisted of 18 pure-looking *A. amathea*, and only one pure-looking *A. fatima* (also *fatima*-like at allozymes and mtDNA). It was assumed that upon moving further into *A. amathea* territory, *A. fatima* alleles would be absent. Thus, the p_{min}-value for all three clines was fixed to be the average index scores at the 268-km site in 1994, ignoring the single *A. fatima* individual that was caught there, giving $p_{min} = 0$ for the mtDNA, and < 0.02 for most of the other clines. The p_{min} and p_{max} values so obtained are thought to reflect the variation present in pure *A. fatima* and *A. amathea* populations. We also assumed a simple single locus tanh cline (the routine can also fit a more complex stepped cline, but this would also require more data) with $F_{ST} = 0$. Butterflies typically have very low local F_{ST} (e.g., Jiggins et al. 1997; Lewis et al. 1997; Joron et al. 2001).

To test for differences between parameter values, we used the result that twice the difference in $\log_e$ likelihood between two models asymptotically follows a χ^2 distribution. Support limits for parameters such as cline centers and widths were obtained where the $\log_e$ likelihood drops to two units below the maximum. These support limits are asymptotically equivalent to 95% confidence limits (Edwards 1972).

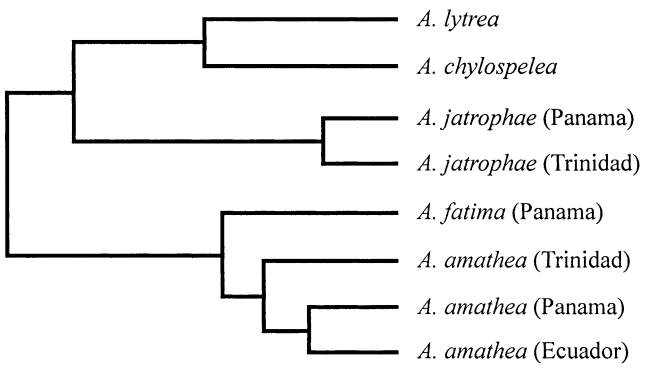

FIG. 3. Phenogram of species of *Anartia*. The UPGMA phenogram was obtained with PHYLIP version 3.5c (Felsenstein 1993), based on Cavalli-Sforza chord distances (see scale bar) from 69 allele frequencies at 17 allozyme loci. The phenogram is robust to distance measures and cluster analysis methodology; for example, analysis based on Nei's D and neighbor joining, or CONTML (restricted maximum-likelihood) give the same topology.

RESULTS

Relationships between Anartia Species

A UPGMA phenogram (Fig. 3) gives an approximate measure of relatedness assuming clocklike evolution of allozyme allele frequencies. These relationships concur approximately with geography where sister taxa occur in allopatry. Thus, the Caribbean sister species *A. lytrea* and *A. chrysopelea* are found on different Caribbean islands, and *A. fatima* and *A. amathea* co-occur only at the hybrid zone in Panama. *Anartia jatrophae*, which has no close relatives, flies together with the other species virtually throughout their combined range. These relationships, at least in unrooted form, do not differ significantly from analysis based on DNA sequence data (Blum et al., unpubl. ms.). The main result of interest from the point of view of this paper is the sister-taxon relationship between *A. fatima* and *A. amathea*.

Cline Shape across the Hybrid Zone in 1994

A summary of all the data is shown in Table 3; the fitted clines for overall wing pattern, allozyme and mtDNA indices for the 1994 collection are shown in Figure 4. The calculated cline centers and widths (together with support limits) are shown in Table 4.

The concordance of the clines (i.e., the similarity of cline shape) for different characters was examined by using ANALYSE to fit a tanh curve to the data for one character while keeping the cline width fixed to that of another character (i.e., allowing only the cline center to vary). If the $\log_e$ likelihood of the resulting cline width was within two units of the maximum, the clines for the two characters were deemed concordant. This analysis showed that wing pattern, allozyme average, and mtDNA clines were all concordant in 1994 (Fig. 4). Similarly the four wing character clines were concordant with one another, as were the three allozyme clines.

TABLE 3. Details of collections made along the hybrid zone between 1977 and 2000 together with the hybrid index scores for each population.

Year	Distance/ km[1]	Sample size[2]	Wing index[2]	mtDNA index	Allozyme index
2000	147	9 (9)	0.97 (0.97)	1.00	—
	221	50 (82)	0.96 (0.98)	0.98	—
	237	13 (43)	0.94 (0.97)	1.00	—
	247	34 (86)	0.83 (0.86)	0.88	—
	268	26 (42)	0.76 (0.76)	0.85	—
1994	190	32	0.98	1.00	—
	214	21	0.95	1.00	0.98
	228	20	0.95	1.00	0.94
	239	26	0.82	0.84	0.87
	251	29	0.23	0.41	0.33
	261	20	0.22	0.26	0.27
	268	20	0.05	0.05	0.10
1980	109	3	1.00	—	—
	159	3	1.00	—	—
	187	75	0.85	—	—
	229	17	0.63	—	—
	246	15	0.08	—	—
1977	186	76	0.71	—	—

[1] Distances are kilometers from Panama City.
[2] Figures in parentheses include individuals from the August 2000 collection. The mitochondrial haplotype of these individuals was not established.

The coincidence of the clines (i.e., the similarity in position) for different characters was investigated by using ANALYSE to fit the maximum likelihood curve to the data for one character while keeping the cline center fixed to that of another character (i.e., allowing only the cline width to vary). In no case was the change in $\log_e$ likelihood > 2 compared to the maximum likelihood cline; thus all the clines in 1994 were considered to be coincident.

Hardy-Weinberg Equilibrium, and Linkage Disequilibrium

Some of the values at the *Got*-2 locus in Table 5 indicate significant positive deviations from Hardy-Weinberg equilibrium (i.e. heterozygote deficit), especially in the more *amathea*-like hybrid zone populations. The other two loci tested show similarly positive values of F_{IS}, but the deviations are not significant. However, it is notoriously difficult to detect deviations from Hardy-Weinberg with sample sizes as small as those available. Deviations from Hardy-Weinberg are not surprising as it is known that *A. fatima* and *A. amathea* show strong assortative mating and that F_2 survivorship is reduced (Silberglied 1984; Davies at al. 1997), which should reduce the fraction of heterozygotes.

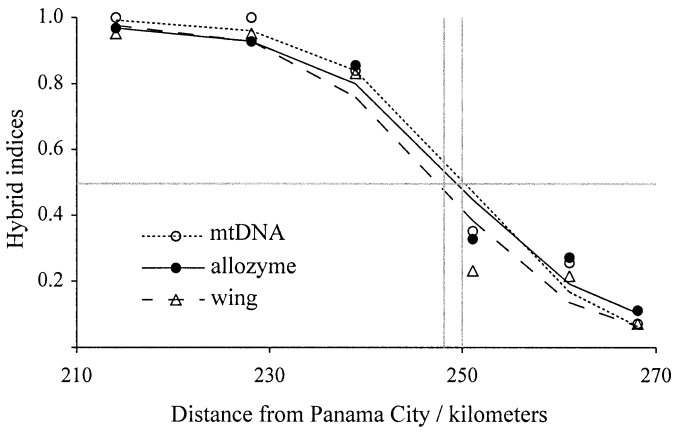

FIG. 4. Clines associated with the hybrid zone in 1994. The allozyme cline is the hybrid index, an average of the three loci measured, and the wing cline is the average of the four wing characters scored. The two vertical lines represent the cline centers for the outer clines.

TABLE 4. Cline centers and widths obtained by fitting maximum-likelihood curves to the 1994 data. Figures in parentheses are support limits. The value for the cline center is the distance from Panama City.

		Center (km)	Width (km)
Wing pattern	1. Band solidity	246 (242–250)	25 (16–37)
	2. Red upper fw	248 (243–252)	30 (19–45)
	3. Red upper hw	247 (242–252)	31 (20–49)
	4. Spot under fw	249 (245–253)	26 (18–38)
	Total wing index	248 (243–252)	28 (19–44)
Allozymes	*Pk*	248 (244–253)	28 (17–44)
	Got-2	249 (245–253)	27 (19–41)
	Hbdh	251 (246–256)	26 (15–44)
	Total allozyme index	249 (245–254)	28 (17–44)
mtDNA		250 (246–254)	27 (19–40)

Table 6 contains the results of the linkage disequilibrium analysis for 1994. Significant positive gametic correlations are present between all loci in the hybrid zone. Table 7 contains the correlation coefficients and the linkage disequilibria values between the wing, mtDNA and averaged allozyme indices, which all show a similar pattern. These correlations and disequilibria scores are strong even between nuclear loci and mtDNA that are unlinked, suggesting that gametic disequilibria are not dependent on physical linkage, but must be due to gene flow between taxa. Again, disequilibria are typically stronger in the more *amathea*-like populations of the hybrid zone.

Cline Movement over 20 Years

The cline centers and widths (based on wing-color patterns) calculated for 1980, 1994, and 2000 are shown in Table 8. The data, along with their fitted curves, are shown in Figure 5. ANALYSE was used as in the previous analysis to show that these three clines were significantly noncoincident, indicating a genuine shift of the cline centers to the east during the 20-year sampling period ($G = -2\Delta\log_e L = 30$ and 38, from comparing the 1994 cline center with 1980 and 2000 respectively, df $= 2$, $P < 0.001$).

TABLE 5. Heterozygote deficit (F_{IS}) values by locus at each population sampled in 1994.

Locus	No. of alleles	F_{IS} at each sampled population					
		214	228	239	251	261	268
Got-2	3	−0.03	−0.12	0.22	0.62*	0.57*	1.00*
Hbdh	4	−0.03	—	0.22	0.28	0.12	0.28
Pk	2	−0.03	0.31	−0.11	−0.13	0.28	0.63

* Significant F_{IS} (likelihood ratio tests, $P < 0.05$).

TABLE 6. Gametic correlations (linkage disequilibria) in hybrid zone populations of *Anartia fatima* × *A. amathea* sampled in 1994. Correlations are shown between all pairs of allozyme loci as well as with mtDNA haplotype.

	Site	*Got-2*	*Pk*	*Hbdh*
Pk	214	−0.03		
	228	0.12		
	239	0.55*		
	251	0.61*		
	261	0.86*		
	268	0.80*		
Hbdh	214	−0.03	−0.03	
	228	—	—	
	239	0.60*	0.30	
	251	0.47*	0.31	
	261	0.60*	0.46*	
	268	0.60*	0.45*	
mtDNA	214	—	—	—
	228	—	—	—
	239	0.43*	0.73	0.03
	251	0.46*	0.33	0.01
	261	0.88*	0.83*	0.83*
	268	0.99*	0.80*	0.60*

"—" indicates one or both loci involved are fixed so that disequilibrium calculation is impossible. Tests were not done at site 190 because of a lack of allozyme data. (* = $P < 0.05$).

The figures shown in Table 8 must be interpreted with some caution. The cline center information is of particular importance, as it reveals the position of the hybrid zone over the course of 20 years. The relatively large support limits for the cline center in 1980 is due to the collection sites not adequately covering the hybrid zone. Movement of the hybrid zone towards inaccessible habitat means that in 2000, collections over only a small part of the hybrid zone were possible. As only the western end of the hybrid zone was captured in 2000, the lower support limits of 274 km and 279 km for the wing and mtDNA data, respectively, are used as conservative values for this cline center. These data give an average hybrid zone movement rate of 2.5 km per year over the period 1980–2000. Between 1980 and 1994, the cline appears to have moved at 1.9 km per year, and at 3.8 km per year between 1994 and 2000 (but probably higher, as this is based on the lower support limit for cline center in 2000).

The information on cline widths is of limited use due to the large support limits for the 1980 and 2000 collections caused by the paucity of data. For the accurate calculation of cline width and its support limits, ANALYSE requires sufficient data points within the hybrid zone. As we have few samples within the hybrid zone, and because some of them are clearly out of line with the majority of the data at all other loci (e.g., km 251 in 1994), the support limits for cline widths are questionable. Therefore, apparently significant changes in cline width with time (Table 8) are in our view untrustworthy.

Figure 6 demonstrates that the movement of the wing pattern cline from 1994 to 2000 was accompanied by a virtually identical shift in the mtDNA cline. Even though the hybrid

TABLE 7. Correlation coefficients, *r*, and disequilibrium, *D**, between the wing, allozyme and mtDNA indices in the 1994 and 2000 populations.

		r		*D**	
	Site	mtDNA	wing	mtDNA	wing
Allozymes 1994	190	—	—	—	—
	214	—	0.10	—	0.00
	228	—	−0.18	—	0.00
	239	0.27	0.44*	0.04	0.04
	251	0.25	0.29	0.07	0.05
	261	0.78**	0.92**	0.19	0.18
	268	0.95**	0.94**	0.07	0.06
Wing 1994	190	—		—	
	214	—		—	
	228	—		—	
	239	0.50*		0.09	
	251	0.56**		0.16	
	261	0.87**		0.23	
	268	0.99**		0.06	
Wing 2000	147	—		—	
	221	0.09		0.00	
	237	—		—	
	246	0.74*		0.07	
	268	0.85**		0.12	

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

TABLE 8. Cline centers and widths obtained by fitting curves to the collection data. Figures in parentheses are support limits. The value for the cline center is the distance from Panama City.

	Year	Center (km)	Width (km)
Wing pattern	1980	224 (213–239)	74 (52–118)
	1994	247 (243–251)[1]	27 (18–40)[1]
	2000[2]	*291* (273–*420*)	*84* (*48–322*)
	2000	*283* (272–*316*)	*57* (*35–116*)
mtDNA	1994	250 (247–254)	26 (19–38)
	2000[2]	*303* (279–*543*)	*89* (*45–454*)

Italicized values should be interpreted with caution; see text for details.

[1] The small differences between these values and the values shown in Table 4 for the total wing index are a result of a slightly larger data set being used here, including the 190 km site, and more individuals for some of the other sites.

[2] Excluding individuals from the August 2000 collection whose mitochondrial haplotype was not established.

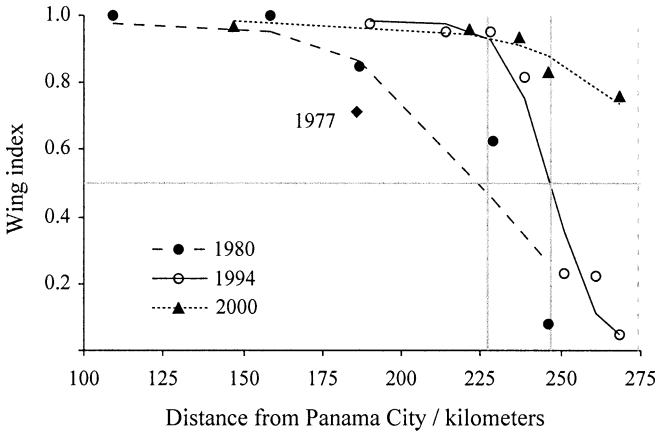

FIG. 5. Clines fitted to the wing data for the years 1980, 1994, and 2000. Only a single data point was available from 1977. The vertical lines represent calculated cline centers for 1980 and 1994, respectively. The broken vertical line represents the lower support limit for the cline center in 2000 (see text for details).

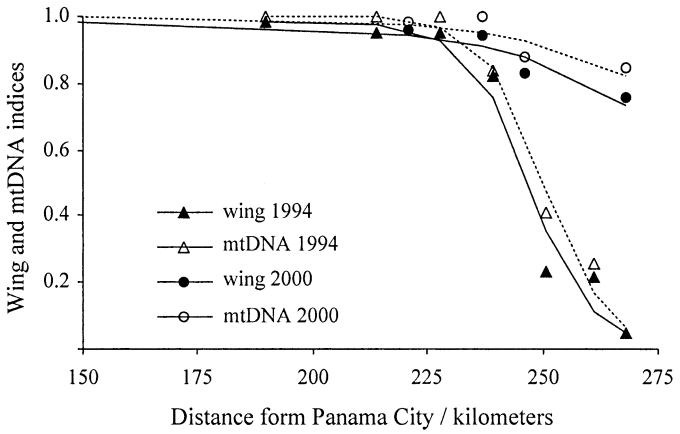

FIG. 6. Clines fitted to the wing and mtDNA data from 1994 and 2000. The wing and mtDNA cline coincide even though the clines have moved.

zone has moved, the mtDNA and wing clines were found to be coincident and concordant in both years.

DISCUSSION

Absence of mtDNA Introgression

Coincident clines are a common feature of hybrid zones. In the case of the *Anartia amathea*/*fatima* hybrid zone, the wing morphology, allozyme, and mtDNA clines associated with the hybrid zone are not only coincident, but also overlie one another very closely. This is similar to the *Bombina* hybrid zone where the clines in allozyme, belly pattern, mtDNA, and mating call are very similar (Szymura and Barton 1986). However, mtDNA clines at hybrid zones are often discordant with the clines associated with nuclear loci. Many believe that mitochondrial markers are selectively neutral, and that the selective interactions between mtDNA and nuclear loci are weaker since mtDNA segregates independently of the chromosomes. A number of analyses of hybrid zones with asymmetrical mtDNA introgression have implicitly assumed neutrality of mtDNA markers (Marchant et al. 1988; Gyllensten et al. 1985; Takahata and Slatkin 1984; Rohwer et al. 2001).

The *Anartia* hybrid zone shows no evidence of mtDNA introgression, with the mtDNA cline coinciding very closely with the allozyme clines. Of the 132 pure-looking *A. fatima* and *A. amathea* caught in 2000 within 100 km of the hybrid zone, only two had a foreign haplotype (one from each species). The asymmetrical reduced tendency to mate for F_1 hybrid females from the cross *A. amathea* (female) × *A. fatima* (male) (Davies et al 1997) might be expected to cause introgression of the *A. fatima* haplotype into *A. amathea*. Unfortunately, it was not possible to carry out any sampling in the *A. amathea* side because of guerrilla activity. But the shape of the mtDNA cline, and its concordance with nuclear clines, reveals no differential introgression (Fig. 4), and the ~80–90% cytonuclear correlation on the *amathea* side of the zone (km 261, 268; Tables 7, 8) suggests a general lack of introgression.

In the center of the hybrid zone and westwards (e.g., km 251 in 1994), at least 30% of the individuals are hybrids (i.e., have allozyme hybrid indices between 2 and 4, Fig. 7). Therefore, it is not the case that rare sporadic hybridization is preventing introgression. Davies et al. (1997) have shown that hybrid fitness is greatly reduced as a result of reduced fertility of F_1 females and low F_2 larval survivorship. Thus, despite hybrids being formed in relatively high numbers, strong selection against the hybrids and their progeny, coupled with further immigration from the *fatima* side of the zone, must cause the observed loss of all *amathea* genomic markers on the western *fatima* side of the zone.

If this hybrid zone is indeed as ancient as the Great Faunal Interchange three million years ago, *A. amathea* and *A. fatima* could have been exchanging genes through the hybrid zone for a long time. This length of time seems long enough for all truly neutral alleles to have diffused across and to have reached a state of equilibrium near the hybrid zone. Therefore, any loci at present showing marked differences in allele frequencies between the two species across the narrow width of the hybrid zone are probably either under differential selection or are tightly linked to loci under selection. The lack of mitochondrial introgression therefore suggests that the divergent mitochondrial haplotypes are selected against in a foreign nuclear type; in other words, some genes on the mitochondrial genome are under epistatic selection in these species. Studies suggest that similar nuclear-cytoplasmic epistasis occurs in *Drosophila* (Hutter and Rand 1995; Kilpatrick and Rand 1995). The case for this becomes more compelling when the cline movement is taken into account. Figure 6 shows the surprising result that the movement of the wing pattern from 1994 to 2000 is matched by an equivalent movement of mtDNA, such that their clines remain highly concordant. If mtDNA is neutral, we should to be able to detect *A. amathea* haplotypes in the ''wake'' of the moving hybrid zone (cf. Rohwer et al. 2001). No such individuals were found. Even though this *A. amathea* ''wake'' would in time be diluted by immigration of *A. fatima* haplotypes into the region, since only 14 and 6 years have passed between the three sampling periods, if such a ''wake'' existed it probably would have been detected.

A number of studies have inferred hybrid zone motion from observed asymmetrical introgression across hybrid zones, assuming that the mitochondrial or other genetic wake is a relict of that movement (Parsons et al. 1993; Rohwer et al. 2001). However, we have a situation that is the complete converse: the *Anartia* hybrid zone has demonstrably moved, yet has left no mitochondrial wake behind. Thus, asymmetry of introgression seems as likely to be due to asymmetrical permeability (for example, due to asymmetrical Haldane's Rule effects, as in *Anartia*) or asymmetrical selection (global advantage of one haplotype), resulting in introgression of mitochondrial or nuclear DNA across a stationary hybrid zone (Ferris et al. 1983). We therefore propose that asymmetry of introgression, or lack of introgression of molecular markers, is relatively unconvincing evidence either for or against hybrid zone movement. Asymmetry of gametic disequilibria, on the other hand, may provide better evidence (see next section).

Gametic Correlations and Genotypic Clustering

Figure 7 illustrates another interesting feature of this particular hybrid zone. The distribution of individuals' allozyme

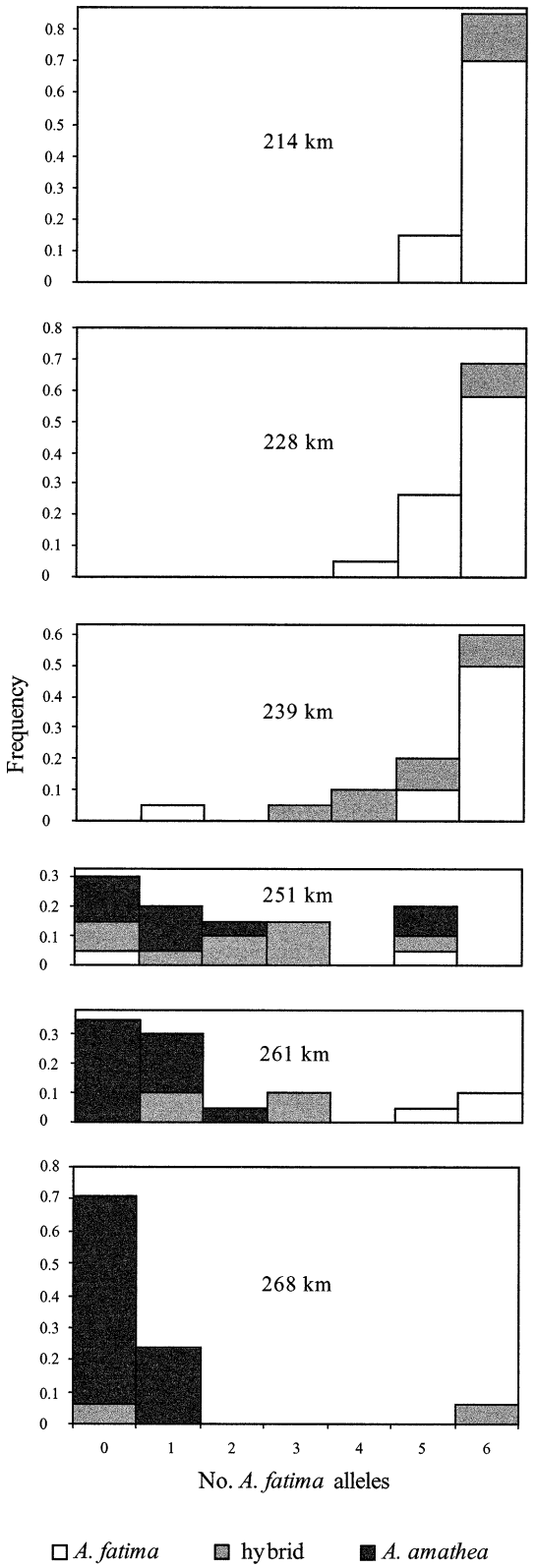

FIG. 7. Distribution of allozyme hybrid index scores in populations sampled in 1994. A hybrid index of 6 represents pure *A. fatima*, while 0 represents pure *A. amathea*. The different shades indicate whether individuals had *A. fatima*, hybrid (wing index between 0.15 and 0.85), or *A. amathea* wing coloration.

scores at the center of the hybrid zone (the 251-km population) is flat to bimodal, as opposed to unimodal, as expected in a randomly mating population. A bimodally distributed population is characteristic of a situation where hybridization between the two species is occasional and selection against hybrids is strong (Jiggins et al. 1997, Jiggins and Mallet 2000). In such cases the two taxa can be considered separate species, even though there may be evidence for gene flow in the form of hybrids and recombinants. In contrast, unimodally distributed populations are characteristic of a situation where disruptive selection is relatively weak and hybridization is common; this can be the case even in very strongly divergent and apparently ancient pairs of taxa, as in *Oniscus* woodlice (Bilton et al. 1999). Here the two forms in contact cannot be called separate species, because it is not possible to identify distinct forms in mixed populations. The *A. fatima*/*A. amathea* hybrid zone, with its flat to bimodal distribution of allozyme scores at the hybrid zone center (Fig. 7), represents a borderline case: moderate reproductive barriers are present together with frequent although not panmictic hybridization. If distinguishable genotypic clusters are taken as evidence for separate species, these *Anartia* are at or just beyond the threshold of speciation.

There is an interesting pattern of gametic and phenotypic correlations across the hybrid zone. In general, heterozygote deficits at single allozyme loci (Table 5), nuclear and cytonuclear disequilibria (Table 6 and 7), and correlations between phenotypes (Table 7) are greatest on the *amathea* (eastern) side of the hybrid zone, at sites 261 and 268. The disequilibria are due to a few virtually pure *fatima* individuals within eastern populations consisting mainly of *amathea*, whereas, on the *fatima* (western) side of the hybrid zone, most individuals have intermediate hybrid indices (Fig. 7), and heterozygote deficits, disequilibria, and correlations are much lower (Tables 5–7). This is exactly the pattern expected if the cline is moving eastwards. Mixing would first take place when *fatima* individuals move eastwards into the *amathea* range, leading to initially high disequilibria and heterozygote deficits on the eastern side of the zone. Subsequently, in these populations this would be followed by a period of hybridization and recombination that reduce disequilibria and heterozygote deficits in the western end of the hybrid zone, as the cline center moves on. Finally, the hybrid genomes are outcompeted by further immigrants of pure *fatima* from the west. We should, however, be somewhat cautious of drawing too many conclusions from this data: first the disequilibria at different loci and hybrid deficits are not statistically independent and second, the 1994 sample sizes are not large enough for such tests. It is also true that certain patterns of asymmetric hybridization or asymmetric selection other than cline movement may also cause asymmetric patterns of disequilibria. Nonetheless, it is intriguing that the pattern of correlations appears to be as expected for a hybrid zone that is advancing to the east, as is in fact occurring.

Cline Movement

This study shows conclusively that the *Anartia* hybrid zone is moving eastwards in favor of *A. fatima*. The evidence is

based on three observation periods (four if the 1977 single location sample is counted) and reveals a consistent story.

In estimating hybrid zone movement, we have ignored possible seasonal fluctuations of the hybrid zone position. Local *Anartia* populations are known to fluctuate: populations diminish during the drier months (mid-December to mid-May), and local extinctions are frequent (Silberglied et al 1979). However, the 1994 collection was based on two field trips: 20–21 December 1993 and 21 February 1994; and the 2000 collection on three trips: 4–7 May 2000, 2–4 June 2000, and 27–28 August (dry to wet season transition). This has allowed measurement of any seasonal variation that may be associated with the hybrid zone. Information collected in different months from these years shows no evidence for seasonal variation of cline position within the same collecting year.

It is difficult to guess the ecological causes of hybrid zone movement, especially as *A. fatima* and *A. amathea* have very similar ecological requirements (Silberglied et al 1979). It is also not known for how long the observed motion has been occurring. If the hybrid zone is three million years old (corresponding to the rise of the Panama isthmus, see Silberglied et al. 1979), the current direction and speed of the movement must have begun very recently. Alternatively, in view of the rapid movement we observe, the hybrid zone could have originated far away and may have nothing to do with the rise of the Isthmus of Panama.

The rate of motion appears to have accelerated from 1.9 to $>$ 3.8 km per year over the study period. A possible correlate is with forest cover change. Deforestation of the Darién Province, where the hybrid zone occurs, has followed the development of the Pan-American Highway through the center of Darién. Subsequently this led to continued logging and clear-cutting of the seasonally dry forest for crops and pasture. It is possible that this threw a previously stationary hybrid zone into disequilibrium. *Anartia* butterflies are known to prefer open habitats, and are specialists on open grassy areas and forest clearings. Deforestation in Panama has probably made both species more abundant. *Anartia fatima* now inhabits the entire length of Panama (except the more highly forested east Darién) in higher densities than possible when forest was the dominant vegetation. *Anartia amathea*, in contrast, is found east of the hybrid zone in the Atrato region of Colombia, in land that is still chiefly under forest. Presumably, it is found here in lower densities than the *A. fatima* population to the west of the hybrid zone. The movement of the hybrid zone may therefore reflect the higher numbers of *A. fatima* migrating eastwards relative to the fewer *A. amathea* emerging from scattered clearings and migrating westwards, as a result of their differing population densities. This hypothesis is supported by the asymmetry found in the linkage disequilibria and gametic correlations, suggesting the pure *fatima* colonizes *amathea* sites more readily than vice versa.

Recently, however, another moving hybrid zone has been found in the Darién, between color-pattern races of the butterfly *Heliconius erato*. This zone is moving in the opposite direction to the *Anartia* hybrid zone, but at similar speed (Mallet 1986; M. J. Blum, unpubl. ms.). Like *Anartia*, *H. erato* also benefits from human activities and deforestation, and is abundant throughout degraded habitats in the Darién. The opposite direction of cline movement in these two pairs of human-associated taxa suggests that movement is controlled more by ecological and genetic factors peculiar to each pair of taxa, rather than by a single external cause.

Evidence for and Measurement of Selection

In this hybrid zone, there is strong selection against heterozygotes (s), and there is evidence for a competitive advantage of *fatima* (S). A simple single-locus model incorporating both effects gives the *FF*, *FA*, and *AA* genotypes fitnesses of $1 + 2S$, $1 - s + S$, and 1 (Barton 1979), where *F* and *A* alleles are *fatima* and *amathea* alleles. Taking the gene flow rate (measured as the standard deviation in distance between parent and offspring) to be σ, the width (i.e., inverse of the maximum gradient) of such a cline will be approximately $\sqrt{8\sigma^2/s}$ (Eq. 1), and it will move at a speed of approximately $(S/2)\sqrt{2\sigma^2/s}$ per generation (Eq. 2) toward the *amathea* side (Barton 1979). The hybrid zone here is clearly not dependent on a single-locus difference, but because there are extremely high gametic disequilibria and correlations between traits, we can treat the clines as though they were a single locus under extremely strong selection (in fact the selection acts on the whole genome).

Egg hatch, larval survivorship, and hybrid mating propensity are all reduced in F_1, F_2, or backcross broods between *A. fatima* and *A. amathea* (Davies et al. 1997). This reduction in fitness, coupled with the strong linkage disequilibria found in the hybrid zone (Tables 7, 8) indicates that $s \approx 0.5$ (or possibly greater). In a number of hybrid zones, linkage disequilibria can be used to estimate the rates of gene flow, so that selection pressures can be estimated indirectly using cline theory (e.g., Szymura and Barton 1986, 1991; Mallet et al. 1990). However, these analyses depend on the assumption of random mating and unimodality of genotypes. In this study, linkage disequilibria (Tables 7, 8) are enhanced by assortative mating (Davies et al. 1997) as well as gene flow, a situation for which there is as yet no applicable theory (but see Nurnberger et al. 1996 for an example where analysis of clines was performed under conditions of high F_{IS}). We have some idea of the value of s, and also have estimates of both cline width and velocity. By rearranging Equations 1 and 2, and substituting these known parameters into them, we can gain an approximate idea of the competitive advantage, S, and the gene flow rate, σ.

Reliable estimates of cline width range from 27 km in 1994 to 74 km in 1980 (the upper estimate is less certain due to the paucity of data in that year). Thus, from Equation 1, the gene flow rate, $\sigma = 6.8$–18.5 km generation$^{-1/2}$. The hybrid zone is moving at a speed of about 2.5 km/year, and there are approximately eight generations a year (Silberglied et al. 1979). From Equation 2 this would suggest a selective advantage of *fatima* over *amathea* of $S = 0.02$–0.05. The theory underlying these calculations is dependent on several approximations, such as small selection pressures, that are probably violated in this example (though simulations have shown that for values of s up to 0.5, the results deviate by only 10–20% from their true value). However, they give an order-of-magnitude idea of the forces that must be involved in maintaining the hybrid zone and powering its movement.

Evolutionary and Biogeographic Implications of a Moving Hybrid Zone

Although most hybrid zones are thought to be stationary (Barton and Hewitt 1981, 1985), few studies have investigated suitable species with as many as eight generations per year. Thus, the absence of apparent movement in most studies to date may be due to the slow speed of any movement with respect to human lifetimes, rather than to an absence of movement on a geological time scale. It would also be the case that if movement of the sort we measure is common and rapid, most hybrid zones would either quickly move to the edge of the taxon pair's range and become extinguished, or become trapped at some population structural boundary (Barton and Hewitt 1981). Thus, even if hybrid zone movement is an important component of evolutionary change, most hybrid zones found today could still actually be stationary.

The existence of moving hybrid zones casts doubt on the reliability of associating Pleistocene refugia with current centers of species endemism in the Neotropics (Mallet 1993). Given that a hybrid zone between a closely related pair of *Anartia* species has been shown to move at about 2.5 km/year, biogeographic patterns of endemism set up in refugia tens of thousands of years ago may by now have become very blurred or undetectable. As well as casting doubt on maps of refugia based on the current positions of hybrid zones, this result has strong implications for conservation measures which concentrate on protecting areas thought to have been refugia during the Pleistocene (e.g., Brown 1979), but which are not currently centers of endemism: it may be better to concentrate on conserving areas with high current species diversity than to trust inferences about the past from current patterns of distribution.

The possibility of evolutionary change occurring due to a shifting balance between different adaptive peaks as suggested by Wright (1932, 1977) is often rejected, in part due to lack of evidence for Phase III, the spread of a new adaptive peak (Coyne et al. 2000). Whether or not the movement of the *Anartia* hybrid zone is due to human-caused changes in the environment, the existence of such moving hybrid zones shows that the key process equivalent to Phase III occurs naturally. Moving hybrid zones and resultant range changes which are rapid, at least when viewed on a geological timescale, could be important in evolution, biogeography, and speciation.

Acknowledgments

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