Genetic and phenotypic population structure of the *Coenonympha tullia* complex (Lepidoptera: Nymphalidae: Satyrinae) in California: no evidence for species boundaries

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Received January 20, 1988

PORTER, A. H., and GEIGER, H. 1988. Genetic and phenotypic population structure of the *Coenonympha tullia* complex (Lepidoptera: Nymphalidae: Satyrinae) in California: no evidence for species boundaries. Can. J. Zool. **66**: 2751–2765.

Decisions regarding species status of taxa showing geographic replacement are explicit hypotheses about population structure. The structure of 21 populations of the *Coenonympha tullia* group from northern California, southwestern Oregon, and northern Nevada was analyzed for evidence of reproductive isolation. These samples included five subspecies (*california*, *eryngii*, *ampelos*, *eunomia*, and *mono*) nominally placed in three species (*california*, *ampelos*, and *ochracea*). We found very high intra- and inter-population variability in the "diagnostic" wing pattern characters used by previous authors. There is evidence of intergradation between *eryngii* and *eunomia* in southwestern Oregon, and between *california* and *ampelos* in the eastern Feather River drainage in California. A complex cline involving *california*, *eryngii*, and *ampelos* occurs in the Pit River drainage of northeastern California. The taxon *mono* appears distinct, apparently because of an absence of *Coenonympha* populations in the expected *mono–ampelos* contact area. Electrophoretic analysis of the same 21 populations showed very high intrapopulation genetic variability (expected heterozygosity = 13.5–20.4%, percentage of polymorphic loci (most common allele <99%) = 35.5–58.8%; 14 alleles at the locus for phosphoglucose isomerase (one population with 11 alleles)). However, interpopulation (geographic) variability was extremely low. Standardized genetic variance among populations (F_{ST}, using Wright's formulation) in contact zones indicates that gene flow is probably uninterrupted between the subspecies *california*, *eryngii*, *ampelos*, and *eunomia*. F_{ST} values for the isocitrate dehydrogenase locus indicate that present-day gene flow is probably unimportant in maintaining similarity between *ampelos* and *mono*. The genetic population structure is reminiscent of highly vagile colonizing species, but this may be largely historical, due to post-Pleistocene range changes rather than high present-day interpopulation migration rates. We conclude that all California populations are conspecific. Only the subspecies *mono* is clearly separated from the others, at the level 0.034 (Nei's unbiased distance), approximating that of weak subspecies in other taxa. The North American entities should all be provisionally classified as subspecies of the holarctic species *tullia* unless evidence is found to support their separation.

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Les décisions concernant le statut spécifique des taxons de remplacement géographique sont des hypothèses explicites sur la structure des populations. La structure de 21 populations de *Coenonympha* du groupe *tullia* provenant du nord de la Californie, du sud-ouest de l'Oregon et du nord du Nevada a fait l'objet d'une analyse propre à mettre en lumière l'isolement génétique potentiel. Les échantillons analysés représentaient cinq sous-espèces (*california*, *eryngii*, *ampelos*, *eunomia* et *mono*) appartenant à trois espèces nominales (*california*, *ampelos* et *ochracea*). Il existe une très grande variabilité, au sein des populations et d'une population à l'autre, dans les caractéristiques «diagnostiques» des ailes utilisées par les taxonomistes. Il y a intergradation de ces caractéristiques entre *eryngii* et *eunomia* dans le sud-ouest de l'Oregon et entre *california* et *ampelos* dans l'est du bassin de la rivière Feather en Californie. Un gradient complexe impliquant à la fois *california*, *eryngii* et *ampelos* a été observé dans le bassin de la rivière Pit dans le nord-est de la Californie. Le taxon *mono* semble une entité distincte, mais c'est probablement parce qu'il n'y a pas de populations de *Coenonympha* dans l'aire où l'on s'attendrait à trouver la transition *mono–ampelos*. L'électrophorèse des 21 populations a mis en lumière une très grande variabilité génétique au sein de chaque population (hétérozygotie prévue = 13,5–20,4%, pourcentage de locus polymorphes (allèle la plus fréquente à <99%) = 35,5–58,8%; 14 allèles au locus pour phospho-glucose isomérase dont une population a 11 allèles). En dépit de cela, la variabilité (géographique) entre les populations est très faible. La variance génétique standardisée entre les populations (F_{ST}, d'après la formule de Wright) dans les zones de contact indique que la transmission des gènes est probablement interrompue entre les sous-espèces *california*, *eryngii*, *ampelos* et *eunomia*. Les valeurs de F_{ST} au locus isocitrate déshydrogénase indiquent que la transmission actuelle des gènes ne joue probablement pas de rôle dans le maintien de la similarité entre *ampelos* et *mono*. La structure génétique des populations rappelle celle d'espèces colonisatrices très mobiles, mais il s'agit peut-être là d'un phénomène historique, sans doute dû aux changements de répartition après le Pléistocène plutôt qu'à des taux actuels élevés de migration d'une population à une autre. Il faut conclure que toutes les populations de la Californie sont conspécifiques. Seule la sous-espèce *mono* se sépare clairement des autres, à un niveau de 0,034 (distance de Nei), niveau voisin de faibles sous-espèces chez d'autres taxons. Les entités nord-américaines devraient donc être classifiées, de façon provisoire, comme des sous-espèces de l'espèce holarctique *tullia*, au moins jusqu'à ce que des preuves formelles justifient leur separation.

[Traduit par la revue]

Introduction

Decisions regarding the taxonomic status of closely related entities that show geographic replacement are problematic, and in practice whether to group such taxa as one polytypic species or as separate species is often arbitrary. Once made, these taxonomic decisions represent explicit and testable predictions about population structure: gene flow is absent where species boundaries exist. Slatkin (1987) reviews methods and assumptions for estimating gene flow among populations: at the geographic boundaries that separate two taxonomic entities, sharp

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Printed in Canada / Imprimé au Canada

distinctions between diagnostic genetic features of each taxon constitute evidence for reproductive isolation. This is not logically different from the methods of the more traditional taxonomy based on phenotypic characters. A lack of sharp genetic boundaries indicates that the taxa may be united by at least partial gene flow (Barton 1979).

Herein, we apply the methods of assessing phenotypic and genetic population structure to the problematic *Coenonympha tullia* group, a holarctic complex of small, weak-flying satyrine butterflies which frequent grassland habitats. Currently in the North American butterfly literature, some authors prefer to place the whole complex under the specific epithet *tullia* (Müller) (Dornfield 1980; Shapiro 1974; Miller 1981; Shapiro et al. 1981; Scott 1986), while others consider the group to comprise five or six parapatric, polytypic species (Emmel 1975; Miller and Brown 1981; Pyle 1981; Hodges et al. 1983; Austin 1985; Garth and Tilden 1986; Tilden and Smith 1986). Our aim is to describe in detail the phenotypic variation in wing pattern within and among populations, and compare these data with the population genetic structure, to test these taxonomic hypotheses. We expect that these two types of evidence will support one another regarding the status of reproductive isolation among taxa.

Taxonomic background

Davenport (1941) provided the only complete taxonomic revision of *Coenonympha*, based on characters of wing pattern and wing venation, and on genital morphology. He recognized 14 geographic entities in the North American *tullia* group, and placed them as subspecies of the polytypic species *tullia*. Their conspecificity was based on morphological similarity in venation and genitalia, and the presence of what appeared to be intermediate forms in contact areas between the ranges of the named taxa. Figures or exact descriptions of intermediate specimens were not given; however, Davenport alluded to the ambiguous material in the subspecies accounts. Information of this nature was also given to support the conspecificity of the Eurasian *tullia*-group taxa. The Old World and New World entities were considered to be conspecific because available specimens from both sides of the Bering Strait were morphologically indistinguishable, indicating a continuous transition of forms from Europe through Asia to Alaska, and thence throughout North America. In all, Davenport recognized 31 subspecies of *tullia*, and three more have since been added in North America.

Davenport suggested that some groups of subspecies appeared to be related on the basis of wing pattern similarity, but did not give characters to support his groupings. He also proposed that the current biogeography of subspecies is intimately related to post-Pleistocene range changes, based on the fact that much of North American range occurs in areas that were under extensive ice sheets 18 000 years ago.

Brown (1955) expanded Davenport's three groups of similar North American subspecies to five, and provided characters by which these groups could be recognized (Table 1). Brown (1955, p. 365) then invoked the original superspecies definition of Mayr (1942) to designate these subspecies groups as separate species, but he presented no biological evidence of reproductive isolation between his groupings to support his taxonomic change. The use of superspecies in the *tullia* group classification initially arose as a taxonomic convenience to express phenetic relationships of geographic entities.

Brown and Heineman (1961) gave some data indicating partial reproductive isolation between the taxa *heinemani* and *inornata* on islands in the St. Lawrence River, where they are sympatric. The isolation they described was apparently mediated by diapause differences in the larvae, so that *inornata* fly only in early summer and *heinemani* only in late summer. However, Shapiro (1974) states that both *heinemani* and *inornata* are double brooded in New York State, including the islands in the St. Lawrence River, with flights in early and late summer. Eberlie (1979) and W. Kiel (*in litt.*) reared the second flight of *inornata* from the first, although they worked with "pure" *inornata* populations. Neither showed *inornata* and *heinemani* to be seasonal forms of the same population. However, Brown and Heineman (1961) may have confounded a polyphenism with reproductive isolation; their populations require attention using more modern techniques.

After 1974, there were no further published attempts to contribute data to resolve the taxonomic problem. A series of regional faunal works, mostly published as guides to butterflies of North America or subregions of North America (Dornfield 1980; Emmel 1975; Garth and Tilden 1986; Hodges et al. 1983; Miller and Brown 1981; Pyle 1981; Scott 1986; Tilden and Smith 1986), treated the taxonomy of the *tullia* group ambiguously and often arbitrarily. Some erected new phenetic species based on scanty life history data, others moved subspecies among species without any attempt at justification, while still other considered the whole group to be conspecific. None made important contributions to the knowledge of the group and, taken together, they have almost entirely obscured the real advances made by earlier workers.

Even though Brown's (1955) superspecies taxonomy has never been supported by evidence, it has never actually been refuted, either. Most workers concentrate on "typical" forms, and do not look for areas of intergradation. Four accounts come closest to addressing this problem: Dornfield (1967) stated that "there is no good evidence that [*ampelos* and *eunomia*] overlap or that they show clinal intergradation or natural hybridization," and Shapiro et al. (1981) stated that "no two named entities in *Coenonympha* appear to be sympatric anywhere in northern California, although strays of both *california* and *ampelos* have been found near Donner Pass." We will describe areas of contact subsequently discovered between both pairs of taxa. Austin and Murphy (1987) cite distributional data documenting hybridization between "*ochracea*" *brenda* and "*ampelos*" *elko* in northeastern Nevada, and distributional gaps separating "*ochracea*" *mono* from both "*ochracea*" *brenda* and "*ampelos*" *ampelos*. In a review of the Rocky Mountain members of the genus, Miller (1981) stated that the entities *benjamini* and *ochracea* co-occur in "some places" where they "behave as separate species." This situation is potentially very important, but requires thorough documentation before conclusions can be drawn.

Three of Brown's nominal species, *california*, *ampelos*, and *ochracea*, have subspecies that occur in northern California. This situation has provided us with an opportunity to test for reproductive isolation in areas where these taxa meet. We used two methods to look for such evidence. First, we scored the wing pattern characters used by Brown to identify populations to their respective subspecies and putative species, and to describe the phenotypic structure of the populations in areas of contact. If more than one species exists in this group, we expect the phenotypic analysis to show discrete boundaries in areas of contact. Second, we used electrophoresis to assay for allelic differences at loci coding for metabolic enzymes. These

TABLE 1. The groupings of subspecies into putative species following Brown (1955), and the characters that define these groupings

Putative species	Subspecies	Diagnostic characters
inornata	*inornata* Edwards *benjamini* McDunnough *mcisaaci* dos Passos *nipisiquit* McDunnough *heinemani* Brown	Few or no ventral hind wing eyespots; lacks basal patches on ventral hind wing; usually single brooded,[a] flying in early summer (last two subspecies fly in late summer)
ochracea	*ochracea* Edwards *mackenziei* Davenport *brenda* Edwards *subfusca* Barnes & Benjamin *furcae* Barnes & Benjamin *mono* Burdick[b]	Two light basal patches on ventral hind wing; well-marked ventral hind wing eyespots; usually single brooded, with second or partial second brood occurring more frequently than among *inornata*
ampelos	*ampelos* Edwards *elko* Edwards *insulanus* McDunnough *columbiana* McDunnough *eunomia* Dornfield	Few or no ventral hind wing eyespots; lacks basal patches on ventral hind wing; double brooded at least in south of range, ground color much lighter than *inornata*
kodiak	*kodiak* Edwards *mixturata* Alpheraky *yukonensis* Holland	Lacks ventral hind wing eyespots and basal patches; single brooded; areas of strongly contrasting colors on ventral hind wing
california	*california* Westwood *eryngii* Edwards	Ground color whitish; ventral hind wing lacks basal patches; eyespots on ventral hind wing well marked in *california*, few or lacking in *eryngii*; double brooded

NOTE: Subsequently described subspecies have been included, grouped on the basis of these characters. Synonomy follows Miller and Brown (1981).

[a]The populations from Ontario to New England are apparently at least partially double brooded (Scott 1986), including subspecies *heinemani* in upstate New York (Shapiro 1974).

[b]The subspecies *mono* has poorly developed eyespots on the ventral hind wing, and is sometimes classified with *ampelos*.

data were used to find additional characters to define taxa, and to describe the genetic structure of the populations.

The presence of characteristics defining two or more taxonomic entities uncorrelated in contact populations can also be used to infer intergradation. If the characters are highly correlated, then coexistence of reproductively isolated populations is indicated. This principle holds for both morphological and enzyme characters. However, because some wing pattern characteristics in *Coenonympha* are known to be polyphenic, the assumptions linking wing phenotype to genotype are probably more tenuous than the assumptions linking enzyme staining pattern to allelic differences. Until laboratory crosses are done under a variety of environmental conditions, our conclusions (as in most taxonomic studies) will have to rely on these assumptions.

Materials and methods

Study populations

We surveyed 21 populations from northern California and adjacent Oregon and Nevada (Fig. 1, Table 2), representing three nominal species, five subspecies, and areas of contact. Most samples represent fall broods, collected from late August to early September 1986. However, Mono Lake was sampled in June 1986 (this population is univoltine or facultatively bivoltine; none were flying in the fall of 1987), Mix Canyon was sampled in late May 1987 (this was probably the first brood; Weissman 1972), and Woodfords was sampled in July 1987 (a year with a late flight season, thus probably the first brood).

The four samples from northeastern California, at Burney, MacArthur, Adin, and Goose Lake (Fig. 1), are taken from an extraordinarily interesting area biogeographically. The Pit River drains most of the deserts of northeastern California westward into the Pacific Ocean, and provides a low elevation corridor for migration and contact between biotas. There are places along Highway 299 in eastern Shasta County where elements of the low-elevation east slope Northern Juniper Woodland plant community co-occur with species of the low-elevation west slope Foothill Woodland community (after Munz and Keck 1973): these communities are separated elsewhere by high mountains. Because of the mixing of biotas, we expected these samples to provide strong tests of Brown's superspecies taxonomy. Notably, samples from this region were taken from cow pastures and artificially irrigated alfalfa fields, and may represent more recent evolutionary events than the surrounding habitats would indicate.

Three populations from southwestern Oregon were sampled in the presumed area of contact between *eryngii* and *eunomia* (Fig. 1A). The Grant's Pass and Sunny Valley populations are part of the Rogue River drainage, and are separated by a low (550 m) pass from the Riddle population in the Umpqua River drainage. Grassland habitats in this region are patchily distributed, being confined to floodplains and farmland in the valleys and roadsides and scattered rocky outcrops on the well-forested ridges.

A series of populations were sampled in southeastern Plumas County (Fig. 1B) to look for evidence of introgression between the

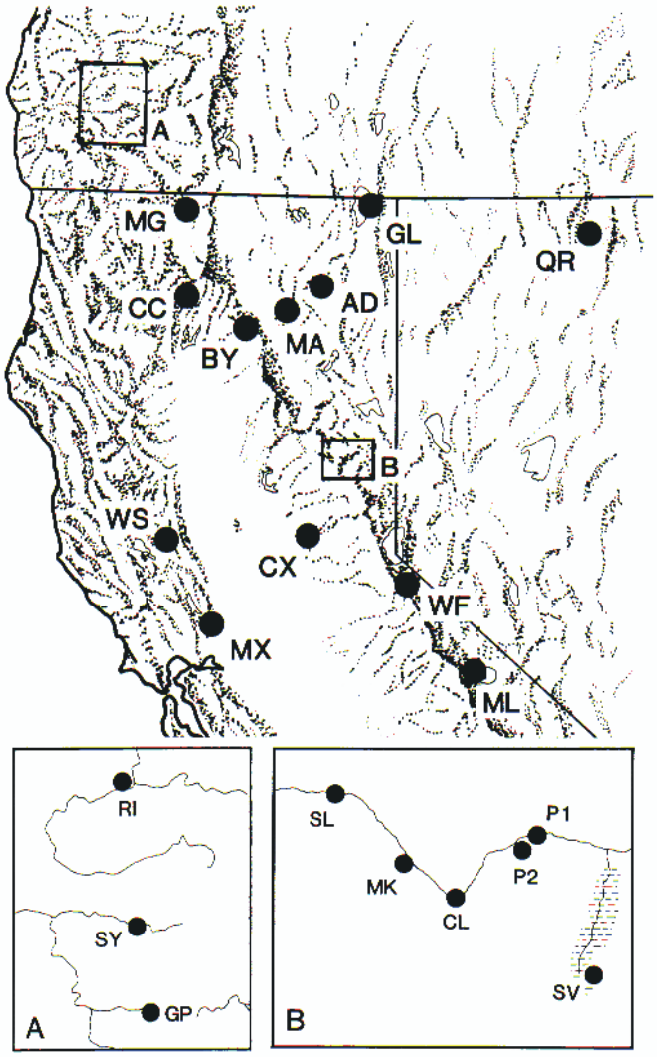

FIG. 1. Locations of populations used in this study. Insets A and B show sampling areas in Oregon and eastern California, respectively. Further locality data and a key to abbreviations are found in Table 2.

west-slope *california* and the east-slope *ampelos* along the Feather River. This river drains a region with an eastern Sierran biota westward via a deep canyon through the Sierra–Cascade Range, providing a potential corridor for migration and contact. The sample from Colfax is not on the Feather River, but is representative of Sierran west-slope *california*.

The taxon *ampelos* appears to have a southern limit in the Carson River drainage of western Nevada and eastern California, while *mono* appears to the south in the Walker River drainage and Mono Basin. There were no *Coenonympha* populations in the expected area of contact between *ampelos* and *mono* in Antelope Valley, vicinity of Coleville, Mono County, CA, in 1987, although individual specimens with *mono* phenotypes have been collected there in the past (S. O. Mattoon, *in litt.*).

All specimens were stored individually in glassine envelopes immediately upon capture, and kept in resealable plastic bags on wet ice for transport back to Davis. They were then frozen alive and stored at −80°C until electrophoretic analysis in the spring and summer of 1987. Wings were removed before electrophoresis and stored for morphological analyses. These have been deposited as vouchers in the Bohart Museum at the University of California, Davis.

Morphological analyses

Wing characters used are derived from those given in Table 1 to separate subspecies and putative species. The ground color of the dorsal forewing was scored from 1 to 4 based on the comparison of each specimen to a series of specimens chosen as color standards (these are also in the Bohart Museum with appropriate labels). Care was taken to avoid the influence of the scattered, heavily melanized scales in arriving at these scores, to avoid as much as possible the biases of polyphenic variation (potentially induced by microhabitat differences) or sexual differences in melanization. A score of 1 corresponds to the whitish to pale cream color of *california* and *eryngii*, 2 corresponds to a deeper cream to straw yellow color, 3 represents the pale to medium ochre color of typical *ampelos*, and 4 represents the deeper ochre seen in *mono* and *ochracea*.

The number of ventral hind wing eyespots was counted only with reference to those with dark pupils. Unpupiled eyespots were often present, but could not be distinguished reliably on relatively unmelanized individuals, so they were omitted from the analysis. The pale basal patches on the ventral hind wing were scored as to their degree of expression. The posterior basal patch invariably showed a degree of expression less than or equal to that of the anterior patch. A score of 0 represented the absence of patches, 1 represented the presence of at least a faint anterior patch, and 2 represented a well-developed anterior patch and at least a partial posterior patch.

The actual scores of each population are compared with those derived from the literature descriptions to characterize each population taxonomically. The combinations of these characters used to diagnose the five subspecies are given in Table 3.

Electrophoretic analyses

We assayed 21 enzyme loci using horizontal starch gel electrophoresis following the protocol of Ayala et al. (1972) and Geiger and Shapiro (1986), with minor modifications to improve staining. Of these, 17 loci were considered easily scorable and were used: adenylate kinase (*Ak-1*, *Ak-2*), adolase (*Aldo*), fumarase (*Fum*), glutamic-oxaloacetic transaminase (*Got-1*, *Got-2*), glutamic-pyruvic transaminase (*Gpt*), glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), α-glycerophosphate dehydrogenase (α-*Gpdh*), hexokinase (*Hk*), isocitrate dehydrogenase (*Idh-1*), malate dehydrogenase (*Mdh-1*), malic enzyme (*Me-2*), phosphoglucomutase (*Pgm*), phosphoglucose isomerase (*Pgi*), pyruvate kinase (*Pk*), and superoxide dismutase (*Sod-1*).

Zymograms were scored by giving the most common electromorph at each locus on the first gel the arbitrary score of 100. All other electromorphs were given scores corresponding to the distance (in millimetres) migrated relative to the most common: for example, an electromorph migrating 10 mm further cathodally than the most common was given the score 110; a more annodal electromorph might be given the score 92. Data are given in this form in this paper. These designations were converted to letter scores (A, B, C, etc.) for analysis using the computer program BIOSYS-1 (version 1.6) (Swofford and Selander 1981). For the purposes of this study, all electromorphs were assumed to be allelic variants, and will subsequently be referred to as alleles.

Values for F_{ST}, a statistic describing the component of overall genetic variance due to among-population differences, were calculated following Wright's original formula using BIOSYS-1. These were taken over selected groups of populations within the data set to look for regions of interrupted gene flow (Wright 1978). In the infinite island model, gene flow is defined by the parameter Nm, where N is the effective population size and m is the effective migration rate between populations. Following Wright (1931), when $Nm \ll 0.5$, genetic drift is the significant factor influencing neutral gene frequency among populations. When $Nm \gg 0.5$, gene flow is the primary factor. At $Nm = 0.5$, the outcome is unpredictable. In the island model, $Nm = (1/F_{ST} - 1)/4$, and $Nm < 0.5$ when $F_{ST} > 0.333$. The neutral locus with the highest F_{ST} value estimates the upper bound of Nm, which is the maximum possible level of *present-day* gene flow among populations within the group. Present-day gene flow within the group is negligible when $F_{ST} > 0.333$.

Results

Morphological variation and phenotypic population structure

The frequencies of color scores among populations are

TABLE 2. Key to population abbreviations, with localities and collection dates

Abbreviation	Population name	Location	Date
AD	Adin	Modoc Co., CA	1986-09-02
BY	Burney	Shasta Co., CA	1987-08-06
CC	Castle Crags	Near Castella, Shasta Co., CA	1986-09-12
CL	Clio	Plumas Co., CA	1986-09-04
CX	Colfax	Placer Co., CA	1986-09-04
GL	Goose Lake	E shore; Modoc Co., CA	1987-08-23
GP	Grant's Pass	Josephine Co., OR	1987-08-30
MA	MacArthur	Shasta Co., CA	1986-09-09
MG	Montague	Siskiyou Co., CA	1986-09-10
MK	Mohawk	Plumas Co., CA	1986-09-04
ML	Mono Lake	Mono Co., CA	1986-06-06, 1986-06-07, 1986-06-14, 1986-06-15
MX	Mix Canyon	Near Vacaville, Solano Co., CA	1987-05-28, 1987-05-31
P1	Portola 1	Portola, Plumas Co., CA	1986-09-04
P2	Portola 2	5 km S Portola, Plumas Co., CA	1986-09-04
QR	Quinn River	25 km N Orovada, Humboldt Co., NV	1987-08-05
RI	Riddle	Douglas Co., OR	1987-08-30
SL	Sloat	Plumas Co., CA	1987-09-04
SV	Sierraville	Sierra Co., CA	1986-08-27
SY	Sunny Valley	Josephine Co., OR	1987-08-30
WF	Woodfords	Alpine Co., CA	1987-07-07
WS	Wilbur Springs	Colusa Co., CA	1986-09-02

shown in Fig. 2. The phenotypic population structure of this character shows variation within most populations, underlying a major trend of geographic variation. All populations west of the Sierra–Cascade divide except Riddle (i.e., nominal *california* and *eryngii*) showed high frequencies of white individuals, with some individuals of a cream color. The populations at Quinn River, Woodfords, and the eastern Feather River region (Fig. 2B; nominal *ampelos*) were predominantly ochre, with a low frequency of darker ochre individuals, and some yellow individuals at Portola 2 and Clio. Riddle (nominal *eunomia*) was mostly ochre, with one lighter individual. The Mono Lake population (*mono*) was almost entirely dark ochre.

Populations from the Pit River drainage (Burney, Adin, McArthur, Goose Lake), where nominal *ampelos* and *california–eryngii* are expected to contact, showed a wide range of color scores. These grade from a high frequency of white individuals at Burney to predominantly ochre individuals at Goose Lake. A similar situation occurred at the contact zone between *eryngii* and *eunomia* in southwestern Oregon. The Grant's Pass population was predominantly white, with some yellow and ochre members; Sunny Valley had a higher frequency of yellow individuals (and one ochre individual was seen but not caught), while Riddle was predominantly ochre.

The frequencies of hind wing eyespot numbers among populations are shown in Fig. 3. Again, the phenotypic population structure of this character shows well-marked intrapopulation variation underlying a major trend of geographic variation. The populations at Mix Canyon, Colfax, and Wilbur Springs all had high frequencies of butterflies with two or more eyespots, characteristic of subspecies *california*. Burney tended to have relatively more individuals with one or zero eyespots, suggesting some influence from *eryngii* and (or) *ampelos*. All of the remaining populations (nominal *eryngii*, *eunomia*, *ampelos*, and *mono*) showed a predominance of individuals with zero or one eyespot. However, the frequency of butterflies with two or three eyespots in populations east of the Sierra–Cascade divide (nominal *ampelos*) was considerably higher than expected from discussions in the literature. In the Feather River drainage (Fig. 3B), this may be due to introgression from *california*.

TABLE 3. Wing pattern scores that characterize the taxonomic groupings used in this study, based on literature descriptions summarized in Table 1

	Ground color[a]	No. of hind wing eyespots	Basal patches[b]
ampelos	3[c]	0 or 1	0
eryngii	1	0	0
eunomia	3	0	0
california	1	2–5	0
mono	4	0	2

[a]1, white; 2, straw yellow; 3, pale ochre; 4, deep ochre.

[b]0, no patch visible; 1, anterior patch at least weakly expressed; 2, anterior patch well expressed and posterior patch at least weakly expressed.

[c]There is some confusion about this character, dating back to Davenport's (1941) sinking of *elko* into *ampelos*. Additionally, the type locality of *ampelos* may be an intergrade population as defined in this study (see Discussion). Many would consider the appropriate color scores to be 2–3. We follow Austin (1985) in restricting the name to "pure" populations, but for the purposes of discussing intergradation rather than for strictly taxonomic reasons.

The basal wing patch is reported to be diagnostic for *ochracea* and *mono*. Figure 4 shows that this character is very well expressed in the Mono Lake population, but occurs at low frequency in most populations throughout the region.

All three "diagnostic" characters have phenotypic population structures characterized by high levels of both intrapopulation and interpopulation (geographic) variation. The geographic boundaries where the three "diagnostic" characters change states are not congruent except between *ampelos* and *mono*, where both wing color and basal patches change frequency. There is quite a bit more intrapopulation variability than has been previously reported in the literature.

The data sets for Adin, Goose Lake, MacArthur, Portola 1, and Sierraville are large enough to examine for correlations between diagnostic characters among individuals, which would indicate coexistence of reproductively isolated nominal

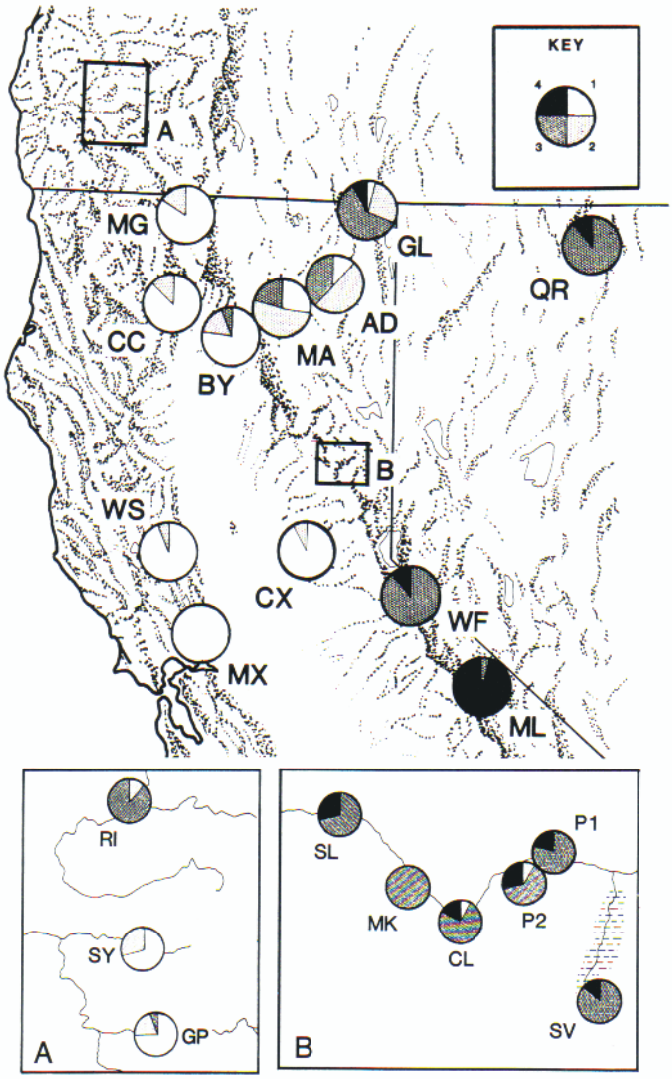

FIG. 2. Frequency distributions of dorsal wing color scores in study populations. Insets A and B show sampling areas in Oregon and eastern California, respectively. Color scores as follows: 1, white; 2, straw yellow; 3, pale ochre; 4, deep ochre. Melanic scales were ignored in this analysis because they vary seasonally and are probably strongly influenced by local environment.

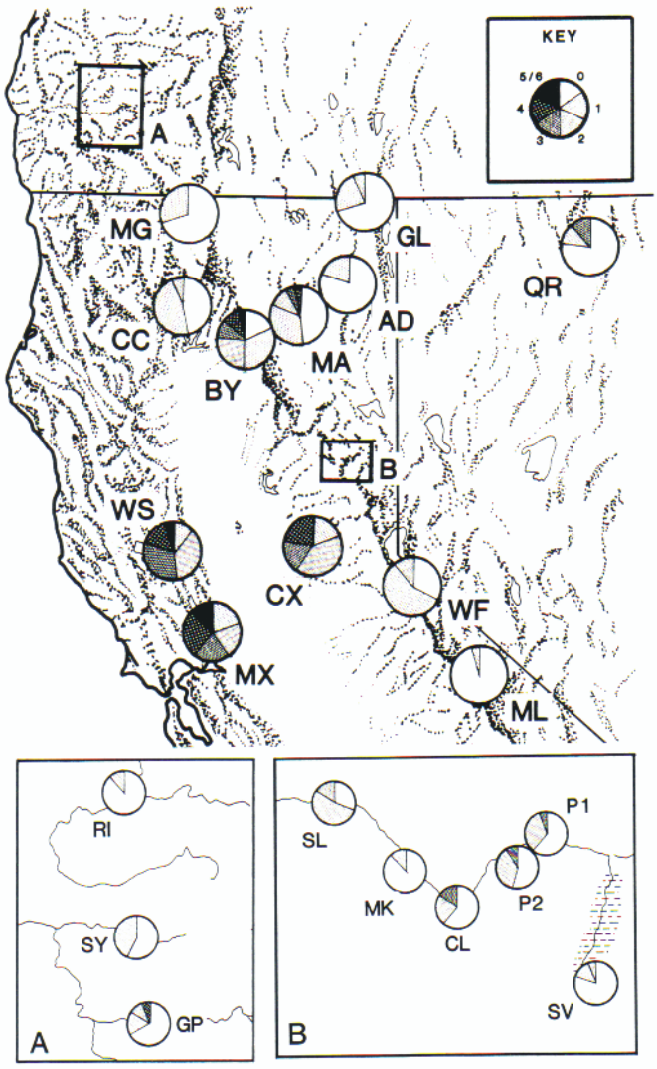

FIG. 3. Frequency distributions of ventral hind wing eyespot values in study populations. Insets A and B show sampling areas in Oregon and eastern California, respectively. Values scored by direct count of eyespots with dark pupils: 0, no eyespots; 1, one eyespot, etc. Unpupiled eyespots were ignored in this analysis because they could not be resolved in unmelanized individuals.

species taxa. Examining Table 3, we expect that if *ampelos* and *california* coexist in the same population, a significant negative correlation coefficient (r) should be found between values for color and hindwing eyespots. Likewise, if *california* and (or) *ampelos* and *mono* coexist, we expect a positive r between color and basal patches. For *california* and *mono*, a significantly negative r is expected. Table 4 shows the negative results of this analysis. In summary, we find no evidence from wing pattern that these populations are made up of coexisting species.

Genetic variability

Allele frequencies of polymorphic loci for each population are given in Table 5. All populations studied showed a striking degree of polymorphism at several loci. *Pgi* had a total of 14 alleles among populations, ranging from 4 to 11 alleles in each population. *Idh-1* and *Mdh-1* each had nine alleles; all occurred at both loci in the MacArthur sample, but only a single *Idh-1* allele was found at Mono Lake. There were eight alleles at *Got-1*, seven at *Ak-1*, and six at *Pgm*. Other loci showed fewer alleles, and only four loci were monomorphic (*Ak-2*, *Aldo*, *Fum*, *Gpt*).

Heterozygosity (H_{obs}, H_{exp}) and percent polymorphic loci (P) values for each population are given in Table 6. Observed heterozygosity (H_{obs}) scores ranged from a low of 12.3% at Mono Lake to 18.5% at Sloat. Heterozygosity calculated from Hardy–Weinberg proportions (H_{exp}) was slightly higher, ranging from 13.5% at Mono Lake to 20.4% at Montague. The heterozygote deficiency may reflect selection at some loci, or perhaps mating behaviors that promote local inbreeding. Percent of loci polymorphic at the 99% level ranged from 35.5 to 58.8%. All of these values are exceedingly high, approaching *Drosophila* (the group with the overall highest average values for genetic diversity; Nevo 1978) in H values and surpassing them in values of P. This is an indication that even populations in isolated habitat patches in mountainous regions, such as southwestern Oregon and Woodfords, have not undergone appreciable bottleneck effects in their recent past.

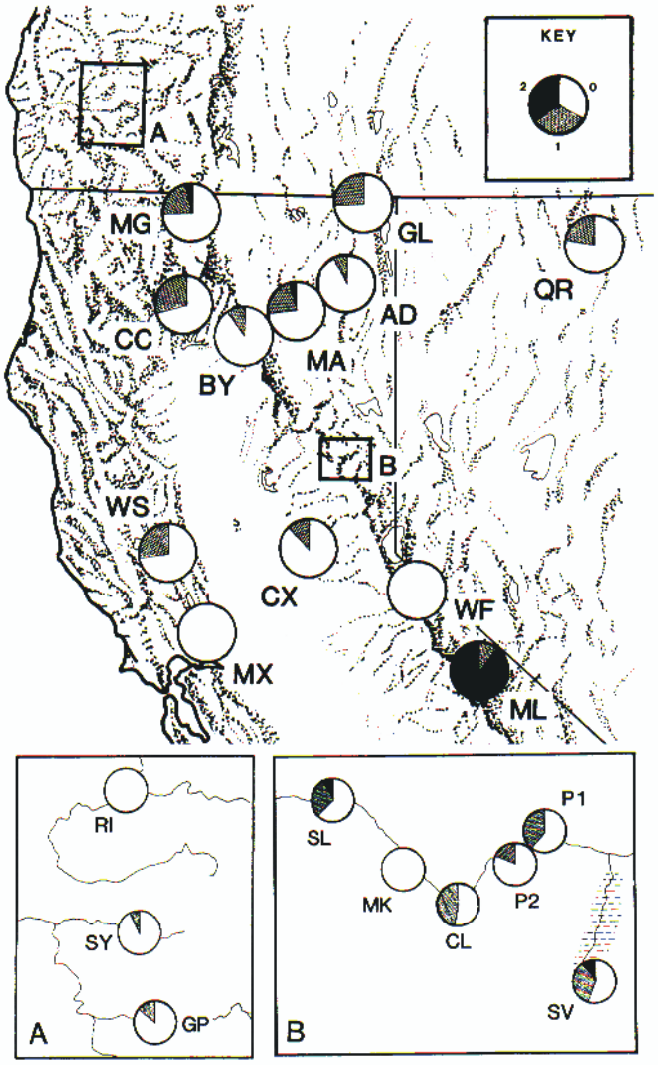

FIG. 4. Frequency distributions of ventral hind wing basal patches in study populations. Insets A and B show sampling areas in Oregon and eastern California, respectively. Values scored by direct count of basal patches: 0, no patch visible; 1, anterior patch at least weakly expressed; 2, anterior patch well expressed and posterior patch at least weakly expressed.

Most loci did not differ significantly from Hardy–Weinberg equilibrium, except *Got-1* at Clio, Adin, and Mono Lake, *Got-2* at Adin, and *Ak-1* at Portola 1. Each showed an excess of homozygotes. All of these loci are easy to score, so the deviations are apparently real. None of the alleles at these loci correlated with wing pattern characters in a way that would suggest selection against "hybrid" individuals or a Wahlund effect. As a precaution, all analyses requiring the assumption of neutrality were performed without *Got-1*.

Despite the very high variability within populations, interpopulation variability was very low. Loci showing significant geographic variabillity (χ^2 test for heterogeneity across populations) were *Ak-1*, *Idh-1*, *Pgi*, *Pgm*, *Got-1*, *Got-2*, and *Hk*. However, no subspecies or putative species could be characterized by the presence of any allele in high frequency. The Mono Lake population (subspecies *mono*) showed a lower average number of alleles per locus than other populations, and otherwise notable differences at two loci. *Idh-1* was fixed for allele 100. However, this was the most common allele in all other populations, despite the high number of alleles at this locus (up to nine). The only two alleles of *Pgm* at Mono Lake were 94 and 100, both at relatively high frequency. In all other populations, *Pgm* alleles 100 and 106 were highest in frequency, although allele 94 was found in 16 of the 21 populations studied. There were no fixed allelic differences to support the taxonomic separation of *california*, *eryngii*, and *ampelos*.

TABLE 4. Correlation of diagnostic characters among individuals in variable populations

	Correlation coefficient (r)		
	c vs. h	c vs. b	h vs. b
Expected	−	+	−
Adin	0.330	0.026	0.360
Goose Lake	−0.013	0.227	0.020
MacArthur	0.094	−0.039	−0.108
Portola	0.705	−0.394	−0.191
Sierraville	0.333	−0.322	−0.085

NOTE: Expected: sign of the correlation expected if species are coexisting in the same population; c, color; h, hind wing eyespots; b, basal patches. No correlation coefficient is significant (one-tailed test, $\alpha \leq 0.05$).

Genetic population structure

F_{ST} values for each locus among different groups of populations are given in Table 7. Within the nominal species *california*, the maximum F_{ST} value is 0.108, which is very close to 0.103, the maximum within the subspecies *california*, and much higher than 0.069, the maximum for subspecies *eryngii*. This indicates that gene flow is probably not interrupted between these subspecies.

Within the nominal species *ampelos*, the maximum F_{ST} value was 0.155. This drops to 0.095 in subspecies *ampelos* and to 0.073 in phenotypically "pure" subspecies *ampelos* (i.e., without the Feather River populations, which show hints of introgression from *california*), and is 0.079 in the Feather River group. Gene flow is apparently uninterrupted within the subspecies *ampelos*.

When the nominal species *california* and *ampelos* are grouped as conspecific, the maximum F_{ST} value is 0.104. This value is almost identical with the F_{ST} within the nominal species *california*, and is much lower than within the nominal species *ampelos*. This result suggests that there is no lack of gene exchange between these nominal taxa, and supports their conspecificity.

Population groups in the *california*–*ampelos* contact zone provide further tests of species boundaries. The Pit River group transects the contact between these nominal species. It shows a maximum F_{ST} value of 0.049, again suggesting that gene flow is fairly strong between these nominal species. The southwestern Oregon group transects the boundary between nominal subspecies *california eryngii* and *ampelos eunomia*. The maximum F_{ST} value among these populations is 0.102, equivalent to the gene flow within nominal subspecies *california california* or *ampelos ampelos*, and well below the 0.333 cutoff. This suggests that *eunomia* is at least connected to *ampelos* via *eryngii*. *Ampelos*–*eunomia* contact areas should be sought along the Snake River at the Oregon–Washington border or across low passes in the Oregon Cascade Range.

A contact area between the nominal species *ampelos* (sub-

TABLE 5. Allelic frequencies of all polymorphic

	AD	BY	CC	CL	CX	GL	GP	MA	MG	MK
n	36	31	15	13	17	31	40	66	27	8
Ak-1										
76	0.014	0.016				0.016	0.034	0.023		
80	0.042			0.038				0.015		0.063
86	0.014		0.100					0.038		
90	0.125	0.242	0.167	0.077	0.206	0.306	0.414	0.129	0.352	0.063
100	0.806	0.710	0.733	0.846	0.794	0.661	0.517	0.765	0.648	0.875
102										
110		0.032		0.038		0.016		0.030		
120										
Gapdh										
90						0.032				
100	1.00	1.00	0.967	1.00	1.00	0.968	1.00	0.985	1.00	1.00
105			0.033							
109								0.015		
Got-1										
84							0.013		0.019	
89				0.269				0.008		
91	0.083	0.258	0.033			0.129	0.262	0.182	0.056	
94	0.014		0.067		0.176			0.045	0.185	0.250
100	0.722	0.661	0.800	0.654	0.676	0.677	0.575	0.574	0.800	0.679
102										
104							0.025	0.008		
108	0.097	0.081	0.033	0.077	0.118	0.129	0.013	0.053	0.037	0.063
110	0.083		0.067		0.029	0.065	0.112	0.015	0.130	
Got-2										
100	0.917	1.00	1.00	1.00	0.853	1.00	1.00	0.977	0.981	1.00
105	0.083				0.147			0.023	0.019	
α-Gpdh										
90	0.028				0.029		0.013	0.015	0.019	
100	0.972	1.00	0.933	1.00	0.971	1.00	0.987	0.977	0.944	0.938
107			0.033						0.037	
110								0.008		0.063
119			0.033							
Hk										
94	0.014									
100	0.986	1.00	0.900	1.00	1.00	1.00	1.00	0.985	1.00	1.00
103			0.100					0.015		
Idh-1										
90	0.069	0.032		0.115	0.029	0.081	0.038	0.045		0.118
95	0.014		0.033			0.081		0.030		
98					0.029			0.008		
100	0.569	0.645	0.567	0.692	0.529	0.532	0.525	0.568	0.481	0.438
103	0.236	0.210		0.192	0.147	0.129	0.125	0.144	0.167	0.375
106	0.097	0.081	0.333		0.235	0.177	0.287	0.167	0.333	
108			0.067		0.029			0.015		
111	0.014	0.032					0.025	0.015		
114								0.008		
Mdh-1										
88	0.014							0.008		
91		0.081			0.029		0.038	0.023	0.037	
93	0.014							0.008		
100	0.917	0.887	0.967	0.885	0.882	0.952	0.925	0.841	0.870	0.813
105	0.014					0.016	0.013	0.008	0.019	
110	0.014	0.032		0.115	0.029	0.032	0.013	0.038	0.037	0.188
112	0.028		0.033		0.059			0.061	0.019	
114							0.013	0.008		
120								0.008	0.019	
Me-2										
100	1.00	1.00	0.967	1.00	1.00	0.935	1.00	1.00	1.00	1.00
105			0.033			0.065				

loci scored by populations

ML	MX	P1	P2	QR	RI	SL	SV	SY	WF	WS
21	15	19	11	9	17	14	21	14	9	19
				0.056	0.088	0.107				
	0.100			0.056						
0.452	0.033	0.132		0.111	0.471		0.143	0.393	0.389	0.158
0.548	0.867	0.868	1.00	0.722	0.412	0.893	0.857	0.607	0.611	0.789
					0.029					
				0.056						0.026
										0.026
				0.056			0.024	0.038		0.026
1.00	1.00	1.00	1.00	0.944	1.00	1.00	0.976	0.962	1.00	0.974
	0.033									
		0.026				0.036	0.024			
		0.026		0.056	0.471		0.048	0.071	0.111	0.158
0.571	0.133	0.132	0.318			0.250	0.143			0.079
0.405	0.700	0.789	0.636	0.889	0.382	0.679	0.667	0.857	0.889	0.684
					0.059					
	0.033					0.036				
0.024	0.033	0.026		0.056			0.095			0.026
	0.067		0.045		0.088		0.024	0.071		0.053
0.976	1.00	0.974	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.024		0.026								
0.024		0.026	0.045			0.071	0.024		0.167	
0.976	1.00	0.947	0.955	1.00	1.00	0.893	0.952	1.00	0.833	0.933
		0.026				0.036	0.024			0.033
										0.033
0.024		0.053		0.056		0.036	0.024			
0.976	1.00	0.947	1.00	0.944	1.00	0.964	0.976	1.00	1.00	1.00
		0.079	0.182	0.167		0.071	0.048	0.036	0.278	
	0.033									
1.00	0.533	0.605	0.636	0.667	0.882	0.571	0.714	0.429	0.333	0.711
		0.079	0.182	0.167	0.029	0.107	0.048	0.107	0.333	0.158
	0.400	0.184			0.059	0.250	0.190	0.357	0.056	0.132
		0.026								
	0.033	0.026								
	0.033									
0.024					0.118		0.024			0.053
0.952	0.933	0.816	0.864	0.944	0.882	0.929	0.810	0.964	0.944	0.933
		0.105	0.091	0.056		0.036	0.024			
0.024						0.036	0.024	0.036	0.056	
	0.033	0.079	0.045				0.119			0.026
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

TABLE 5

	AD	BY	CC	CL	CX	GL	GP	MA	MG	MK
Pgi										
81	0.014								0.019	
85						0.016		0.015		
88	0.028		0.067	0.115	0.029	0.048		0.015		0.063
94	0.056	0.097	0.033		0.029	0.032	0.050	0.076	0.093	
97	0.014	0.032			0.059		0.013	0.008	0.019	
100	0.514	0.419	0.267	0.308	0.412	0.516	0.463	0.364	0.481	0.313
103	0.028		0.067					0.008	0.037	
105							0.025			
107	0.222	0.339	0.467	0.500	0.411	0.323	0.300	0.439	0.241	0.375
111		0.016						0.008		
114	0.111	0.081	0.033		0.029	0.048	0.125	0.038	0.056	
117	0.014	0.016	0.067	0.077				0.023	0.037	0.250
121						0.016	0.025	0.008	0.019	
Pgm										
80	0.014									
94	0.014			0.038	0.059	0.032	0.063	0.015	0.037	
100	0.458	0.355	0.500	0.346	0.417	0.597	0.512	0.515	0.185	0.438
106	0.417	0.516	0.500	0.577	0.441	0.339	0.350	0.417	0.537	0.438
110	0.097	0.113		0.038	0.029	0.032	0.050	0.053	0.222	0.125
112		0.016							0.019	
Pk										
94	0.014									
100	0.986	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sod-1										
89								0.008		
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.992	1.00	1.00

NOTE: Population abbreviations follow Table 2; enzyme abbreviations and allelic designations are described in the text.

species *ampelos*) and *ochracea* (subspecies *mono*) was expected in northern Mono County, CA, but was not found. F_{ST} values between populations at Woodfords (*ampelos*) and Mono Lake (*mono*) show a maximum of 0.313 at *Idh-1*, which is very close to the 0.333 cutoff, and suggests that gene flow is very weak or recently interrupted between *ampelos* and *mono*.

The Feather River group represents the only set of geographically adjacent populations sampled. The maximum F_{ST} value is 0.079. This value is almost as high as values within subspecies and species, suggesting that interpopulation genetic differentiation on a large scale, even between nominal species, is not much greater than on a very local scale.

Phenograms

Phenograms were constructed using UPGMA (see Sneath and Sokal 1973) on several different genetic distance measures. Figure 5 shows the phenogram for Nei's (1978) unbiased distance and identity, the most appropriate measures when sample sizes are variable among populations. The Mono Lake population deviates significantly from the others, reflecting the differences discussed above. The deviation shown is at a level corresponding to subspecies in other butterfly studies (Geiger and Scholl 1985; H. J. Geiger, unpublished data). Portola 1 and Sierraville (nr. *ampelos*), Colfax (*california*) and MacArthur (*california* × *eryngii* × *ampelos* intergrades), and Goose Lake (*california* × *eryngii* × *ampelos* intergrades) and Sunny Valley (*eryngii* × *eunomia* intergrades) each cluster at a distance of 0.00000. On the other hand, the adjacent populations at Portola 1 (P1) and Portola 2 (P2) cluster at a distance of 0.008. Using other genetic distance measures (unpublished data), the distances between populations are somewhat greater, but distances between clusters of populations remain quite small. Excluding *mono*, clusters of populations do not fall out, using any method, as expected from the taxonomy based on wing pattern.

Discussion

Population structure

The wing pattern analyses demonstrate that the diagnostic characters used in *Coenonympha* taxonomy show considerable intrapopulation variability, and that the "diagnostic" characters are not as geographically restricted as previously supposed. Introgression is indicated in three areas despite the fact that the scoring methods we used should tend to emphasize discrete boundaries. There appears to be a complex cline involving three taxa in northeastern California. The taxa *california* and *eryngii*, which differ in the number of hind wing eyespots, appear to intergrade in the north–south direction at Burney. This subspecies pair and *ampelos*, which differ in ground color and (in part) in eyespot number, appear to form a long east–west cline from Burney to at least Goose Lake along the Pit River. The taxa *eryngii* and *eunomia*, which differ in ground color, appear to intergrade in southwestern Oregon. There does not appear to be a cline between *ampelos* and *mono* as there is a relatively sharp differentiation in ground color and basal patch scores between Woodfords and Mono Lake. However, this appears to be associated with an absence of any permanent *Coenonympha* populations in the expected contact area at Coleville, Mono County, CA, rather than with any behavioral barriers to gene flow.

The electrophoretic analysis shows a very high degree of polymorphism in populations, yet a surprising lack of interpopulation differentiation. In many cases, the genetic relationships among populations between taxa are closer than among populations within a taxon. This is supported by the F_{ST} values

(*concluded*)

ML	MX	P1	P2	QR	RI	SL	SV	SY	WF	WS
							0.048			
										0.026
	0.200	0.079					0.024			0.026
0.262		0.026	0.182	0.111	0.029	0.107	0.048	0.036	0.167	0.105
								0.036	0.056	
0.048	0.400	0.263	0.273	0.333	0.412	0.214	0.405	0.393	0.389	0.316
	0.033								0.056	0.026
					0.029					0.026
0.643	0.267	0.500	0.364	0.333	0.382	0.643	0.452	0.393	0.167	0.395
								0.071		0.026
0.024		0.026		0.222	0.118			0.036	0.056	0.053
0.024	0.100	0.105	0.182			0.036	0.024		0.111	
					0.029			0.036		
0.452	0.033	0.028			0.088	0.036	0.048	0.036	0.056	0.026
0.548	0.400	0.500	0.227	0.444	0.382	0.607	0.405	0.536	0.667	0.658
	0.500	0.472	0.773	0.500	0.500	0.214	0.500	0.429	0.167	0.263
	0.067			0.056	0.029	0.143	0.048		0.111	0.053
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
							0.024			
1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.976	1.00	1.00	1.00

for population groups, which indicate that the subspecies *california*, *eryngii*, and *eunomia* are conspecific, and are not separated by gene flow barriers. Standard genetic distance phenograms do not even recognize these taxa as separate subspecies.

The taxon *mono* does appear to be isolated genetically at the level of a weakly defined subspecies. Intergrade populations between *ochracea* and *mono* have not been reported in the literature. The possibility remains that *mono* is geographically isolated from the remaining North American *Coenonympha*, and the evidence presented thus far does not preclude reproductive isolation. The widespread distribution of the basal hind wing patches, previously thought to be limited to the subspecies *mono* and to *ochracea* further east, suggests a number of plausible explanations: (*i*) this character has never been entirely lost in other subspecies, (*ii*) low-level unidirectional gene flow is occurring from *mono* into the other taxa, (*iii*) this character confers adaptive advantages under some conditions, or (*iv*) *mono*-like populations were much more widespread in northeastern California before being swamped by range extensions of other taxa from the north and west. The first explanation appears untestable, and the second can probably be ruled out: *mono* characters do not turn up at the nearby Woodfords population, where they would be expected to be most frequent following diffusion principles, and F_{ST} values imply that present-day gene flow levels are not strong enough to maintain similarity. Woodfords may have been through a recent bottleneck, which could remove *mono* characters, but the high P and H values suggest that this has not been the case. Experiments to test the third explanation could be constructed, and perhaps should be designed using intertaxon hybrids to avoid biases from genes not involved in the basal patch trait.

The last scenario is consistent with the available climatological data for the Great Basin in the Quaternary, reviewed by Mehringer (1977). Much of the Great Basin was filled with large pluvial lakes during the Ice Ages, which began to evaporate 12 000 − 10 000 years ago. These were almost entirely

TABLE 6. Genetic variability measures for each population

	n	H_{obs}	H_{exp}	P
Adin	36	15.5	18.4	58.8
Burney	31	15.9	15.1	35.3
Castle Crags	15	16.9	18.4	58.8
Clio	13	13.1	16.1	35.3
Colfax	17	16.3	19.4	47.1
Goose Lake	31	13.3	15.2	41.2
Grant's Pass	40	15.9	15.7	35.3
MacArthur	66	17.9	18.9	58.8
Montague	27	16.6	20.4	47.1
Mohawk	8	15.4	19.1	41.2
Mono Lake	21	12.3	13.5	47.1
Mix Canyon	15	15.3	16.5	35.3
Portola 1	19	15.3	17.7	52.9
Portola 2	11	13.9	15.0	35.3
Quinn River	9	16.3	15.8	41.2
Riddle	17	12.8	14.2	35.3
Sloat	14	18.5	16.8	47.1
Sierraville	21	17.6	18.0	58.8
Sunny Valley	14	15.2	15.3	35.3
Woodfords	9	16.3	17.7	35.3
Wilbur Springs	19	15.8	16.4	41.2

NOTE: H_{obs}, % mean observed heterozygosity over all loci; H_{exp}, % mean expected heterozygosity over all loci, assuming Hardy–Weinberg proportions; P, % polymorphic loci, where most common allele has a frequency <99%.

TABLE 7. F_{ST} values for each polymorphic locus in populations grouped according to taxonomy (nominal species within a superspecies) and by geographic region for contact areas and groups of geographically adjacent populations

	Ak-1	*Gapdh*	*Got-2*	*α-Gpdh*	*Hk*	*Idh-1*	*Mdh-1*	*Me-2*	*Pgi*	*Pgm*	*Pk*	*Sod-1*	Mean
By taxonomy													
california (''species'')[a]	0.043	0.023	**0.108**	0.027	0.085	0.047	0.016	0.028	0.031	0.059			0.047
california (subspecies)[b]	0.029	0.018	**0.103**	0.020		0.044	0.009		0.028	0.039			0.036
eryngii (subspecies)[c]	0.043	0.022	0.012	0.013	**0.069**	0.014	0.016	0.022	0.033	0.064			0.031
ampelos (''species'')[d]	**0.155**	0.038	0.023	0.058	0.028	0.089	0.049		0.054	0.083		0.021	0.060
ampelos (subspecies)[e]	**0.095**	0.036	0.023	0.053	0.025	0.071	0.046		0.056	0.091		0.021	0.052
ampelos (''pure'' ssp.)[f]	0.050	0.029		0.091	0.029	0.061	0.014		0.026	**0.073**			0.047
california + *ampelos* (as a single species)[g]	**0.104**	0.028	0.088	0.054	0.050	0.075	0.039	0.049	0.046	0.061	0.013	0.018	0.052
All study populations	**0.109**	0.028	0.081	0.052	0.047	0.092	0.039	0.049	0.058	0.086	0.013	0.018	0.051
In contact areas													
Pit River[h]	0.023	0.018	0.045	0.012	0.009	0.011	0.019	**0.049**	0.018	0.022	0.010	0.006	0.020
Southwestern Oregon[i]	0.018	0.026		0.008		**0.102**	0.027		0.009	0.016			0.029
ampelos–*mono*[j]	0.004		0.012	0.059	0.012	**0.313**	0.004	0.125	0.095				0.122
Geographically adjacent populations													
Feather River[k]	0.038	0.020	0.022	0.023	0.023	0.059	0.040		0.037	**0.079**		0.020	0.036

NOTE: Missing values indicate that the locus was monomorphic within the population group. Values in bold type are the maxima for each row. Fotnotes indicate component populations for each group (according to abbreviations in Table 2). When $F_{ST} > 0.333$, estimated gene flow is negligible among populations.

[a]BY, CC, CX, GP, MG, MX, WS.
[b]CX, MX, WS.
[c]CC, GP, MG.
[d]CL, MK, P1, P2, QR, RI, SL, SV, WF.
[e]CL, MK, P1, P2, QR, SL, SV, WF.
[f]QR, WF.
[g]All except ML.
[h]AD, BY, GL, MA.
[i]GP, RI, SY.
[j]ML, WF.
[k]CL, MK, P1, P2, SL, SV.

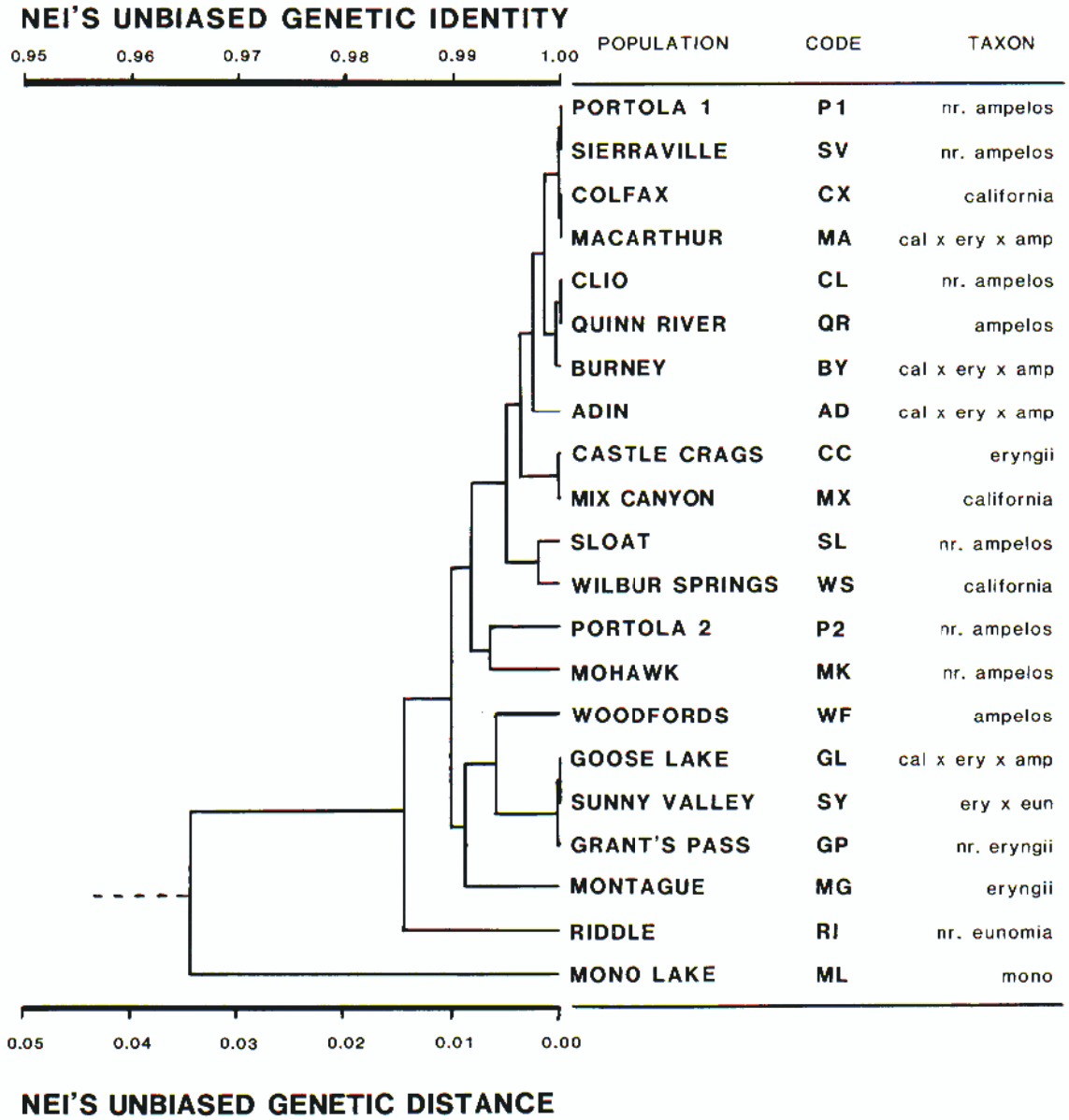

FIG. 5. Phenogram constructed using UPGMA on Nei's (1978) unbiased genetic identity and distance matrices. Population codes refer to Figs. 1–4 and Table 2. Populations are classified based on wing pattern, as discussed in the text. *cal*, *california*; *ery*, *eryngii*; *amp*, *ampelos*; *eun*, *eunomia*.

dry 7500 years ago, as they are today. From 7000–4000 years ago, there was an extremely dry period, such that Lake Tahoe dropped to a level below its outlet into the Truckee River (Davis et al. 1976). During these periods, biological changes were manifold. Many ecologically dominant plant species underwent major range fluctuations (Wells 1983), and many large vertebrates from these areas are now extinct. The subspecies *mono* appears to be restricted to wet meadows; these were presumably much more widespread in the eastern Sierra–Cascade range and the northern Great Basin during the Pleistocene than today. During the extremely dry periods, a widespread *mono* would have been restricted to wet meadows in high valleys and around permanent lakes, while more xerophilic subspecies such as *ampelos* or *eryngii* could colonize less predictable habitats in areas with intermittent water. For example, perhaps *mono* requires more succulent hosts for larval development than *ampelos* or *eryngii*. Such a scenario may explain the high frequencies of well-developed basal patches in, for example, the wet meadows in the Sierraville region and at Goose Lake, and perhaps the widespread occurrence of dark ochre butterflies in *ampelos* populations generally. Although this hypothesis is not directly testable, predictions about habitat requirements could be tested directly, and compared with palynological data on past distributions of habitat associates (perhaps even host plants), to provide an indirect test. The phenotypic similarity between *eryngii* and the northeastern Nevada subspecies *elko* could perhaps be explained by a similar scenario.

Neither the phenotypic population structure, using the morphological characters originally provided by Brown (1955), nor the genetic population structure reported here, support the hypothesis that species boundaries exist in the *tullia* complex in the region we studied. The striking lack of electrophoretic differences among *california*, *eryngii*, *ampelos*, and *eunomia*

suggests that the wing patterns of these subspecies are not good predictors of genetic (or phylogenetic) relationships. The geographic ranges of "diagnostic" wing pattern characters appear to overlap independently except where they are confined by gaps in the distribution of the species (e.g., in the high elevations of the Sierra Nevada Range, or between Woodfords and Mono Lake). Given the recency of the climatic events, the occurrence of *mono* characters in *ampelos* populations, and the maintenance of both high genetic variability and similarity to other taxa at Mono Lake, it appears unlikely that the taxon *mono* has achieved reproductive isolation. The distribution pattern is more consistent with primary rather than secondary contact between taxa.

The genetic population structure, characterized by high intrapopulation variability and low geographic differentiation, is very similar to what is found in highly vagile, colonizing species such as *Pontia protodice*, *Pontia occidentalis*, *Pieris rapae*, and even *Danaus plexippus* (Shapiro and Geiger 1986; Vawter and Brussard 1983; Eanes and Koehn 1978). *Pontia protodice* maintains geographic variation in clearly adaptive diapause characteristics despite otherwise high levels of gene flow (Shapiro and Geiger 1986). The possibility that wing ground color and numbers of hind wing eyespots have adaptive relevance should be investigated in *Coenonympha*. For example, the dorsal surfaces of the wings are exposed only during flight, and ground color could be used as a stimulus for release of courtship behaviors. Different numbers of hind wing eyespots are known to correlate with different male mating behaviors in the satyrine butterfly *Pararge aegeria* (L.) in Europe (Shreeve 1987). We do not mean to imply that *tullia* must be a highly vagile animal; past range fluctuations associated with climatic changes may have been sufficient for genetic homogenization if the population sizes have remained large.

Taxonomy

The data on phenotypic population structure indicate an important problem concerning the definition of the name *ampelos*. According to Brown (1964), it is most likely that the type specimen of *ampelos* came from southeastern Oregon, near the California border. This is very close to the Goose Lake locality, which shows a very broad spectrum of phenotypes consistent with introgression from the west. If nominate *ampelos* came from an intergrade population, it explains much of the taxonomic difficulty associated with the name. Nevertheless, in this paper we have treated the name *ampelos* as phenotypically pure for the purposes of discussion of geographic variation and speciation. An in-depth study supported by large sample sizes is needed in southeastern Oregon and northwestern Nevada to provide an adequate definition of *ampelos*.

Davenport (1941) synonomized the pale *elko* Edwards with *ampelos* on the basis of pale specimens in a series from Portola. Austin (1985) restricts the name *elko* to the predominantly pale populations in the eastern Humboldt River drainage in northeastern Nevada. Portola is well separated from the range of *elko* by intervening populations without pale phenotypes. Rather, the occurrence of pale (color = 2) butterflies, and individuals with high numbers of eyespots, in the Feather River region (including population 2 at Portola) suggests gene flow from *california* from the west through this low-elevation corridor. A transect to look for clinal variation along the Feather River west of Sloat could test this hypothesis.

The oldest name available for the holarctic species is *tullia*, which applies to a European taxon (Davenport 1941). Based on Davenport's statement that there are no discernible morphological differences between Alaskan and eastern Siberian populations, there is currently no reason to doubt that the North American and Eurasian subspecies are conspecific. On the evidence reviewed and presented here, there is currently no indication that more than one species exists in the North American *tullia*-group taxa: the species is not the appropriate taxonomic level for the classification of geographic variation within this group. Even though the oldest valid name for North America is *california* Westwood, the species name *tullia* should be retained for the North American subspecies unless evidence is found to support their separation.

Acknowledgements

The electrophoresis was carried out in F. J. Ayala's laboratory; we thank him and the members of his laboratory for advice and hospitality. Collecting was supported in part by a Jastro-Shields Research Scholarship and a Graduate Research Award to A.H.P. from the University of California—Davis. D. Olson, K. N. Jones, and A. M. Shapiro also provided live specimens. C. Fox helped run many of the gels. S. Karl provided technical advice; computer time was funded in part by the Zoology Department. E. M. Jakob provided access to a personal computer which greatly aided in the preparation of this manuscript. A.H.P.'s work was supported in part by a research scholarship from the Committee on Population Biology at the University of California—Davis. H.J.G.'s work at Davis was supported by National Science Foundation grant BSR-8306922 to A. M. Shapiro. A. M. Shapiro funded the electrophoresis through California Agricultural Experiment Station project CA-D*-AZO-3994-H, *Climatic Range Limitation of Phytophagous Lepidopterans*, and gave advice and encouragement throughout. A. M. Shapiro, S. O. Mattoon, and an anonymous reviewer provided comments or information that clarified this manuscript considerably.

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