Syst. Zool., 39(2):131–147, 1990

TESTING NOMINAL SPECIES BOUNDARIES USING GENE FLOW STATISTICS: THE TAXONOMY OF TWO HYBRIDIZING ADMIRAL BUTTERFLIES (*LIMENITIS*: NYMPHALIDAE)

ADAM H. PORTER*

Department of Zoology, University of California, Davis, California 95616

Abstract.—Gene flow estimates between groups of populations (Nm_{GT}) derived from Wright's hierarchical *F*-statistics can be used to test taxonomic hypotheses about the location of biological species boundaries. The interpretation of Nm_{GT} is limited by the assumptions of the island model: balancing selection, time to equilibrium, and the limits to detectability of alleles are biases acting in the same direction as gene flow to cause similarity among populations. However, confidence limits of $0.01 \leq Nm_{GT} \leq 0.15$ between sympatric sibling species suggest that these biases are weak relative to gene flow in causing genetic similarity. Nm_{GT} also compares favorably to gene flow estimated from a mark-recapture study, and Nm_{GT} values across a well-studied grasshopper hybrid zone suggest that gene flow is limited but not stopped by the barrier.

The nominal species *Limenitis lorquini* and *L. weidemeyerii* hybridize along their contact areas in western North America. Their hybrid zone was mapped using wing pattern characters, and the same populations were analyzed electrophoretically at 18 enzyme loci. Gene flow is strong within these taxa ($1.56 \leq Nm \leq 15.23$). Across the hybrid zone, $0.57 \leq Nm_{GT} \leq 1.97$, suggesting that although there is a partial barrier to genetic exchange, these taxa share significant portions of their gene pools. Small population sizes and other circumstantial evidence argue that much of the similarity observed among populations is actually due to gene flow, rather than balancing selection, although undetected allelic differences may also be partially responsible. *L. weidemeyerii* and its "subspecies" are best considered as univoltine subspecies of *lorquini*, and all should be treated as a single genetic unit when cladistic analyses are performed. The ease with which these results were obtained suggests that systematists should pay more attention to the genetic population structure of taxonomic boundaries. [*Nm*, genetic population structure, hybrid zone, Lepidoptera, genetic isolation.]

Mayr's (1942, 1963) biological species concept recognizes populations as conspecific if their members (can) share a common gene pool, but this has been a particularly difficult criterion to measure (Ehrlich, 1961). Despite the promise of mitochondrial characters (Avise et al., 1987), cladistic methods are not suited a priori to the definition of evolutionary units (taxa) at or below the level of species. Cladistic methods use particular characters as markers for the history of the whole genome. To the extent that different characters are functionally independent, they may acquire independent geographic ranges within a species. Thus, derived characters are not necessarily good markers for the whole genomes of populations within a species. Cladistics works well for the phylogenetic arrangement of reproductively (and genetically) isolated groups, but phenetically based methods are the only methods available to define such groups. Among phenetic methods, it makes sense to use those statistics which describe gene flow among populations. Recent advances in the estimation of gene flow using allelic frequency data (Slatkin, 1985a, 1987; Slatkin and Barton, 1989) suggest an empirical approach to the verification of species boundaries in nature.

Gene Flow and Taxonomic Boundaries

Wright (1931, 1969, 1978) and Slatkin (1981, 1985b) provide procedures for the estimation of gene flow based on the island model of genetic population structure,

* Present address: Department of Zoology, University of Canterbury, Christchurch 1, New Zealand. Address after 1 January 1991: Zoological Museum, Universität Zurich. Winterhurerstr. 190, 8057 Zürich, Switzerland.

which assumes no selection among an infinite number of equal-sized populations, all exchanging an equal proportion of individuals each generation. Under this scenario, an equilibrium is established between the differentiating effects of genetic drift and the homogenizing effects of gene flow, such that the degree of genetic differentiation among populations has a simple relationship to gene flow. By measuring genetic differentiation, and assuming the conditions of the model, it is possible to get an indirect estimate of gene flow. These estimates measure a weighted average of gene flow if there is spatial or temporal heterogeneity. Wright's methods measure genetic differentiation using a value (F) which is based on the variance in gene frequencies among populations. Following Wright (1931, 1978),

$$F_{ST} = \frac{\sigma_{ST}^2}{q_T(1 - q_T)}, \qquad (1)$$

where q_T is the average frequency of an allele q among all populations, the subscript S represents [sub]populations, and T represents the total set of populations. The numerator measures the observed variance among populations; the denominator represents the maximum possible variance among populations (which only occurs if populations are all either fixed for q or completely lacking in q).

If populations are grouped (e.g., into geographic regions or taxa), one can determine the component of genetic differentiation due to intergroup effects by first partitioning the variance of the numerator (Wright, 1978):

$$F_{ST} = \frac{\sigma_{SG}^2 + \sigma_{GT}^2}{q_T(1 - q_T)}, \qquad (2)$$

where here the subscript SG represents the component of overall genetic variance attributable to populations within groups, and GT represents the component attributable to differentiation among groups. When sample sizes are equal, $q_T = \Sigma\, q_G/n$, where q_G is the average gene frequency within groups and n is the number of groups, so that

$$F_{ST} = \frac{1}{n}\,\Sigma_G\left(\frac{\sigma_{SG}^2}{q_G(1 - q_G)}\right) + \frac{\sigma_{GT}^2}{q_T(1 - q_T)}.$$

This simplifies and rearranges to provide a measure of intergroup differentiation:

$$F_{GT} = F_{ST} - \bar{F}_{SG}. \qquad (3)$$

It is usually possible to use standard population genetic computer packages to obtain values for F_{ST} and F_{SG}, and simulation studies suggest that these statistics provide the best estimators of gene flow when gene flow is homogeneous among all populations (Slatkin and Barton, 1989). However, because Wright's F-statistics are population parameters rather than statistical estimators of those parameters (inasmuch as they do not correct for sampling errors [Weir and Cockerham, 1984]), I also have used statistical estimators θ_{ST}, θ_{SG}, and θ_{GT} in the data analyses, using the formulae given in Weir and Cockerham (1984:1364). My θ_{ST}, θ_{SG}, and θ_{GT} correspond to their θ, θ_1, and θ_2, respectively.

Under the island model, the approximation

$$Nm_{ST} \approx (1/F_{ST} - 1)/4 \qquad (4)$$

describes the relationship between gene flow and genetic differentiation (Wright, 1931), where N is the effective population size and m is the effective proportion of migrants between populations. This approximation is derived from a larger equation which incorporates second-order terms in m, but is accurate when m is small enough that m^2 is negligible; it is also accurate in simulations under an isolation-by-distance model (Slatkin and Barton, 1989). When values of N among populations are approximately equal, Nm represents the actual number of individuals exchanged among populations each generation. When $Nm < 0.5$ (i.e., $F_{ST} > 0.33$), genetic drift is the primary cause of the observed genetic differentiation among populations; when $Nm > 0.5$, gene flow is the primary determinant (Wright, 1931). Extending this result to groups of populations (and sub-

stituting subscripts GT for ST in approximation [4]), if the genetic differentiation between groups is small ($F_{GT} < 0.33$), then gene flow across the group boundary is likely to be present; if F_{GT} is larger, then gene flow across the boundary is likely to be small or nonexistent.

Interpretation of gene flow statistics in systematics.—Deviations in nature from the genetic population structure of the island model should, in principle, introduce biases in the accuracy of the gene flow estimates. These biases may be found by relaxing the island model's assumptions, which include: (i) equal gene flow among all populations (which is the impetus of this study), (ii) demic population structure, (iii) large number of populations, (iv) equal effective population sizes, (v) negligible selection, (vi) negligible mutation, (vii) gene flow and drift having reached equilibrium, and (viii) all alleles having been identified.

Just how limiting are these assumptions? Slatkin and Barton (1989) provide mathematical parameters describing the continuum between continuous and demic population structures, and their simulations show that Nm estimates derived from F_{ST} are accurate even when population structure is not demic (assumption [ii]). When there are fewer than ~10 actual (not sample) populations in a group (assumption [iii]), then Nm estimates based on F_{ST} (and θ_{ST}) are biased upward (Nei et al., 1977). If effective population sizes differ among populations (assumption [iv]), the genetic constitutions of larger populations will be less influenced by immigrants than are smaller populations, but larger populations will more strongly affect the genetic makeup of satellite populations. The biases introduced in gene flow estimation with heterogeneous population sizes have not been worked out except in one very simple case (Porter, 1989a), and may even be situation-specific. It is therefore necessary to defend assumptions (iii) and (iv) for each case study.

Natural selection (assumption [v]) presents a somewhat more complex set of difficulties, because each locus is likely to have its own set of selection pressures (e.g., Carter and Watt, 1988). However, since these Nm estimates are based on overall genetic similarity measures, averaged over loci and alleles (Wright, 1978; Weir and Cockerham, 1984), it is appropriate to look also at the effects of selection averaged over loci and alleles. Selection may either have no net effect (≈neutrality), it may promote net similarity among populations (balancing selection), or it may promote net differentiation among populations (diversifying selection). Diversifying selection mimics the net effect of drift by promoting differentiation: gene flow estimates under this situation are likely to be underestimates. However, because of linkage effects, intrinsic barriers to effective gene flow at all loci are set up under diversifying selection (Barton, 1983; Barton and Hewitt, 1983), and these barriers tend to reduce the bias of the Nm estimation. Balancing selection acts in the same direction as gene flow by promoting similarity: Nm estimates derived under balancing selection are necessarily overestimates of the true gene flow.

Like diversifying selection, mutation (assumption [vi]) acts to cause net differentiation among populations. However, given the low background mutation rate, mutation alone is not likely to be an important source of bias unless gene flow is quite small. Slatkin (1989) provides methods for the estimation of very low levels of gene flow using the history of mutations in mitochondrial clades.

When effective population sizes are large, violation of assumption (vii) is likely to generate overestimates of Nm_{GT} if populations have recently become separated. Nm_{GT} will tend to be underestimated in recent secondary contact, but this effect may be ephemeral: in a two-population model, the initial trajectory of approach to equilibrium of the most divergent alleles is quite steep (Porter, 1989a). Hidden alleles (assumption [viii]) hide differentiation, promoting the illusion of genetic similarity (Coyne, 1982). Like balancing selection and non-equilibrium, they provide a source of overestimation of gene flow.

Of all of the assumptions, net balancing selection and hidden variation are the most likely to cause significant bias in gene flow

estimation. In natural populations where the selection regime and the extent of hidden variation are unknown, Nm estimates in principle provide an approximate upper bound to the actual gene flow value.

In the remaining sections of this paper, I will first present tests of both the accuracy and the strength of biases in gene flow estimators between pairs of taxa where gene flow is reasonably well known. I will then turn to the analysis of a hybrid zone between two parapatric nominal species of admiral butterflies in California and Nevada in order to show how Nm_{GT} can be used to address questions of species-level classification in less well-studied systems.

TESTS OF THE ESTIMATION TECHNIQUE

The strength of the biases in Nm estimation can be approximated by obtaining Nm_{GT} estimates between sympatric sibling species pairs, as well as by comparisons of Nm_{GT} with gene flow estimates obtained from mark-recapture studies. I will also present the analysis of gene flow across a well-studied hybrid zone to demonstrate the utility of this method in the taxonomy of parapatric taxa.

Sympatric sibling species are not presently in genetic contact, so estimates of $Nm_{GT} > 0$ are in fact attributable to those factors besides gene flow which promote observed genetic similarity between species, and the Nm_{GT} value estimates the importance of these biases. Further, Nm_{GT} estimates derived from F_{GT} and θ_{GT} can be compared to determine which gives the more accurate value (i.e., the value closer to zero).

F values were obtained by simply averaging over alleles and loci, as recommended by Slatkin and Barton (1989); θ values were computed by averaging over alleles and loci following Weir and Cockerham's (1984) weighted averages method, using a program written in Basic on a Macintosh Plus® computer (Porter, unpubl.). Hardy-Weinberg heterozygosities were used rather than observed heterozygote frequencies, both to simplify the calculation of θ values and because the data sources reported allelic frequency data. Weir and Cockerham (1984) also provide a degrees of freedom correction for population groups which I did not employ, because the groups analyzed were not considered to be samples of a larger set of groups. Variance estimates of F and θ were obtained by jackknifing over loci (Efron, 1982; Weir and Cockerham, 1984). From these, standard errors and 95% confidence intervals were calculated. These confidence intervals were then converted directly to Nm confidence intervals using approximation (4). This procedure tends to make the Nm confidence intervals seem unreasonably large, especially when Nm is large, but this is typically attributable to the inverse relationship between F (or θ) and Nm rather than to limitations in the data. Gene flow is interpreted as being panmictic when values of F or $\theta \leq 0$, as well as when Nm exceeds the average effective population size.

I calculated F-statistics and their corresponding Nm estimates from eight sets of sympatric sibling species, three of which I report here. *Euphydryas editha* and *E. chalcedona* data from McKechnie et al. (1975) were culled to include only population samples collected in microsympatry. The enzymes of these populations are likely to be under relatively similar (though obviously not identical) conditions of natural selection from both their internal and external environments, and at least in *E. editha* and probably in *E. chalcedona* as well, the populations are discrete enough that genetic drift will have reached an equilibrium with gene flow. No cases of natural hybridization between these taxa have been reported despite extensive study by Ehrlich and his associates for over 25 years. The second data set is from Harrison (1979) on *Gryllus pennsylvanicus* and *G. veletis*, morphologically indistinguishable species which nevertheless have very different life histories. The selective regimes experienced by these species are likely to be less similar than between the *Euphydryas* species above, and one may predict a lesser degree of bias from balancing selection. The last data set (Guttman and Weigt, 1989) compares unnamed sympatric species of the

Enchenopa binotata complex, recognized by their hostplant associations. Of the four sympatric host-differentiated *Enchenopa* species they studied, I arbitrarily chose one pair for presentation. Results from the three analyses presented here are representative of all the analyses I have performed using this method.

In all data sets involving sympatric sibling species (Table 1), the Nm_{GT} estimate obtained from θ_{GT} is much closer to zero than the estimate from F_{GT}. Indeed, in *Euphydryas*, Nm_{GT} estimates from F_{GT} absurdly indicate that these species are members of a single panmictic population. The greater accuracy of θ_{GT} in estimating (intergroup) Nm_{GT} is in marked contrast to Slatkin and Barton (1989), who showed that F_{SG} is superior to θ_{SG} for estimating intragroup gene flow (Nm_{SG}). Throughout the remainder of this paper, all Nm_{GT} values reported are calculated from θ_{GT}.

Nm_{SG} among *Euphydryas editha* populations (Table 1) suggests that average gene flow among California populations of this species is approximately one individual exchanged per population per generation. For *E. chalcedona* this value is much higher, as expected from their more open population structure (Ehrlich and Murphy, 1987) and affinity for a wider range of habitats (Scott, 1986). Between species, the 95% confidence limits show that the Nm_{GT} estimate (from θ_{GT}) is significantly greater than zero, indicating contributions to genetic similarity from factors other than gene flow—a result in agreement with the analyses of McKechnie et al. (1975). However, these biases are quite small relative to gene flow within these species, and alone appear to provide only minor contributions to overall genetic similarity. Similar results obtain for *Gryllus* and *Enchenopa*, but the biases in Nm_{GT} appear to be even weaker in these taxa.

One criticism of using sympatric sibling species to quantify biases in Nm estimation is that balancing selection may be somewhat stronger among conspecific populations than between species. To test the accuracy of estimates when the groups are clearly conspecific, it is appropriate to compare estimates of Nm_{GT} against gene flow estimates obtained from mark-recapture studies. Such studies are difficult to carry out, and I know of only one which reports the necessary information. Baker (1975) gives limited allelic frequency data on differentiation between two song dialect "races" of white-crowned sparrows (*Zonotrichia leucophrys*). Baker and Mewaldt (1978) reported that 5–15 natally banded birds crossed the same dialect boundary to set up territories on the opposite side. The value $2.19 \leq Nm_{GT} \leq 8.87$ (Table 1) from Baker's (1975) data is reasonably consistent with results from the banding study, especially since not all the movements of banded birds necessarily result in breeding (i.e., effective gene flow). The high Nm_{SG} estimates within dialect races also agree qualitatively with dispersal patterns reported in Baker and Mewaldt (1978). Although their data are somewhat limited for the purposes of this study, the analysis suggests that balancing selection among population groups within a species is small relative to gene flow in promoting genetic similarity.

How well does Nm_{GT} estimate gene flow across well-studied hybrid zones? Chromosomal races of the grasshopper *Podisma pedestris* show considerable introgression in a narrow hybrid zone despite relatively strong selection against hybrids, but with limited genetic differentiation between the races in allozyme frequencies (Halliday et al., 1983, 1984). Their comparisons of average genetic distances within vs. among races suggested that gene flow was uninterrupted across the cline, indicating that these races are not genetically isolated. Reanalysis using Nm_{GT} (Table 1) sharpens the acuity somewhat, suggesting that gene flow is considerably stronger within each chromosomal race than between races, but indeed, the level of differentiation between races is consistent with gene flow of one genome exchanged every one to two generations. The result agrees with the general conclusions of Halliday et al. (1983, 1984), and is in rough agreement with studies of dispersal (Barton and Hewitt, 1982) and selection in the cline (Barton and Hewitt,

TABLE 1. Estimates of gene flow between population groups using previously published data sets. Subscripts: SG = within species; ST = all populations pooled; GT = between groups. Confidence limits of Nm are obtained from the 95% confidence limits of the appropriate F-statistic (calculated from estimates jackknifed over loci) using approximation (4). Note that the actual gene flow between sympatric sibling species is zero ($Nm_{GT} = 0$), so the deviations observed below are attributable to factors other than gene flow.

	Value	SE	Nm	95% confidence limits Lower	Upper
Sympatric sibling species pairs					
Euphydryas editha and *E. chalcedoma*:microsympatric population pairs only (McKechnie et al., 1975)					
F_{SG} (*editha*)	0.204	0.006	0.97	0.91	1.05
F_{SG} (*chalcedona*)	0.042	0.000	5.74	5.74	5.74
F_{ST}	0.105	0.007			
F_{GT}	−0.018	0.004	panmictic	panmictic	panmictic
θ_{GT}	0.679	0.028	0.12	0.09	0.15
Gryllus pennsylvanicus and *G. veletis* (Harrison, 1979)					
F_{SG} (*pennsylvanicus*)	0.054	0.009	4.40	3.25	6.67
F_{SG} (*veletis*)	0.028	0.008	8.74	5.53	20.03
F_{ST}	0.161	0.011			
F_{GT}	0.120	0.003	1.84	1.74	1.94
θ_{GT}	0.916	0.026	0.02	0.01	0.04
Enchenopa sympatric host races: ex *Cercis* and *Juglans* (Guttman and Weigt, 1989)					
F_{SG} (ex *Cercis*)	0.136	0.000	1.59	1.59	1.59
F_{SG} (ex *Juglans*)	0.197	0.000	1.02	1.02	1.02
F_{ST}	0.261	0.000			
F_{GT}	0.094	0.000	2.40	2.40	2.40
θ_{GT}	0.906	0.015	0.03	0.02	0.04
Group boundaries studied using mark-recapture and electrophoretic techniques					
Zonotrichia leucophrys song dialect races (Baker, 1975)					
F_{SG} (RCA dialect)	0.007	0.011	36.32	8.32	panmictic
F_{SG} (DP dialect)	0.005	0.004	49.18	19.57	panmictic
F_{ST}	0.016	0.010			
F_{GT}	0.010	0.008	25.17	9.63	panmictic
θ_{GT}	0.065	0.019	3.60	2.19	8.87
Population groups separated by hybrid zones					
Podisma pedestris XO/XX and neo-XY/neo-XX races (Halliday et al., 1983)					
F_{SG} (XO/XX)	0.055	0.006	4.32	3.47	5.69
F_{SG} (neo-XY/neo-XX)	5.85	0.055	0.007	4.29	3.36
F_{ST}	0.065	0.007			
F_{GT}	0.010	0.000	23.71	23.65	23.77
θ_{GT}	0.283	0.039	0.63	0.45	0.96

1981), as well as current taxonomy which recognizes the races as conspecific.

Together, these analyses demonstrate that Nm_{GT} (from θ_{GT}) provides a reasonable estimate of the rate of genetic exchange between groups of populations, except when the estimate falls below $Nm_{GT} = 0.5$. When gene flow is lower than this, biases from other factors which promote genetic similarity among groups of populations begin to exert significant influence. For the purposes of species-level systematics, the qualitative interpretation is especially strong: When $Nm_{GT} > \sim 1$ then gene flow is likely to be an important factor promoting genetic similarity between groups; when $\sim 1 > Nm_{GT} > \sim 0.5$ then gene flow is weak but probably present at levels

strong enough to at least permit rapid exchange of favorable genes (see Barton and Bengsston, 1986); when $\sim 0.5 > Nm_{GT} > 0$ then the groups are almost or fully genetically isolated, and gene flow is likely to be unimportant relative to genetic drift, overestimation notwithstanding. In the first two scenarios, it is desirable to examine any secondary evidence pertaining to the proportion of similarity that can be attributed to other factors besides gene flow. In the latter case, more sensitive analyses of introgression can provide more definite answers to the status of genetic isolation, but by the rules of inductive logic no test can show that a complete boundary exists.

The important point for systematists is that this method is reasonably accurate and easy to apply, and can provide insights into the status of genetic isolation which may be quite difficult to obtain using other methods. The following case study provides an example of the application of this approach, here used to resolve the systematic relationship between two hybridizing butterfly taxa long treated as separate species in the taxonomic literature.

CASE STUDY

The nymphalid butterfly genus *Limenitis*, well known for its mimetic species in North America (Platt, 1983), shows a number of hybrid zones between pairs of parapatric nominal species (Remington, 1968; Platt and Brower, 1968; Brown, 1934). The facultatively multivoltine nominal species *L. lorquini* occurs from southern California to Canada in the Pacific drainage, spilling over to the western Great Basin in Nevada, and to the east slope of the Bitterroot Mts. in Montana. The univoltine nominal species *L. weidemeyerii* occurs in the Rocky Mountains and in montane habitats in the Great Basin. Both species occur in small populations in canyons and riparian areas. The immature stages of these butterflies are relatively sedentary, and all interpopulation dispersal occurs as adults.

In western Nevada and eastern California, *L. lorquini* and *L. weidemeyerii* hybridize wherever they contact, and this hybridization has been extensive for at least 55 years (Brown, 1934). In the laboratory, the F_1 between these taxa yields approximately normal sex ratios (Platt, 1983), and in the field, hybrid phenotypes of both sexes are commonly encountered. These taxa have remained classified as species (and I shall use the traditional taxonomy initially in this paper) based on slight genitalic differences (Platt, 1970) and on the inference that there should be intrinsic barriers to genetic exchange, since they have apparently maintained their phenotypic "integrity" in the face of introgressive hybridization (Remington, 1968; Platt, 1970). However, these criteria are not particularly good measures of gene flow: (i) models of gene flow in clines (Endler, 1977; Barton and Hewitt, 1983, 1985; Barton and Bengsston, 1986) suggest that phenotypic markers used by taxonomists to delineate parapatric taxa are not necessarily reliable markers for reproductive isolation, and (ii) Porter and Shapiro (1990) demonstrated that, as one might expect, genitalic differentiation alone is not a reliable indicator of reproductive isolation between parapatric taxa. Here, I approach the taxonomic problem using population genetic methods for the estimation of gene flow, to independently determine the level of genetic exchange between these taxa. The null hypothesis is that *L. lorquini* and *L. weidemeyerii* are effectively genetically isolated, despite occasional hybridization. Estimates of gene flow not significantly greater than those of typical sympatric sibling species would lend support to the proposition that these taxa maintain separate gene pools.

METHODS

The study populations (Table 2) comprised two transects of the hybrid zone between *L. lorquini lorquini* and *L. weidemeyerii latifascia* (Fig. 1). Also included were three populations of the subspecies *L. w. weidemeyerii*, and a single population of *L. l. burrisoni*. Butterflies were haphazardly netted and held individually in glassine envelopes, then stored on wet ice in resealable plastic bags. They were hand-carried or

TABLE 2. Key to *Limenitis* populations.

Code	Population	Collection date
L. l. lorquini populations W of Sierra Nevada crest		
GC	Gates Canyon, 12 km NNW Vacaville, Solano Co., California	3 and 10 May 1987
MX	Mix Canyon, 9 km NW Vacaville, Solano Co., California	4 and 9 May 1987, and 14 May 1988
WS	West Sacramento, Yolo Co., California	15 and 17 May 1988
L. l. lorquini populations E of Sierra Nevada crest		
LV	Lee Vining Creek, 7,700 ft, 10 km W Lee Vining, Mono Co., California	28 June 1986
MP	Slinkard Creek, E side of Monitor Pass, 2 mi NW Topaz, Mono Co., California	27 June 1986
SZ	Schurz, Lyon Co., Nevada	14 July 1986
VC	Virgin Creek, Sheldon Antelope Range, Humboldt Co., Nevada	21 July 1988
L. lorquini burrisoni population		
BS	Bass Creek, 10 km S Florence, Ravalli Co., Montana	8 August 1988
Hybrid populations		
BC	Bodie Canyon, 7–20 km NE Bodie, Mono Co., California and Mineral Co., Nevada	5, 9 and 29 July 1987
BP	Bridgeport Canyon, 18 km N Mono Lake, Mono Co., California	6 July 1986
CC	Corey Creek Canyon, 35 km W Hawthorne, Wassuk Mts., Douglas Co., Nevada	14 July 1986
ML	Mono Lake County Park, N shore Mono Lake, Mono Co., California	27–29 June 1986
PF	Alta Creek, Pine Forest Mts., Humboldt Co., Nevada	15 July 1988
L. w. latifascia populations		
JM	Meadow Creek, 10 km S Rowland, Jarbridge Mts., Elko Co., Nevada	13 July 1988
KC	Kingston Canyon, 18 km W Kingston, Toiyabe Mts., Lander Co., Nevada	30 July 1987
MM	4 km E South Fork Pass, Monitor Mts., Eureka Co., Nevada	25 July 1987
L. w. weidemeyerii populations		
AS	Aspen, Pitkin Co., Colorado	12 July 1988
RC	Red Canyon, 10 km SW Manzano, Torrance Co., New Mexico	3 July 1988
SI	San Isabel, Custer Co., Colorado	5 July 1988

mailed back to Davis and frozen alive at −80°C until electrophoretic analysis.

The ventral half of the thorax of each butterfly was removed and prepared for starch gel electrophoresis following Ayala et al. (1972) and Porter (1989a, b). Eighteen metabolic enzyme loci were considered scorable: adenylate kinase (two loci: AK-1, AK-2; Enzyme Commission number: 2.7.4.7), aldolase (ALDO; 4.1.2.13), fumarase (FUM; 4.2.1.2), glutamic-oxaloacetic transaminase (GOT-1; 2.6.1.1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1.2.1.12), α-glycerophosphate dehydrogenase (α-GPD; 1.1.1.8), hexokinase (HK-1; 2.7.1.1), isocitrate dehydrogenase (IDH-1, IDH-2; 1.1.1.42), malate dehydrogenase (MDH-1, MDH-2; 1.1.1.37), malic enzyme (ME-1, ME-2; 1.1.1.40), phosphoglucose isomerase (PGI; 5.3.1.9), phosphoglucomutase (PGM; 2.7.5.1), and superoxide dismutase (SOD-1, SOD-2; 1.15.1.1). Sample sizes were sometimes small for particular loci because of poor resolution on some gels.

Butterflies were also scored for diagnostic wing pattern traits to quantify the limits of the hybrid zone. These traits were (Fig. 2) (*lorquini* state first): wing band color (cream or white); dorsal wing apex color (red or black); dorsal wing subapical area color (red or black); ventral submarginal line (red or black); and ventral median ground color (red or black). All traits but the first also showed an intermediate (red/black) state. Voucher specimens from each population are deposited in the Bohart Museum of Entomology at U. C. Davis.

The computer program BIOSYS-1 (Swofford and Selander, 1981) was used to obtain standard genetic variability scores (heterozygosities and percent polymorphic loci). Calculations of *F*-statistics and *Nm* estimates follow the previous section.

RESULTS

Wing pattern phenotypic frequencies from both transects are shown in Figures 3 and 4. From west to east in the Mono Lake transect (Fig. 3), there is a sharp change in diagnostic traits from *L. lorquini* to *L. weidemeyerii* at Mono Lake County Park, with a tail of *L. lorquini* introgression out to Bodie Creek. The Corey Creek sample contained no individuals with *L. lorquini* characters, but specimens of hybrid phenotype collected there in the past are in the Nevada State Museum in Las Vegas (G. T. Austin, pers. comm.). There is evidence of very weak introgression from the hybrid zone westward into *L. lorquini* in the Lee Vining Canyon sample. Specimens of hybrid and "pure" *L. weidemeyerii* phenotypes from this locality are also contained in museums (e.g., Bohart Museum). The Mono Lake hybrid zone region is therefore defined here to include the populations from Mono Lake to Corey Creek. There is some precedent in the literature to define the zone more narrowly (e.g., Endler's [1977] 80% rule), but the fact that populations in this region are geographically separated from other *L. weidemeyerii* populations by lowland desert and riparian areas (including the phenotypically "pure" *L. lorquini* population at Schurz) argues for a less mathematical definition (cf. Harrison, 1986; Rand and Harrison, 1989).

Allelic frequencies of variable loci are given in Table 3. In addition, all populations were fixed for alleles $ALDO_{100}$, FUM_{100}, $GAPDH_{100}$, $SOD\text{-}1_{100}$, $SOD\text{-}2_{100}$, and $ME\text{-}2_{100}$. The intrapopulation genetic variability scores (Table 4) are normal for butterflies (H. J. Geiger and A. H. Porter, unpubl. data) and other insects (Nevo, 1978). No significant deviations from Hardy-Weinberg proportions were detected, although small sample sizes precluded discovery of all but the grossest deviations.

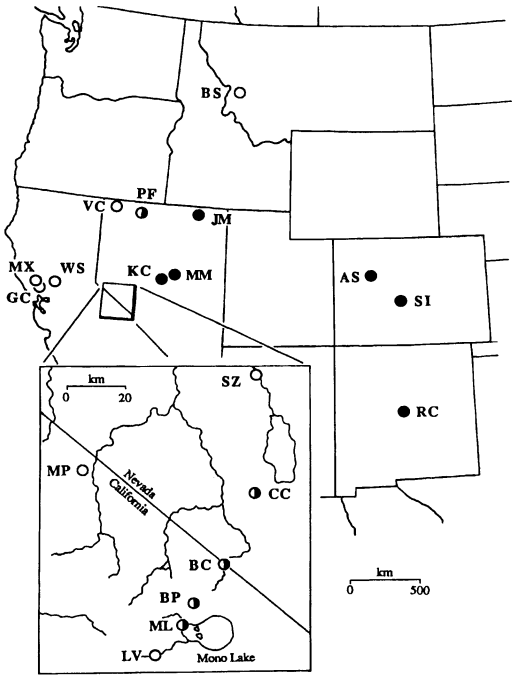

FIG. 1. Study populations. Open circles, *lorquini* populations; half circles, hybrid populations; closed circles, *weidemeyerii* populations. Population abbreviations in Table 2.

Nevertheless, my method of θ calculation using expected rather than observed heterozygosities should be reasonably unbiased.

Genetic differentiation (F_{SG}) and gene flow estimates within population groups (Nm_{SG}) are shown in Table 5. In all cases, the gene flow estimates are significantly greater than $Nm_{SG} = 1$, indicating that the gene pools within these population groups are quite cohesive.

Genetic differentiation (θ_{GT}) and gene flow estimates between groups (Nm_{GT}) are shown in Table 6. In all cases, Nm_{GT} is significantly greater than zero, rejecting the null hypothesis that *L. lorquini* and *L. weidemeyerii* are genetically isolated. Within nominal species, small samples of populations within groups and (sometimes) individuals within populations have greatly inflated the confidence limits, but gene flow does appear to be strong for *L. l. lorquini*,

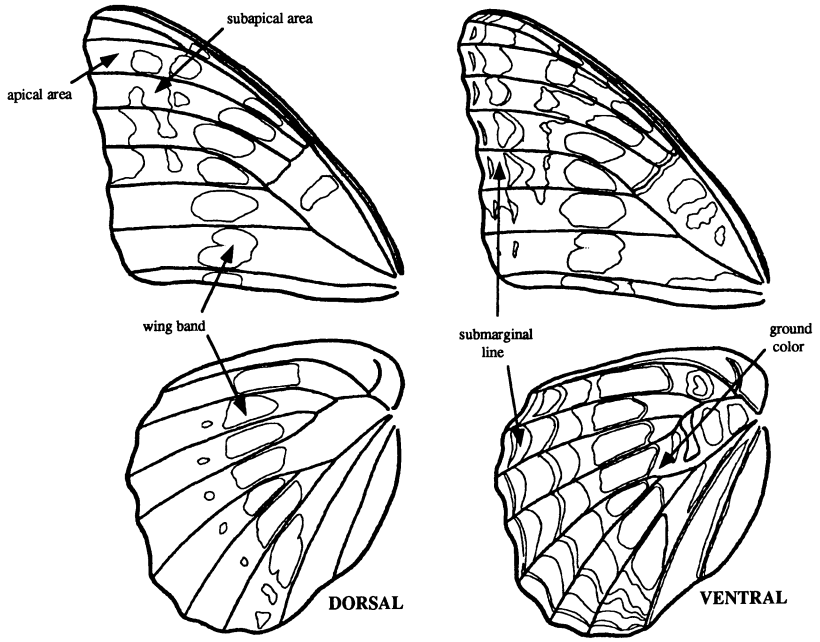

FIG. 2. Wing pattern characters scored in Figures 3 and 4. *L. lorquini lorquini* character states shown. See text for explanation.

even across the Sierra Nevada crest. This estimate (and the high Nm_{SG} values for *L. lorquini*) is consistent with *L. lorquini*'s multivoltinism (two to three episodes of gene flow per year), and with occasional observations of rapidly flying individuals up to 5 kilometers from suitable habitat (unpubl. data; A. M. Shapiro, pers. comm.).

Between nominal species, Nm_{GT} is smallest when all populations of *L. lorquini* and *L. weidemeyerii* are compared, with the confidence interval overlapping $Nm_{GT} = 0.5$. When only the adjacent subspecies *L. l. lorquini* and *L. w. latifascia* are compared, gene flow jumps up to almost one individual exchanged per year, a jump consistent with the relatively low mean gene flow estimate found between nominal *L. weidemeyerii* subspecies.

Estimates of gene flow between "pure" Nevadan *L. w. latifascia* and the Mono Lake hybrid zone suggest that effectively one breeding individual per year is able to make this crossing (Nm_{GT} is even higher when the Corey Creek sample is grouped with *L. w. latifascia*), despite intervening deserts, dry mountain ranges, and the presence of "pure" *L. l. lorquini* in riparian habitats near Schurz, Nevada (Austin, 1985). Again however, this rate is consistent with the average gene flow estimated between subspecies of *L. weidemeyerii*, which must cross similar barriers in Utah.

L. l. lorquini has relatively easy access to the Mono Lake hybrid zone from the northwest in continuous riparian and montane habitat. However, gene flow from *L. lorquini* into the hybrid zone is low (again approximating one successfully breeding individual per year) relative to the rates of gene flow within "pure" *L. l. lorquini*. Despite the evidence of significant gene flow between *L. lorquini* and *L. weidemeyerii*, there does appear to be a partial intrinsic barrier to genetic exchange. This interpretation is consistent with the steep cline in wing pattern traits between Lee Vining Creek and Mono Lake, and with evidence of a tail of introgression in wing characters from *L. lorquini* into *L. weidemeyerii* in the Mono Lake region, but only little introgression in the opposite direction.

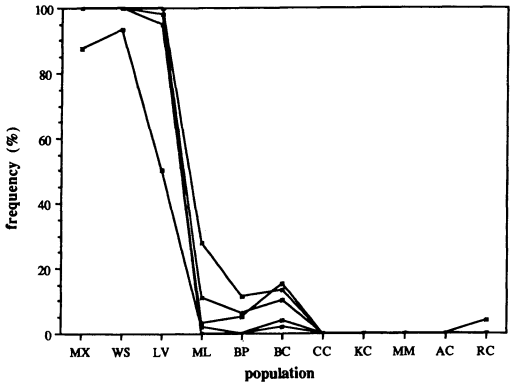

FIG. 3. Wing pattern frequencies in the Mono Lake transect. Note the sharp cline between Lee Vining Creek and Mono Lake. Population abbreviations in Table 2; characters shown in Figure 2; map in Figure 1.

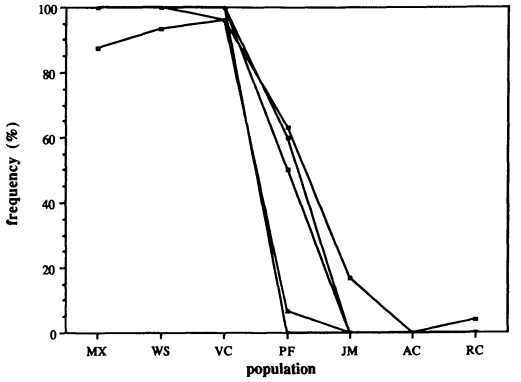

FIG. 4. Wing pattern frequencies in the northern Nevada transect. Population abbreviations in Table 2; characters shown in Figure 2; map in Figure 1.

DISCUSSION

Accuracy of gene flow estimation.—Taken at face value, the gene flow estimates presented here suggest that *L. lorquini* and *L. weidemeyerii* do not maintain entirely separate gene pools, although there is evidence of partial genetic isolation. To what extent are the assumptions of the analysis met? I roughly approximate deme sizes in both nominal species to be on the order of $\sim 10 < N < \sim 200$ in individual canyons (with much smaller effective population sizes likely) (Porter, unpubl. data), and both taxa are usually present wherever suitable habitat is found. Below 500 m west of the Sierra Nevada crest, *L. lorquini* populations seem larger ($\sim 30 < N < \sim 300$) and more continuously distributed in the spring, but summer drought produces small populations centered around permanent moisture. Given these small demes, effective population sizes are not likely to vary geographically by more than an order of magnitude. Thus, the island model conditions (ii), (iii), and (iv) are met to a first order of approximation.

Balancing selection, time since divergence, and failure to uncover hidden allelic differentiation (Coyne, 1982) may contribute to the observed genetic similarity, running up the estimate of gene flow. However, given the small local population sizes, if gene flow were negligible among all populations, genetic drift would bring most loci to fixation within a few generations. There is almost surely substantial gene flow among demes within mountain ranges, but the effective population sizes within mountain ranges in Nevada seem small enough that abnormally slow drift or very strong selection (the intervening habitats in the Great Basin dried up 10,000 years ago [Mehringer, 1977]) would be required to maintain the overall genetic similarity observed. One would also expect that the detected private alleles would have a higher average frequency if the geographic barriers among populations or mountain ranges were not being crossed (Barton and Slatkin, 1986). Thus, it seems likely that the biases induced by the biological assumptions of the estimation techniques contribute a relatively small proportion to the estimated gene flow. Hidden allelic variation between nominal species may also be present in the loci studied, but unless this can be demonstrated by further study, it is parsimonious to use the data at hand. The important conclusion from a systematist's standpoint is that the partial intrinsic barrier does not appear strong enough to prevent evolutionarily significant (i.e., $Nm_{GT} > 0.5$) neutral gene flow to occur between these nominal species.

Hybrid zone maintenance.—*L. lorquini* and *L. weidemeyerii* have not changed detect-

TABLE 3. Allelic frequency data for *Limenitis* populations. Locus abbreviatons in text; population abbreviations in Table 2; sample sizes in parentheses.

Locus & allele	Population																		
	AS	BC	BO	BP	CC	GC	JM	KC	LV	ML	MM	MP	MX	PF	RC	SI	SZ	VC	WS
AK-1	(2)	(22)	(9)	(1)	(1)	(4)	(7)	(19)	(5)	(1)	(10)	(7)	(8)	(10)	(12)	(5)	(1)	(7)	(18)
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.9	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.972
112											0.1								0.028
GOT-1	(7)	(22)	(26)	(36)	(15)	(11)	(7)	(19)	(9)	(13)	(10)	(7)	(26)	(15)	(12)	(5)	(11)	(12)	(18)
90	0.071	0.045						0.026							0.125		0.045		0.028
93	0.071	0.156	0.038	0.056	0.1	0.091	0.042	0.026		0.077	0.05	0.214	0.096	0.033				0.083	0.083
100	0.857	0.727	0.788	0.806	0.833	0.909	0.917	0.895	1.000	0.769	0.95	0.786	0.885	0.933	0.875	1.000	0.955	0.875	0.889
109		0.045																	
112		0.023	0.173	0.139	0.067		0.042	0.053		0.154			0.019	0.033				0.042	
α-GPDH	(7)	(2)	(26)	(36)	(15)	(12)	(12)	(19)	(9)	(13)	(10)	(7)	(21)	(15)	(12)	(5)	(11)	(12)	(18)
95														0.033					
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.967	1.000	1.000	1.000	1.000	1.000
HK	(7)	(22)	(26)	(36)	(15)	(12)	(11)	(19)	(7)	(13)	(10)	(7)	(28)	(9)	(12)	(5)	(11)	(8)	(18)
100	1.000	1.000	1.000	1.000	1.000	1.000	0.864	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
105							0.136												
IDH-1	(5)	(22)	(26)	(26)	(13)	(11)	(5)	(19)	(6)	(12)	(10)	(7)	(21)	(5)	(5)	(5)	(11)	(5)	(18)
77				0.028															
85				0.028															
94		0.023	0.077	0.028									0.024		0.1		0.273		0.028
100	1.000	0.977	0.923	0.917	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.976	1.000	0.9	1.000	0.727	1.000	0.972
IDH-2	(5)	(20)	(26)	(36)	(14)	(10)	(5)	(19)	(6)	(12)	(10)	(7)	(20)	(5)	(11)	(1)	(11)	(5)	(18)
80			0.019	0.208	0.25														
94							0.1							0.3	0.364				0.028
100	0.7	0.975	0.981	0.792	0.75	1.000	0.9	1.000	1.000	1.000	1.000	1.000	1.000	0.7	0.636	1.000	1.000	1.000	0.972
112	0.3	0.025																	
MDH-1	(7)	(22)	(26)	(36)	(15)	(9)	(12)	(19)	(9)	(13)	(10)	(7)	(22)	(15)	(12)	(5)	(11)	(12)	(18)
87	0.143						0.125	0.237			0.1				0.042				0.083
100	0.857	1.000	1.000	1.000	1.000	1.000	0.833	0.763	1.000	1.000	0.9	1.000	1.000	1.000	0.958	1.000	1.000	0.958	0.917
110							0.042											0.042	
MDH-2	(7)	(22)	(26)	(35)	(15)	(1)	(12)	(19)	(6)	(1)	(10)	(7)	(1)	(15)	(12)	(5)	(11)	(12)	(18)
100	1.000	1.000	1.000	0.943	0.967	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
107				0.057	0.033														

TABLE 3. Continued.

Locus & allele	Population																		
	AS	BC	BO	BP	CC	GC	JM	KC	LV	ML	MM	MP	MX	PF	RC	SI	SZ	VC	WS
ME-1	(7)	(22)	(26)	(36)	(15)	(9)	(12)	(19)	(9)	(11)	(10)	(7)	(21)	(15)	(12)	(5)	(11)	(12)	(18)
95													0.024					0.042	
100	1.000	1.000	1.000	1.000	0.933	1.000	1.000	1.000	1.000	1.000	1.000	0.929	0.976	1.000	1.000	1.000	0.955	0.958	1.000
105					0.067							0.071					0.045		
PGI	(7)	(22)	(26)	(36)	(15)	(12)	(12)	(19)	(9)	(13)	(10)	(7)	(28)	(15)	(12)	(5)	(11)	(12)	(18)
83		0.273					0.042												
87	0.071		0.019					0.158			0.05		0.018		0.042	0.2			
90		0.091				0.083	0.083		0.056		0.05	0.143	0.089	0.067		0.1	0.227	0.083	0.083
100	0.786	0.636	0.962	0.972	1.000	0.917	0.875	0.842	0.944	0.962	0.9	0.857	0.893	0.933	0.917	0.7	0.773	0.875	0.806
107				0.028						0.038								0.042	0.111
109	0.143														0.042				
112			0.019																
PGM	(7)	(22)	(26)	(36)	(15)	(12)	(12)	(19)	(9)	(13)	(10)	(7)	(24)	(15)	(12)	(5)	(11)	(12)	(18)
92	0.071	0.023	0.019																
94		0.023		0.014	0.1	0.125			0.111		0.05	0.214	0.146	0.033			0.136	0.042	0.056
97							0.042												0.028
100	0.929	0.864	0.923	0.944	0.8	0.833	0.917	0.947	0.889	0.962	0.95	0.714	0.813	0.933	1.000	1.000	0.773	0.875	0.806
104		0.068																	
107		0.023	0.038	0.042	0.033	0.042	0.042			0.038		0.071	0.042				0.091	0.083	0.111
109			0.019		0.067			0.053						0.033					

TABLE 4. Genetic variablity measures for all study populations. N = mean sample size per locus; $\bar{x}_{alleles}$ = mean number of alleles per locus; H_{obs} = observed heterozygosity (standard errors in parentheses); H_{exp} = heterozygosity calculated from Hardy-Weinberg proportions; P = % of loci polymorphic (more than one allele detected). Population abbreviations follow Table 2.

Population	N	$\bar{x}_{alleles}$	H_{obs}	H_{exp}	P
MX	21.9	1.4	0.046 (0.022)	0.046 (0.023)	27.8
GC	9.8	1.2	0.033 (0.018)	0.035 (0.020)	16.7
WS	18.0	1.6	0.074 (0.031)	0.068 (0.027)	38.9
VC	10.7	1.4	0.051 (0.022)	0.049 (0.021)	27.8
SZ	10.4	1.3	0.061 (0.034)	0.076 (0.035)	27.8
LV	7.3	1.1	0.019 (0.013)	0.018 (0.013)	11.1
PF	13.3	1.4	0.059 (0.034)	0.051 (0.027)	27.8
BP	33.8	1.6	0.053 (0.022)	0.061 (0.026)	33.3
BO	25.0	1.5	0.028 (0.018)	0.042 (0.022)	27.8
ML	10.7	1.2	0.034 (0.026)	0.030 (0.022)	16.7
CC	13.9	1.4	0.072 (0.033)	0.069 (0.032)	27.8
KC	19.0	1.3	0.061 (0.032)	0.053 (0.026)	22.2
MM	10.0	1.3	0.044 (0.018)	0.043 (0.018)	27.8
JM	10.9	1.6	0.063 (0.022)	0.073 (0.026)	33.3
RC	11.6	1.3	0.069 (0.034)	0.064 (0.031)	27.8
BC	21.9	1.7	0.076 (0.040)	0.074 (0.038)	27.8

ably in wing pattern characters over the last 140 years since they were described, nor have the hybrid frequencies changed substantially at Mono Lake (Brown, 1934). Hypotheses concerning the factors responsible for maintaining the stability of this hybrid zone include (i) genetic incompatibility during development (e.g., effects described by Haldane's rule [Haldane, 1922; Orr and Coyne, 1989]), (ii) genetic disruption of the physiological mechanism regulating voltinism (as reported in *Pieris* [Bowden, 1953, 1957] and *Papilio* [Clarke and Willig, 1977] butterfly hybrids), and (iii) sexual selection (note also that human "predation" in the Mono Lake region cannot be ruled out: hybrid phenotypes are prized by amateur butterfly collectors). The same range of hostplants is shared by both taxa (Tietz, 1972; Scott, 1986). Both sexes of hybrid phenotypes are frequently produced in the hybrid zone, although hybrid females I have seen are either backcrosses to *L. weidemeyerii* or of more complex ancestry. Females are the heterogametic sex in butterflies, and the hybrid females in the field may have been produced from backcrossing male F_1's.

Regarding sexual selection, it is notable that the white wing band in *L. weidemeyerii* and in most hybrid zone phenotypes strongly reflects ultraviolet light, while the cream-colored band of *lorquini* reflects only weakly (Scott, 1973). Males of the Euro-

TABLE 5. Estimated population differentiation (F_{SG}) and corresponding gene flow within population groups (Nm_{SG}).

Group	n_{loci}	$n_{alleles}$	F_{SG}	SE	Nm_{SG}	95% confidence limits Lower	Upper
L. lorquini[a,b,c] pooled	8	29	0.054	0.000	4.41	4.41	4.41
L. l. lorquini[a,b] pooled	8	23	0.049	0.000	4.90	4.90	4.90
Sierran west slope[a]	8	22	0.019	0.000	12.99	12.99	12.99
Sierran east slope[b]	5	12	0.079	0.000	2.93	2.93	2.93
L. weidemeyerii[d,e] pooled	8	28	0.075	0.033	3.11	1.56	22.83
L. w. weidemeyerii[d]	6	15	0.064	0.024	3.68	2.00	15.23
L. w. latifascia[e]	7	22	0.036	0.000	6.70	6.70	6.70
Mono hybrid zone[f]	7	22	0.034	0.000	7.04	7.04	7.04

[a] Populations GC, MX, WS. [b] LV, MP, SZ. [c] BS, VC. [d] AS, RC, SI. [e] JM, KC, MM. [f] BC, BP, CC, ML.

TABLE 6. Estimated population differentiation (θ_{GT}) and corresponding gene flow between population groups (Nm_{GT}).

Group	n_{loci}	$n_{alleles}$	θ_{GT}	SE	Nm_{GT}	95% confidence limits Lower	Upper
Within nominal species							
Sierran west[a] vs. east slope[b] *l. lorquini*							
	8	23	0.002	0.035	121.13	3.26	panmictic
w. latifascia[c] vs. *w. weidemeyerii*[d]							
	8	26	0.272	0.140	0.67	0.21	panmictic
Between nominal species							
All *lorquini*[a,b,e] vs. all *weidemeyerii*[c,d]							
	9	34	0.313	0.049	0.55	0.36	0.90
l. lorquini[a,b] vs. *w. latifascia*[c]							
	9	28	0.208	0.049	0.95	0.57	1.97
Between hybrid zone and nominal species							
l. lorquini[a,b] vs. Mono hybrid zone[f]							
	9	31	0.214	0.030	0.92	0.67	1.36
Sierran east slope *l. lorquini*[b] vs. Mono hybrid zone[f]							
	7	24	0.156	0.033	1.35	0.88	2.50
w. latifascia[c] vs. Mono hybrid zone[f]							
	10	34	0.207	0.050	0.96	0.57	2.05

[a] Populations GC, MX, WS. [b] LV, MP, SZ. [c] JM, KC, MM. [d] AS, RC, SI. [e] BS, VC. [f] BC, BP, CC, ML.

pean *Limenitis camilla* use the wing band as a courtship stimulus (Lederer, 1960). Other coloration differences across the hybrid zone mostly involve red vs. black wing pigmentation patterns; these differences are invisible to *Limenitis*, which see little of the red end of the spectrum (G. Bernard, pers. comm.).

Whatever the mechanism, the sharp cline between the Lee Vining Creek and Mono Lake populations points to the likelihood that the Mono Lake region of the hybrid zone is maintained by a migration/selection balance (Bigelow, 1965; Moore, 1977; Barton and Hewitt, 1985). A drift/migration balance remains as a possibility in northern Nevada, where hybrid and "pure" populations are separated by inhospitable habitat. J. A. Endler (pers. comm.) has pointed out that the small deme sizes will make it extremely difficult to distinguish between the effects of drift and selection in the field.

Taxonomy.—Barton's (1979, 1983; Barton and Hewitt, 1983; Barton and Bengsston, 1986) models of gene flow across clines imply that intrinsic gene flow barriers (from which we may hope to define taxonomic units) may range from complete impermeability to complete permeability. In the final analysis, decisions regarding the location of biological species boundaries must be statistical and subjective by nature—where this line should be drawn remains a matter for discussion. However, because cladistic analyses should be undertaken using genetically isolated units, the conservative systematist should tend to lump questionable species-level taxa.

The neotropical butterfly genus *Heliconius* represents a model system for the taxonomy of North American *Limenitis*: both show well-differentiated subspecies hybridizing in narrow contact areas (Platt and Brower, 1968; Platt, 1983; Brown et al., 1974; Turner, 1971; Mallet, 1986; Mallet and Barton, 1989). In light of the evidence of substantial gene flow across the *L. lorquini-weidemeyerii* hybrid zone, I recommend that the nominal *L. weidemeyerii* subspecies *weidemeyerii* Edwards, *angustifascia* (Barnes and Benjamin), *latifascia* Perkins and Perkins,

nevadae (Barnes and Benjamin), *oberfoelli* Brown, and *sinefascia* Austin be recognized as univoltine subspecies of *L. lorquini* Boisduval (taxonomic references in Miller and Brown [1981]). In subsequent cladistic treatments of this group, these taxa should be treated as a single genetic unit. This nomenclature may be further modified if significant gene flow occurs between hybridizing *L. lorquini* (sens. lat.) and *L. arthemis* in southwestern Canada (Remington, 1968; Porter, 1989b).

ACKNOWLEDGMENTS

This study was part of my dissertation research, and was supported in part by a grant entitled *Cross-disciplinary Studies of Population Structure* from the Albert E. Sloan Foundation to the Institute of Ecology at U. C. Davis, three Jastro-Shields Research Scholarships from the Division of Agricultural and Environmental Sciences, and two Graduate Research Awards from U. C. Davis. F. J. Ayala and H. B. Shaffer kindly provided laboratory facilities, and H. J. Geiger showed me how to run gels. J. A. Endler, N. H. Barton, P. S. Ward, E. M. Jakob, Q. Wheeler, an anonymous reviewer, and especially A. M. Shapiro provided insightful comments or discussions during the fermentation of this manuscript. G. T. Austin and J. Emmel directed me to important study populations; A. M. Shapiro, E. M. Jakob, and M. Reddy helped in sampling. I would also like to thank members of Lepidopterists' Society for not collecting in the hybrid zone following the annual meeting in 1987.

REFERENCES

AUSTIN, G. T. 1985. Lowland riparian butterflies of the Great Basin and associated areas. J. Res. Lepid., 24:117-131.

AVISE, J. C., J. ARNOLD, R. M. BALL, E. BERMINGHAM, T. LAMB, J. E. NEIGEL, C. A. REEB, AND N. C. SAUNDERS. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. Ann. Rev. Ecol. Syst., 18:489-522.

AYALA, F. J., J. R. POWELL, M. L. TRACEY, C. A. MOURAO, AND S. PEREZ-SALAS. 1972. Enzyme variability in the *Drosophila willistoni* group. IV. Genetic variation in natural populations of *Drosophila willistoni*. Genetics, 70:113-139.

BAKER, M. C. 1975. Song dialects and genetic differences in white-crowned sparrows (*Zonotrichia leucophrys*). Evolution, 29:226-241.

BAKER, M. C., AND L. R. MEWALDT. 1978. Song dialects as barriers to dispersal in white-crowned sparrows, *Zonotrichia leucophrys nuttalli*. Evolution, 32: 712-722.

BARTON, N. H. 1979. Gene flow past a cline. Heredity, 43:333-339.

BARTON, N. H. 1983. Multilocus clines. Evolution, 37:454-471.

BARTON, N. H., AND B. O. BENGSSTON. 1986. The barrier to genetic exchange between hybridising populations. Heredity, 56:357-376.

BARTON, N. H., AND G. M. HEWITT. 1981. The genetic basis of hybrid inviability between two chromosomal races of the grasshopper *Podisma pedestris*. Heredity, 47:367-383.

BARTON, N. H., AND G. M. HEWITT. 1982. A measurement of dispersal in the grasshopper *Podisma pedestris*. Heredity, 48:237-249.

BARTON, N. H., AND G. M. HEWITT. 1983. Hybrid zones as barriers to gene flow. Pages 341-359 *in* Protein polymorphism: Adaptive and taxonomic significance (G. S. Oxford and D. Rollinson, eds.). Academic Press, New York.

BARTON, N. H., AND G. M. HEWITT. 1985. Analysis of hybrid zones. Ann. Rev. Ecol. Syst., 16:113-148.

BARTON, N. H., AND M. SLATKIN. 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. Heredity, 56:409-415.

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

BOWDEN, S. R. 1953. Timing of imaginal development in male and female hybrid Pieridae (Lep.). Entomologist, 86:255-264.

BOWDEN, S. R. 1957. Diapause in female hybrid *Pieris napi aldawinda* and related subspecies (Lep.). Entomologist, 90:247-328.

BROWN, C. 1934. Notes on *Basilarchia lorquini* Bdv., form *fridayi* Gun. (Lepid.: Nymphalidae). Ent. News, 14:205-206.

BROWN, K. S., L. M. COOK, AND J. R. G. TURNER. 1974. Quaternary refugia in tropical America: Evidence from race formation in *Heliconius* butterflies. Proc. Roy. Soc. Lond. B, 187:369-378.

CARTER, P. A., AND W. B. WATT. 1988. Adaptation at specific loci. V. Metabolically adjacent enzyme loci may have very different experiences of selective pressures. Genetics, 119:913-924.

CLARKE, C. A., AND A. WILLIG. 1977. The use of α-ecdysone to break permanent diapause of female hybrids between *Papilio glaucus* L. female and *Papilio rutulus* Lucas male. J. Res. Lepid., 16:245-248.

COYNE, J. A. 1982. Gel electrophoresis and cryptic protein variation. Pages 1-32 *in* Isozymes: Current topics in biological and medical research. Volume 6. Alan R. Liss, Inc., New York.

EFRON, B. 1982. The jackknife, the bootstrap, and other resampling plans. Society for Industrial and Applied Mathematics, Philadelphia.

EHRLICH, P. R. 1961. Has the biological species concept outlived its usefulness? Syst. Zool., 10:167-176.

EHRLICH, P. R., AND D. D. MURPHY. 1987. Conservation lessons from long-term studies of checkerspot butterflies. Conserv. Biol., 1:122-131.

ENDLER, J. A. 1977. Geographic variation, speciation, and clines. Princeton University Press, Princeton, New Jersey.

GUTTMAN, S. I., AND L. A. WEIGT. 1989. Macrogeographic genetic variation in the *Enchenopa binotata* complex (Homoptera: Membracidae). Ann. Entomol. Soc. Am., 82:156-165.

HALDANE, J. B. S. 1922. Sex-ratio and unisexual sterility in hybrid animals. J. Genetics, 12:101-109.

HALLIDAY, R. B., N. H. BARTON, AND G. M. HEWITT.

1983. Electrophoretic analysis of a chromosomal hybrid zone in the grasshopper *Podisma pedestris*. Biol. J. Linn. Soc., 19:51–62.

HALLIDAY, R. B., N. H. BARTON, AND G. M. HEWITT. 1984. Genetic and chromosomal polymorphisms in hybridizing populations of the grasshopper *Podisma pedestris*. Biol. J. Linn. Soc., 21:299–305.

HARRISON, R. G. 1979. Speciation in North American field crickets: Evidence from electrophoretic comparisons. Evolution, 33:1009–1023.

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

LEDERER, G. 1960. Verhaltensweisen der Imagines und der Entwicklungsstadien von *Limenitis camilla camilla* L. (Lep. Nymphalidae). Z. Tierpsych., 17: 521–546.

MALLET, J. 1986. Hybrid zones of *Heliconius* butterflies in Panama and the stability and movement of warning colour clines. Heredity, 56:191–202.

MALLET, J., AND N. H. BARTON. 1989. Strong natural selection in a warning-color hybrid zone. Evolution, 43:421–431.

MAYR, E. 1942. Systematics and the origin of species. Columbia University Press, New York.

MAYR, E. 1963. Animal species and evolution. Harvard University Press, Cambridge, Massachusetts.

MCKECHNIE, S. W., P. R. EHRLICH, AND R. R. WHITE. 1975. Population genetics of *Euphrydryas* butterflies. I. Genetic variation and the neutrality hypothesis. Genetics, 81:571–594.

MEHRINGER, P. J. 1977. Great Basin late Quaternary environments and chronology. Pages 113–167 *in* Models and Great Basin prehistory: A symposium; Dallas TX, 1975. Volume 12 (D. D. Fowler, ed.). Desert Res. Inst. Publ. Soc. Sci.

MILLER, L. D., AND F. M. BROWN. 1981. A catalogue/checklist of the butterflies of America north of Mexico. Lepid. Soc. Mem., 2:1–280.

MOORE, W. S. 1977. Evaluation of narrow hybrid zones in vertebrates. Quart. Rev. Biol., 52:263–277.

NEI, M., A. CHAKRAVARTI, AND Y. TATENO. 1977. Mean and variance of Fst in a finite number of incompletely isolated populations. Theor. Pop. Biol., 11: 291–306.

NEVO, E. 1978. Genetic variation in natural populations: Pattern and theory. Theor. Pop. Biol., 13: 121–177.

ORR, H. A., AND J. A. COYNE. 1989. The genetics of postzygotic isolation in the *Drosophila virilis* group. Genetics, 121:527–537.

PLATT, A. P. 1970. Statistical comparisons of valval structure within and between populations of North American *Limenitis* (Nymphalidae). Can. Entomol., 102:513–533.

PLATT, A. P. 1983. Evolution of North American admiral butterflies. Bull. Entomol. Soc. Am., 29:10–22.

PLATT, A. P., AND L. P. BROWER. 1968. Mimetic versus disruptive coloration in intergrading populations of *Limenitis arthemis* and *astyanax* butterflies. Evolution, 22:699–718.

PORTER, A. H. 1989a. Gene flow statistics and species-level systematics in butterflies. Ph.D. Thesis, University of California, Davis.

PORTER, A. H. 1989b. Genetic evidence for reproductive isolation between hybridizing *Limenitis* butterflies (Lepidoptera: Nymphalidae) in southwestern New Mexico. Amer. Midl. Natur., 122:275–280.

PORTER, A. H., AND A. M. SHAPIRO. 1990. The lock-and-key hypothesis: Lack of mechanical isolation in a butterfly hybrid zone (Lepidoptera: Pieridae). Ann. Entomol. Soc. Am., 83:107–114.

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 joined biotas. Evol. Bio., 2:321–428.

SCOTT, J. A. 1973. Survey of ultraviolet reflectance of Nearctic butterflies. J. Res. Lepid., 12:151–160.

SCOTT, J. A. 1986. The butterflies of North America: A natural history and field guide. Stanford University Press, Stanford, California. 486 pp.

SLATKIN, M. 1981. Estimating levels of gene flow in natural populations. Genetics, 99:323–335.

SLATKIN, M. 1985a. Gene flow in natural populations. Ann. Rev. Ecol. Syst., 16:393–430.

SLATKIN, M. 1985b. Rare alleles as indicators of gene flow. Evolution, 39:53–65.

SLATKIN, M. 1987. Gene flow and the geographic structure of natural populations. Science, 236:787–792.

SLATKIN, M. 1989. Detecting small amounts of gene flow from the phylogenies of alleles. Genetics, 121: 609–612.

SLATKIN, M., AND N. H. BARTON. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution, 43:1349–1368.

SWOFFORD, D. L., AND R. B. SELANDER. 1981. A computer program for the analysis of allelic variation in genetics. J. Hered., 72:281–283.

TIETZ, H. M. 1972. An index to the described life histories, early stages and hosts of the macrolepidoptera of the continental United States and Canada. Volumes 1 and 2. Allyn Museum of Entomology, Sarasota, Florida. 1041 pp.

TURNER, J. R. G. 1971. Two thousand generations of hybridization in a *Heliconius* butterfly. Evolution, 38:233–243.

WEIR, B. S., AND C. C. COCKERHAM. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution, 38:1358–1370.

WRIGHT, S. 1931. Evolution in Mendelian populations. Genetics, 16:97–159.

WRIGHT, S. 1969. Evolution and the genetics of populations. Volume 2. The theory of gene frequencies. University of Chicago Press, Chicago.

WRIGHT, S. 1978. Evolution and the genetics of populations. Volume 4. Variability within and among natural populations. University of Chicago Press, Chicago.

Received 12 June 1989; accepted 13 December 1989