Evolution, 56(11), 2002, pp. 2296–2312

A FINE-SCALE SPATIAL ANALYSIS OF THE MOSAIC HYBRID ZONE BETWEEN *GRYLLUS FIRMUS* AND *GRYLLUS PENNSYLVANICUS*

CHARLES L. ROSS[1,2] AND RICHARD G. HARRISON[1]
[1]*Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York 14853*

Abstract.—The pattern of character variation within a hybrid zone, the hybrid zone structure, has been used to infer the processes that maintain hybrid zones. Unfortunately it is difficult to infer process from structure alone because many different processes can produce the same pattern of character variation. Mosaic hybrid zones may be maintained by exogenous selection in a heterogeneous environment and/or endogenous selection against hybrid individuals; habitat preference, premating isolating barriers and/or fertility selection can also contribute. The spatial scale at which a hybrid zone is sampled affects its apparent structure; a hybrid zone may appear clinal at one scale and mosaic at another. Here, we sample the mosaic hybrid zone between two field crickets, *Gryllus firmus* and *G. pennsylvanicus*, at a scale that spans the boundaries between individual soil-habitat patches. From our analysis, we find that at fine scales, the mosaic hybrid zone resolves into a set of steep clines across patch boundaries. Both morphological and molecular traits exhibit sharp and generally concordant clines. However, clines for mitochondrial DNA and one anonymous nuclear marker are clearly displaced as a result of current hybridization or past introgression (the ''ghost of hybridization past''). Thus, scale is important for the structure of this and probably other hybrid zones. The extremely sharp, concordant clines across patch boundaries indicate that the cricket hybrid zone is undoubtedly structured by selection. However, the detailed mechanisms responsible for the maintenance of the hybrid zone—whether endogenous selection against hybrids, exogenous selection by the environment, and/or behavioral preferences for mates or habitats—remain to be elucidated. Determining these mechanisms will depend on closer inspection of the organisms themselves and their interactions, as is the case for all hybrid zones.

Key words.—Clines, crickets, *Gryllus*, hybrid zone, mosaic, spatial scale.

Received March 25, 2002. Accepted July 21, 2002.

Although hybrid zone structure, the spatial distribution of phenotypes and genotypes, has been described for numerous pairs of hybridizing taxa (Harrison 1993; Arnold 1997), discovering the processes that are responsible for observed patterns has proved more difficult. Elucidating hybrid zone structure is a relatively straightforward endeavor of identifying the spatial distribution of traits and alleles among individuals and populations. Spatially, hybrid zones may be broadly characterized as clinal or mosaic in nature (Harrison 1990). In clinal hybrid zones, pure individuals (or populations) meet and hybridize to form a smooth transition (or stepped cline) from one parental type to the other. Mosaic hybrid zones are characterized by a patchwork of alternating pure parental types (or populations) throughout the zone of hybridization, with abrupt transitions and reversals in the character of individuals and populations through space. The relationship between these structural types is not clear. Certainly, one basic difference between them is their dimensionality. Clinal zones generally are treated as one-dimensional (although see Sites et al. 1995; Bridle et al. 2001; Marshall and Sites 2001), whereas mosaic zones require two dimensions to describe. However, it is uncertain whether these hybrid zones differ only in this aspect (mosaic zones representing sets of clinal zones for numerous pairs of pure populations) or whether there are different forces maintaining the patterns as well.

Structure has been used to infer the processes that maintain many hybrid zones (Endler 1977; Barton and Hewitt 1985; Harrison 1990, 1993; Barton and Gale 1993; Arnold 1997). Tension zones, for example, which have a clinal pattern, are zones maintained by intrinsic (endogenous) selection against hybrids, balanced by dispersal and recombination (Key 1968; Barton and Hewitt 1985, 1989; Barton and Gale 1993). Other clinal hybrid zones may be maintained by extrinsic (exogenous) selection, in which case the position of the hybrid zone clines should coincide with environmental gradients, ecotones, or other natural barriers to dispersal (Endler 1977), although tension zones may also settle in these places. Independent of the nature of the selective forces acting, the shape of character clines across a hybrid zone can be used to estimate the strength of selection (Szymura and Barton 1986, 1991; Barton and Gale 1993).

Many mosaic hybrid zones are presumably maintained by extrinsic selection in a heterogeneous environment. Different habitat patches, which favor one parental form or the other, structure the hybrid zone into a mosaic pattern, and hybrids, which have lower fitness in either habitat, are repeatedly produced and selected against. Consequently, the environment structures the hybrid zone as well as providing the framework to maintain it. Unfortunately, from pattern alone, it may be difficult to discern the specific mechanisms that control the structure of many hybrid zones (Moore and Price 1993; Ross 2000). Indeed, many hybrid zones may be maintained by a combination of endogenous and exogenous selection, perhaps coupled with habitat preferences. By understanding more about the structure of hybrid zones in general, however, a link between structure and maintenance can be elaborated.

Spatial scale can be an important issue when looking at the structures of hybrid zones because patterns of variation will appear only at specific spatial scales. Different patterns may be revealed by sampling at different spatial resolutions, and some patterns may be overlooked because they exist on

[2] Present address: Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721; E-mail: clross@email.arizona.edu.

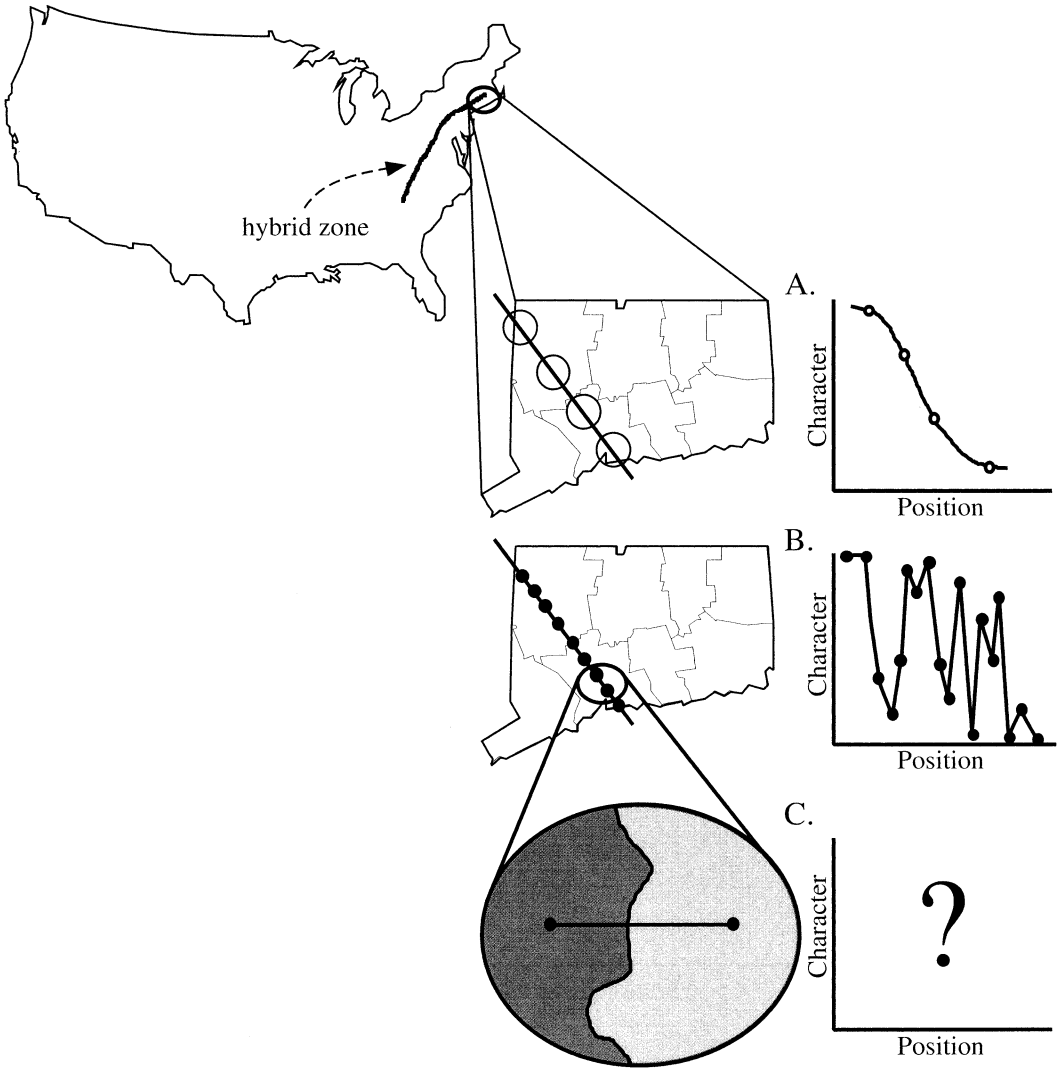

FIG. 1. Effect of spatial scale on cline shape in the *Gryllus* hybrid zone. At continental scales, the hybrid zone represents a long but narrow region of overlap and hybridization between two widespread cricket species. At regional scales (e.g., across the state of Connecticut; A), the hybrid zone forms a smooth cline from *G. firmus*–like to *G. pennsylvanicus*–like crickets. When the hybrid zone is sampled at smaller spatial scales (a few kilometers; B), the hybrid zone is mosaic in nature. It is not clear what the structure of the hybrid zone is at very fine scales (C), across patch boundaries.

a scale not resolved by the sampling (Bridle et al. 2002). Although the issue of scale has rarely been studied explicitly, many hybrid zones may vary in structure at different spatial scales (e.g., Searle 1986; Cruzan and Arnold 1993; Hewitt 1993; Patton 1993; Szymura 1993; Sites et al. 1995).

The mosaic hybrid zone between the two North American field crickets, *Gryllus pennsylvanicus* and *G. firmus* (Harrison and Arnold 1982; Harrison 1986), varies in structure over different spatial scales (Fig. 1). At regional scales, from Connecticut to Virginia, the hybrid zone appears clinal in nature (Harrison and Arnold 1982; Harrison 1986). Across the state of Connecticut, there are gradual transitions in numerous characters from northwestern Connecticut to the central coast (Harrison 1986; Harrison and Rand 1989). The mosaic nature of the hybrid zone is revealed at intermediate spatial scales, among habitat patches within Connecticut (Harrison 1986; Harrison and Bogdanowicz 1997). At this scale, the hybrid zone is presumably structured by an underlying heterogeneity in soil type across the state (Rand and Harrison 1989). For the hybrid zone, the association of *G. pennsylvanicus* with loam soils and *G. firmus* with sand soils suggests that each species has the highest fitness on the soil with which it is associated (Harrison and Rand 1989; Rand and Harrison 1989). In this scenario, hybrid individuals do not have the highest fitness in any habitat patch.

The fine-scale structures of hybrid zones are rarely described in conjunction with descriptions at larger spatial scales. Fine-scale sampling allows resolution of variation on scales equal to and less than the dispersal distances of individuals (defined as the standard deviation of distances between the sites where parents and offspring reproduce). For mosaic hybrid zones, this scale must include the boundaries between adjacent patches of different habitats (tens of meters for this cricket hybrid zone). Analysis of fine-scale structure

allows for the appropriate comparison between clinal and mosaic hybrid zones. Given limited dispersal, it is at fine scales in mosaic hybrid zones that parental types meet, mate, and form hybrid offspring.

Modeling of the structure and maintenance of mosaic hybrid zones has lagged behind modeling of clinal hybrid zones. Much attention has been given to modeling clinal hybrid zone structure by looking at the shape of single and multilocus clines for traits across hybrid zones (Slatkin 1973, 1975, 1976; Nagylaki 1975, 1976; Barton 1979a,b, 1983; Szymura and Barton 1986, 1991; Barton and Gale 1993; Sites et al. 1995; Porter et al. 1997; Kruuk et al. 1999). By assuming hybrid zones are tension zones maintained by a balance between dispersal and selection, clinal models can predict parameters such as dispersal and selection against hybrids that are otherwise difficult to estimate (Barton and Gale 1993). Barton and colleagues also have shown that these models are robust to variations in the nature of selection (Barton and Gale 1993; Kruuk et al. 1999) and are useful even when selection is due to environmental heterogeneity in different habitats. Examining mosaic hybrid zones at fine spatial scales may allow these hybrid zones to be equated to clinal hybrid zones, thus allowing the application of cline shape models to mosaic hybrid zones.

For the *Gryllus* hybrid zone, parental types presumably meet, mate, and form hybrids at habitat patch boundaries. Therefore, understanding how selection maintains this hybrid zone depends on knowing the distributions of crickets, the interactions among crickets and their environments, and the fitnesses of genotypes at patch boundaries on a fine scale. Examination of the fine-scale structure of the *Gryllus* hybrid zone also allows further tests of the hypothesis that soil type is structuring the mosaic hybrid zone (Rand and Harrison 1989). If soil (or associated habitat) is responsible for the mosaic structure, then the soil type-by-genotype association that has been characterized at intermediate scales should exist at fine scales as well, even at the boundaries between distinct soil patches.

In this study, we describe the structure of the cricket mosaic hybrid zone at a fine spatial scale. We examine how both cricket characters and soil characters change across the boundaries of habitat patches in the hybrid zone. From the analysis, we show that the cline shapes of different cricket traits vary from each other but in a consistent manner across different patch boundaries. Additionally, patterns of variation for these traits resemble those seen in many clinal hybrid zones, suggesting that mosaic hybrid zones at fine scales can be viewed as clinal in nature.

The Gryllus Hybrid Zone

Gryllus firmus, the beach cricket, lives in coastal and lowland habitats along the North American eastern seaboard from Florida to Connecticut (Lutz 1908; Fulton 1952; Alexander 1957, 1968). It is found on sands and other soils with high sand content. *Gryllus pennsylvanicus* lives in inland and upland areas from Ontario south along the Appalachian Mountains into northern Georgia (Lutz 1908; Fulton 1952; Alexander 1957, 1968). It is found on loam and other soils with loamy character, frequently in old fields or pastures. *Gryllus firmus* is a larger, lighter colored cricket and females have relatively long ovipositors compared with their body length. *Gryllus pennsylvanicus* is smaller and darker, and females have relatively short ovipositors.

These two field cricket species hybridize in a long, narrow zone that extends at least from North Carolina through Connecticut, approximately along the eastern edge of the Appalachian Mountains (Harrison and Arnold 1982; Fig. 1). In Connecticut, this hybrid zone has been described as mosaic in nature (Harrison 1986). Relatively pure parental types are found throughout the hybrid zone, and habitat heterogeneity allows these parental types to interact directly within the zone, forming populations with bimodal distributions for many characters and rapid transitions in character frequencies through space (Harrison 1986). The mosaic nature of the hybrid zone has been demonstrated for morphological characters (Harrison 1986), allozymes (Harrison 1986), mitochondrial DNA (mtDNA; Harrison et al. 1987), and anonymous nuclear markers (Harrison and Bogdanowicz 1997). Within the hybrid zone, few F_1 hybrids exist, and the distribution of multilocus genotypes is bimodal (Harrison and Bogdanowicz 1997), which is typical for many hybrid zones (Jiggins and Mallet 2000). In addition, the two parental types are associated with different soil types; *G. firmus*–like individuals and alleles are associated with sandy soils within the hybrid zone and *G. pennsylvanicus*–like individuals and alleles are associated with loamy soil patches within the hybrid zone (Harrison and Rand 1989; Rand and Harrison 1989). It is not clear what mechanisms maintain this association.

Materials and Methods

Cricket Sampling

This study was designed to sample crickets at the borders between sand and loam patches in the hybrid zone. Appropriate sampling sites in Connecticut were chosen by first identifying soil type boundaries using statewide and county soil survey maps (Gonick 1978; Reynolds 1979a,b). After candidate sites were investigated to confirm soil types and to assess the probability of sampling crickets continuously across the presumptive patch boundaries, two sites, UT and GHWA, were sampled extensively. UT is located southeast of Middletown in Middlesex County, Connecticut, along a 500-m segment of River Road near the Connecticut River (latitude: 41°33.30′N; longitude: 72°35.18′W). GHWA is located north of East River in New Haven County, Connecticut, along a 762-m length of Warpas Road between Copse and Greenhill Road (latitude: 41°18.02′N; longitude: 72°37.37′W). Additional details of locations are available from C. L. Ross.

For each site, adults and nymphs were collected during September 1996 and 1997. Crickets were collected along both sides of the road as well as in suitable surrounding habitat, occasionally up to 20 m from the road. Over two years, 571 crickets were collected along the UT transect, and 242 crickets were collected along the GHWA transect. For the transects, considerably more effort was used to collect crickets in the perceived middle of the transects where the habitat-soil transitions occurred. This uneven effort was designed to bolster numbers of crickets and achieve better resolution in

the expected density trough that exists at the center of hybrid zone step clines (Barton and Hewitt 1985; Hewitt 1988). Despite this effort, few crickets were collected along some areas of the transects. Additionally, very few crickets were collected at the *G. firmus* endpoint of the GHWA transect during 1997 because this area had been transformed from a vacant lot to a housing development. Crickets were kept alive in individual 50-ml conical tubes until they could be placed at −80°C in the laboratory. Immature crickets were raised in the laboratory to adulthood before they were frozen.

The position along the transects at which each cricket was first seen was recorded to within 0.1 m. For each transect, 0 m indicates the start of the transect at the *G. pennsylvanicus* end, and 500 m (UT) or 762 m (GHWA) indicates the *G. firmus* end of the transect. The sampling resolution of these transects (the distance between collected crickets) varies from less than 1 m to several meters. Thus, sampling was done on a much finer scale than in previous studies (Harrison 1986; Harrison and Bogdanowicz 1997), which sampled populations a few kilometers apart rather than individuals a few meters apart. Rand and Harrison (1989) also sampled adjacent *G. pennsylvanicus*–like and *G. firmus*–like populations that were less than 1 km apart, but these samples were not continuous across patch boundaries.

Soil Samples

Soil samples were collected along each transect every 50 m. For each sample, the top 10 cm of soil was collected. Female crickets deposit eggs only into the top 2 cm of soil (approximately); however, the top 10 cm was collected to ensure an adequate representation of the soil from the parent material for each sample and to average over the inevitably large amount of variation which is typical of the top few centimeters of soil at any site. Soil was also collected every 100 m on both sides of the UT transect 10–15 m from road. These samples were collected to assess the consistency of the soil samples along additional linear paths parallel to the road, to sample away from the road to avoid any possible contamination of fill used in road construction, and to get a two-dimensional picture of the soil in the area. Soil samples were characterized for organic content and particle size distribution, which are two distinct physical differences between many loams and sands (McKeague 1978). Organic content was determined by weighing dried samples before (dry weight) and after (ash weight) burning in a kiln at 500°C for 2 h. Particle size distribution was determined using the hydrometer method (Bouyoucous 1926; Day 1965; Sheldrick and Wang 1993). For comparison to the transect sites, soil samples also were collected from five reference populations within the hybrid zone that harbor well-characterized, relatively pure populations of *G. pennsylvanicus* and *G. firmus* (Harrison and Arnold 1982; Harrison 1986; Rand and Harrison 1989; Harrison and Bogdanowicz 1997). In addition, soil samples were collected from two sites, one on each side of the hybrid zone, where *G. pennsylvanicus* and *G. firmus* populations reside.

Characterizing Morphological Traits

Three morphological characters were measured for all adult crickets from the transects: femur length, pronotum width, and tegmina color. A fourth character, ovipositor length, was measured for all adult females. *Gryllus firmus* is generally larger than *G. pennsylvanicus*, and femur length and pronotum width were used to reflect overall size differences. Ovipositor length is the character that most clearly differentiates the two species. All size measurements were made by C. L. Ross to the nearest 0.1 mm with the same pair of vernier calipers. Tegmina color was assessed by comparing individuals to a standard array of tegmina colors, ranging from a score of 1, which represented dark black tegmina typical of *G. pennsylvanicus*, to 9, which represented the light tan tegmina frequently found in *G. firmus*.

Molecular Markers

All individuals were scored for mtDNA haplotype, and a subset of individuals was scored for three anonymous nuclear loci. For mtDNA, total DNA was extracted from femoral muscle using Qiagen DNeasy tissue kits (Qiagen, Inc., Valencia, CA). An approximately 2-kb segment of mtDNA spanning the cytochrome oxydase I and II (COI and COII) region was amplified using polymerase chain reaction (PCR) with the Harrison lab primers Ron (5′-GCATCACCTGATATAGCATTCCC-3′) and Eva (5′-GAGACCATTACTTGCTTTCAGTCATCT-3′; Simon et al. 1994; Willett et al. 1997). For 10-μl PCR reactions, 1 μl of a 1/1000 dilution of total DNA was added to a reaction mixture (1 × PCR buffer [Gibco-BRL, Rockville, MD], 3 mM $MgCl_2$, 0.2 mM of each primer, 0.05 units TAQ [Gibco-BRL], and 0.2 mM dNTPs). After an initial 2-min soak at 95°C, the reaction was run 35 times through a temperature cycle of 95°C for 30 sec, 47°C for 60 sec, and 72°C for 90 sec, followed by a 5-min extension at 72°C.

Amplification products were digested with the restriction enzyme EcoRV at 37°C for 1 h. Digests were run on 2% TAE agarose gels, and fragments visualized with ethidium bromide. EcoRV cuts the PCR product once in *G. pennsylvanicus* from Connecticut, producing fragments of 1100 bp and 900 bp from the original 2000-bp PCR product. For *G. firmus*, EcoRV cuts the PCR product twice, producing fragments approximately 900 bp, 800 bp, and 300 bp in size (for DNA sequence see Willett et al. 1997). This restriction site difference is diagnostic for populations of *G. pennsylvanicus* and *G. firmus* outside of the hybrid zone in the Northeast (Harrison et al. 1987; Harrison and Rand 1989).

Three anonymous nuclear DNA loci were also scored: GpUC 5, GpUC 279, and GpUC 351 (Harrison and Bogdanowicz 1997). For the UT transect, 125 individuals along the transect were scored for GpUC 5, 81 individuals were scored for GpUC 279, and 123 individuals were scored for GpUC 351. For the GHWA transect, 100 individuals along the transect were scored for GpUC 5, but GpUC 279 and GpUC 351 were not scored. These nuclear DNA markers were initially developed as restriction fragment length polymorphisms (RFLPs) from Southern hybridizations of a cricket genomic DNA library (Harrison and Bogdanowicz 1997). GpUC 5 behaves as a dispersed repeat, whereas GpUC 279 and GpUC 351 most likely represent single-copy loci. GpUC 351 appears to be X-linked because males, which are XO in crickets, never show any heterozygotes (Harrison and Bogdanowicz

1997). All three markers exhibit fixed or nearly fixed restriction site differences between allopatric populations of *G. pennsylvanicus* and *G. firmus* (Harrison and Bogdanowicz 1997). To assay these markers, total DNA was extracted from femurs, thoraces, and/or heads using phenol/chloroform (Sambrook et al. 1989) and digested with appropriate restriction enzymes. Southern blots were performed as in Harrison and Bogdanowicz (1997).

Results

Variation in Morphology across the Transects

Both transects show similar patterns of variation for each morphological trait measured (Fig. 2). All of the morphological characters show a transition from *G. pennsylvanicus*–like to *G. firmus*–like. Groups of individuals on each side of both transects show the same range of variation as pure populations bordering the hybrid zone.

Although all morphological traits change from *G. pennsylvanicus*–like to *G. firmus*–like in the same direction, the traits do not change in a uniform manner. Using a cubic spline method (Eubank 1988), which fits a set of third-degree polynomials to the data with a smoothing function, cline shapes can be approximated across the transect for each of the traits (Fig. 2). Ovipositor lengths have a very sharp transition, or stepped cline, centered at the 300-m position for the UT transect and at the 350-m position for the GHWA transect (Fig. 2A, E). Tegmina color also shows a sharp transition from the *G. pennsylvanicus* side of the transects to the *G. firmus* side of the transects (Fig. 2D, H). With this trait, however, individuals on the *G. pennsylvanicus* side of the transects exhibit only dark tegmina, but individuals on the *G. firmus* side display the full range of scores. Transitions occur approximately at the same position along the transects as the transitions for ovipositor length, but shifted slightly into the *G. firmus* side of the transects (at 320 m for UT and 410 m for GHWA). The clines for both body size measures are decidedly shallower than for ovipositor length or tegmina color (Fig. 2B, C, F, G). Pronotum width and femur length distributions overlap substantially between the two species. However, the gradual transitions along the transects are not solely a function of this overlap. For these characters, the average pronotum width and femur length increase steadily and gradually along the transects, indicating shallow clines. Nonetheless, for both of these traits, the transitions across the transects from *G. pennsylvanicus*–like to *G. firmus*–like character states are centered roughly at the same position as for ovipositor length and tegmina color.

Variation in Molecular Markers across the Transects

The four molecular markers also show striking transitions across the two transects, from alleles or haplotypes characteristic of *G. pennsylvanicus* to alleles or haplotypes characteristic of *G. firmus* (Fig. 3, 4). These transitions are most easily visualized by superimposing the single-locus genotype of each individual on a graph of ovipositor length by transect position.

GpUC 5 and GpUC 351 display transition patterns across the transects similar to those seen for the morphological traits (Figs. 3B, 3D, 4B). In both transects GpUC 5 displays a discrete transition from the *G. pennsylvanicus* allele to the *G. firmus* allele (Figs. 3B, 4B). The positions of these transitions along the transects, approximately 325 m for UT and 400 m for GHWA, are similar to those for the morphological characters. Like the ovipositor length cline, this cline exhibits an abrupt, steep step at both UT and GHWA. Each transect contained fewer heterozygotes than expected given Hardy-Weinberg expectations (UT: observed = 10 [8%], expected 62.4 [50%], $\chi^2_1 = 88.146$, $P \ll 0.0001$; GHWA: observed = 1 [1%], expected 50 [50%], $\chi^2_1 = 95.950$, $P \ll 0.0001$). This deficit of heterozygotes may be represented by F_{IS}, where $1 - F_{IS}$ equals the ratio of observed heterozygote frequency to expected heterozygote frequency given Hardy-Weinberg. Pooling individuals over the entire transect, F_{IS} for GpUC 5 is 0.84 and 0.98 for UT and GHWA, respectively. In addition, both sides of the transects considered separately also show deficits of heterozygotes (*G. pennsylvanicus* side: $F_{IS} = 0.78$ and 0.40, for UT and GHWA, respectively; *G. firmus* side: $F_{IS} = 0.10$ and 1.00 for UT and GHWA). Only one heterozygote was located in the middle of the transects (at 325 m in the GHWA transect). This is where heterozygotes would be expected if the cline is a result of the matings between *G. pennsylvanicus*–like crickets and *G. firmus*–like crickets, producing hybrid offspring. It should be noted that the diagnostic *G. pennsylvanicus* and *G. firmus* alleles are not completely fixed in most populations sampled in the hybrid zone (Harrison and Bogdanowicz 1997), so a few heterozygotes should exist even in seemingly pure populations of each species and at the ends of the transects.

The distribution of GpUC 351 alleles in the UT transect is similar to the distribution of GpUC 5 alleles. Individuals with *G. pennsylvanicus* alleles show a sharp transition to individuals with *G. firmus* alleles at the same approximate position as the morphological clines (Fig. 3D). As with GpUC 5, GpUC 351 shows a deficit of heterozygotes over the entire UT transect (UT: observed = 9 [7%], expected 15 [12%], $\chi^2_1 = 46.453$, $P \ll .0001$; $F_{IS} = 0.41$). However, unlike GpUC 5, there is an excess of heterozygotes when each side of the transect is considered separately (*G. pennsylvanicus* side, $F_{IS} = -5.3$; *G. firmus* side, $F_{IS} = -1.5$). All but one of the heterozygous individuals were located at the two ends of the transect.

Two loci, mtDNA and GpUC 279, show transition patterns that are markedly different from the other characters measured. The *G. firmus* mtDNA haplotype is only found in individuals on the *G. firmus* sides of the transects (Figs. 3A, 4A) and never on the *G. pennsylvanicus* sides of the transects (with one exception in the GHWA transect). In contrast, individuals with the *G. pennsylvanicus* mtDNA haplotype exist throughout the transects. The proportion of *G. pennsylvanicus* mtDNA is 0.75 and 0.17 at the *G. firmus* ends of the UT and GHWA transects, respectively. As a consequence, the clines for mtDNA are shifted toward the *G. firmus* side of the transects relative to morphological traits.

The distribution of GpUC 279 alleles in the UT transect parallels the distribution of mtDNA haplotypes (Fig. 3C). The transition from areas dominated by *G. pennsylvanicus* alleles to areas dominated by *G. firmus* alleles is abrupt, as with the GpUC 5 locus, but is offset from the morphological

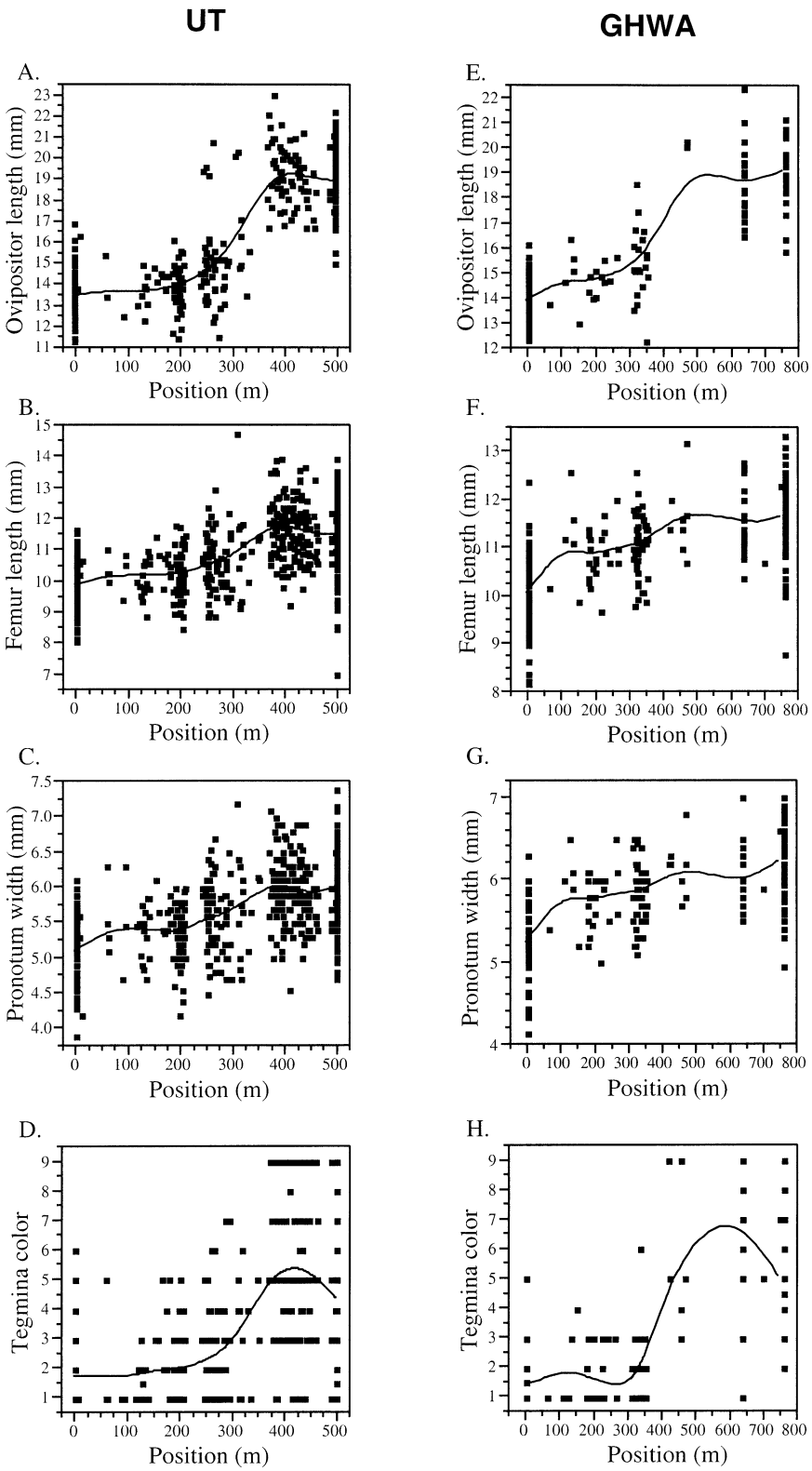

FIG. 2. Morphological characters by position along the transects. Each point on the transects represents a single individual (UT: ovipositor length, $N = 322$; femur length, $N = 557$; pronotum width, $N = 565$; tegmina color, $N = 529$; GHWA: ovipositor length, $N = 130$; femur length, $N = 239$; pronotum width, $N = 240$; tegmina color, $N = 242$). *Gryllus pennsylvanicus*–like individuals are located on the left side of the transects (0 m). *Gryllus firmus*–like individuals are located on the right side of the transects (500 m and 762 m for UT and GHWA, respectively). Cline shape is fit using a cubic spline method (Eubank 1988), with lambda (λ) = 1×10^6. Lambda determines the distance window over which a cubic polynomial is estimated from the data, minimizing the sum of squares error. The cubic regressions for each window are then spliced together over the entire transect to produce the overall cline shape. This λ value produces a stiff cline (less curved).

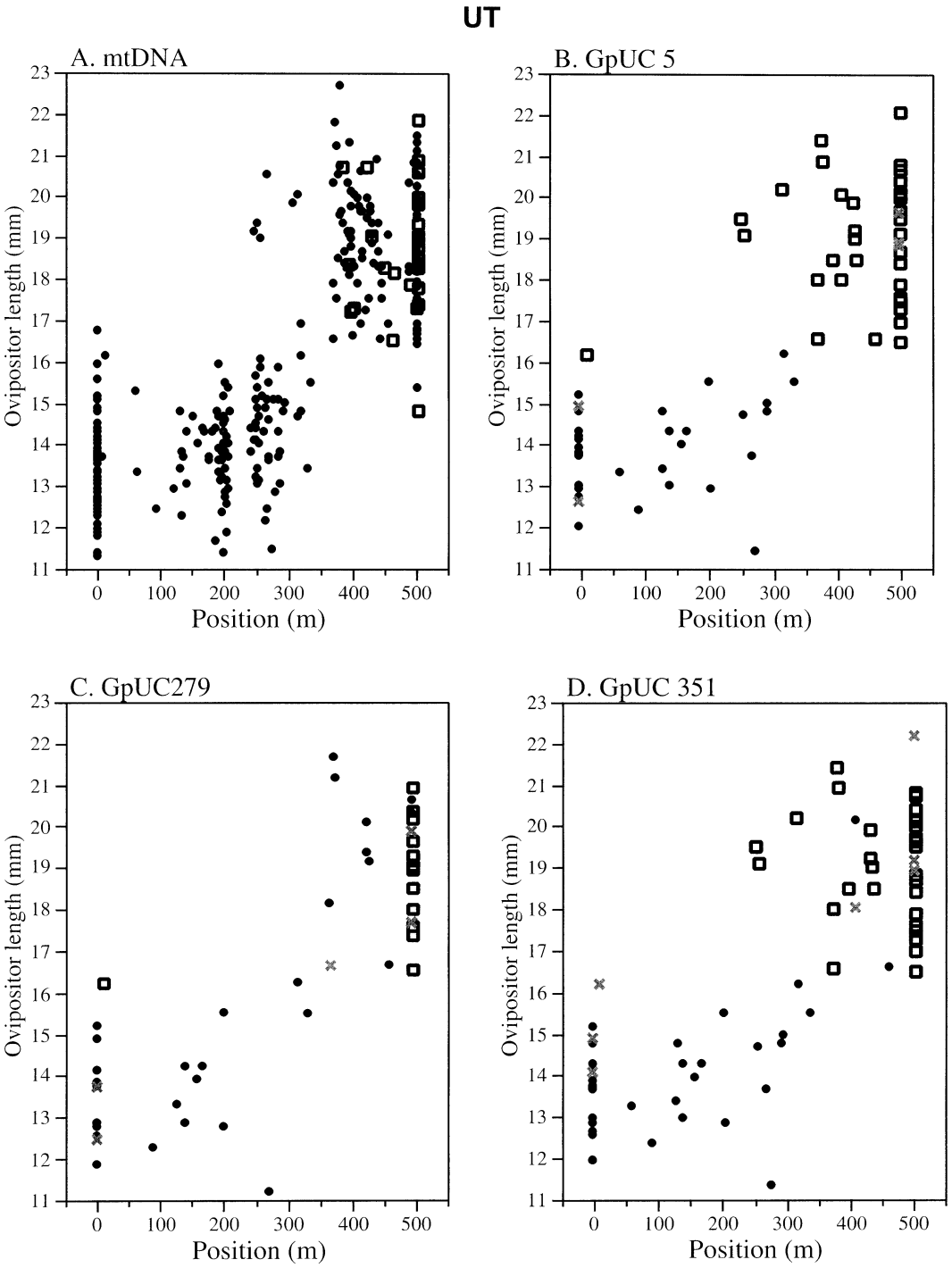

FIG. 3. Molecular characters by position along the UT transect. Molecular genotypes for females are superimposed on the ovipositor by position graphs (to show spread). Each point on the transects represents a single female individual homozygous for the *Gryllus pennsylvanicus* allele (●), a single individual homozygous for the *G. firmus* allele (□), or a heterozygous individual (**X**). For mtDNA, each point represents an individual with the *G. pennsylvanicus* haplotype (●) or *G. firmus* haplotype (□). Although males are not shown on these graphs, the distribution of genotypes is identical. Sample sizes for females (and overall): A, 322 (567); B, 77 (125); C, 47 (81); D, 77 (123).

clines toward the *G. firmus* side of the transect. Similar to the other anonymous nuclear loci, fewer heterozygotes are present than expected with Hardy-Weinberg assumptions, and the population is not in Hardy-Weinberg proportions when the entire transect is considered (UT: observed heterozygotes = 8 [10%], expected = 9 [11%], χ^2_1 [for all HW proportions] = 27.159, $P < .0001$; $F_{IS} = 0.14$). As with GpUC 351, when the two sides of the transects are considered separately, more heterozygotes than expected are observed on both the *G. pennsylvanicus* side ($F_{IS} = -2.3$) and the *G.*

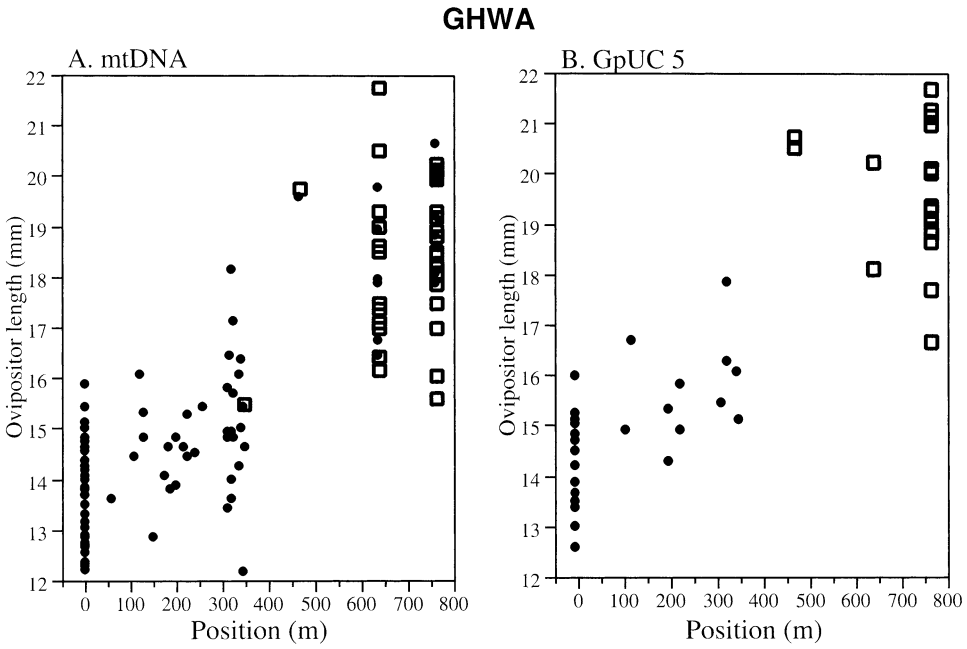

FIG. 4. Molecular characters by position along the GHWA transect. See Figure 3 for details. Sample sizes for females (and overall): A, 129 (240); B, 45 (100).

firmus side ($F_{IS} = -3.9$). Not all heterozygotes are located at the ends of the transects, although most are. Two heterozygous individuals are located near the step in the cline for this locus.

Average Cline for All Characters

The average clines for both transects are plotted in Figure 5. The average cline is generated by first standardizing the scores for all morphological and molecular characters to a scale from 0 to 1. For each morphological character, standardization is based on the range of observed values. For molecular markers, the alternative homozygotes or haplotypes are scored as 0 or 1, respectively, and heterozygotes are scored as 0.5. The average trait score for each individual is then plotted against position, and a cubic spline polynomial is fit to the data (as before) to produce a cline shape function. As a result, the shape of the cline is dependent on the traits and individuals that are included in the analysis, and a different suite of traits, which may experience a different balance of evolutionary forces, will produce a different cline. From Figure 5, both transects show similar average clines, with abrupt steps in the middle of the transects, and gradual decays on either side of the steps to the extremes in scores at the ends of the transects.

Covariance and Linkage Disequilibrium

Hybrid zones are often characterized by increased phenotypic variance in the middle of the zone (Endler 1977). This phenomenon may occur as a result of mixing distinct genomes and/or increasing environmental variability. The covariance between quantitative traits also increases in the middle of hybrid zones due to increased linkage disequilibrium (Szymura and Barton 1986, 1991; Barton and Gale 1993). At the fine scale of the transects in this study, where individuals are sampled without the benefit of population affiliation, variance and linkage disequilibrium are inappropriate measures unless individuals along the transects are grouped into pseudo-populations. Grouping individuals is not necessarily desirable for analysis of fine-scale transects because spatial information is lost through the binning process. Nonetheless, to compare our fine-scale transects with transects over larger spatial scales, where each observation is a population instead of an individual, it is useful to have a sense of how the variance in traits and linkage disequilibrium change over the transects. Consequently, the variance/covariance structures in three areas of the transects, the *G. pennsylvanicus* end, the middle, and the *G. firmus* end, were estimated, along with D^*, the average linkage disequilibrium for diagnostic alleles of phenotypic traits (see Nürnberger et al. 1995).

For all morphological traits except tegmina color, variance is highest in the middle of the UT transect (Table 1A). For the GHWA transect (Table 1B), ovipositor length shows a pattern similar to that seen in the UT transect, but the body size characters and tegmina color show less variance in the middle of the transect than at the ends.

Covariance is highest in the middle of the UT transect for all pairwise combinations of morphological traits (Table 1A) and for four of six combinations at the GHWA transect (Table 1B). For the UT transect femur length and pronotum width show a high pairwise phenotypic correlation ($r^2 = 0.89$). Also, ovipositor length is correlated with both body size traits ($r^2 = 0.88$ with femur length, $r^2 = 0.82$ for pronotum width). If the increase in covariance in the middle of the hybrid zone is the result of high linkage disequilibrium, then the relative increase in covariance can be used to estimate D^* for quantitative phenotypic traits. Using the equation, $cov(z, z') =$

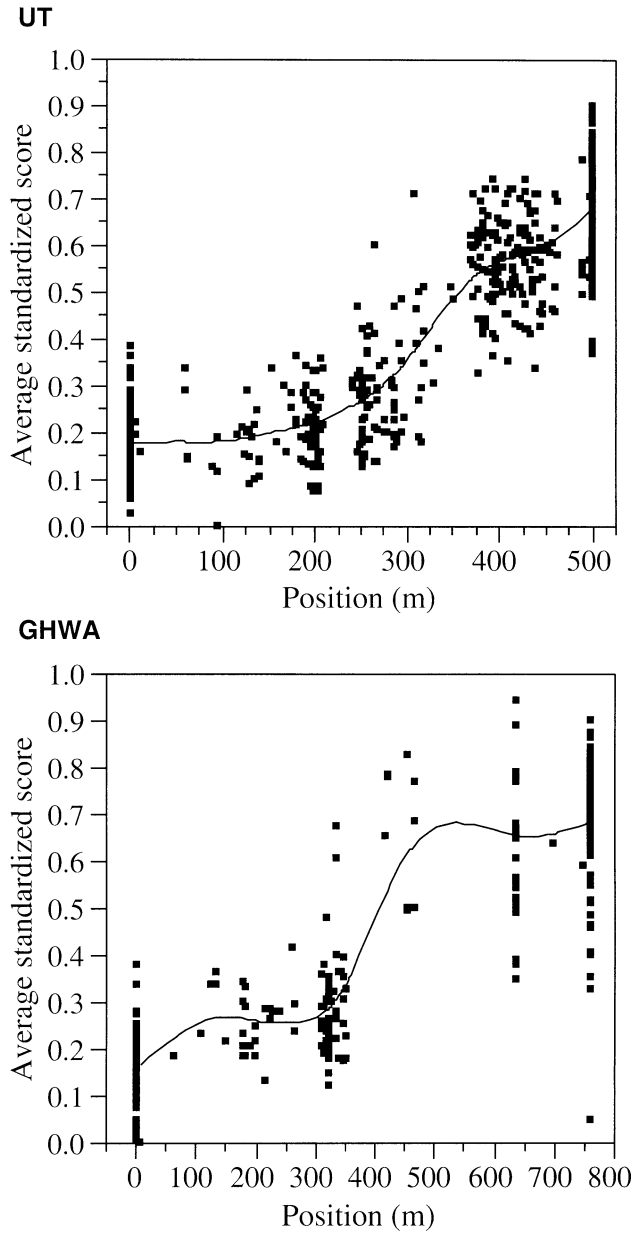

FIG. 5. Average trait score for individuals by position along the transects. Each point on the transects represents the average value for a single individual of all morphological and molecular traits measured. All traits were standardized to a scale between zero and one before averaging. Cline shape is fit using a cubic spline method (Eubank 1988), with lambda (λ) = 1×10^6 (see caption to Fig. 2 for details).

$\Delta z \Delta z' D^*/2$ (Nürnberger et al. 1995), the covariance between two quantitative traits, z and z', is proportional to D^*, the average linkage disequilibrium between diagnostic alleles, where Δz and $\Delta z'$ are the maximum trait differences across the transects. Table 1 lists the estimates of D^* for three areas of both transects. As with covariance, D^* for every pairwise case of morphological traits measured along the UT transect is highest in the middle of the transect. For GHWA, D^* for four of six combinations is highest in the middle, with combinations involving femur length having higher D^* at the *G. firmus* end of the transect.

We also estimated pairwise two-locus linkage disequilibrium, D_{AB}, and gametic phase cytonuclear disequilibrium, *CND*, between molecular loci using GDA version 1.0 (Lewis and Zaykin 2001) and CND (Asmussen and Basten 1994; Basten and Asmussen 1997), respectively. D_{AB} and *CND* are highly significant among all pairwise combinations (Table 2) because most individuals in the transects contained either *G. pennsylvanicus* alleles at all loci or *G. firmus* alleles at all loci. As reported above, very few heterozygotes were found throughout the transect (see Harrison and Bogdanowicz 1997).

We summarize the distribution of multilocus genotypes across the entire UT transect using histograms of genotype scores for males and females (Fig. 6). Genotype scores are calculated by assigning a value of 0 for each *G. pennsylvanicus* allele or mtDNA haplotype and a value of 1 for corresponding *G. firmus* alleles or haplotypes. Because GpUC351 is assumed to be sex-linked, the range of scores is 0–6 for males and 0–7 for females. The histograms emphasize the bimodal nature of the hybrid zone and also the clear signature of introgression of *G. pennsylvanicus* alleles into *G. firmus*. Although each histogram has a large peak at 0 (representing pure *G. pennsylvanicus*), the *G. firmus* peak is broad with relatively few individuals with scores of 6 (males) or 7 (females). F_1 hybrids (i.e., heterozygotes at all nuclear loci and *G. pennsylvanicus* mtDNA) would have scores of 2 (males) and 3 (females), but no actual F_1 hybrids were collected along the transects (individuals with these genotype scores are other multilocus genotypes).

Across the two transects, there is a strong association between morphological traits and nuclear gene alleles or mtDNA haplotypes in spite of introgression at GpUC279 and mtDNA (Table 3). This can be seen clearly by examining the plots of ovipositor length by position (Figs. 3, 4), with individuals coded by the alleles they possess. Interestingly, heterozygotes at autosomal loci are intermediate in character for nearly all of the morphological traits (Table 3).

Changes in Soil across the Transects

Based on two soil characters that differ between sands and loams, particle size distribution and organic content, our data indicate that each transect crosses a patch boundary (Fig. 7). Soils transition from areas with high sand content at the *G. firmus* ends of the transects to areas of moderate sand content at the *G. pennsylvanicus* ends of the transects (Fig. 7). Likewise, organic content, which is typically low in sands and high in loams, is low at the *G. firmus* end of the transect and high at the *G. pennsylvanicus* end of the transect (Fig. 7). The changes in soil attributes are not abrupt but are nonetheless conspicuous. Using the U.S. Department of Agriculture guide for textural classification (Bureau of Plant Industry, Soils, and Agricultural Engineering) and grouping samples by the morphology of crickets collected on them, soil samples where *G. firmus*–like crickets were found are classified as sands, with some samples classified as loamy sands (Fig. 8). Soil samples where *G. pennsylvanicus*–like crickets were found are classified as loams or sandy loams (Fig. 8).

TABLE 1. Estimates of variance, covariance, and D^*. (A) UT transect. (B) GHWA transect. Covariance estimates are listed above the diagonal, with variance estimates listed on the diagonal, for four morphological traits at the *Gryllus pennsylvanicus* end (top), middle (middle), and *G. firmus* end (bottom) of the transect. These values are the residuals of individual estimates after subtracting the respective transect mean for each trait. Sample sizes are given in parentheses. Estimates of D^* are listed below the diagonal for each section of the transect. Δz-values are the maximum trait difference across the transect (see text for details).

A

Δz:	Ovipositor length 11.70	Femur length 7.75	Pronotum width 3.50	Tegmina color 8
Ovipositor length	2.101 (158)	0.808 (155)	0.465 (157)	0.797 (141)
	8.045 (25)	2.615 (25)	1.307 (25)	2.844 (22)
	1.981 (139)	0.800 (137)	0.438 (138)	0.096 (125)
Femur length	0.018	0.631 (242)	0.332 (241)	0.347 (223)
	0.058	1.335 (45)	0.638 (45)	1.310 (45)
	0.018	1.057 (270)	0.421 (269)	0.059 (256)
Pronotum width	0.023	0.011	0.229 (245)	0.210 (225)
	0.064	0.042	0.367 (46)	0.710 (42)
	0.021	0.002	0.253 (274)	−0.043 (260)
Tegmina color	0.017	0.011	0.015	1.650 (226)
	0.061	0.042	0.051	4.820 (42)
	0.002	0.002	−0.003	4.887 (261)

B

Δz:	Ovipositor length 10.10	Femur length 5.15	Pronotum width 2.85	Tegmina color 8
Ovipositor length	1.091 (58)	0.694 (58)	0.354 (58)	0.052 (58)
	3.798 (22)	0.865 (22)	0.606 (22)	1.496 (22)
	1.878 (50)	0.701 (50)	0.462 (50)	0.515 (50)
Femur length	0.027	0.747 (91)	0.387 (91)	0.091 (91)
	0.033	0.421 (57)	0.193 (57)	0.273 (57)
	0.027	0.647 (91)	0.299 (91)	0.299 (91)
Pronotum width	0.025	0.004	0.232 (91)	0.049 (91)
	0.042	0.013	0.140 (57)	0.177 (57)
	0.032	0.015	0.187 (91)	0.057 (91)
Tegmina color	0.001	0.004	0.004	0.760 (91)
	0.037	0.013	0.016	3.784 (57)
	0.013	0.015	0.005	4.018 (91)

These samples are consistent with samples collected from reference populations, albeit not as distinctly sand or loam as sites that harbor pure cricket populations. They are similar, however, to soil samples from reference populations of *G. pennsylvanicus*–like crickets and *G. firmus*–like crickets found within the hybrid zone (Fig. 8).

DISCUSSION

Patterns of Variation across Patch Boundaries in a Mosaic Hybrid Zone

The field cricket hybrid zone was initially described on the basis of morphological and allozyme variation in populations sampled from throughout the northeastern United States (Harrison and Arnold 1982). Analysis of variation revealed a broad zone of hybridization and overlap on a spatial scale of tens to hundreds of kilometers, with *G. firmus* populations found along the coast and at low elevations and *G. pennsylvanicus* populations occupying inland and upland sites. More intensive sampling within small regions (kilometers to tens of kilometers) of the hybrid zone in Connecticut produced a very different picture; the hybrid zone appeared to be a patchwork of populations (a mosaic), with adjacent populations exhibiting substantial differences in morphology, allozyme allele frequencies, mtDNA haplotype frequencies, and frequencies of diagnostic anonymous nuclear gene markers (Harrison 1986; Harrison et al. 1987; Harrison and Rand 1989; Rand and Harrison 1989; Harrison and Bogdanowicz 1997). The mosaic structure of the cricket hybrid zone in Connecticut reflects an underlying patchy distribution of sand and loam soils (Rand and Harrison 1989).

Here we have intensively sampled crickets along transects

TABLE 2. Two-locus linkage disequilibrium, D_{AB}, and cytonuclear disequilibrium, *CND*. Values above the diagonal are estimates of D_{AB} or *CND* and *P*-values of Fisher exact tests (in parentheses) for the UT transect. Values below the diagonal are for the GHWA transect. See text for details.

	mtDNA	GpUC 5	GpUC 279	GpUC 351
mtDNA	—	0.88 (≪0.0001)	0.77 (≪0.0001)	0.76 (≪0.0001)
GpUC 5	0.89 (≪0.0001)	—	0.26 (≪0.0001)	0.40 (≪0.0001)
GpUC 279	—	—	—	0.26 (≪0.0001)

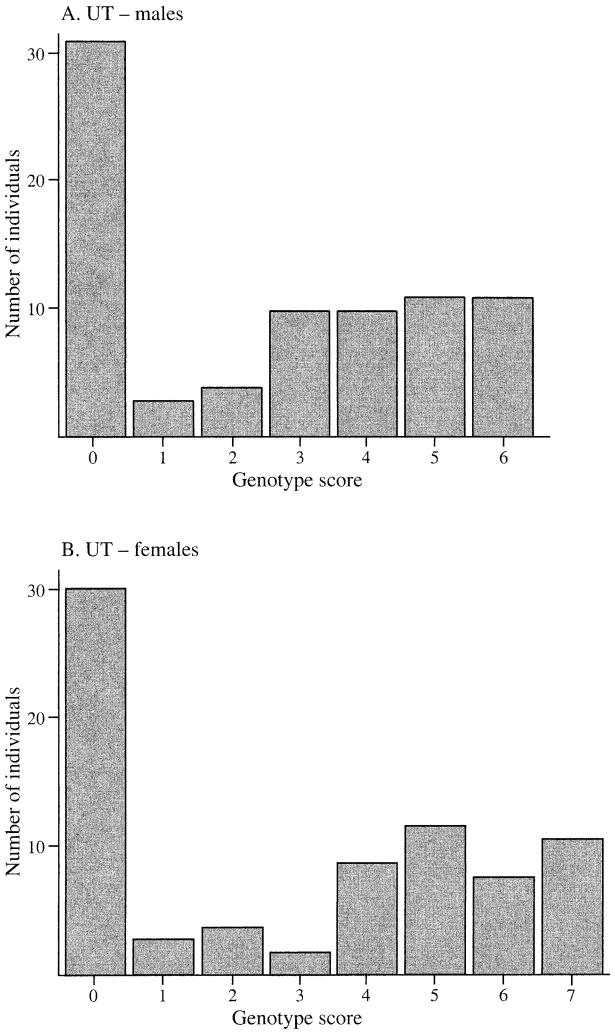

FIG. 6. Histograms of genotype scores for (A) males and (B) females sampled along the entire UT transect. To determine an individual's score, each *Gryllus firmus* allele (or haplotype for mtDNA) contributed one point, so that for nuclear loci, *G. pennsylvanicus* homozygotes scored zero points, *G. firmus* homozygotes scored two points, and heterozygotes scored one point. MtDNA haplotypes were scored as one point (*G. firmus*) or zero points (*G. pennsylvanicus*). See text for details.

across the boundaries between sand and loam patches. Rather than comparing trait, allele, and haplotype frequencies within arbitrarily defined populations, we examined the distribution of morphological and molecular characters in individual crickets over distances of less than a kilometer. At this fine spatial scale, both morphological and molecular characters show abrupt transitions from *G. pennsylvanicus*–like to *G. firmus*–like scores and alleles. These step clines show substantial concordance, especially considering the scale over which they are measured. In addition, both transects show similar patterns even though they are geographically distant and are at different positions in the hybrid zone—UT deep within the hybrid zone and GHWA near the coast where *G. firmus* populations are more common. Accordingly, the cricket mosaic hybrid zone appears clinal in nature when examined at a fine spatial scale across the patch boundaries between habitats. Because the hybrid zone appears clinal at very large spatial scales, the mosaic nature of this hybrid zone is revealed only at intermediate scales, which presumably correspond to the scale of patchiness of the relevant environmental heterogeneity. Thus, scale clearly matters when examining the structure of this and other hybrid zones (Bridle et al. 2002).

Most remarkable is that concordant changes in morphology and diagnostic molecular markers occur over distances less than 100 m at both UT and GHWA. In Connecticut, individuals of both *G. pennsylvanicus* and *G. firmus* are nearly always flightless. From mark-recapture studies it appears that many individuals move only tens of meters from where they are first marked, but a fraction of marked individuals are never recovered and some of these may represent longer-distance dispersal (Harrison and Rand 1989; unpubl. data). The ability to detect pattern in the hybrid zone on a scale equivalent to or less than the dispersal distance of an individual cricket suggests that this spatial resolution is appropriate and useful for elucidating process in this hybrid zone.

It is possible that the transects we sampled represent non-equilibrium situations, for example, very recent contacts between populations of the two species. Under these circumstances, the observed discontinuities may be transient phenomena and a balance between dispersal and selection need not be invoked to explain the sharp discontinuity. If the transects are at equilibrium, then either crickets show strong preferences for different soil types (thereby effectively reducing dispersal across patch boundaries) or strong selection (either exogenous or endogenous; see Moore and Price 1993) counteracts the effects of dispersal and maintains the steep clines.

Because crickets occupy disturbed habitats and because the cricket hybrid zone in Connecticut exists within a heavily populated and developed region, suitable habitat patches tend to be ephemeral. Many interactions at patch boundaries may be relatively recent and probably have not reached equilibrium. Assuming a neutral diffusion model, the abrupt discontinuities and strong disequilibrium that we see would only be consistent with very recent contact between the two cricket species. For randomly mating populations, linkage disequilibrium decays by one-half every generation, so the probability of D^*, D_{AB}, and *CND* values as large as those found in the transects is extremely small unless populations arrived only a few generations ago. It is unlikely that observed patterns are simply a consequence of our encountering such recent contacts, given that we find nearly identical patterns at two independent sites and that we have sampled each of those sites in two successive years. The patterns that we see are also not likely a consequence *solely* of strong habitat choice in the absence of selection, given that the observed clines in morphology and allele and haplotype frequencies are much steeper than the soil gradient. Also, although *G. pennsylvanicus* females in the laboratory prefer to oviposit in loam soils, *G. firmus* females do not show a preference for sand and indeed lay more eggs in loam than in sand when given a choice in the laboratory (Ross 2000).

Therefore, selection likely plays an important role in maintenance of the abrupt discontinuities. However, it is difficult from pattern alone to identify the nature of the selection regime. Both single-locus and multilocus models suggest that

TABLE 3. Means for morphological traits as a function of molecular alleles. (A) UT transect. (B) GHWA transect. Means for four morphological traits (±SE) are listed for each genotype/haplotype of four molecular loci. Sample sizes are listed in parentheses. *Penn.* refers to homozygotes with alleles that are fixed or nearly fixed in pure *Gryllus pennsylvanicus* populations. *Firmus* refers to homozygotes with alleles that are fixed or nearly fixed in pure *G. firmus* populations.

A

	Ovipositor length (mm)	Femur length (mm)	Pronotum width (mm)	Tegmina color
mtDNA				
penn.	16.05 ± 0.17 (291)	10.83 ± 0.05 (501)	5.62 ± 0.03 (508)	3.35 ± 0.11 (473)
firmus	18.81 ± 0.27 (31)	11.48 ± 0.15 (55)	5.99 ± 0.07 (56)	4.28 ± 0.26 (55)
GpUC 5				
penn.	13.91 ± 0.25 (35)	10.19 ± 0.11 (60)	5.26 ± 0.06 (60)	1.48 ± 0.12 (48)
heterozygote	17.06 ± 0.65 (5)	11.01 ± 0.43 (10)	5.88 ± 0.27 (10)	3.57 ± 0.75 (7)
firmus	19.11 ± 0.23 (37)	11.74 ± 0.15 (55)	6.08 ± 0.08 (55)	4.71 ± 0.27 (42)
GpUC 279				
penn.	15.65 ± 0.56 (29)	10.67 ± 0.20 (45)	5.46 ± 0.09 (45)	2.03 ± 0.32 (31)
heterozygote	16.16 ± 1.28 (5)	11.18 ± 0.29 (8)	5.97 ± 0.25 (8)	6.33 ± 0.74 (6)
firmus	18.64 ± 0.39 (13)	11.16 ± 0.26 (28)	5.89 ± 0.12 (28)	4.33 ± 0.37 (24)
GpUC 351				
penn.	14.12 ± 0.26 (37)	10.33 ± 0.11 (62)	5.31 ± 0.07 (62)	1.71 ± 0.20 (49)
heterozygote	17.72 ± 1.05 (7)	10.99 ± 0.44 (9)	5.56 ± 0.18 (9)	2.14 ± 0.54 (7)
firmus	19.18 ± 0.23 (33)	11.70 ± 0.19 (52)	6.13 ± 0.07 (52)	4.90 ± 0.23 (39)

B

	Ovipositor length (mm)	Femur length (mm)	Pronotum width (mm)	Tegmina color
mtDNA				
penn.	15.06 ± 0.21 (89)	10.71 ± 0.07 (162)	5.63 ± 0.04 (162)	2.17 ± 0.15 (162)
firmus	18.91 ± 0.23 (40)	11.64 ± 0.09 (76)	6.19 ± 0.05 (77)	5.17 ± 0.22 (78)
GpUC 5				
penn.	14.63 ± 0.23 (26)	10.51 ± 0.12 (57)	5.53 ± 0.07 (75)	1.39 ± 0.10 (57)
heterozygote	(0)	11.20 (1)	5.90 (1)	1.00 (1)
firmus	19.26 ± 0.28 (19)	11.78 ± 0.14 (41)	6.27 ± 0.06 (42)	5.29 ± 0.28 (42)

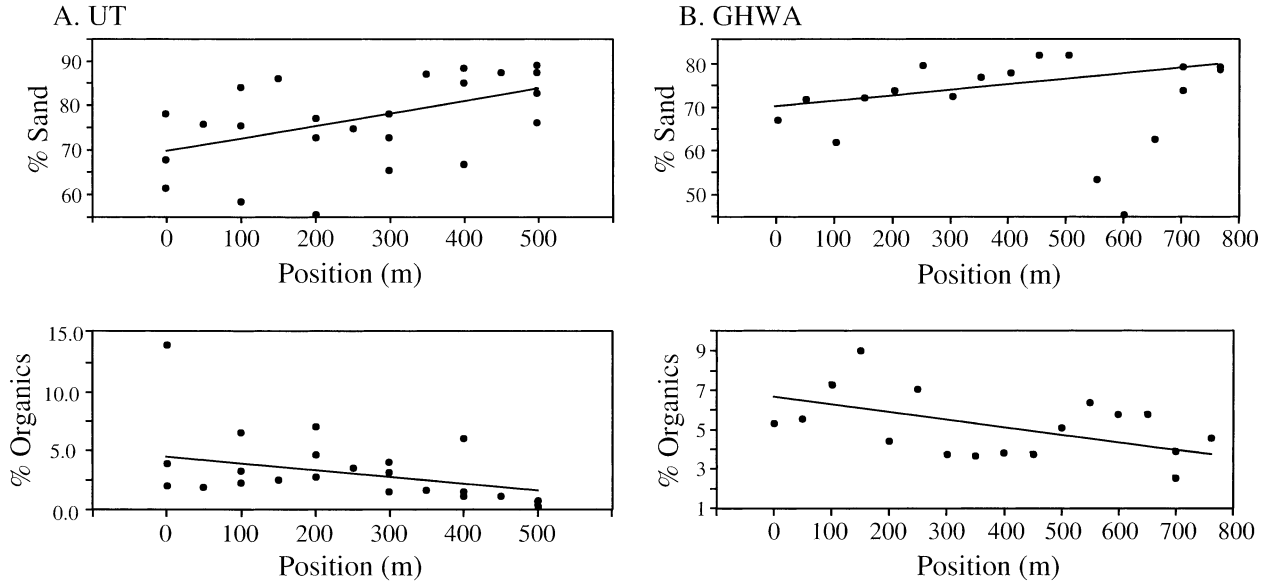

FIG. 7. Percentage sand and organics by position along the transects. In addition to sampling on the roadside, the UT transect was sampled 10 m east (toward the Connecticut River) and 10 m west (uphill) of the road at 100-m intervals. This is indicated by three soil samples at these distances. The lines show the linear regressions of percentage sand or organics on distance. For UT, sand $r^2 = 0.25$, organics $r^2 = 0.26$ (after excluding high value at 0 m). For GHWA, sand $r^2 = 0.45$ (after excluding samples at 550 m, 600 m, and 650 m), organics $r^2 = 0.27$.

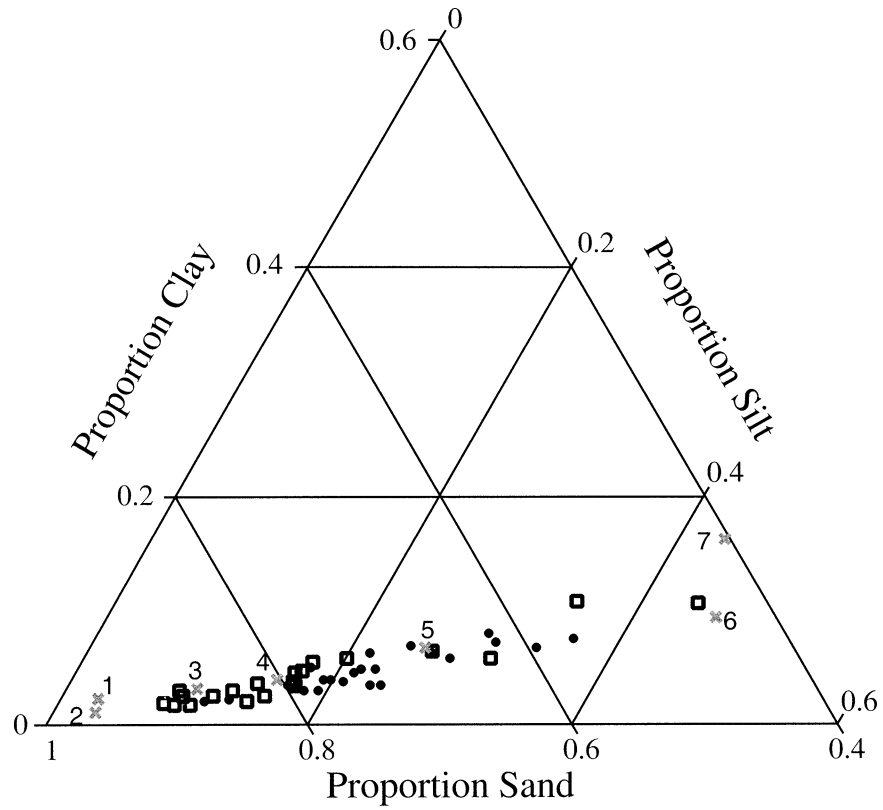

FIG. 8. Ternary plot of particle size distribution for soil samples in the transects. The proportions of the three component particle sizes for each soil sample are shown for the UT and GHWA transects, as well as for seven reference populations. In the graph, soil samples where *Gryllus pennsylvanicus*–like individuals (based on morphology) were collected are indicated by •, soil samples where *G. firmus*–like individuals (based on morphology) were collected are indicated by □, and reference populations are indicated by **X**. In addition, reference populations are numbered: 1–4: locations with *G. firmus* populations (Seaside Park, NJ; Guilford 2, CT; Saybrook Pt., CT; and Canaan 2, CT, respectively); 5–7: locations with *G. pennsylvanicus* populations (Housatonic Meadows, CT; Sharon 1, CT; and Ithaca, NY, respectively). From the graph, *G. firmus*–like crickets are found on soils with higher sand content, whereas *G. pennsylvanicus* are found on loamier soils. The graph does not show the complete range of proportions for each particle size (i.e., the axes do not go from 0 to 1), but all soil samples are shown within the graph.

clines produced by exogenous and endogenous selection cannot be distinguished (Barton and Gale 1993; Kruuk et al. 1999). Furthermore, premating isolating barriers and/or fertility selection may combine to create strong barriers (Gavrilets and Cruzan 1998). Nonetheless, it is useful to explore the possible explanations for the patterns that we see, in the context of our understanding of cricket population structure, ecology, and behavior.

Divergent selection in different soil environments might explain the slope and position of sharp-stepped clines for ovipositor length and/or tegmina color. Soil gradients along the Connecticut transects are not nearly as steep as the clines for ovipositor length and tegmina color, but gradient models of selection indicate that ''stepped clines can evolve in the absence of stepped environments'' (Endler 1977). Ovipositor length determines the maximum depth at which cricket females can place eggs in the soil, although behavioral plasticity may have a strong influence on the actual depth at which eggs are deposited (Masaki 1979, 1986; Bradford et al. 1993). Selection for a long ovipositor in habitats with sandy soils has been invoked to explain longer ovipositors (relative to neighboring populations) in North American field crickets living on sandy soils (Lutz 1908; Alexander 1968). Similarly, it has been proposed that crickets with light tegmina would have an advantage on light colored (sandy) soils (Alexander 1968). However, if tegmina color is adaptive on different soil patches, it is unclear why crickets with dark tegmina would be found on sandy soils, as is the case for both the UT and GHWA transects.

It is difficult to explain the remarkably steep and concordant clines for all morphological and DNA markers on the basis of environmental selection resulting from variation in the nature of the soil substrate. There is no a priori reason to suspect that femur length and pronotum width should directly affect fitness differently in the two soil environments. Furthermore, variation in soil substrate alone cannot readily explain the abrupt step clines for the anonymous nuclear gene markers GpUC5 and GpUC351, which are presumably neutral genetic markers with respect to habitat. To explain these step clines, we might assume that our markers are all closely linked to nuclear genes under strong environmental selection (an unlikely proposition) or that, as a consequence of strong selection at multiple loci across the genome, many chromosomal regions experience strong effective selection, that is, there is ''cohesion of the genome'' (Barton 1983; Kruuk et al. 1999).

An alternative (but not mutually exclusive) selective explanation for the maintenance of steep clines across patch boundaries in the mosaic hybrid zone is that these represent tension zones (Barton and Hewitt 1981, 1985), in which dispersal is balanced by selection against individuals of mixed ancestry. Tension zone models clearly allow for steep clines to be maintained (Barton 1979b, 1983; Barton and Gale 1993), with cline width a function of the magnitude of dispersal, the effective strength of selection on each locus, the number of loci, and the rate of recombination (Barton 1983). If hybrids are less fit, then random colonization of local patches by individuals of two species or races could result in a patchy distribution with narrow clines across patch boundaries maintained by endogenous selection (for an example in chromosomal races of house mice see Hauffe and Searle 1993).

We have no direct evidence for the reduced fitness of hybrids between *G. pennsylvanicus* and *G. firmus*. In the laboratory, egg-to-adult survivorship is much higher in *G. firmus* than in *G. pennsylvanicus*; hybrids exhibit intermediate survivorship and no apparent reduction in fertility relative to the parentals (R. Harrison, unpubl. data). Tension zone models also do not easily explain the position of the cricket step clines, unless density troughs are invoked at the soil patch boundaries. Hence, endogenous selection alone is not likely to explain the patterns of variation in the field cricket hybrid zone. More likely, as emphasized by Kruuk et al. (1999) ''the exclusive action of either endogenous or exogenous selection is undoubtedly an unlikely simplification,'' and the patterns that we see may well derive from a combination of selective forces.

Hybrid zone models that incorporate fertility selection and prezygotic isolation (Gavrilets 1997; Gavrilets and Cruzan 1998) suggest that these factors may also constitute strong barriers to the flow of neutral alleles across hybrid zones. Crosses between *G. firmus* females and *G. pennsylvanicus* males do not give rise to any hybrid offspring (Harrison 1983). The reciprocal cross produces viable, fertile offspring in the laboratory, but prezygotic barriers appear to limit gene exchange in the field (Harrison 1986; Harrison and Rand 1989). In spite of opportunities for hybridization and gene exchange, no F_1 hybrids and very few heterozygotes for the nuclear gene markers are found along the transect.

The cricket hybrid zone is clearly bimodal (Harrison and Bogdanowicz 1997), in the sense that most individuals are very much like one or the other parent species. The distribution of genotype scores shows this most clearly (Fig. 6). Furthermore, comparisons of the associations of traits in crickets—morphological/morphological (Table 1), molecular/molecular (Table 2), and morphological/molecular (Table 3)—suggest high levels of linkage disequilibrium across the transects, especially in the center of the clines. In an earlier study, Harrison and Bogdanowicz (1997) found no F_1 hybrids and strong linkage disequilibrium in three mixed populations in Connecticut. The implication of bimodality and high linkage disequilibrium is that individuals and populations remain pure (i.e., little or no mixing), either because few hybrids are produced or because hybrids have greatly reduced fitness. However, both the earlier study and the data reported here also reveal evidence of introgression at one or more marker loci.

Displacement of Clines for Mitochondrial DNA and GpUC279

The clines for mtDNA and GpUC279 are clearly not concordant with clines for morphological characters or those for the other nuclear gene markers. For both mtDNA and GpUC279, the majority of crickets at the *G. firmus* end of the transects carry *G. pennsylvanicus* alleles or haplotypes. This pattern may reflect ongoing introgression across the UT and GHWA transects or it may be a consequence of past hybridization and introgression (the ''ghost of hybridization past''). The latter scenario suggests that the crickets that initially colonized the *G. firmus* ends of the two transects came from source populations that already were introgressed as a consequence of previous episodes of hybridization at other sites within the mosaic hybrid zone. Whatever the origin of the pattern, the observation of differential introgression argues for differences in the nature of the selection regime experienced by the chromosomal regions (or mtDNA) in which the marker loci reside (Nürnberger et al. 1995).

Because we assay both mtDNA and nuclear gene haplotypes based on single restriction-site differences, it might be the case that the presence of *G. pennsylvanicus* alleles at the *G. firmus* end of the transects represents independent gains or losses of single restriction sites. Several observations argue against this scenario. First, there is strong disequilibrium between mtDNA and GpUC279 at the *G. firmus* end of the UT transect. Crickets that carry *G. pennsylvanicus* mtDNA also carry *G. pennsylvanicus* GpUC279 alleles. This is expected if introgression accounts for the observed pattern, but not if it is simply due to homoplasy. Furthermore, crickets with *G. firmus* morphology that carry *G. pennsylvanicus* mtDNA also carry a strain of *Wolbachia* characteristic of *G. pennsylvanicus* but absent in *G. firmus* (Mandel et al. 2001). Because mtDNA and *Wolbachia* are both maternally transmitted, this pattern is expected if introgression explains the displacement of the mtDNA cline.

Asymmetric introgression of mtDNA has been observed in previous studies of the cricket hybrid zone at larger spatial scales (Harrison et al. 1987; Harrison and Bogdanowicz 1997). The asymmetry (introgression of *G. pennsylvanicus* mtDNA into *G. firmus* but not the reverse) is a consequence of an asymmetry in the outcome of the two reciprocal crosses (Harrison 1983). Because all F_1 hybrids are derived from the cross between *G. firmus* males and *G. pennsylvanicus* females, individuals of mixed ancestry almost always carry *G. pennsylvanicus* mtDNA. *Gryllus firmus* mtDNA can introgress into *G. pennsylvanicus* only if a lineage involves initial backcrossing to a *G. firmus* female followed by repeated backcrossing to *G. pennsylvanicus* males. Extensive introgression of *G. pennsylvanicus* mtDNA may reflect an inherent advantage of this mtDNA throughout the hybrid zone independent of nuclear gene background (positive selection), transient hitchhiking of neutral mtDNA with positively selected nuclear genes (Kilpatrick and Rand 1995), or simply the absence of selection against mtDNA because mtDNA is unlinked to

chromosomal regions that are under negative selection (either exogenous or endogenous).

Within the hybrid zone, many of the populations in which crickets are morphologically like *G. firmus* show evidence of significant introgression of *G. pennsylvanicus* GpUC279 alleles (Harrison and Bogdanowicz 1997). However, introgression of GpUC5 and GpUC351 alleles (from *G. pennsylvanicus* into *G. firmus*) has also occurred, although not as consistently. Furthermore, previous data from populations sampled on a regional scale provide evidence of introgression of *G. firmus* alleles into *G. pennsylvanicus*–like populations, which has not occurred along the UT or GHWA transects. Possible explanations for the differential introgression of nuclear gene markers are the same as invoked for mtDNA; alleles that introgress more readily are either favored across the entire transect or reside in chromosomal regions that are effectively neutral (with introgression of other markers [chromosomal regions] either prevented or delayed by exogenous or endogenous negative selection; Szymura and Barton 1986, 1991; Barton and Gale 1993). Patterns of introgression across hybrid zones often vary among loci (or traits; Harrison 1990; Arnold 1997). The most elegant demonstration of this phenomenon is by Rieseberg et al. (1999), who documented a remarkable consistency across independent, replicate sunflower hybrid zones in the introgression of chromosomal segments in spite of substantial variation among markers in the rates of introgression. These results suggest that introgression is largely controlled by natural selection and that hybrid zones are indeed semipermeable barriers to genetic exchange. Data from the cricket hybrid zone are consistent with both of these conclusions.

Changes in Soil across the Transects

For both the UT and GHWA transects, analyses of soil samples indicate that these transects represent boundaries between sand and loam patches. However, the patches are not discrete, and at fine scales the transition from a sand patch to a loam patch is gradual (Fig. 7). The gradual soil transition may reflect the fuzzy nature of patch boundaries for this hybrid zone, the limits of resolving soil patches using very simple soil characters, and/or that our transects are not necessarily orthogonal to the patch boundary (see below). At intermediate spatial scales, the patchwork association of cricket genotypes and soil types strongly suggests that soil must be structuring and maintaining the field cricket hybrid zone, whereas at fine scales, this association, while apparent, is not nearly as distinct. However, even the gradual transition of soil characters observed at fine scales may produce step clines in cricket characters due to divergent selection (Endler 1977) or behavioral preferences.

Three soil samples along the GHWA transect, at 550 m, 600 m, and 650 m (Fig. 7B), have loamlike characteristics even though they are from the *G. firmus* end of the transect and *G. firmus*–like crickets are found at these locations. That *G. firmus*–like crickets are found at these locations suggests that this area represents a soil patch too small to be recognized by the crickets. The size of a local habitat patch that can support a population of one species embedded in a larger area occupied by a second species depends on the dispersal distance of individuals and on the strength of selection in the different patch types (Levins 1968; Slatkin 1973, 1975; Nagylaki 1975). Thus, patches must be at least some minimum size (Slatkin 1973), and the environment must be relatively coarse grained (Levins 1968) to support a cricket-soil association.

For the UT transect, soil samples taken approximately 10 m to the east and west of the road (which defines the major axis of the UT transect) show that soil characters in less disturbed environments generally correspond to those near the road. Each side of the road, however, has a slightly different character. Samples to the east of the road, near the Connecticut River, generally are slightly sandier in character than the corresponding samples on the road. Samples to the west of the road, which are at slightly higher elevations, are more loamy than corresponding road samples (Fig. 7A). This, of course, is not unexpected, and is consistent with the hypothesis that *G. firmus* may invade upland areas using riverbanks as favorable, sandy corridors.

Comparison of samples east and west of the UT transect also suggest that this transect may not be strictly orthogonal to a sand-loam patch boundary. Furthermore, using cricket characters as a judge of habitat character, the extreme ends of the transects (0 m and 500 m) may not actually represent extremes in soil type. For example, the section of the transect at 200 m appears to be a relatively pure upland area jutting down to the Connecticut River, and the section of the transect at 400 m is a sandy bank deposited from the river. These observations confirm that the real world is far more complex than a discrete two-patch model might suggest and that defining an appropriate scale for patch structure requires detailed observations at multiple spatial scales.

Finally, the shape of step clines for cricket characters will reflect whether samples are collected along transects that are orthogonal to habitat patch boundaries. Because many models use cline shape to estimate evolutionary parameters such as dispersal and selection in hybrid zones, it is essential to map transects onto underlying patterns of environmental heterogeneity. Ideally, mosaic hybrid zones will be sampled in two dimensions (see Marshall and Sites 2001), and two-dimensional models of cline shape will be developed.

Conclusions

In the last few decades, the study of hybrid zones has focused on using pattern to elucidate process. Here we have shown that the pattern of the North American Gryllus hybrid zone—and thus the interpretation of process—is dependent on spatial scale, environmental heterogeneity, sampling of individuals and loci, and the particular history of the system. The cricket hybrid zone is clinal at coarse and fine scales, but it is mosaic at intermediate scales. Many hybrid zones may vary in structure at different scales. The sharp, concordant clines across soil habitat for nuclear, cytoplasmic, and phenotypic markers indicate that this hybrid zone is undoubtedly structured by selection. However, the elucidation of detailed mechanisms for the maintenance of the hybrid zone—whether endogenous selection against hybrids, exogenous selection by the environment, behavioral preferences for mates or habitats, or pre- or postmating barriers—will

depend on closer inspection of the organisms themselves and their interactions.

Acknowledgments

We thank S. Bogdanowicz for help with all aspects of molecular techniques, C. Roberts and J. Roberts for use of and assistance with their phosphor screens, P. Marks for transect equipment, and R. Bryant and staff for the use of soil processing equipment and the use of and assistance in their soil lab. M. Goodisman helped with using CND. This study benefited greatly from discussions with A. Porter. The Zuckers and the Rankins generously allowed us to collect crickets on their land. This project was supported by National Science Foundation grant DEB-981530 from Population Biology to RGH.

Literature Cited

Alexander, R. D. 1957. The taxonomy of the field crickets of the eastern United States (Orthoptera: Gryllidae: *Acheta*). Ann. Entomol. Soc. Am. 50:584–602.

———. 1968. Life cycle origins, speciation, and related phenomena in crickets. Q. Rev. of Biol. 43:1–41.

Arnold, M. L. 1997. Natural hybridization and evolution. Oxford Univ. Press, Oxford, U.K.

Asmussen, M. A., and C. J. Basten. 1994. Sampling theory for cytonuclear disequilibrium. Genetics 138:1351–1363.

Barton, N. H. 1979a. Gene flow past a cline. Heredity 43:333–339.

———. 1979b. The dynamics of hybrid zones. Heredity 43: 341–359.

———. 1983. Multilocus clines. Evolution 37:454–471.

Barton, N. H., and K. S. Gale. 1993. Genetic analysis of hybrid zones. Pp. 13–45 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, Oxford, U.K.

Barton, N. H., and G. M. Hewitt. 1981. Hybrid zones and speciation. Pp. 341–359 *in* W. R. Atchley, and D. S. Woodruff, eds. Evolution and speciation: essays in honour of M. J. D. White. Cambridge Univ. Press, Cambridge, U.K.

———. 1985. Analysis of hybrid zones. Annu. Rev. Ecol. Syst. 16:113–148.

———. 1989. Adaptation, speciation, and hybrid zones. Nature 341:497–503.

Basten, C. J., and M. A. Asmussen. 1997. The exact test for cytonuclear disequilibria. Genetics 146:1165–1171.

Bouyoucous, G. J. 1926. Estimation of the colloidal material in soils. Science 64:362.

Bradford, M. J., P. A. Guerette, and D. A. Roff. 1993. Testing hypotheses of adaptive variation in cricket ovipositor lengths. Oecologia 93:263–267.

Bridle, J. R., S. J. E. Baird, and R. K. Butlin. 2001. Spatial structure and habitat variation in a grasshopper hybrid zone. Evolution 55:1832–1843.

Bridle, J. R., J. Vass-de-Zomba, and R. K. Butlin. 2002. Fine-scale ecological and genetic variation in a *Chorthippus* grasshopper hybrid zone. Ecol. Entomol. 27:499–504.

Cruzan, M. B., and M. L. Arnold. 1993. Ecological and genetic associations in an *Iris* hybrid zone. Evolution 47:1432–1445.

Day, P. R. 1965. Particle fractionation and particle-size analysis. Pp. 545–567 *in* C. A. Black, ed. Methods of soil analysis. Agronomy no. 9, Part 1. American Society of Agronomy, Madison, WI.

Endler, J. A. 1977. Geographic variation, speciation, and clines. Princeton Univ. Press, Princeton, NJ.

Eubank, R. L. 1988. Spline smoothing and nonparametric regression. Marcel Dekker, New York.

Fulton, B. B. 1952. Speciation in the field cricket. Evolution 6: 283–295.

Gavrilets, S. 1997. Single locus clines. Evolution 51:979–983.

Gavrilets, S., and M. B. Cruzan. 1998. Neutral gene flow across single locus clines. Evolution 52:1277–1284.

Gonick, W. N. 1978. General soil map of Connecticut. U.S.D.A. Soil Conservation Service, Connecticut Agricultural Experiment Station, New Haven, CT.

Harrison, R. G. 1983. Barriers to gene exchange between closely related species. I. Laboratory hybridization studies. Evolution 37:245–251.

———. 1986. Pattern and process in a narrow hybrid zone. Heredity 56:337–349.

———. 1990. Hybrid zones: windows on evolutionary process. Oxford Surv. Evol. Biol. 7:69–128.

———. 1993. Hybrid zones and the evolutionary process. Oxford Univ. Press, Oxford, U.K.

Harrison, R. G., and J. Arnold. 1982. A narrow hybrid zone between closely related cricket species. Evolution 36:535–552.

Harrison, R. G., and S. M. Bogdanowicz. 1997. Patterns of variation and linkage disequilibrium in a field cricket hybrid zone. Evolution 51(2):493–505.

Harrison, R. G., and D. Rand. 1989. Mosaic hybrid zones and the nature of species boundaries. Pp. 111–133 *in* D. Otte and J. Endler, eds. Speciation and its Consequences. Sinauer Associates, Sunderland, MA.

Harrison, R. G., D. M. Rand, and W. C. Wheeler. 1987. Mitochondrial DNA variation in field crickets across a narrow hybrid zone. Mol. Biol. Evol. 4:144–158.

Hauffe, H. C., and J. B. Searle. 1993. Extreme karyotypic variation in a *Mus musculus-domesticus* hybrid zone: the tobacco mouse story revisited. Evolution 47:1374–1395.

Hewitt, G. M. 1988. Hybrid zones: natural laboratories in for evolutionary studies. Trends Ecol. Evol. 3:158–167.

———. 1993. After the ice: *parallelus* meets *erythropus* in the Pyrenees. Pp. 140–164 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, Oxford, U.K.

Jiggins, C. D., and J. Mallet. 2000. Bimodal hybrid zones and speciation. Trends Ecol. Evol. 15:250–255.

Key, K. H. L. 1968. The concept of stasipatric speciation. Syst. Zool. 17:14–22.

Kilpatrick, S. T., and D. M. Rand. 1995. Conditional hitchhiking of mitochondrial DNA: frequency shifts of *Drosophila melanogaster* mtDNA variants depend on nuclear genetic background. Genetics 141:1113–1124.

Kruuk, L. E. B., S. J. E. Baird, and N. H. Barton. 1999. A comparison of multilocus clines maintained by environmental adaptation or by selection against hybrids. Genetics 153: 1959–1971.

Levins, R. 1968. Evolution in changing environments. Princeton Univ. Press, Princeton, NJ.

Lewis, P. O., and D. Zaykin. 2001. Genetic data analysis: computer program for the analysis of allelic data. Ver. 1.0 (d16c). Free program available via http://lewis.eeb.uconn.edu/lewishome/software.html.

Lutz, F. E. 1908. The variation and correlation of certain taxonomic characters of *Gryllus*. Carnegie Institute Washington Publication 101:3–63.

Mandel, M. J., C. L. Ross, and R. G. Harrison. 2001. Do *Wolbachia* infections play a role in unidirectional incompatibilities in a field cricket hybrid zone? Mol. Ecol. 10:703–709.

Marshall, J. C., and J. W. Sites Jr. 2001. A comparison of nuclear and mitochondrial cline shapes in a hybrid zone in the *Sceloporus grammicus* complex (Squamata; Phrynosomatidae). Mol. Ecol. 10:435–449.

Masaki, S. 1979. Climatic adaptation and species status in the lawn ground cricket. III. Ovipositor length. Oecologia 43:207–219.

———. 1986. Significance of the ovipositor length in life cycle adaptation of crickets. Pp. 20–34 *in* F. Taylor and R. Karbin, eds. The evolution of insect life cycles. Springer, Berlin.

McKeague, J. A., ed. 1978. Manual on soil sampling and methods of analysis. 2d ed. Canadian Society of Soil Sciences, Ottawa, Ontario.

Moore, W. S., and J. T. Price. 1993. Nature of selection in the northern flicker hybrid zone and its implications for speciation

theory. Pp. 196–225 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, Oxford, U.K.
Nagylaki, T. 1975. Conditions for the existence of clines. Genetics 80:595–615.
———. 1976. Clines with variable migration. Genetics 83:867–886.
Nürnberger, B. D., N. H. Barton, C. MacCallum, J. Gilchrist, and M. Appleby. 1995. Natural selection on quantitative traits in the *Bombina* hybrid zone. Evolution 49:1224–1238.
Patton, J. L. 1993. Hybridization and hybrid zones in pocket gophers (Rodentia, Geomyidae). Pp. 290–308 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, Oxford, U.K.
Porter, A. H., R. Wenger, H. Geiger, A. Scholl, and A. M. Shapiro. 1997. The *Pontia daplidice-edusa* hybrid zone in northwestern Italy. Evolution 51(5):1561–1573.
Rand, D. M., and R. G. Harrison. 1989. Ecological genetics of a mosaic hybrid zone: mitochondrial, nuclear, and reproductive differentiation of crickets by soil type. Evolution 43:432–449.
Reynolds, C. A. 1979a. Soil survey of New Haven County, Connecticut. U.S.D.A. Soil Conservation Service, Connecticut Agricultural Experiment Station. New Haven, CT.
———. 1979b. Soil survey of Middlesex County, Connecticut. U.S.D.A. Soil Conservation Service, Connecticut Agricultural Experiment Station. New Haven, CT.
Rieseberg, L. H., J. Whitton, and K. Gardner. 1999. Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. Genetics 152:713–727.
Ross, C. L. 2000. Ecological and environmental factors affecting the structure and maintenance of a mosaic hybrid zone between the field crickets, *Gryllus firmus* and *Gryllus pennsylvanicus*. Ph.D. diss., Cornell University, Ithaca, NY.
Sambrook, J., E. F. Fritsch, and T. Maniatus. 1989. Molecular cloning. Cold Spring Harbor Press, Cold Spring Harbor, NY.
Searle, J. B. 1986. Factors responsible for a karyotypic polymorphism in the common shrew, *Sorex araneus*. Proc. R. Soc. Lond. B 229:277–298.
Sheldrick, B. H., and C. Wang. 1993. Particle size distribution. Pp. 499–511 *in* M. R. Carter, ed. Soil sampling and methods of analysis. Lewis Publishers, Boca Raton, FL.
Simon, C., F. Frati, A. Beckenbach, H. Liu, and P. Flook. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am., 87:651–701.
Sites, J. W., N. H. Barton, and K. M. Reed. 1995. The genetic structure of a hybrid zone between two chromosomal races of the *Sceloporus grammicus* complex (Sauria, Phrynosomatidae) in central Mexico. Evolution 49:9–36.
Slatkin, M. 1973. Gene flow and selection in a cline. Genetics 75: 733–756.
———. 1975. Genetic drift in a cline. Genetics 81(1):209–222.
———. 1976. Gene flow and selection in a 2 locus system. Genetics 81(4):787–802.
Szymura, J. M. 1993. Analysis of hybrid zones with *Bombina*. Pp. 261–289 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, Oxford, U.K.
Szymura, J. M., and N. H. Barton. 1986. Genetic analysis of a hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*, near Cracow in Southern Poland. Evolution 45: 237–261.
———. 1991. The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*: comparisons between transects and between loci. Evolution 45:237–261.
Willett, C. S., M. J. Ford, and R. G. Harrison. 1997. Inferences about the origin of a field cricket hybrid zone from a mitochondrial DNA phylogeny. Heredity 79:484–494.

Corresponding Editor: G. Wallis