TOPOGRAPHIC DIVERSITY, ZONE WIDTH, AND THE STRENGTH OF REPRODUCTIVE ISOLATION IN A ZONE OF OVERLAP AND HYBRIDIZATION

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Abstract. —Two closely related species of ground crickets, *Allonemobius fasciatus* and *A. socius,* overlap and hybridize in a contact zone in the eastern United States. In earlier work, Howard (1986) described geographic variation in the width of the zone and in the strength of reproductive isolation between the two ground crickets. The zone was wider in the hills and mountains of southeastern Ohio and West Virginia than along the eastern coastal plain, and reproductive isolation appeared to be stronger where the zone was wider. Howard attributed the greater width in the mountains to the wide intermingling and patchy distribution of habitats appropriate for a species adapted to a northern climate and for a species adapted to a southern climate. He also pointed out that the mosaicism and the increased breadth of the zone in the mountains enhanced the probability of occurrence of reinforcement. We tested three predictions that emerged from Howard's hypothesized links among topographic diversity, zone width, and the strength of reproductive isolation. The first two predictions were fulfilled. The northern cricket, *A. fasciatus,* occurred in the high mountains south of its previously known distributional limit; and the zone narrowed considerably in Illinois, an area of low topographic diversity. These results provide further evidence for the importance of the environment in determining the structure of the zone. The third prediction was falsified. Contrary to the prediction, the strength of reproductive isolation between the two species was as strong in Illinois as in the Appalachian Mountains. This result suggests that if reinforcement has occurred in the zone, the width of the zone has not been a major factor in the process.

Key words. —Electrophoresis, hybrid zone, mosaic, reinforcement, reproductive isolation, topographic diversity.

Over the past two decades, a rich theoretical and empirical literature on hybrid zones has grown up, fueled by interest in questions ranging from the nature of species boundaries to the role of natural selection in the evolution of premating isolating mechanisms. As disparate as the questions are, there are some viewpoints common to most hybrid zone studies. Perhaps the most pervasive is that hybrid zones can be interpreted as clines. Indeed, Barton and Hewitt (1985) point out that the term "hybrid zone" is now synonymous with "cline" and, rightly or wrongly, may be recognized as such. However, even as this interpretation of hybrid zones has hardened into tradition during the recent past, an ever increasing number of empirical studies describe zones in which patterns of variation do not resemble simple monotonic clines (Gartside, 1980; Harrison, 1986; Howard, 1986; Sperling, 1987; Rand and Harrison, 1989). Instead, these zones are characterized by a "broken pattern" of variation with clear reversals of gene frequency changes along linear tran-sects running through them. Harrison (1986) has coined the term "mosaic hybrid zone" to describe such zones.

The existence of mosaic hybrid zones has important implications for several contentious debates in evolutionary biology. One concerns the role of the environment in determining the position and maintenance of hybrid zones. Endler (1977) believes that hybrid zones or clines often result from the balance between selection along environmental gradients and dispersal. Barton and Hewitt (1981, 1985) also emphasize the balance between dispersal and selection, but they stress hybrid unfitness rather than genotype-specific responses to the environment as the important selective factor. One prediction made by the environmental gradient model that is not made by the hybrid unfitness model is the existence of hybrid zones with broken patterns of distribution (Barton and Hewitt, 1985), i.e., the existence of mosaic hybrid zones.

Another debate central to studies of hybrid zones is the question of whether rein-

forcement (the strengthening of premating isolating mechanisms in zones of overlap due to selection against hybridization) ever occurs in hybrid zones. Although reinforcement was an important part of a widely accepted allopatric speciation model (Dobzhansky, 1940, 1970), the concept recently has come under widespread criticism. Opponents of reinforcement argue that, unless sexual isolation and ecological differences have evolved in allopatry, secondary contact is much more likely to lead to a stable hybrid zone, to fusion of the two populations, or to the extinction of one of the populations, than to the evolution of reproductive barriers (J. A. Moore, 1957; Mayr, 1963; Paterson, 1978, 1982; W. S. Moore, 1979; Templeton, 1981; West-Eberhard, 1983; Butlin, 1989). However, some of the major criticisms of reinforcement were formulated with narrow tension zones in mind and are less applicable to broad mosaic hybrid zones (Littlejohn, 1981; Howard, 1986; Harrison and Rand, 1989). Moreover, mosaic hybrid zones lessen the likelihood of fusion and extinction and therefore increase the likelihood of reinforcement (Harrison and Rand, 1989).

The sister species, *Allonemobius fasciatus* and *A. socius,* are small ground-inhabiting crickets in the subfamily Nemobiinae. The former species occurs in the northeastern United States; the latter is found in the southeastern United States (Howard, 1983; Howard and Furth, 1986). They meet and hybridize along an extensive contact zone reported to stretch from the East Coast to Ohio (Howard, 1986). The zone varies in width, being comparatively narrow along a north-south transect through the coastal plain of New Jersey and relatively broad through the Appalachian Mountains of West Virginia and Virginia. Along the mountain transect the zone is clearly mosaic with populations of the northern species, *A. fasciatus,* and mixed populations of both species occurring to the south of some *A. socius* populations. In such instances, *A. fasciatus* and mixed populations are found at higher elevations than the *A. socius* populations. Howard (1986) described geographic variation in levels of hybridization within the zone. The single mixed population from the east-coast transect contained a higher pro-

portion of hybrid individuals than mixed populations from the mountain transect. Adopting Short's (1969) terminology for hybrid zones, which emphasizes interactions between the hybridizing taxa, the best description of this contact zone is a zone of "overlap and hybridization," because pure-species individuals occur with high frequency in most populations.

Based on the above findings, Howard (1986) suggested that the mosaicism of the zone results from the adaptation of the two cricket species to different climatic regimes (northern and southern) and from the patchy distribution of these regimes in the area of contact. Moreover, Howard (1986) suggested that the lessening of hybridization in mixed populations from the wider part of the zone (the mountain transect) may reflect the outcome of reinforcement. He pointed out that barriers to gene flow are more likely to evolve where a hybrid zone is broad because gene flow from parental populations is reduced and so is less likely to swamp the effects of natural selection inside the zone for the development of premating isolating mechanisms. The mosaicism of the zone in the Appalachian Mountains further enhances the possibility of reinforcement by allowing for many relatively independent contacts between the two taxa. With many trials, there is an increased chance that reproductive barriers will develop (Littlejohn, 1981). Once a trait difference responsible for premating isolation evolves locally, its carriers would enjoy increased reproductive success, and the variant could spread through the contact zone due to a combination of individual and interdemic selection, a process envisioned by Wright (1978, 1980).

If Howard's hypothesized links between topographic diversity, the width of the zone, and the strength of reproductive isolation are correct, the following predictions should be fulfilled. (1) Additional *A. fasciatus* and mixed populations should occur south of Charleston in West Virginia because the highest mountains in the state are found in that area. (2) In the states west of the Appalachian Mountains, such as Illinois, where there is little altitudinal diversity and hence little climatic mosaicism, the zone of overlap and hybridization will narrow. (3) Where
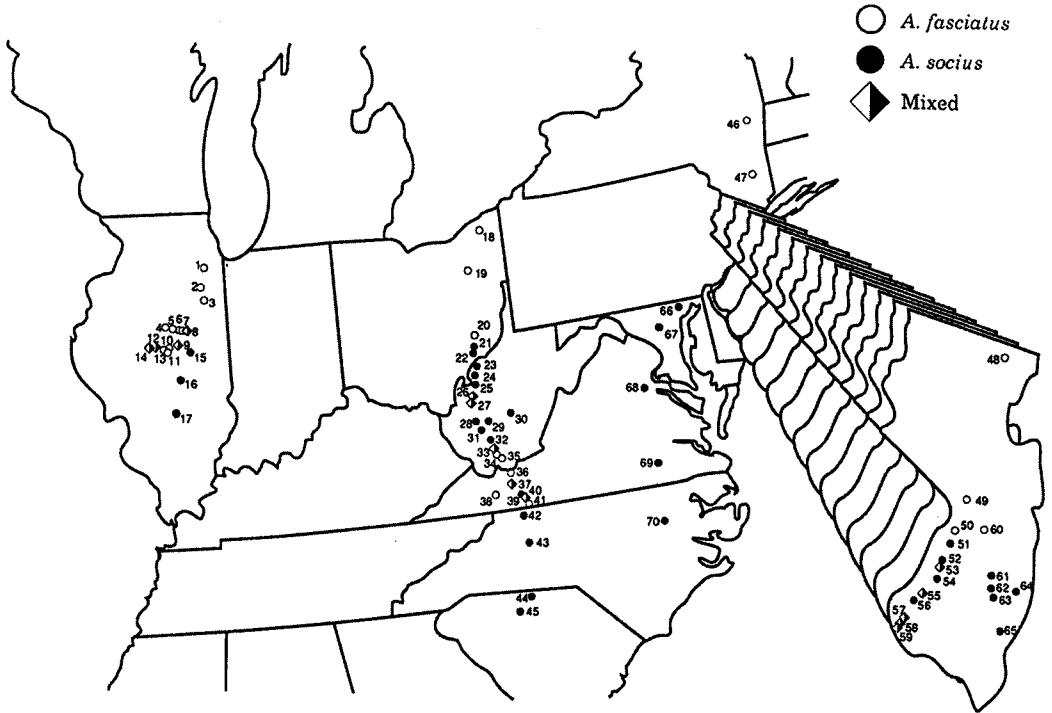
Fig. 1. Map of the eastern United States showing the geographic structure of the zone of overlap and hybridization between *A. fasciatus* and *A. socius*. The Illinois transect is represented by populations 1–17, the mountain transect by populations 18–45, and the east-coast transect by populations 46–70.

the zone has narrowed in Illinois, levels of hybridization will increase. We tested these predictions and further mapped the zone by extensive sampling and genetic analyses of populations from Illinois; the coastal plain of eastern North America; and the mountainous areas of southeastern Ohio, West Virginia, and Virginia.

MATERIALS AND METHODS

Populations along three transects were sampled in 1986 or 1987. The east-coast transect (EC) stretched from Rotterdam, New York to Rocky Mount, North Carolina and included 25 populations. The majority of populations were located in the southern half of New Jersey, the area in which *A. fasciatus* and *A. socius* meet (Howard, 1986). The mountain transect (MTN) extended from Madison, Ohio to Chester, South Carolina and contained 28 populations. The Illinois transect (ILL) spanned the area from Kankakee, Illinois to Farina, Illinois. Seventeen populations were sampled (Fig. 1). Detailed locality information is available

upon request from the senior author. We froze crickets in liquid nitrogen immediately upon collection and subsequently stored them in the laboratory at $-80°C$.

We employed horizontal starch gel electrophoresis to study four enzymes in each cricket. Each enzyme is encoded by a separate genetic locus: hexokinase (*Hk*), isocitrate dehydrogenase-1 (*Idh-1*), peptidase-3 (*Pep-3*), or glutamic-oxalacetic transaminase (*Got*). There is a fixed electrophoretic difference between the two species at *Hk*. At the other three loci, one or more alleles are specific to the gene pool of a single species. At two loci, *Pep-3* and *Got*, the predominant alleles in *A. fasciatus* populations (1.0 and 1.0, respectively) occur with a frequency of less than 0.05 in allopatric *A. socius* populations (Appendix 1). At one locus, *Idh-1*, the most common alleles in *A. socius* populations (2.2, 2.0, 1.8) occur with a frequency of less than 0.05 in allopatric populations of *A. fasciatus*. Electrophoretic methods are described by Howard (1982).

TABLE 1. Summary of scoring procedure for the calculation of character index scores. Allele designations refer to mobility relative to the most common allele in *A. fasciatus*. See text for details.

| Locus | A. fasciatus | | A. socius | |
	Allele	Maximum positive score	Allele	Maximum positive score
Hk	1.00(+1)	+2	0.80(−1)	−2
Pep-3	1.00(+1)	+2	—	0
Got	1.00(+1)	+2	—	0
Idh-1	—	0	2.20, 2.00, 1.80(−1)	−2
Total		+6		−4

We computed a character index score for each individual based on its genotype at the four loci surveyed. Character index profiles provide a good way to visualize the genotypic composition of a population and hence the level of isolation between two taxa. Alleles specific to *A. fasciatus* were assigned a score of +1, whereas alleles specific to *A. socius* were assigned a score of −1. The character index score of an individual represented the sum of its scores over all four loci. Because three loci (*Hk*, *Pep-3*, and *Got*) had unique alleles in *A. fasciatus* but only two loci had unique alleles in *A. socius*, the score of an individual could range from −4 to +6 (Table 1). Individuals lacking alleles of *A. socius* were regarded as pure *A. fasciatus*, and individuals without alleles of *A. fasciatus* were considered pure *A. socius*. Individuals harboring alleles of both species were considered to be of mixed ancestry and will be referred to as hybrid individuals.

We tested agreement of single locus genotype frequencies with Hardy-Weinberg equilibrium expectations by using the formula of Levene (1949), which corrects for small sample sizes. The inbreeding coefficient for a locus in a population (F_{IS}) was calculated via BIOSYS-1 (Swofford and Selander, 1981). Where allele frequencies were sufficiently intermediate, we calculated Burrows' composite measure of linkage disequilibrium (Cockerham and Weir, 1977; Weir, 1979; Weir and Cockerham, 1989) for pairwise combinations of the four marker loci to determine whether independent assortment occurred. This measure does not require that two loci be in Hardy-Weinberg equilibrium; however, it is frequency dependent. The value of this measure could range from +0.50 (complete linkage disequilibrium, no heterozygosity, and equal genotype frequencies) to 0.0 (no linkage disequilibrium) to −0.50 (complete linkage disequilibrium of alternative homozygotes, no heterozygosity, and equal genotype frequencies). The *Idh-1* data were reduced to a two allele classification to perform the calculation. The hypothesis that the composite measure of linkage disequilibrium equals zero was tested via a chi-square test statistic with one degree of freedom (Weir and Cockerham, 1989)

RESULTS

Our prediction that the zone of overlap and hybridization would extend south below Charleston, West Virginia was fulfilled. Populations of the northern species, *A. fasciatus*, and mixed populations of both species continued to occur at least 215 kilometers south of Charleston (Figs. 1 and 2; Appendix 1). A mixed population is a population that contains both pure *A. fasciatus* and pure *A. socius*. Topographic diversity accounted for the mosaic nature of the zone and for the great breadth of the zone in this area. In all cases where *A. fasciatus* or mixed populations occurred south of *A. socius* populations, they occurred at a higher elevation than the *A. socius* populations (Table 2).

The second prediction was also fulfilled. In Illinois where there is little altitudinal diversity (the elevation of collecting sites along the entire transect ranged from 140 m to 238 m) and hence less climatic mosaicism, the zone of overlap narrowed. Because of the mosaic nature of the zone and the wide geographic rage covered by the sampling, accurately estimating the width is difficult, but it is probably less than 40 km (Fig. 1). This upper range has been supported by additional electrophoretic anal-

TABLE 2. Elevations of collecting sites along the mountain transect.

Site desig.	Species composition	Town/locality	Elevation (m)
MTN18	A. fasciatus	Madison, Ohio	246
MTN19	A. fasciatus	Akron, Ohio	326
MTN20	A. fasciatus	Belle Valley, Ohio	244
MTN21	A. socius	Macksburg, Ohio	213
MTN22	A. socius	Marietta, Ohio	238
MTN23	A. socius	Williamstown, W. Virginia	225
MTN24	A. socius	Rockport, W. Virginia	177
MTN25	A. socius	Sherman, W. Virginia	183
MTN26	Mixed	Kenna, W. Virginia	244
MTN27	Mixed	Kenna2, W. Virginia	293
MTN28	A. socius	Sissonville, W. Virginia	201
MTN29	A. socius	Clendinin, W. Virginia	183
MTN30	A. socius	Frametown, W. Virginia	335
MTN31	A. socius	Chesapeake, W. Virginia	219
MTN32	A. socius	Paint Creek, W. Virginia	219
MTN33	Mixed	Mossy, W. Virginia	427
MTN34	A. fasciatus	North Beckley, W. Virginia	756
MTN35	A. fasciatus	Ghent, W. Virginia	878
MTN36	A. fasciatus	Rocky Gap, Virginia	643
MTN37	Mixed	Wytheville, Virginia	707
MTN38	A. fasciatus	Rural Retreat, Virginia	731
MTN39	A. socius	Austinville, Virginia	683
MTN40	Mixed	Hillsville, Virgnia	823
MTN41	A. fasciatus	Fancy Gap, Virginia	835
MTN42	A. socius	Bottom, N. Carolina	347
MTN43	A. socius	Oswalt, N. Carolina	298
MTN44	A. socius	Fort Mill, S. Carolina	185
MTN45	A. socius	Chester, S. Carolina	200

yses of small population samples from other localities along this transect (Howard and Waring, unpubl. data).

Howard (1986) speculated that the width of the contact zone along the east-coast transect may be as little as 5 to 20 km. In light of the results from populations studied as part of this investigation, the upper range should be revised to about 55 km (Fig. 1). Even though the zone is considerably narrower along this transect than along the mountain transect, the mosaic nature of the zone is still evident. The five mixed populations occurred south of some A. socius populations. It is likely that climatic mosaicism accounts for this pattern, but there is little altitudinal diversity in southern New Jersey (elevations of collecting sites ranged from 0 to 37 meters) so the location of the mixed and pure populations within the contact zone was not related to elevational gradients.

The third prediction, that levels of hybridization would be greater in populations from the Illinois transect than in populations from the mountain transect, was not supported by the data. Consider the mountain transect first. Figure 2 displays character index profiles from this transect. Unless one species greatly predominates, with random mating and no differential selection among genotypes, scores from a mixed population should cluster in the middle of the histogram. This pattern of clustering was not found in any of the five mixed populations (the five mixed populations were: MTN26, MTN27, MTN33, MTN37, and MTN40) with possible exception of MTN37. In general, the majority of individuals had scores near one of the two extremes, -4 or $+6$. The proportion of hybrid individuals ranged from 0.204 in MTN26 to 0.556 in MTN37 (Fig. 2). Most of these hybrids were not highly intermediate in genotype. Rather they possessed a genotype characteristic of one of the two species, with the exception of one or two aberrant alleles. Hexokinase and Pep-3 were out of Hardy-Weinberg equilibrium in all five populations (x^2, $P < 0.05$). Isocitrate dehydroge-

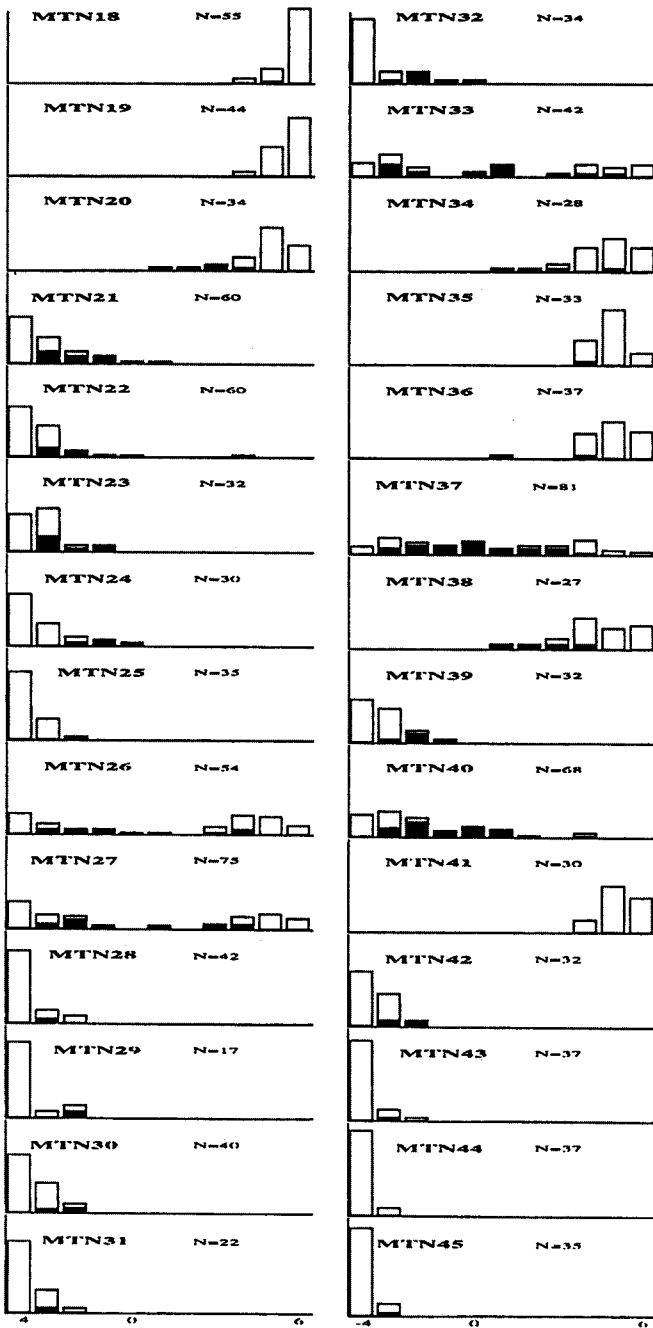FIG. 2. Character index profiles for populations collected along the mountain transect (MTN18–MTN45). The height of the bar gives the proportion of individuals in a population having that score. The solid part of the bar represents the proportion of individuals with that character-index score having a genotype indicative of mixed ancestry. Each graph represents a different population. the sample size is given by N.

nase-1 was out of equilibrium in all populations but MTN40. Glutamic-oxalacetic transaminase was in equilibrium in all populations but MTN27. At each locus that was out of equilibrium, lack of agreement with Hardy-Weinberg expectations could be attributed to a deficiency of "hybrid" heterozygotes. F_{IS} values were positive in all such cases (Table 3).

The anomalous results for *Got* may be explained by differential introgression or by the relative lack of differentiation between the two species at this locus. The allele fixed in *A. socius* (1.60) occurs with a frequency ranging from 0.05 to 0.42 in populations of *A. fasciatus* located more than 150 km north of the contact zone (Appendix 1). Thus, allopatric populations of the two ground cricket species are more similar in allele frequencies at this locus than at any other examined in this investigation. At the same time, some evidence points to the importance of two-way gene flow between the species. The nearest allopatric *A. fasciatus* population along this transect (MTN20) had a relatively high frequency of the 1.6 allele (0.44). The same was true for the four populations of *A. fasciatus* within the zone, MTN34 (0.53), MTN35 (0.54), MTN36 (0.47), and MTN38 (0.41). In some mixed populations (MTN26, MTN32, and MTN40), the *Got*[1.60] allele predominated among pure *A. fasciatus*. Similarly, the *Got*[1.00] allele, which is very rare or absent in samples of *A. socius* south of the contact zone, was present in 8 of 11 pure populations of *A. socius* within the mountain transect part of the contact zone (absent from MTN25, MTN29, and MTN30), occurring with a frequency ranging from 0.01 to 0.11. Among individuals from mixed populations that had pure *A. socius* genotypes at *Idh, Pep-3,* and *Hk,* the frequency of the *Got*[1.00] allele ranged from 0.16 in MTN40 to 0.03 in MTN27.

The lack of deviation from Hardy-Weinberg expectations at *Idh-1* in MTN40 can be be attributed to the preponderance of a *A. socius*-like genotypes in this population, coupled with a smaller amount of inter-taxon differentiation at this locus than at *Hk* and *Pep-3.*

The composite measures of linkage disequilibrium support the picture framed by

TABLE 3. The inbreeding coefficients (F_{IS} values) of marker loci in mixed populations.

Locus	Idh-1	Hk	Pep-3	Got
Site				
ILL8	0.399	0.763	0.573	0.385
ILL9	0.560	0.753	0.721	0.559
ILL13	0.608	0.606	0.595	0.329
ILL14	0.682	0.843	0.928	0.129
MTN26	0.646	0.812	0.546	0.189
MTN27	0.550	0.725	0.892	0.402
MTN33	0.332	0.714	0.514	0.260
MTN37	0.211	0.365	0.458	-0.113
MTN40	0.092	0.697	0.414	0.045
EC57	0.109	0.419	0.596	0.246
EC58	0.135	0.664	0.453	0.388

the character-index profiles and single locus data. In three mixed populations (MTN26, MTN27, and MTN33), highly significant linkage disequilibrium was present for all pairwise combination of the four marker loci (Table 4). The pure *A. fasciatus* and pure *A. socius* genotypes occurred more often than expected if assortments were random. In the MTN37 population, the three pairwise combinations involving *Got* did not exhibit linkage disequilibrium. In the MTN40 population, only *Idh-1* and *Hk* were in significant linkage disequilibrium. The MTN40 results can be attributed to a preponderance of *A. socius* in the sample.

The results from the Illinois transect resemble the results from the Mountain transect. Figure 3 displays character index profiles from the Illinois transect. Once again, the majority of individuals had character-index scores near one of the two extremes, −4 or +6. The proportion of hybrid individuals in the four mixed populations (ILL8, ILL9, ILL13, and ILL14) ranged from 0.226 in ILL14 to 0.529 in ILL13. Isocitrate dehydrogenase, *Hk,* and *Pep-3* were out of Hardy-Weinberg equilibrium in all four populations ($\chi^2, P < 0.05$). Glutamic-oxalacetic transaminase was out of equilibrium ($\chi^2, P < 0.05$) in all mixed populations but ILL14 ($\chi^2, P > 0.40$). F_{IS} values were positive for all four loci in the mixed populations (Table 3). Highly significant linkage disequilibrium was present for all pairwise combinations of the four marker loci (Table 4). Once again, pure *A. socius* and pure *A. fasciatus* genotypes occurred more often than expected.

TABLE 4. Burrow's composite measure of linkage disequilibrium (Weir and Cockerham, 1989) for mixed populations of *A. fasciatus* and *A. socius*. The numbers in the row beneath the estimates of linkage disequilibrium represent χ^2 values and test the hypothesis $D = 0$. There is one degree of freedom.

Site	Idh-1/Hk	Idh-1/Pep-3	Idh-1/Got	Hk/Pep-3	Hk/Got	Pep-3/Got
ILL8	0.229	0.229	0.238	0.286	0.219	0.206
	15.208**	16.912**	22.644**	21.458**	18.140**	15.799**
ILL9	0.323	0.270	0.271	0.317	0.309	0.295
	100.968**	56.571**	62.075**	65.853*	65.407**	68.521**
ILL13	0.239	0.265	0.198	0.289	0.218	0.223
	18.080**	22.207**	24.600**	26.421**	19.493**	37.843**
ILL14	0.308	0.341	0.182	0.320	0.185	0.211
	15.204**	17.374**	9.778**	14.872**	9.396**	9.903**
MTN26	0.350	0.320	0.146	0.377	0.164	0.166
	122.048**	101.375**	15.094**	168.916**	16.138**	17.389**
MTN27	0.324	0.334	0.235	0.350	0.223	0.232
	132.606**	112.741**	68.255**	108.934**	59.123**	46.449**
MTN33	0.234	0.213	0.149	0.297	0.218	~0.1985
	16.987**	16.241**	11.303**	23.606**	18.279**	17.505**
MTN37	0.164	0.145	0.0378	0.198	0.026	0.020
	21.811**	17.513**	2.481 NS	36.796**	1.117 NS	0.692 NS
MTN40	0.067	0.044	0.016	0.042	0.012	0.006
	3.970*	2.570 NS	0.535 NS	2.659 NS	0.317 NS	0.103 NS
EC57	0.025	0.053	0.092	0.051	0.099	0.007
	9.612**	2.575 NS	5.991*	2.695 NS	6.568*	0.027 NS
EC58	0.178	0.035	0.110	0.065	0.156	0.057
	33.994**	3.270 NS	18.269**	11.526**	30.450**	11.253**

NS = not significant, * $P < 0.05$, ** $P < 0.005$.

Thus, there is no evidence of increased hybridization between *A. fasciatus* and *A. socius* in the Illinois portion of the contact zone despite considerable narrowing of the zone. If anything, barriers to gene flow appear stronger, especially at or near the *Got* locus.

Results from the east-coast transect, another region where the zone narrows, reinforce the findings from the Illinois transect. One species or the other greatly predominated in the five mixed populations from the EC transect (Fig. 4). Only two populations, EC57 and EC58, displayed sufficiently intermediate allele frequencies to allow for complete statistical analysis of the genotypic data. The proportion of hybrids in EC58 was 0.22. The proportion of hybrids in EC57 was 0.37. Glutamic-oxalacetic transaminase, *Idh-1*, *Pep-3*, and *Hk* all displayed significant deviations from Hardy-Weinberg expectations in EC58 (χ^2, $P < 0.05$) and F_{IS} values were positive (Table 3). Hexokinase and *Pep-3* were out of Hardy-Weinberg equilibrium in EC57 (χ^2, $P < 0.05$), and again F_{IS} values were positive

(Table 3). Pairwise measures of linkage disequilibrium were significant in EC58, with the exception of *Idh-1/Pep-3*. In contrast, only three pairwise combinations of genes exhibited significant linkage disequilibrium in EC57 (Table 4). The differences between the results from EC57 and EC58 may be attributed to a larger proportion of hybrids in the EC57 population, to a smaller sample size from this population, and to gene flow from *A. socius* to *A. fasciatus* at the *Pep-3* locus in this population. Overall, there is evidence of strong isolation between the two species in mixed populations from the EC transect.

The *Pep-3* results from the EC transect are worthy of further comment. In general, the frequency of the *Pep-3*[1.30] allele is higher in *A. fasciatus* populations from the EC transect than in *A. fasciatus* populations from the other two transects (Appendix 1). The higher frequency may be due to gene flow from *A. socius* populations. The *Pep-3*[1.30] allele predominates in all mixed populations from the EC transect, even those in which the predominant species is *A. fas-*

FIG. 3.   Character index profiles for populations collected along the Illinois transect (ILL1–ILL17). The height of the bar gives the proportion of individuals in a population having that score. The solid part of the bar represents the proportion of individuals with that character-index score having a genotype indicative of mixed ancestry. Each graph represents a different population. The sample size is given by *N*.

ciatus. Despite the apparent gene flow, the *Pep-3*[1.00] homozygote occurs more frequently than expected in EC57 and EC58. One possible explanation for this finding is selection against heterozygotes at the *Pep-3* locus or selection acting at a closely linked locus. Another possible explanation is occasional dominance of the *Pep-3*[1.00] allele,

something we have observed in the offspring of heterospecific matings (Howard and Gregory, unpubl. data). The two *Pep-3* alleles are in Hardy-Weinberg equilibrium in allopatric *A. fasciatus* populations.

DISCUSSION

As Barton and Hewitt (1985) have pointed out, a broken pattern of distribution of genotypes within a hybrid zone is strong prima facie evidence for the importance of environmental gradients in determining the position, width, and maintenance of the zone. An even stronger case for the role of the environment can be made when major characteristics of the zone, such as position and width, can be successfully predicted in unexplored areas on the basis of environmental characteristics. Here we predicted the occurrence of additional *A. fasciatus* south of Charleston, West Virginia because the high mountains in the region provide a climatic regime typical of the northern latitudes at which this cricket occurs. The prediction was fulfilled. Not only did *A. fasciatus* occur south of Charleston (Fig. 1), but the pattern of distribution of the species matched the pattern described by Howard in 1986. Where *A. fasciatus* or mixed populations occurred south of *A. socius* populations, the former two were at higher elevations than the latter (Table 2). The pattern is so consistent that there can be little doubt it is real. As Howard (1986) pointed out, this is the pattern of distribution one would expect to find if two closely related species, one adapted to a northern climate and one adapted to a southern climate, met in a mountainous area at the limits of their ranges as set by climatic factors.

We also predicted that in Illinois, where the topography is less varied and hence climatic mosaicism less pronounced, the zone of overlap and hybridization would narrow. This prediction too was fulfilled, providing additional support for the importance of the external environment in determining the distribution of the two species and the width of the zone. Assigning an actual width to the zone in this area is difficult in light of the clear mosaicism (Fig. 1). It is probably less than 40 km. In contrast, the distance between areas inhabited entirely by *A. fasciatus* and areas inhabited entirely by *A. socius* is approximately 350–400 km along the mountain transect.

By claiming a role for the environment in determining the position and characteristics of the *A. fasciatus - A. socius* hybrid zone, we are not denying a role for biotic factors, especially interspecific competition. For example, it is possible that climatic factors limit the distribution of only one of these species and that interspecific competition limits the distribution of the second species.

At present, we do not know the climatic condition to which the crickets are sensitive. The most likely candidate is temperature, which demonstrates the clearest latitudinal and elevational gradients in the eastern United States (Boucher, 1975; Stringer, 1972). Temperature is most likely to play a role during the winter when north-south differences are most exaggerated. *Allonemobius socius* eggs may not be able to survive winter temperatures tolerated by *A. fasciatus* eggs. Alternatively or concomitantly, egg diapause may not be as intense in *A. fasciatus*, resulting in untimely development during warming periods at lower elevations or further south.

In earlier work, Howard (1986) reported geographic variation in the proportion of hybrids in mixed populations. The patterns of variation suggested stronger isolation between the two ground cricket species in broader parts of the hybrid zone, an outcome consistent with theory (Bigelow, 1965; Remington, 1968; Barton and Hewitt, 1981, 1985). These patterns have not held up under further investigation, falsifying the third prediction put forth in the Introduction. Despite a great narrowing of the zone in Illinois, the level of isolation between the species appears to be no less than the level of isolation in the Appalachian Mountains of West Virginia and Virginia. Also, contrary to earlier results from a different New Jersey population (no longer available for examination due to interstate highway modification), a character index profile and patterns of genetic disequilibrium from a New Jersey mixed population (EC58) resemble the profiles and patterns found in populations from the mountain transect. Taken together, the above results do not run counter to the possibility that reinforcement
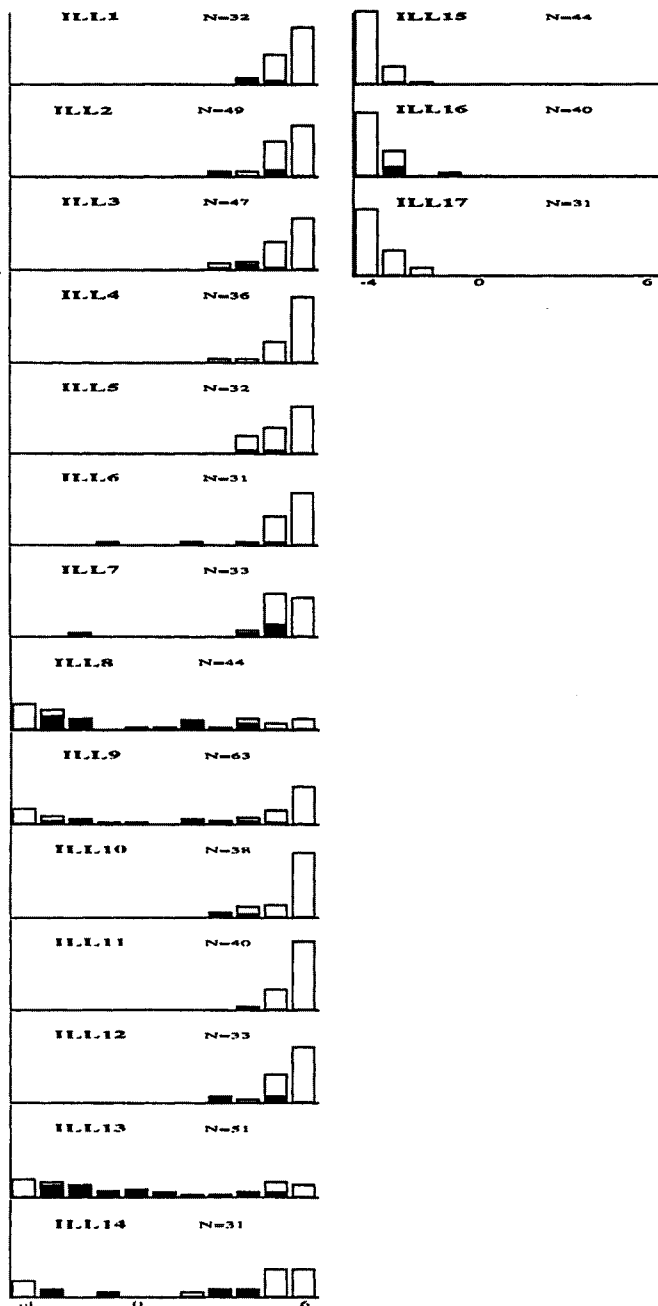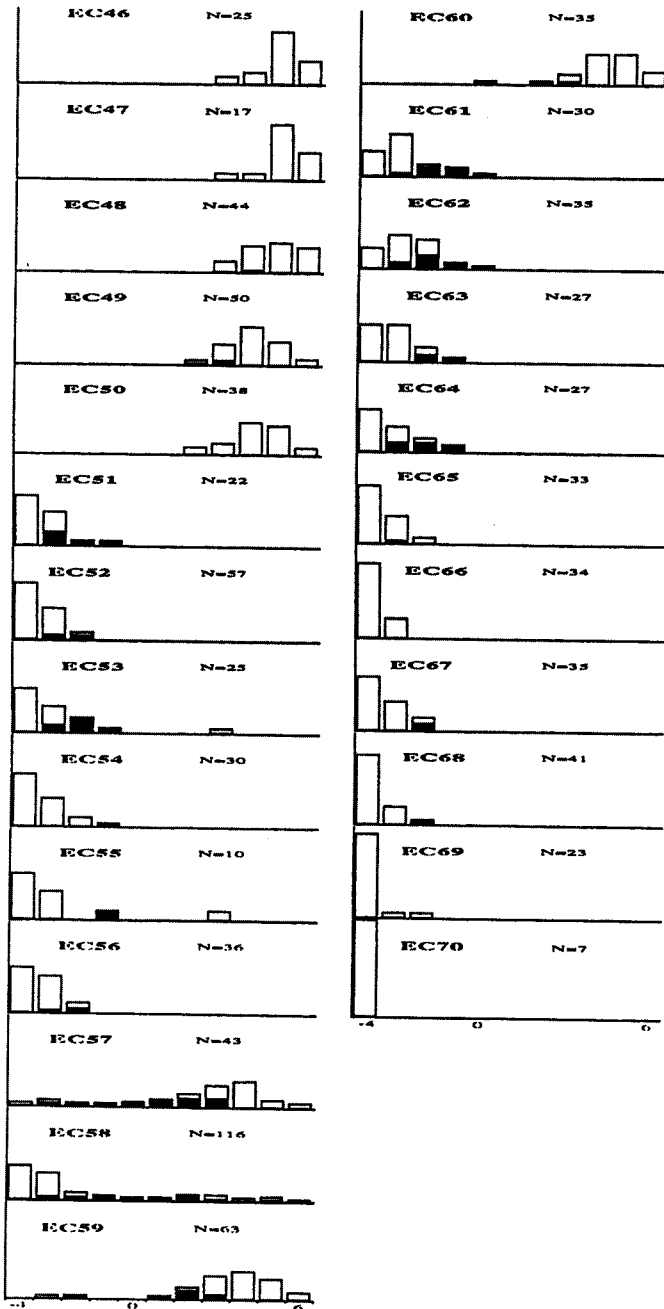
FIG. 4. Character index profiles for populations collected along the east-coast transect (EC46–EC70). The height of the bar gives the proportion of individuals in a population having that score. The solid part of the bar represents the proportion of individuals with that character-index score having a genotype indicative of mixed ancestry. Each graph represents a different population. The sample size is given by N.

has occurred in the zone, but they do suggest that the width of the zone has not been a major factor in this process.

The patch size of this hybrid zone is sometimes quite large, especially in the Appalachian Mountains (see Fig. 1) raising the question of whether clinal hybrid zones occur at the boundaries of the patches. Our

impression is that they do not; this is based largely on the island-like distribution of the moist, short grass habitat preferred by these crickets (Howard and Harrison, 1984). But much more detailed microgeographic sampling will be needed to answer this question.

The large patch size in the Appalachian Mountains also raises the question of whether the hybrid zone in this area could be more realistically viewed as three or four zones separated by broad patches of parental types. More detailed lateral sampling will be needed to answer this question.

In general, the width of a zone may be a less important factor in the development of reproductive barriers in mosaic hybrid zones characterized by large patch sizes, than in mosaic hybrid zones characterized by small patch sizes. When pure species patches are large, individuals within the patches are unlikely to be exposed to selection against hybridization. If such patches occur throughout the zone, the swamping effect of gene flow from pure species populations will occur throughout the zone, not just near the borders of the zone.

The overwhelming impression left by the results of the electrophoretic analysis is the similarity in interspecific interactions throughout the zone. Pure species individuals were abundant in all mixed populations, predominating in all but three populations. Most individuals classified as hybrid were not highly intermediate in genotype, rather they possessed genotypes more characteristic of backcrosses. Thus, successful hybridization between pure species individuals appears to be a relatively rare event in most mixed populations throughout the zone. Whether this can be attributed to the strengthening of premating isolating mechanisms in areas of sympatry awaits further study. However, with the foregoing question in mind, it is worth noting that Benedix and Howard (submitted) have found evidence of calling song displacement in *A. fasciatus* males from the zone of overlap and hybridization.

LITERATURE CITED

BARTON, N. H., AND G. M. HEWITT. 1981. Hybrid zones and speciation, pp. 109–145. *In* W. R. Atchley and D. S. Woodruff (eds.), Evolution and Speciation: Essays in Honor of M. J. D. White. Cambridge Univ. Press, Cambridge UK.

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

BENEDIX, J. H., JR., AND D. J. HOWARD. 1991. Calling song displacement in a zone of overlap and hybridization. Evolution, *In press.*

BIGELOW, R. S. 1965. Hybrid zones and reproductive isolation. Evolution 19:449–458.

BOUCHER, K. 1975. Global Climate. Wiley & Sons, N.Y.

BUTLIN, R. 1989. Reinforcement of premating isolation, pp. 158–179. *In* D. Otte and J. A. Endler (eds.), Speciation and its consequences. Sinauer Associates, Sunderland, MA.

COCKERHAM, C. C., AND B. S. WEIR. 1977. Digenic descent measures for finite populations. Genet. Res. Camb. 30:121–147.

DOBZHANSKY, T. 1940. Speciation as a stage in evolutionary divergence. Am. Nat. 74:312–321.

———. 1970. Genetics of the Evolutionary Process. Columbia Univ. Press, N.Y.

ENDLER, J. A. 1977. Geographic Variation, Speciation and Clines. Princeton Univ. Press, Princeton, NJ.

GARTSIDE, D. F. 1980. Analysis of a hybrid zone between chorus frogs of the *Pseudacris nigrita* complex in the southern United States. Copeia 1980:56–66.

HARRISON, R. G. 1986. Pattern and process in a narrow hybrid zone. Heredity 56:337–349.

HARRISON, R. G., AND D. M. RAND. 1989. Mosaic hybrid zones, pp. 111–133. *In* D. Otte and J. A. Endler (eds.), Speciation and its Consequences. Sinauer Associates, Sunderland, MA.

HOWARD, D. J. 1982. Speciation and coexistence in a group of closely related ground crickets. Ph.D. Diss. Yale University, New Haven, CT.

———. 1983. Electrophoretic survey of eastern North American *Allonemobius* (Orthoptera: Gryllidae): Evolutionary relationships and the discovery of three new species. Ann. Entomol. Soc. Am. 76:1014–1021.

———. 1986. A zone of overlap and hybridization between two ground cricket species. Evolution 40:34–43.

HOWARD, D. J., AND D. G. FURTH. 1986. Review of the *Allonemobius fasciatus* (Orthoptera: Gryllidae) complex with the description of two new species separated by electrophoresis, songs, and morphometrics. Ann. Entomol. Soc. Am. 79:472–481.

HOWARD, D. J., AND R. G. HARRISON. 1984. Habitat segregation in ground crickets: The role of interspecific competition and habitat selection. Ecology 65:69–76.

LEVENE, H. 1949. On a matching problem arising in genetics. Ann. Math. Stat. 20:91–94.

LITTLEJOHN, M. J. 1981. Reproductive isolation: A

critical review, pp. 298–334. *In* W. R. Atchley and D. S. Woodruff (eds.), Evolution and Speciation: Essays in Honor of M. J. D. White. Cambridge Univ. Press, Cambridge.

MAYR, E. 1963. Animal Species and Evolution. Belknap Press, Cambridge.

MOORE, J. A. 1957. An embryologist's view of the species concept, pp. 325–338. *In* E. Mayr (ed.), The Species Problem. Am. Assoc. Adv. Sci. Publ. No. 50., Washington, DC.

MOORE, W. S. 1979. A single locus mass-action model of assortative mating, with comments on the process of speciation. Heredity 42:173–186.

PATERSON, H. E. H. 1978. More evidence against speciation by reinforcement. S. Afr. J. Sci. 74:369–371.

———. 1982. Perspective on speciation by reinforcement. S. Afr. J. Sci. 78:53–57.

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.

REMINGTON, C. L. 1968. Suture zones of hybrid interaction between recently joint biotas. Evol. Biol. 2:321–428.

SHORT, L. L. 1969. Taxonomic aspects of avian hybridization. Auk 86:84–105.

SPERLING, F. A. H. 1987. Evolution of the *Papilio machaon* species group in western Canada (Lepidoptera: Papilionidae). Quaest. Entomol. 23:198–315.

STRINGER, E. T. 1972. Foundations of Climatology. W. H. Freeman Company, San Francisco, CA.

SWOFFORD, D. L., AND R. B. SELANDER. 1981. BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72:281–283.

TEMPLETON, A. R. 1981. Mechanisms of speciation—a population genetic approach. Annu. Rev. Ecol. Syst. 12:23–48.

WEIR, B. S. 1979. Inferences about linkage disequilibrium. Biometrics 35:235–254.

WEIR, B. S., AND C. C. COCKERHAM. 1989. Complete characterization of disequilibrium at two loci, pp. 86–110. *In* M. W. Feldman (ed.), Mathematical Evolutionary Theory. Princeton Univ. Press, Princeton, NJ.

WEST-EBERHARD, J. J. 1983. Sexual selection, social competition, and speciation. Q. Rev. Biol. 58:155–183.

WRIGHT, S. 1978. Evolution and the Genetics of Populations, Vol. 4. Variability within and among Natural Populations. Univ. Chicago Press, Chicago, IL.

———. 1980. Genic and organismic selection. Evolution 34:825–843.

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APPENDIX 1. Allele frequencies in populations 1 through 70.

	Populations													
Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Idh-1														
(*N*)	32	61	47	36	32	31	33	44	63	39	40	33	51	31
2.20	0.03	0.04	0.03	—	0.02	0.05	0.06	0.62	0.32	0.01	—	0.03	0.59	0.26
2.00	—	—	—	—	—	—	0.03	0.03	—	—	—	—	0.02	0.02
1.80	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1.00	0.97	0.96	0.97	1.00	0.98	0.95	0.91	0.34	0.68	0.99	1.00	0.97	0.39	0.73
Hk														
(*N*)	32	60	48	36	32	31	33	44	63	40	40	33	51	31
1.00	1.00	1.00	0.99	1.00	0.98	0.98	0.97	0.40	0.66	0.98	0.98	0.97	0.47	0.71
0.80	—	—	0.01	—	0.02	0.02	0.03	0.60	0.34	0.02	0.01	0.03	0.53	0.29
Pep-3														
(*N*)	32	61	47	36	32	31	33	44	63	39	40	33	51	31
1.30	0.06	0.06	0.06	0.06	0.09	0.08	0.06	0.60	0.35	0.04	—	0.02	0.59	0.34
1.00	0.94	0.94	0.94	0.94	0.91	0.92	0.94	0.40	0.65	0.96	1.00	0.98	0.41	0.66
Got														
(*N*)	32	49	48	36	32	31	33	44	63	40	40	33	51	31
2.20	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1.60	0.12	0.12	0.21	0.12	0.19	0.21	0.21	0.72	0.37	0.14	0.11	0.17	0.63	0.40
1.00	0.88	0.88	0.79	0.88	0.80	0.79	0.79	0.28	0.63	0.86	0.89	0.83	0.37	0.60
0.50	—	—	—	—	0.01	—	—	—	—	—	—	—	—	—

	Populations													
Locus	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Idh-1														
(*N*)	44	40	38	55	44	34	41	42	32	30	35	54	75	42
2.20	0.89	0.89	0.75	0.01	—	0.02	0.79	0.80	0.78	0.73	0.89	0.38	0.45	0.88
2.00	0.01	0.01	0.03	—	—	0.02	—	0.02	—	—	—	—	—	—
1.80	—	—	0.03	—	—	—	0.01	0.01	—	—	—	—	0.01	—
1.00	0.10	0.10	0.20	0.99	1.00	0.97	0.20	0.17	0.22	0.27	0.11	0.62	0.53	0.12
Hk														
(*N*)	47	40	38	56	44	34	42	42	33	30	35	54	75	42
1.00	—	0.01	—	1.00	1.00	0.93	0.07	0.06	0.03	0.10	0.01	0.56	0.43	—
0.80	1.00	0.99	1.00	—	—	0.07	0.93	0.94	0.97	0.90	0.99	0.44	0.57	1.00
Pep-3														
(*N*)	47	40	39	56	44	34	42	42	33	30	35	54	75	42
1.30	1.00	1.00	1.00	0.04	0.10	—	1.00	0.96	1.00	0.97	1.00	0.43	0.50	0.99
1.00	—	—	—	0.96	0.90	1.00	—	0.04	—	0.03	—	0.57	0.50	0.01
Got														
(*N*)	47	40	32	56	44	34	42	42	33	30	35	54	75	42
2.20	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1.60	0.99	0.95	1.00	0.05	0.10	0.44	0.90	0.96	0.89	0.97	1.00	0.76	0.72	0.99
1.00	0.01	0.05	—	0.95	0.90	0.56	0.10	0.04	0.11	0.03	—	0.24	0.28	0.01
0.50	—	—	—	—	—	—	—	—	—	—	—	—	—	—

	Populations													
Locus	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Idh-1														
(*N*)	17	41	22	34	42	36	33	37	81	28	32	69	30	32
2.20	0.85	0.80	0.86	0.90	0.48	0.08	0.02	0.05	0.41	0.07	0.73	0.62	—	0.72
2.00	—	—	—	—	—	—	—	—	—	—	0.02	—	—	0.14
1.80	—	—	—	—	—	—	—	—	—	—	—	0.01	—	0.02
1.00	0.15	0.20	0.14	0.10	0.52	0.92	0.98	0.95	0.59	0.93	0.25	0.38	1.00	0.12

APPENDIX 1. Continued.

							Populations							
Locus	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Hk														
(N)	18	41	22	35	42	34	34	37	82	28	32	69	30	32
1.00	—	0.01	—	0.04	0.48	0.96	1.00	0.99	0.49	1.00	0.03	0.17	1.00	0.03
0.80)	1.00	0.99	1.00	0.96	0.52	0.04	—	0.01	0.51	—	0.97	0.83	—	0.97
Pep-3														
(N)	18	41	22	35	42	38	34	37	82	27	32	68	30	32
1.30	0.97	1.00	1.00	0.96	0.57	0.10	0.03	0.01	0.66	0.28	0.98	0.85	—	1.00
1.00	0.03	—	—	0.04	0.43	0.90	0.97	0.99	0.34	0.72	0.02	0.15	1.00	—
Got														
(N)	18	41	22	35	42	38	34	37	82	28	32	69	30	32
2.20	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1.60	1.00	1.00	0.98	0.96	0.70	0.53	0.54	0.47	0.79	0.41	0.97	0.85	0.38	0.97
1.00	—	—	0.02	0.04	0.30	0.47	0.46	0.53	0.21	0.59	0.03	0.15	0.62	0.03
0.50	—	—	—	—	—	—	—	—	—	—	—	—	—	—

							Populations							
Locus	43	44	45	46	47	48	49	50	51	52	53	54	55	56
Idh-1														
(N)	37	37	35	25	37	44	54	42	22	57	25	32	10	36
2.20	0.32	0.39	0.30	—	—	—	—	—	—	0.02	0.08	0.11	0.05	0.18
2.00	0.46	0.34	0.43	—	—	0.01	0.02	—	0.46	0.54	0.36	0.38	0.30	0.33
1.80	0.15	0.23	0.20	—	—	—	0.01	—	0.38	0.26	0.34	0.27	0.35	0.24
1.00	0.07	0.04	0.07	1.00	1.00	0.99	0.97	1.00	0.16	0.18	0.22	0.25	0.30	0.25
Hk														
(N)	40	40	44	25	37	44	54	41	22	58	26	33	10	38
1.00	—	—	0.02	1.00	1.00	1.00	1.00	1.00	0.02	—	0.06	0.02	0.15	0.01
0.80	1.00	1.00	0.98	—	—	—	—	—	0.98	1.00	0.94	0.98	0.85	0.99
Pep-3														
(N)	40	40	44	25	17	44	50	42	22	58	26	31	10	28
1.30	1.00	1.00	1.00	0.10	0.15	0.19	0.48	0.42	0.98	0.99	0.96	1.00	1.00	0.99
1.00	—	—	—	0.90	0.85	0.81	0.52	0.58	0.02	0.01	0.04	—	—	0.01
Got														
(N)	40	40	44	25	17	44	54	40	22	58	26	33	10	38
2.20	—	—	—	—	—	—	—	—	—	—	—	0.03	—	—
1.60	0.99	1.00	0.99	0.42	0.29	0.41	0.49	0.49	0.93	0.97	0.83	0.97	0.95	0.99
1.00	0.01	—	0.01	0.58	0.71	0.59	0.51	0.51	0.07	0.03	0.17	—	0.05	0.01
0.50	—	—	—	—	—	—	—	—	—	—	—	—	—	—

							Populations							
Locus	57	58	59	60	61	62	63	64	65	66	67	68	69	70
Idh-1														
(N)	43	116	64	35	30	35	27	28	33	34	30	41	26	18
2.20	—	0.09	0.02	—	0.05	0.04	0.04	0.04	0.09	0.28	0.14	0.18	0.19	0.28
2.00	0.15	0.28	0.06	—	0.40	0.31	0.46	0.45	0.30	0.15	0.46	0.57	0.60	0.44
1.80	0.07	0.26	0.01	—	0.20	0.20	0.20	0.32	0.41	0.47	0.21	0.15	0.15	0.28
1.00	0.78	0.37	0.92	1.00	0.35	0.44	0.30	0.20	0.20	0.10	0.19	0.09	0.06	—
Hk														
(N)	43	117	66	35	30	35	27	29	34	35	35	35	26	19
1.00	0.86	0.21	0.96	0.96	0.10	0.03	0.04	0.10	—	—	0.03	0.03	—	—
0.80	0.14	0.79	0.04	0.04	0.90	0.97	0.96	0.90	1.00	1.00	0.97	0.97	1.00	1.00

APPENDIX 1. Continued.

Locus	Populations													
	57	58	59	60	61	62	63	64	65	66	67	68	69	70
Pep-3														
(*N*)	43	117	64	35	30	35	27	28	34	35	35	35	23	7
1.30	0.83	0.92	0.66	0.40	0.95	0.90	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.00	0.17	0.08	0.34	0.60	0.05	0.10	—	—	—	—	—	—	—	—
Got														
(*N*)	43	117	66	35	30	35	27	29	34	35	35	35	23	7
2.20	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1.60	0.44	0.87	0.35	0.34	0.98	0.96	0.96	0.91	0.98	1.00	1.00	1.00	1.00	1.00
1.00	0.56	0.13	0.65	0.65	0.02	0.04	0.04	0.09	0.02	—	—	—	—	—
0.50	—	—	—	—	—	—	—	—	—	—	—	—	—	—