

## HYBRIDIZATION AND GEOGRAPHIC VARIATION IN TWO MEADOW KATYDID CONTACT ZONES

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**Abstract.**—In this study, previously unrecognized hybridization was documented between two meadow katydids in each of two disjunct contact zones, in the southeastern United States and along the Potomac River near Washington, DC. These two zones have very different histories and dynamics of interaction between the two taxa. *Orchelimum nigripes* and *O. pulchellum* (Tettigoniidae: Conocephalinae) are distributed west and east, respectively, of the Appalachian Mountains, from the Great Lakes to the Gulf Coast and along the Atlantic Coastal Plain from New York to the Florida Keys, but are not found in the Appalachians themselves. In addition, during this century *O. nigripes* has become established in a small area east of the Appalachians, in the Potomac River basin, where it has completely replaced *O. pulchellum* along the river corridor above Washington, DC. I sampled katydids from 40 sites across both hybrid zones and mapped geographic patterns of genetic variation (allele frequencies at two diagnostic loci) and variation in a morphometric index for males. Although the two taxa are quite distinct over most of their extensive distributions, there is clear evidence of introgression in both contact zones. In the Deep South, samples from a transect along the Gulf Coast define a broad hybrid zone of about 50–100 km, while samples from a transect 200 km to the north define a zone of about 150–250 km in width. Only one Deep South population shows a deviation from Hardy-Weinberg equilibrium at either locus, and there is no evidence of linkage disequilibrium in any Deep South population. In the Potomac region, there is a narrow upstream-downstream hybrid zone along the river. Within the Potomac River floodplains downriver from Washington, DC, as well as outside the floodplains throughout the region, *O. pulchellum* is present in abundance, but *O. nigripes* markers are virtually absent. Within the floodplains upriver from Washington, DC, *O. nigripes* is abundant, but *O. pulchellum* markers are virtually absent. All four mixed ancestry Potomac populations sampled show strong and highly significant linkage disequilibrium, although only one clearly deviates from single-locus Hardy-Weinberg equilibrium. The position of the Deep South hybrid zone is generally consistent with interspecific and intraspecific phylogeographic patterns previously reported for numerous taxa from the southeastern United States. The observed genetic and morphometric clines appear to be the result of neutral introgression over thousands of years. In the much younger Potomac hybrid zone, *O. nigripes* appears to be spreading downriver, interbreeding with *O. pulchellum*, and replacing it. The mechanism for this replacement remains uncertain, but may be clarified by ongoing behavioral, genetic, and breeding studies.

**Key words.**—Clinal variation, hybrid zone, introgression, katydid, *Orchelimum*, Potomac, southeastern United States, species replacement, Tettigoniidae.

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For many evolutionary biologists, questions about the nature of species—what they are, how they arise, how they are maintained, and how they disappear—are among the most fundamentally interesting in all of biology. These questions have been approached in many different ways. One important approach, studying taxa that exchange genes in hybrid zones, has provided considerable insight into these issues (Barton and Hewitt 1985, 1989; Hewitt 1988, 1990a; Harrison 1990, 1993; Arnold 1997). Hybrid zones are regions where genetically distinct groups of organisms interact, leaving at least some offspring of mixed ancestry (Harrison 1990). Because the taxa involved may be partly, but not completely, reproductively isolated, hybrid zones offer unique opportunities to study the nature of species, the origin of species, and behavioral and genetic mechanisms of reproductive isolation.

Following the establishment of a hybrid zone, there are several possible fates for the zone: (1) if hybrids are selectively inferior, the interacting taxa may evolve reproductive isolation by the process of reinforcement; (2) the hybrid zone may be stabilized (on an ecological time scale) by some combination of selection and/or dispersal; (3) if the genetic equilibria represented by the two taxa are selectively neutral, the

two may diffuse into one another, their distinctions gradually fading away as clines become increasingly broad and shallow; (4) under some circumstances, one taxon may replace the other, with little or no introgression.

The first two possibilities have received a great deal of attention from evolutionary biologists. Reinforcement in hybrid zones formed by secondary contact was at one time widely considered to be an important mechanism of speciation (e.g., Dobzhansky 1940; Remington 1968; Lewontin 1974), but its importance has been viewed with increasing skepticism in recent years, on both theoretical and empirical grounds (e.g., Mayr 1963; Hauber and Bloom 1983; Barton and Hewitt 1985; Spencer et al. 1986; Butlin 1989; Sanderson 1989; Hatfield et al. 1992; Ritchie et al. 1992; but see Howard 1993; Kelly and Noor 1996). In contrast, the idea that stable hybrid zones may be maintained for hundreds or even thousands of years has gained much support from recent theoretical work, although except for a very few cases (Barton and Hewitt 1985) such long-term stability is quite difficult to demonstrate in nature.

The third possibility listed above, the fusion of diverged taxa upon secondary contact, may be very common (Hewitt 1989). However, examples of hybrid zones that appear to be decaying following secondary contact are quite rare in the literature (but see Barrowclough 1980; Wynn 1986). This

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striking absence of putatively neutral clines in the literature is somewhat surprising, because decaying hybrid zones must often remain recognizable for hundreds or thousands of years (A. H. Porter et al. 1997). Furthermore, given their greater breadth, decaying hybrid zones would seem to be more likely to be encountered than narrow, stabilized zones. It is possible that these broad hybrid zones tend to be overlooked because they are only apparent from sampling on a fairly large geographic scale. It may also be the case that evolutionary biologists have simply focused on narrow hybrid zones because they are more striking, although they clearly cannot provide a complete picture of the evolutionary role of hybridization.

As with neutral introgression, reports of the replacement of one taxon by another following hybridization are rather scarce in the literature, although Remington (1968) and others have suggested several potential candidates for this scenario. Evidence for the local or global extinction of one taxon following a period of hybridization is most likely to come from contacts that have been initiated in historical times, allowing us to observe their early progress. Two possible examples involve the golden-winged and blue-winged warblers (*Vermivora chrysoptera* and *V. pinus*) in the northeastern United States (Gill 1980) and the two introduced fire ants *Solenopsis richteri* and *S. invicta* in the Southeast (Shoemaker et al. 1996).

Which of the outcomes of secondary contact just discussed actually develops in any particular case is not simply a function of the taxa involved. Based on the few examples for which interactions between the same taxa have been studied in different regions, it is apparent that the historical and ecological context of the interaction may greatly influence its outcome (e.g., Frech and Confer 1987; Bert and Harrison 1988; Hairston et al. 1992; Shoemaker et al. 1996). Additional comparative studies of multiple contact zones involving the same taxa will help clarify the factors determining the consequences of secondary contact.

In this study, I provide evidence for both neutral introgression and replacement in two disjunct katydid hybrid zones I have identified in the southeastern United States (in the "Deep South," specifically, Mississippi and Alabama) and along the Potomac River near Washington, DC. The Deep South zone appears to date back at least to the late Pleistocene or early Holocene, whereas the Potomac zone was apparently established in this century. These two zones provide an unusual opportunity to compare the structure and dynamics of contact zones of very different ages. Although the Deep South zone appears to have been decaying in an effectively neutral fashion for thousands of years, in the recently established Potomac zone there may be selective costs to hybridization and possibly sexual selection favoring one species.

#### BACKGROUND

*Orchelimum nigripes* Scudder and *O. pulchellum* Davis are abundant and widespread conocephaline katydids (Tettigoniidae: Conocephalinae) found throughout much of the eastern United States. They are distributed west and east, respectively, of the Appalachian Mountains. Neither is known from the Appalachians themselves. South of the Appalachians, *O. nigripes* and *O. pulchellum* were believed, prior to

this study, to be neatly parapatric allopecies (e.g., Walker 1971). However, I have documented extensive introgression in this region that defines a very broad hybrid zone. Curiously, in 1974 *O. nigripes* was collected east of the Appalachians along the Potomac River near Cabin John, Maryland (~10 km northwest of Washington, DC), where it was found to be abundant (Morris and Walker 1976), as it is still today. Historically, this stretch of the Potomac has been thoroughly studied by entomologists and other biologists, many of them affiliated with the United States National Museum, so unusually good historical records and collections are available. Based on a comparison of current distributions with museum specimens collected from specific sites during the first half of this century and with an early annotated list of the Orthoptera of Plummer's Island, Maryland (McAtee and Caudell 1918), it is evident that *O. nigripes* has appeared (or, at least, become established) along the Potomac River quite recently, certainly within the last 50–75 years, probably moving eastward from Ohio through West Virginia or Pennsylvania (although it is not known from these intervening regions), perhaps with inadvertent assistance from humans. Thus, in the vicinity of Washington, DC, *O. nigripes* and *O. pulchellum* now form a second hybrid zone, this one rather narrow and apparently of very recent origin.

Both *O. nigripes* and *O. pulchellum* are found on woody and herbaceous vegetation in freshwater marshes and swamps and along the edges of lakes, ponds, and rivers, often in great abundance. Based on extensive field experience with both taxa, no differences in habitat or other aspects of their ecology are apparent, although only *O. nigripes* has been studied in detail in the field (Feaver 1983). Both appear to be univoltine over most or all of their distributions. Morphologically, the two taxa are similar, but over most of their extensive distributions they are easily distinguished, differing conspicuously in a suite of characters including shape and inflexion of the lateral pronotal lobes, cercal morphology, leg coloration, shape of the ovipositor, and shape of the right vein of the stridulatory "mirror frame" in males (Davis 1909; Rehn and Hebard 1915; Blatchley 1920; Walker 1971; L. Shapiro, unpubl. data). Of particular interest is the extreme similarity of their calling songs (Walker 1971; L. Shapiro, unpubl. data), which may in fact be indistinguishable, a highly unusual phenomenon for two such clearly differentiated acoustic insects. Indeed, although *O. nigripes* females given a choice in mating experiments show a strong preference for conspecific males (L. Shapiro, unpubl.; see Discussion below), preliminary phonotaxis experiments suggest that this assortative mating is not based on song discrimination (Shapiro 1996).

In this study I used allozyme electrophoresis and morphometric analysis to examine the structure of the Deep South and Potomac contact zones and the ecological and evolutionary forces shaping them. I consider the structure and history of the Deep South zone in the context of data on other taxa from this region, and compare this contact with the more recent contact in the Potomac region.

#### MATERIALS AND METHODS

##### *Field Collections*

I collected over 1700 *Orchelimum* from (1) three sites in the Midwest (MW), in Ohio and Illinois; (2) 23 sites along

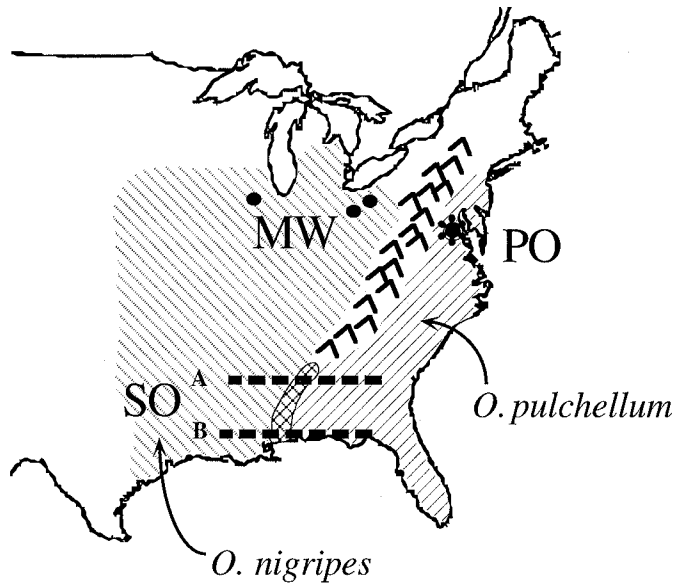


FIG. 1. Sampling locations in the Midwest (MW), in the Potomac region (PO), and along two transects in the Deep South (SO-A and SO-B). Hatching indicates the distributions of *Orchelimum nigripes* and *O. pulchellum*. Cross-hatching shows the Deep South hybrid zone discovered in this study (the two species were formerly believed to have neatly parapatric distributions in this region).

two east-west transects (SO-A and SO-B) through the Deep South, south of the Appalachian barrier; and (3) 17 sites in the Potomac (PO) region, north and south of Washington, DC, both along and away from the Potomac River (Figs. 1–3). Collections were made from the Midwest in 1993 and 1994; in the Deep South in 1991, 1992, and 1993; and from the Potomac region in 1991, 1992, 1993, and 1994. *Orchelimum* are very wary and visually cryptic, but males sing loudly and persistently. At most sites, males were located primarily by tracking their songs. At some sites, both males and females could be located as they jumped when disturbed by my movements through the vegetation. Specimens were captured with a sweep net or by hand, as the situation warranted. Sample sizes from each site (population) ranged from 7 to 103 individuals, but most samples included between 20 and 60 animals (Table 1). All specimens were returned to the lab alive (usually transported in iced coolers, but occasionally mailed overnight via express mail) and frozen at  $-70^{\circ}\text{C}$ .

#### *Allozymes*

Based on a preliminary screening of samples from clearly allopatric pure *O. nigripes* and *O. pulchellum* populations, I identified two loci, isocitrate dehydrogenase (IDH-2, EC 1.1.1.42, cathodal) and malate dehydrogenase (MDH, EC 1.1.1.37, anodal) that are nearly fixed for different alleles in the two taxa (or have alleles unique to *O. pulchellum*, which were pooled for all analyses). I used homogenate from a single hind leg to score each individual's genotype at these two loci (details in Shapiro 1996). From these data, allele frequencies were estimated for each population.

I used the software package GENEPOP (vers. 2, Raymond and Rousset 1995a; see also Raymond and Rousset 1995b) to perform for each population an exact probability test ex-

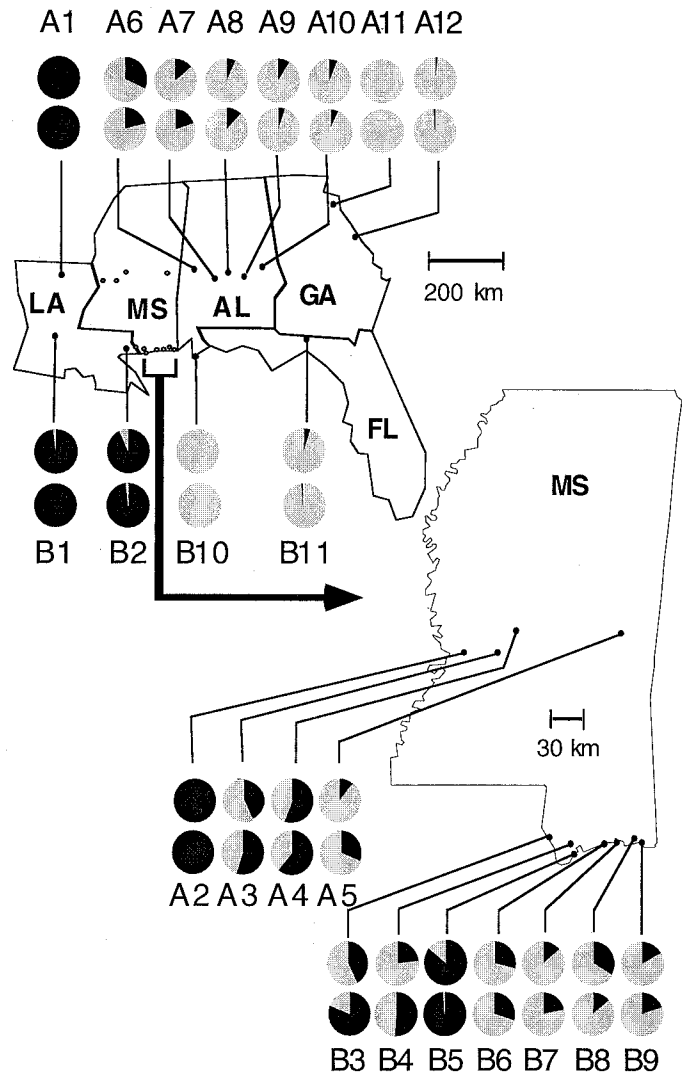


FIG. 2. Locations of populations sampled along transects SO-A and SO-B in the Deep South. Pie charts for each population show allele frequencies for MDH (upper pies) and IDH (lower pies). *Orchelimum nigripes* and *O. pulchellum* alleles are indicated by darker and lighter shades, respectively.

aming deviations from Hardy-Weinberg equilibrium at each locus (using complete enumeration), as well as an unbiased test to estimate the exact probability (and the standard error of this estimate) of nonrandom association between loci in each population (i.e., genotypic linkage disequilibrium).

For each population showing a significant association between loci, I calculated the disequilibrium coefficient,  $D$ , and the standardized disequilibrium coefficient  $D'$  (Hedrick 1987; Lewontin 1988). In addition, I calculated the correlation coefficient  $r$ , which measures the extent to which alleles at different loci are correlated in their occurrence in chromosomes (Hartl and Clark 1989). The test statistic  $nr^2$  has an approximate chi-square distribution with one degree of freedom (Hill 1974), assuming the two loci are not closely linked and effective population sizes are not too small (Golding 1984).

Potomac samples showed significant linkage disequilib-

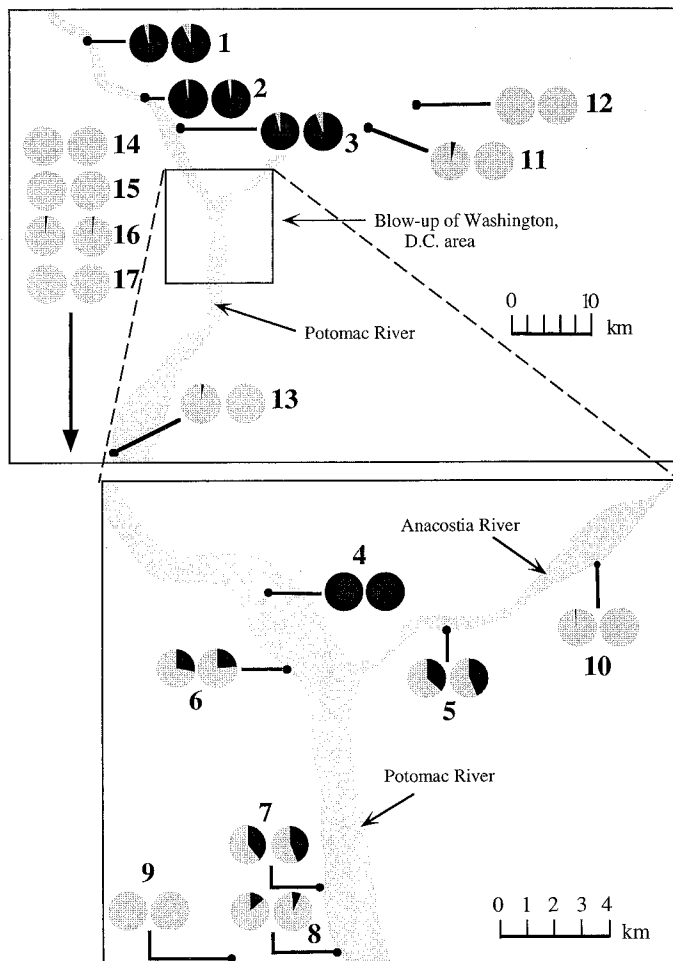


FIG. 3. Locations of populations sampled in the Potomac region. Pie charts for each population show allele frequencies for MDH (left) and IDH (right). *Orchelimum nigripes* and *O. pulchellum* alleles are indicated by darker and lighter shades, respectively. Site PO-14 is about 15 km downstream from PO-13; sites PO-15 to PO-17 are along the Rappahannock River about 60–100 km south of Washington, DC.

rium in all populations where disequilibrium could be estimated (i.e., the four sampled populations with at least two segregating alleles with frequency greater than 0.05 at each locus). No linkage disequilibrium was identified in the Deep South, however, so disequilibrium statistics are not reported for these populations.

### Morphometrics

As noted above, *O. nigripes* and *O. pulchellum* show clear differences in a number of morphological characters that could be analyzed as geographically varying quantitative traits distinguishing the two taxa. For example, in a preliminary study I found that I was able to use ovipositor shape very effectively to separate pure *O. nigripes* and *O. pulchellum* (unpubl. data). However, for this study I focused on the structure of male cerci, both because cerci are easy to handle and to isolate from the rest of the specimen and because the usefulness of cercal characters has already been well established by *Orchelimum* systematists (Rehn and He-

TABLE 1. Frequency of *O. nigripes* alleles for MDH and IDH and mean CVA score for each population sampled along the two Deep South transects, in the Potomac region, and in the Midwest. The frequency of *O. pulchellum* alleles in each population can be obtained by subtracting the frequency of *O. nigripes* alleles from one.

Population	<i>O. nigripes</i> MDH frequency (n)	<i>O. nigripes</i> IDH frequency (n)	Mean CVA score (n)
SO-A1	1.00 (26)	1.00 (26)	0.66 (20)
SO-A2	1.00 (7)	1.00 (7)	0.42 (6)
SO-A3	0.43 (33)	0.55 (32)	1.72 (25)
SO-A4	0.56 (26)	0.62 (26)	2.37 (12)
SO-A5	0.11 (53)	0.31 (53)	2.58 (29)
SO-A6	0.32 (56)	0.21 (56)	2.88 (37)
SO-A7	0.13 (30)	0.20 (30)	4.11 (27)
SO-A8	0.07 (41)	0.12 (41)	4.47 (27)
SO-A9	0.09 (22)	0.05 (22)	4.14 (18)
SO-A10	0.06 (42)	0.07 (43)	4.58 (36)
SO-A11	0.00 (24)	0.00 (24)	4.60 (17)
SO-A12	0.02 (92)	0.01 (92)	4.68 (50)
SO-B1	0.98 (22)	1.00 (22)	0.57 (17)
SO-B2	0.94 (61)	0.98 (61)	0.41 (36)
SO-B3	0.43 (27)	0.81 (27)	1.52 (24)
SO-B4	0.23 (61)	0.51 (61)	2.30 (44)
SO-B5	0.86 (42)	0.99 (42)	0.60 (30)
SO-B6	0.29 (24)	0.30 (23)	2.53 (13)
SO-B7	0.14 (39)	0.22 (39)	1.78 (28)
SO-B8	0.34 (22)	0.14 (22)	2.53 (10)
SO-B9	0.17 (48)	0.20 (48)	2.68 (34)
SO-B10	0.00 (28)	0.00 (28)	3.60 (17)
SO-B11	0.05 (62)	0.01 (69)	5.84 (18)
PO-1	0.96 (102)	0.93 (103)	0.62 (50)
PO-2	0.98 (78)	0.98 (78)	0.72 (45)
PO-3	0.96 (39)	0.95 (38)	0.74 (16)
PO-4	1.00 (30)	1.00 (30)	0.79 (7)
PO-5	0.38 (24)	0.44 (24)	2.98 (24)
PO-6	0.28 (57)	0.25 (57)	3.50 (42)
PO-7	0.39 (46)	0.44 (46)	2.40 (23)
PO-8	0.14 (59)	0.07 (59)	3.97 (38)
PO-9	0.00 (32)	0.00 (32)	4.66 (22)
PO-10	0.01 (85)	0.00 (85)	4.73 (56)
PO-11	0.04 (25)	0.00 (25)	4.69 (19)
PO-12	0.00 (31)	0.00 (31)	4.42 (15)
PO-13	0.03 (34)	0.00 (34)	4.32 (21)
PO-14	0.00 (14)	0.00 (15)	4.73 (9)
PO-15	0.00 (16)	0.00 (16)	4.29 (10)
PO-16	0.02 (22)	0.02 (22)	4.83 (5)
PO-17	0.00 (11)	0.00 (11)	4.87 (6)
MW-1	0.98 (21)	1.00 (21)	0.80 (16)
MW-2	1.00 (31)	1.00 (31)	0.39 (23)
MW-3	1.00 (34)	1.00 (34)	0.83 (20)

bard 1915; Blatchley 1920; Thomas and Alexander 1962; Walker 1971).

To measure cerci, I removed the right cercus from each frozen male's abdomen, placed it in a labeled tube, and returned the animal to the freezer for later molecular analysis. Each cercus was glued to a small piece of acid-free, rag card stock that was pierced by an insect pin. The contour of each cercus was then traced onto a 5 × 8 inch index card using a Wild M5A stereomicroscope fitted with a drawing tube. These contours were digitized with a Sony XC-57 video camera and a PC with an imaging board running the software package MORPHOSYS (Meacham and Duncan 1991).

To quantify the shape difference between *O. nigripes* and *O. pulchellum* cerci, I derived two simple measures using

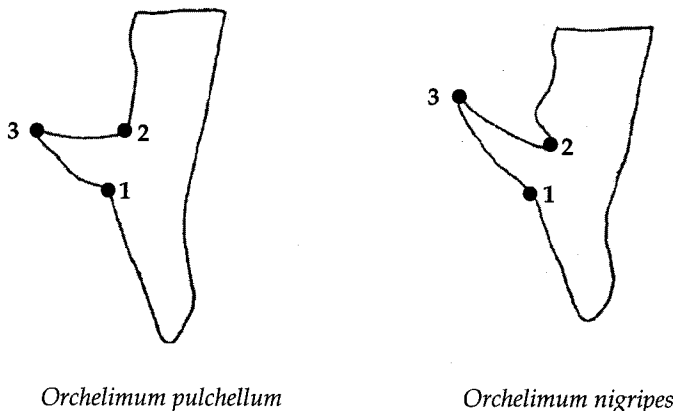


FIG. 4. Cerci of *Orchelimum pulchellum* and *O. nigripes*, with landmarks indicated. Two cercal measures were used in the canonical variates analysis: cercal measure *M1* is angle 1-3-2; measure *M2* is calculated by projecting a line from point 3 onto line 1-2 to establish a new point, point 4, then calculating the ratio between segments 1-2 and 1-4.

three landmarks on the cercal tooth, two at its base and one at its tip. I used MORPHOSYS to calculate these measures for each cercus (Fig. 4). The population means of the two measures were highly correlated among hybrid populations (defined for this purpose as all populations with an average *O. nigripes* allele frequency between 0.20 and 0.80;  $r = 0.83$ ,  $P < 0.01$ ), but within at least some of these populations the two measures were only weakly correlated, implying some degree of independence. A morphological index was constructed from the two measures using a canonical variates analysis (CVA) carried out with NT-SYS (Rohlf 1993) based on 50 pure *O. nigripes* and 50 pure *O. pulchellum* individuals, both museum specimens and samples collected by me, pooled from multiple allopatric populations from throughout their distributions. The discriminant function produced by this analysis performed perfectly when tested with an independent reference set of pure *O. nigripes* and *O. pulchellum*, correctly classifying all 40 *O. nigripes* and 40 *O. pulchellum* in the test dataset. Cerci were measured from nearly 1100 males from within and outside the hybrid zones and their CVA scores calculated. These scores were then averaged for each population to obtain a morphological index score for that population.

## RESULTS

### Allozymes

#### Deep South

The locations of the sampled Deep South populations and their allele frequencies at the two surveyed loci are shown in Figure 2 and Table 1 (precise locations are given in Shapiro 1996). Along both SO-A and SO-B, MDH and IDH exhibit marked east-west clines in allele frequency that are steep relative to the total geographic distributions of *O. nigripes* and *O. pulchellum* (Fig. 5), although SO-B5 represents a local cline reversal (Fig. 2; see Discussion). Along each of the two transects, the MDH and IDH clines are closely concordant. Approximate cline widths, defined as the distance (in some

cases interpolated from a curve fit by eye) between 20% and 80% allele frequencies (Endler 1977), are shown for both loci along both transects in Table 2. Also in Table 2 are the cline widths defined as the inverse of the maximum slope of allele frequencies across the cline, which are more useful for analytical purposes (Barton and Gale 1993). An analogous definition for clines in quantitative traits, the ratio of the difference between the populations on either side and the maximum slope across the cline (Barton and Gale 1993), was used to estimate CVA cline widths (Table 2) from morphometric data (see below). Clines at both allozyme loci define a hybrid zone of about 150–250 km in width along SO-A, and about 50–100 km in width along SO-B, with the hybrid zone along SO-A centered about 200 km farther west than along SO-B (Fig. 5).

Only one of the Deep South populations from either transect showed a significant deviation ( $P < 0.05$ ) from Hardy-Weinberg equilibrium at either locus (SO-B5, MDH,  $P = 0.0224$ ), and none showed significant linkage disequilibrium, even without any correction for multiple tests (data available from author or Shapiro 1996). Any correction for multiple tests would make the single significant Hardy-Weinberg deviation nonsignificant.

#### Potomac

The locations of Potomac populations sampled and their allele frequencies at the two surveyed loci are shown in Figure 3 and Table 1 (precise locations are given in Shapiro 1996). Allele frequencies for both MDH and IDH change abruptly both upstream and downstream from Washington, DC, while populations with intermediate allele frequencies are found only along a short stretch (~20 km) of the Potomac River (Fig. 6). Outside the Potomac floodplains, *O. nigripes* alleles are effectively absent (Fig. 3). As in the Deep South transects, the MDH and IDH clines are closely concordant. Only one population (PO-5) showed a clear deviation from Hardy-Weinberg equilibrium at either locus, but all four mixed ancestry populations along the river showed strongly significant genotypic linkage disequilibrium (Tables 3, 4). Estimated values of  $D$ ,  $D'$ ,  $r$ , and  $nr^2$  for the four mixed ancestry Potomac populations are given in Table 4, along with  $P$ -values for the chi-square test of association. Table 4 also includes the estimated exact probabilities for the genotypic contingency tables, which are consistent with the chi-square tests.

#### Midwest

All three midwestern populations sampled are genetically typical *O. nigripes*, as expected. Allele frequencies at the two surveyed loci are shown in Table 1.

### Morphometrics

#### Deep South

Along both SO-A and SO-B, CVA scores show a relatively sharp decrease from east to west (i.e., they become increasingly *nigripes*-like), consistent with the pattern seen for allozymes (Fig. 7). This transition in population means results from the shifting of score distributions, with no indication

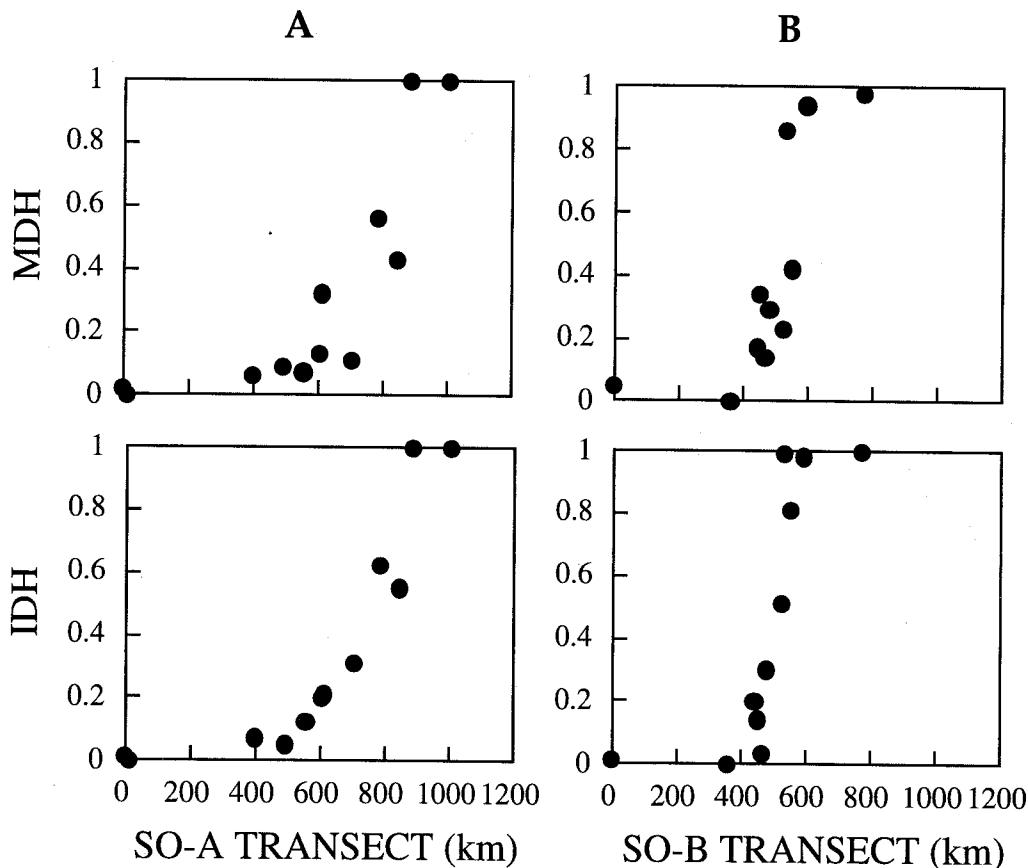


FIG. 5. *Orchelimum nigripes* allele frequencies along two Deep South (SO) transects. (A) MDH and IDH allele frequencies along SO-A. (B) MDH and IDH frequencies along SO-B.

of bimodality within intermediate populations (Shapiro 1996). Table 1 shows the average CVA scores for Deep South populations.

#### Potomac

CVA scores show a sharp increase moving downstream in the general vicinity of Washington, DC, and an even sharper increase moving away from the river, consistent with the lack of *O. nigripes* genetic markers downstream and away from the river. Table 1 shows the average CVA scores for Potomac populations. As in the Deep South, Potomac populations with

intermediate population means show no indication of bimodality (Shapiro 1996).

#### Midwest

All three midwestern populations sampled had CVA scores typical of pure *O. nigripes*. Table 1 shows the average CVA scores for midwestern populations.

#### Concordance of Genetic and Morphometric Patterns

The genetic and morphometric indices covary along both Deep South transects (Fig. 8). The association of morphometric and genetic change along these transects is very tight, the single outlier being the morphometrically extreme population SO-B11. This association is readily apparent in the Potomac region as well, even though there are only four transitional populations to examine here.

#### Additional Populations

In addition to the samples reported on here, very small samples were collected opportunistically at other sites along the eastern seaboard (New York, New Jersey, Maryland, North Carolina, and South Carolina; total  $n = 20$ ). As expected, these additional animals showed the coloration, morphology, and (where assessed) allozyme profile of pure *O. pulchellum*.

TABLE 2. Cline widths along both Deep South transects for MDH allele frequency, IDH allele frequency, mean allozyme allele frequency, and CVA score (morphometric index) estimated using the definitions of both Barton and Gale (1993) and Endler (1977).

Transect	Character	Barton cline width (km)	Endler cline width (km)
SO-A	MDH	180	150
	IDH	261	210
	Mean allozyme	213	140
	CVA score	~150-400	~370
SO-B	MDH	81	50
	IDH	87	100
	Mean allozyme	104	60
	CVA score	~300	~120

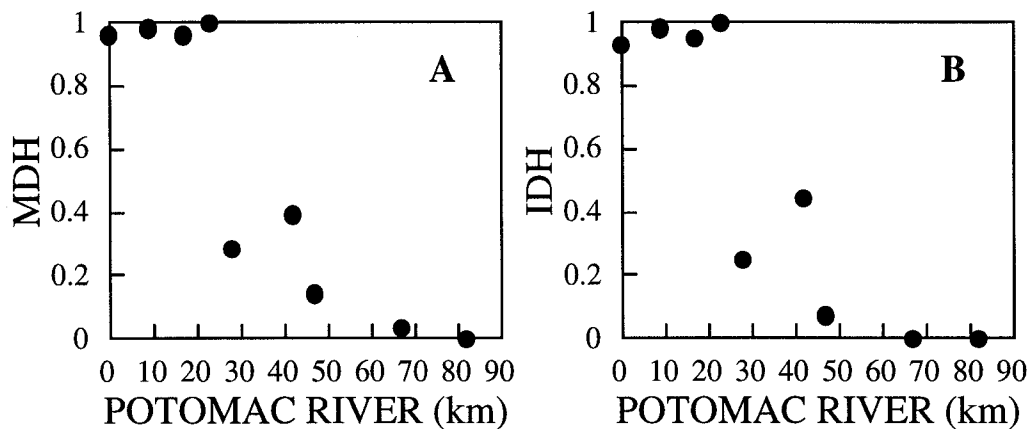


FIG. 6. *Orchelimum nigripes* allele frequencies for populations along the Potomac River. (A) MDH. (B) IDH.

### DISCUSSION

In recent studies of hybrid zones, there has been a great emphasis on analyzing the forces maintaining stable, narrow, multiple-cline zones. This is a consequence of important theoretical developments, combined with detailed empirical data for several hybrid zones, suggesting that many narrow hybrid zones represent a dynamic equilibrium between dispersal into the zone and intrinsic selection against hybrids (Barton and Hewitt 1985, 1989; Barton and Gale 1993). Such hybrid zones have been termed "tension zones" (Key 1968; Barton and Hewitt 1985). The width of a tension zone is determined by the ratio of the dispersal rate ( $\sigma$ , the standard deviation of parent-offspring distance along a linear axis) and the effective strength of selection against hybrids at the center of the cline, with greater dispersal (relative to selection) producing a broader cline (Barton and Gale 1993).

However, not every secondary contact results in a tension zone. In some zones there must be effectively neutral introgression between taxa that diverged in allopatry, and then reestablished contact without having evolved reproductive isolation. Unless they have been established very recently, we should expect such zones to be broad relative to dispersal. For example, a neutral allele that has been spreading by diffusion since the last retreat of the Pleistocene ice ( $\sim 10,000$  years ago) is expected to extend over  $\sim 100 \sigma$  (Barton and

Hewitt 1989). In these nonequilibrium cases of effectively neutral introgression, the parameters determining hybrid zone width are dispersal and time elapsed since secondary contact, rather than dispersal and selection against hybrids (N. H. Barton, pers. comm.).

There are few examples in the literature of hybrid zones for which neutral introgression has been considered a viable explanation for observed patterns of geographic variation. In part, this is because the majority of hybrid zones studied are rather narrow ( $\sim 50 \sigma$ , Barton and Hewitt 1989), making other explanations more plausible unless the establishment of the zone is known to be very recent. However, in recent years even when zones are very broad relative to dispersal estimates, most authors have simply taken this as evidence that their estimates of dispersal were too low (e.g., Barton and Hewitt 1985). Given the tremendous difficulties faced in accurately measuring natural dispersal rates and the tendency for direct approaches to systematically underestimate effective dispersal (Slatkin 1987; Koenig et al. 1996), this conclusion may often be correct. Unfortunately, it can be difficult to distinguish between stabilized clines that are broad because of unexpectedly high dispersal combined with weak selection and effectively neutral clines that are broad because they have been decaying for a long time.

In extreme cases, however, hybrid zones may be so wide that the hypothesis of a dynamic equilibrium is not reasonable. For these clines, a selection-dispersal balance would require either extraordinary levels of gene flow or selection

TABLE 3. Results from exact probability tests for deviation from Hardy-Weinberg equilibrium (HWE) for MDH and IDH (using complete enumeration), and associated inbreeding coefficients ( $F_{IS}$ ) for the four mixed ancestry Potomac populations. In addition to the usual test of HWE, a more powerful test was performed that specifies heterozygote deficit as the alternative hypothesis.

Popula- tion	MDH			IDH		
	HWE test, $P$ -value (MDH)	Hetero- zygote deficit, $P$ -value (MDH)	$F_{IS}$ (MDH)	HWE test, $P$ -value (IDH)	Hetero- zygote deficit, $P$ -value (IDH)	$F_{IS}$ (IDH)
PO-5	0.0019	0.0019	+0.657	0.0000	0.0000	+0.919
PO-6	1.0000	0.7160	-0.034	0.0751	0.0633	+0.251
PO-7	0.0692	0.0544	+0.280	0.0692	0.0383	+0.302
PO-8	0.0824	0.0824	+0.252	1.0000	1.0000	-0.064

TABLE 4. Disequilibrium statistics ( $D$ ,  $D'$ , and  $r$ ), disequilibrium test statistic  $nr^2$  with results from chi-square test, and results with standard errors (SE) from estimated exact probability tests of independence between loci for genotypic contingency tables for the four mixed ancestry Potomac populations.

Popula- tion	$D$	$D'$	$r$	$nr^2$	$P$ for $nr^2$	$P$ for genotypic contingency table (SE)
					(chi square, 1 df)	
PO-5	0.1897	0.900	0.790	14.97	$\ll 0.001$	0.0000 (0.0000)
PO-6	0.0998	0.564	0.516	15.15	$\ll 0.001$	0.0001 (0.0001)
PO-7	0.1514	0.685	0.626	18.02	$\ll 0.001$	0.0000 (0.0000)
PO-8	0.0298	0.512	0.337	6.71	$< 0.01$	0.0158 (0.0008)

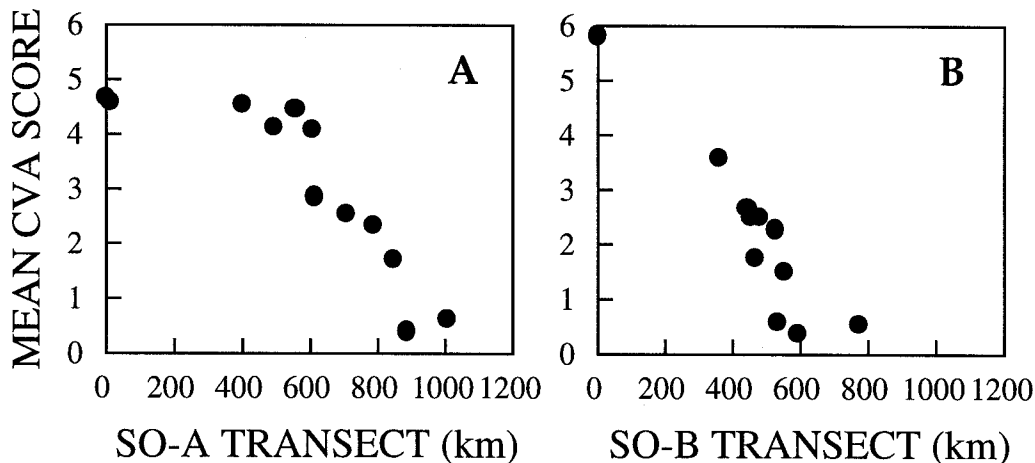


FIG. 7. Mean CVA scores for Deep South (SO) populations. (A) Transect SO-A. (B) Transect SO-B.

so weak as to be effectively neutral. The Deep South contact zone described here falls into this category, and provides a clear example of a hybrid zone that must have been decaying for thousands of years.

#### The Deep South Hybrid Zone

##### Genetic Structure of the Hybrid Zone

The broad east-west hybrid zone between *O. nigripes* and *O. pulchellum* in the Deep South appears to be an example of ongoing neutral introgression. Although the 50–250 km hybrid zone is fairly narrow relative to the distribution of these katydids, which stretches some 2000 km from Florida to Texas, it is quite broad relative to reasonable estimates of dispersal (precise estimates of dispersal rates are not available, but the best available data for other orthopterans suggest values on the order of 100 m/generation or less; Aikman and Hewitt 1972; Barton and Hewitt 1982; Barendse 1990; Kindvall and Ahlén 1992; Mason et al. 1995; Hjermann and Ims 1996). If analyzed as a tension zone established between two advancing populations coming into contact across a broad front, such a wide cline implies unrealistically broad dispersal and/or extremely weak selection (the limit of which is neutral introgression) against recombinants. For example, applying

Barton's methods of cline analysis (Barton and Gale 1993), selection against hybrids at the center of the zone (i.e., the reduction in mean fitness of populations at the center relative to those at the edge) of 0.01 would imply a dispersal rate on the order of 5–10 km/generation, and even selection of just 0.001 would imply dispersal of several kilometers each generation.

In fact, as shown in Table 5, even if we assume neutral introgression the observed clines are probably even broader than would be predicted by a standard diffusion model, assuming reasonable parameter values for gene flow and time since (presumably late Pleistocene) contact. Thus, it is very unlikely that these clines are constrained by selection against hybrids. Rather than having to explain how a very narrow hybrid zone can be maintained in the face of dispersal and recombination (the more familiar problem encountered in the hybrid zone literature), we must explain the width of a hybrid zone that is unexpectedly broad.

Clines that are considerably broader than predicted by a simple diffusion model may develop for neutral characters if the initial colonization of an area by different populations proceeds in jumps, as it will if the distribution of dispersal distances is sufficiently leptokurtic (i.e., with tails thicker than those of a normal distribution; Hewitt 1990b; Nichols

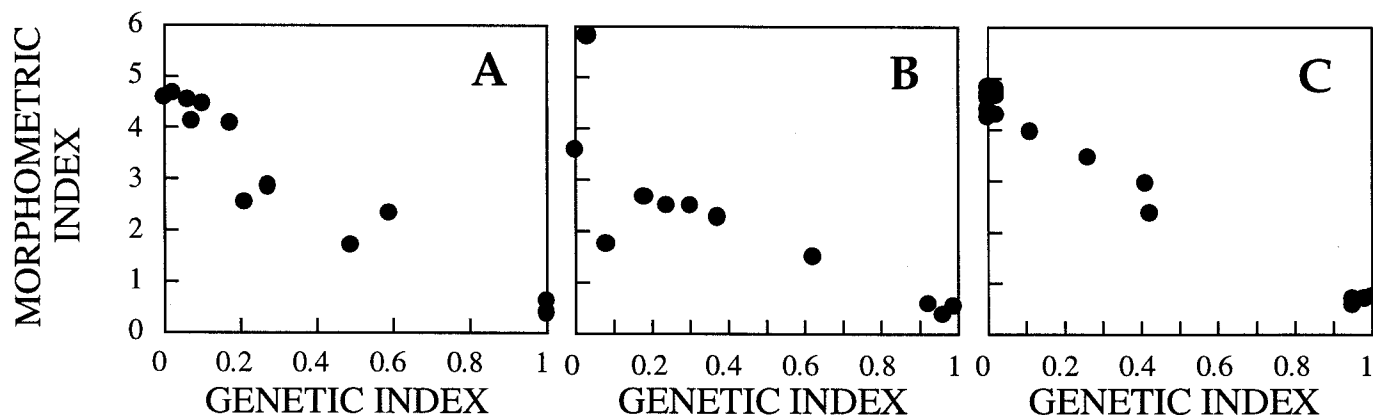


FIG. 8. Mean CVA scores (morphometric index) and mean *Orchelimum nigripes* allele frequencies (genetic index) for populations along two Deep South transects and in the Potomac region. (A) Transect SO-A. (B) Transect SO-B. (C) Potomac region (PO).



TABLE 5. Predicted cline widths resulting from neutral introgression with a range of values for gene flow ( $\sigma$ ) and time ( $T$ ) parameters. Predicted widths are given for the cline width definitions of both Barton and Endler (see text).

$\sigma$ (m)	$T$ (generations)	Predicted Barton cline width (km)*	Predicted Endler cline width (km)**
10	5000	2	1
10 <sup>2</sup>	5000	18	12
10 <sup>3</sup>	5000	178	119
10	10,000	3	2
10 <sup>2</sup>	10,000	25	17
10 <sup>3</sup>	10,000	251	168
10	12,000	3	2
10 <sup>2</sup>	12,000	28	18
10 <sup>3</sup>	12,000	275	184
10	15,000	3	2
10 <sup>2</sup>	15,000	31	21
10 <sup>3</sup>	15,000	307	206

\*  $w = 2.51\sigma(T)^{1/2}$  (Barton and Gale 1993).

\*\*  $w = 1.68\sigma(T)^{1/2}$  (Endler 1977).

and Hewitt 1994). Such dispersal patterns are common (Aikman and Hewitt 1972; Endler 1977; Mason et al. 1995; Ibrahim et al. 1996), and may be especially likely during colonization and range expansion (e.g., S. D. Porter et al. 1988; Shoemaker et al. 1996). If the distribution of suitable habitat results in a very patchy population structure, so that an allele may be locally abundant in a region where it is otherwise rare, unexpectedly broad clines can persist for some time (Nichols and Hewitt 1986, 1994; Hewitt 1993). There are numerous examples of orthopterans and other insects whose dispersal patterns are strongly constrained by a patchy distribution of suitable habitat (e.g., Buren et al. 1974; Kindvall and Ahlén 1992; Preziosi and Fairbairn 1992; Rank 1992; Hewitt 1993; Mason et al. 1995; Nürnberger and Harrison 1995; Gerber and Templeton 1996), which may result in thick-tailed distributions of dispersal distances. It is very likely that such a dispersal pattern characterizes the movements of *O. nigripes* and *O. pulchellum* in the Deep South. Because these katydids are closely associated with water and are weak fliers, their movements must be mostly confined to a relatively small area, with periodic long-distance dispersal between ponds, river systems, or other freshwater bodies (e.g., during major floods). Furthermore, between 5 and 10 thousand years ago, when these katydids were likely recolonizing the Gulf Coast, suitable habitat may have been even more patchy than it is today (Delcourt 1980), causing an even stronger broadening of clines. Related aspects of population structure, such as small effective population size (Nichols 1989) can also produce broadened clines. At the present time, however, it is difficult to assess the likely role of population size in structuring the Deep South hybrid zone, beyond making the qualitative observation that today there is great variation in population size among sites.

If the range expansion of *O. nigripes* and *O. pulchellum* has indeed involved significant long-distance dispersal, resulting in interspersed isolated populations rather than two diffusing fronts (see discussion of "mottled" hybrid zones in Searle 1993), this could leave a genetic trace in the form of a "lumpy" distribution of allele frequencies. For example,

if a single gravid female moves far ahead of the expanding front and establishes a new colony, which then expands into a larger patch, this new patch would be genetically distinct from those around it (Ibrahim et al. 1996). In the dataset reported here, SO-B5 shows local cline reversals consistent with this scenario (see Fig. 2, Table 1).

Thus, the broad clines observed in this study are most likely transitory, continually decaying remnants of historical isolation that resulted in genotypic and phenotypic divergence, but little or no reproductive isolation upon recontact. This conclusion gains additional support from the apparent single-locus Hardy-Weinberg equilibrium and two-locus linkage equilibrium in populations sampled through the hybrid zone. As in many other hybrid zones (Buño et al. 1994 and references therein), cline width differs between transects. This difference is consistent among the two genetic loci and two morphometric characters and may be a consequence of historical differences between the transects: some evidence indicates a steep moisture gradient between a more mesic central Mississippi/Alabama and a xeric Gulf Coast during the early Holocene (Whitehead and Sheehan 1985). If this is correct, it is not surprising that the *Orchelimum* clines have expanded more slowly along the historically less hospitable Gulf Coast.

The geographic pattern of genetic and morphological variation of *O. nigripes* and *O. pulchellum* in the Southeast is consistent with patterns observed in other Gulf Coast taxa, many of which show similar east-west disjunctions (e.g., tiger beetles: Vogler and DeSalle 1993; fishes: Wiley and Mayden 1985; Birmingham and Avise 1986; reptiles and amphibians: Conant and Collins 1991; birds: Moldenhauer 1992; Gill et al. 1993; vertebrates: Blair 1958). The taxonomic and ecological range of organisms exhibiting this pattern suggests that large-scale historical fluctuations in the physical environment of the southeastern United States have had a remarkably broad impact in shaping the biogeography of this region (for discussion and review see Avise 1996; Shapiro 1996).

### The Potomac Hybrid Zone

#### Genetic Structure of the Hybrid Zone

Although *O. nigripes* has apparently become established in the Potomac region only within this century (see Background), it has completely replaced *O. pulchellum* along the Potomac River from at least Great Falls, Virginia (PO-1, just above the Fall Line), to around Roosevelt Island (PO-4, 23 km downstream from Great Falls; Fig. 3). Populations with intermediate allele frequencies and morphology are found along the Potomac from just above the point where the Anacostia River drains into it for ~20 km downstream, as well as along the Anacostia near its confluence with the Potomac (Fig. 3). No populations sampled away from the Potomac show significant introgression of *O. nigripes* alleles.

[NOTE: At least five *O. nigripes* specimens (now in the collections of the United States National Museum) were collected by Henry Fox in 1913 near the Rappahannock River at Tappahannock, Virginia (~175 km south of Washington, DC). However, *O. pulchellum* specimens were collected here, also by Fox, in 1915 (specimens now at the Academy of

Natural Sciences of Philadelphia). *Orchelimum pulchellum* is currently abundant and widespread in this area, and *O. nigripes* is not known to be present (pers. obs.). Single *O. nigripes* specimens were collected at Amherst, Massachusetts, in 1964 and 1993. Thus, it is conceivable that local, transient eastern *O. nigripes* populations have existed in the past (Shapiro 1995).]

Importantly, in all four mixed ancestry river populations deviations from linkage equilibrium are quite strong and highly significant, although deviations from Hardy-Weinberg equilibrium are clearly significant in just a single population (Table 3). These katydids have a high chromosome number,  $2n = 30 + X$  in males (J. Cabrero, L. Shapiro, and J. P. M. Camacho, unpubl. data), making it unlikely a priori that the two enzyme loci used in this study are physically linked. Indeed, there is no indication of physical linkage from the genotypic composition of samples from the Deep South. Thus, the significant linkage disequilibrium found in the mixed ancestry Potomac populations implies some combination of gene flow and one or more forms of selection.

Gene flow is a potentially powerful cause of linkage disequilibrium. Following the mixing of populations, linkage disequilibrium will rapidly decay in subsequent generations if no other factors come into play. Ongoing gene flow into a population, however, may greatly slow or prevent the decay of disequilibrium. Such ongoing gene flow may indeed be important in the Potomac hybrid zone (see below). Furthermore, while a population may reach single-locus Hardy-Weinberg equilibrium after a single generation of random mating, associations *between* loci can be far more persistent (Robbins 1918; Feldman and Christiansen 1975; Mallet and Barton 1989), perhaps accounting for the combination in the Potomac zone of strong linkage disequilibrium with only modest deviations from Hardy-Weinberg equilibrium.

Sexual selection, in the form of both assortative mating and assortative fertilization, may play an important role in the Potomac zone. Mating trials in the laboratory with Potomac *O. nigripes* and *O. pulchellum* have shown that there is strong (but asymmetric) assortative mating between the two taxa (L. Shapiro, unpubl. data). Furthermore, breeding studies have demonstrated that although heterospecific crosses can produce normally viable offspring, heterospecifically mated females exhibit dramatically reduced fecundity relative to conspecifically mated females (L. Shapiro, unpubl. data). Selection against heterozygotes at one or more loci may also be important in structuring this hybrid zone. Preliminary results from laboratory matings using Potomac animals suggest that  $F_1$  males may have abnormally small testes and fail to produce sperm (J. Cabrero, L. Shapiro, and J. P. M. Camacho, unpubl. data).

In summary, although the strong linkage disequilibrium in the Potomac zone may be generated and maintained in part by ongoing gene flow, if this were simply a case of neutral introgression, strong linkage disequilibrium could not be maintained for many generations. Assortative mating and fertilization and selection against hybrids (e.g., reduced fertility of hybrid males) may also be important. These possibilities are being investigated in ongoing studies.

### *Dynamics of the Potomac Hybrid Zone*

Based on historical records of the distribution of *O. pulchellum* and the contemporary structure of the hybrid zone, it seems *O. nigripes* has been steadily replacing *O. pulchellum*, pushing forward a narrow hybrid zone as it advances downriver.

This observation suggests several possibilities. First, the Potomac zone could be a simple tension zone that has moved downstream to its present position, lodging in a density trough and leaving a stream of pure *O. nigripes* in its wake. Under this scenario, *O. nigripes* is no longer replacing *O. pulchellum*. Tension zones are expected to move down density gradients and become trapped in areas of locally low population density and dispersal (Bazykin 1969; Barton and Hewitt 1989). The segment of the river where the hybrid zone is located, along the urbanized river banks of Washington, DC, is relatively developed, with patchily distributed *Orchelimum* habitat, and could conceivably represent such a region of low population density. However, because tension zones are so easily trapped by density and dispersal troughs, they are not expected to move very far from where they are initially formed (Barton and Hewitt 1989). If the Potomac tension zone initially formed upstream (as it apparently did, because it is otherwise difficult to explain the virtual absence of *O. pulchellum* alleles upstream from the hybrid zone), it might be expected to become trapped before moving downstream as far as its present position. Nevertheless, based on currently available data, the scenario of a trapped tension zone cannot be ruled out.

A second possibility is that *O. nigripes* is somehow selectively superior to *O. pulchellum* (and presumably to hybrids) and is continuing to replace *O. pulchellum* as it moves downstream, pushing a hybrid zone in front of it as it advances. This selection could operate in various ways. For example, as noted above, there is some evidence of sexual selection that could play an important role in the replacement of *O. pulchellum* (and the maintenance of genetic disequilibrium). Laboratory experiments indicate that *O. nigripes* females are significantly more discriminating than *O. pulchellum* females in mate selection. As a consequence of such asymmetric assortative mating, hybrid males might be less likely to backcross with *O. nigripes* females than with *O. pulchellum* females, and the *O. nigripes* genome would thus remain relatively "pure," while the *O. pulchellum* genome became increasingly diluted with *O. nigripes* genes. If partial assortative mating plays an important role in maintaining the strong linkage disequilibrium in the Potomac hybrid zone, then we should expect this disequilibrium gradually to break down as mixed-ancestry individuals (with intermediate phenotypes) increase their proportion in these populations, as may have happened thousands of years ago in the Deep South. Other types of selection favoring *O. nigripes* over *O. pulchellum* are also possible, but are so far unsupported. Based on the extensive field experience of several observers, there is currently no evidence for ecological or habitat differences between the two taxa. Laboratory studies suggest that *O. nigripes* may mature slightly faster than *O. pulchellum* (unpubl. data), but the difference is so slight that it is difficult to imagine its being important over the brief time scale of

interest in the Potomac region. Nevertheless, further investigation of life history characteristics of the two taxa is certainly warranted.

An interesting, third possibility to consider as an explanation for the structure of this hybrid zone is that of differential immigration between *O. nigripes* and *O. pulchellum* populations. It is conceivable that the Potomac River itself could promote such an imbalance. Along much of the Potomac River above Washington, DC, near the Fall Line, where *O. nigripes* has replaced *O. pulchellum* and lives right along the river, the river is often fast moving, with rapidly fluctuating water levels and periodic flooding. In contrast, the lower Potomac is far more sluggish, and *O. pulchellum* populations extend into marshy backwaters that are absent upstream. *Orchelimum* that fall into the water can float and swim quite well (pers. obs.), and dispersal by drifting or rafting would clearly be unidirectional downstream. Perhaps more importantly, *Orchelimum* oviposit in herbaceous plant stems, depositing numerous eggs in each plant (Feaver 1983), and these eggs could be transported considerable distances in plants washed downstream by winter and spring floods.

The importance of river-facilitated dispersal, often resulting in extremely asymmetric gene flow, has been documented for a number of plants (e.g., Waser et al. 1982; Schneider and Sharitz 1988; Thébaud and Debussche 1991; Johansson and Nilsson 1993). River dispersal has also been suggested as an important dispersal mechanism for small animals during floods in the Mississippi floodplains (Viosca 1926). Warne and Hartley (1975) have argued that the distribution of the conocephaline katydid *Conocephalus dorsalis* in the British Isles has been strongly affected by the movement of eggs by water. There is little doubt that occasional long-distance dispersal of eggs or adults would tend to move *O. nigripes* genes downriver, but it is not clear how frequent or important this dispersal might actually be. A modest amount of effectively unidirectional, long-distance migration resulting in a net influx of *O. nigripes* individuals (and their genes) into the downstream range of *O. pulchellum* could contribute to a downstream movement of the hybrid zone.

The true explanation for the Potomac hybrid zone may involve some combination of tension zone dynamics, biased dispersal, and perhaps a selective advantage (e.g., generated by asymmetric assortative mating) for *O. nigripes* that would allow it to advance, pushing the hybrid zone over *O. pulchellum*. Although tension zones are expected to become trapped by local dispersal barriers, this tendency could be overcome by sufficiently strong asymmetric dispersal or selection.

Such a scenario of hybridization and advancing replacement has been documented for several other systems, including the ~50-year-old hybrid zone between two introduced fire ants in the southeastern United States (Shoemaker et al. 1996), the geographically complex "hybrid zone" between the golden-winged and blue-winged warblers in the eastern United States (Gill 1980), a hybrid zone between an introduced and a native deer in Scotland (Abernethy 1994), and the hybrid zones between several pairs of bird taxa (orioles, buntings, and crested titmice) with distributions meeting in the Great Plains (Rising 1983). One exciting aspect of the fire ant, warbler, and deer hybrid zones and the katydid hybrid

zone reported on here is that all four have been documented early in the history of contact, so that changes such as cline movements and the progress of introgression and taxon replacement can be followed in coming decades from their earliest stages. The Potomac katydid hybrid zone is of particular interest because it can be compared to the very old zone in the Deep South, allowing us to address questions of how hybrid zone dynamics may vary through time and space. The work reported here establishes a foundation for further study of this system.

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