

# ADAPTIVE DIFFERENTIATION WITH LITTLE GENIC CHANGE BETWEEN TWO NATIVE CALIFORNIA MINNOWS

JOHN C. AVISE, JERRY J. SMITH[1], AND FRANCISCO J. AYALA
*Department of Genetics and [1]Department of Wildlife and Fisheries Biology*
*University of California, Davis, California 95616*

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Recent studies of protein variation using electrophoretic techniques have provided estimates of two parameters of paramount importance in evolutionary theory: (1) the amount of genic variability in natural populations, and (2) the amount of genic differentiation between populations. Two generalizations have emerged from these studies: (1) the amount of genic variation in sexually reproducing, outcrossing species is far higher than had been anticipated according to some models of population structure (reviews in Selander and Kaufman, 1973; Lewontin, 1974), and (2) the amount of genic differentiation between local populations of a species is usually small relative to that between populations of different species (review in Avise, 1974). This second observation is relevant to the data presented in this paper.

A variety of statistics have been devised to measure genetic similarity (or its converse, genetic differentiation) between populations. However, different methods generally give similar numerical values when applied to the same data. As a rule measures of genetic similarity range from 1 (= complete similarity, i.e., the same alleles and in the same frequencies exist in the two populations compared) to 0 (= complete differentiation, i.e., the two populations share no alleles). If a number of gene loci are studied, the values of genetic similarity are averaged over loci. We have surveyed the literature, and found 651 pairwise comparisons between closely related species, each comparison involving 14 or more gene loci (Fig. 1). A total of 615, or 94%, of all pairwise comparisons give genetic similarities no greater than 0.90. Typically the similarities in a group of congeneric species range from 0.30 to 0.80, while the mean usually lies between 0.50 and 0.60. (The lower histogram in Fig. 1 has its mode between 0.10 and 0.20 because it includes a large number of comparisons between the relatively distantly related species of the *Drosophila obscura* and *affinis* subgroups.) This low degree of genic similarity is also observed between species that by other criteria appear very similar, such as sibling species (Johnson and Selander, 1971; Ayala et al., 1974), or hybridizing species (Avise and Smith, 1974). Typical results are shown in Figure 2. In contrast, conspecific populations have similarity values very rarely smaller than 0.80, and generally greater than 0.90. These results suggest that speciation may usually be accompanied by substantial genic differentiation.

We report here results that, to a certain extent, disagree with those just summarized. We have studied allozyme variation at 24 loci in two presumed species of California minnows, *Hesperoleucus symmetricus* and *Lavinia exilicauda*. Most populations of the two species cannot be distinguished at 23 of the loci, while at a single locus the two species are fixed for alternate alleles. Within the Pajaro River system in Central California, where the two species are sympatric, they are polymorphic for alleles at that locus, and the allele frequencies are associated with environmental variables.

## MATERIALS AND METHODS

*Populations studied.* The populations of hitch (*Lavinia exilicauda*) and roach

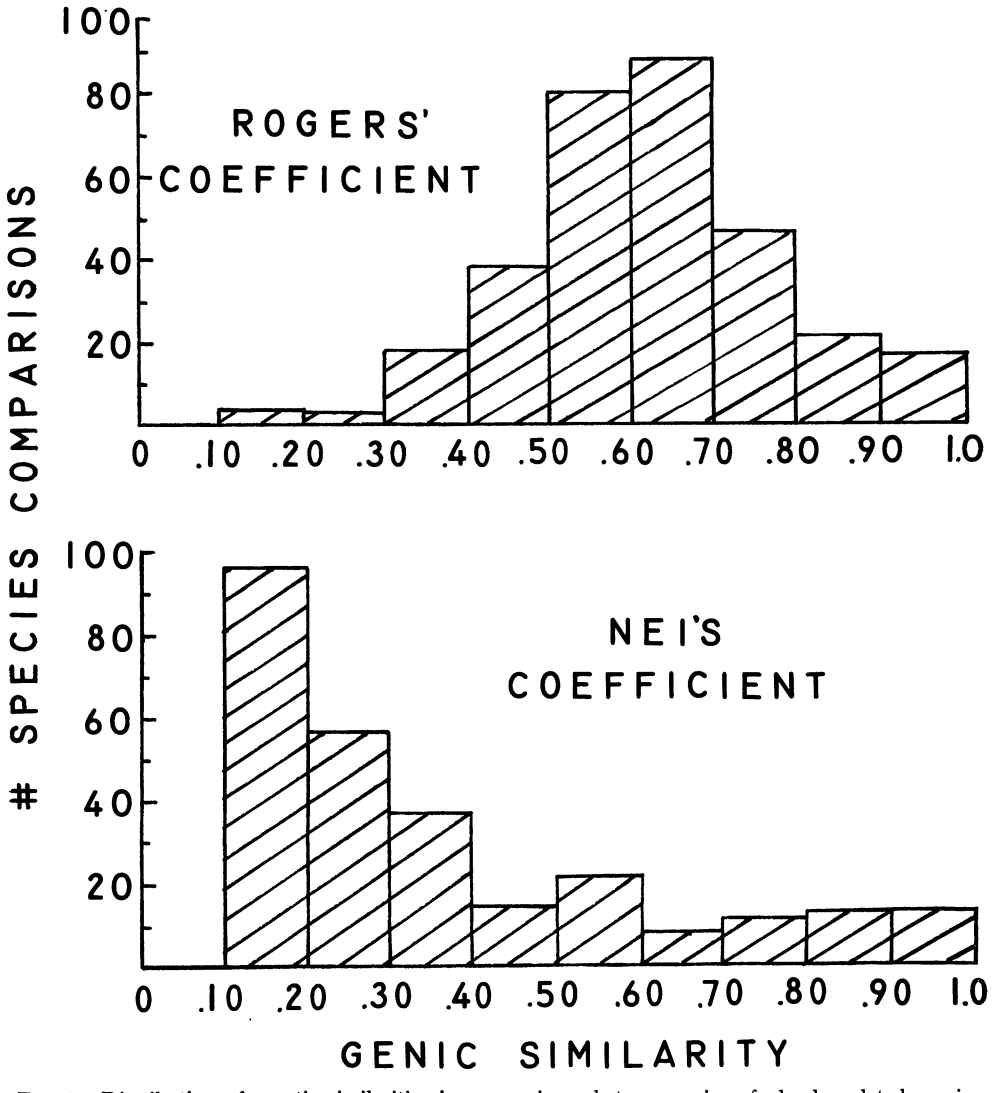


FIG. 1. Distribution of genetic similarities in comparisons between pairs of closely related species. *Upper histogram*: 313 pairwise comparisons using Rogers' (1972) coefficient of similarity (data from Avise et al., 1974*a*, *b*; Avise and Smith, 1974; Hall and Selander, 1973; Johnson and Selander, 1971; Johnson et al., 1972; Kim, 1972; Nevo and Shaw, 1972; Nair et al., 1971; Patton et al., 1972; Webster et al., 1972; Yang et al., 1972). *Lower histogram*: 273 pairwise comparisons using Nei's (1972) coefficient of identity (data from Avise and Ayala, 1975; Ayala et al., 1974; Hedgecock and Ayala, 1974; Lakovaara et al., 1972*a*, *b*; Nevo et al., 1974; Zouros, 1973). The following studies are not included in the histograms because they use other indices of similarity: Hubby and Throckmorton, 1968; Turner, 1974; Utter et al., 1973).

(*Hesperoleucus symmetricus*) sampled for this study are listed in Table 1 (see also Fig. 3). Because evolutionary relationships of populations within and among these taxa are controversial, we include a brief description of their past and present taxonomic status.

*Hitch populations.* On the basis of differences in fin ray counts Snyder (1913) described two species of *Lavinia, exilicauda*

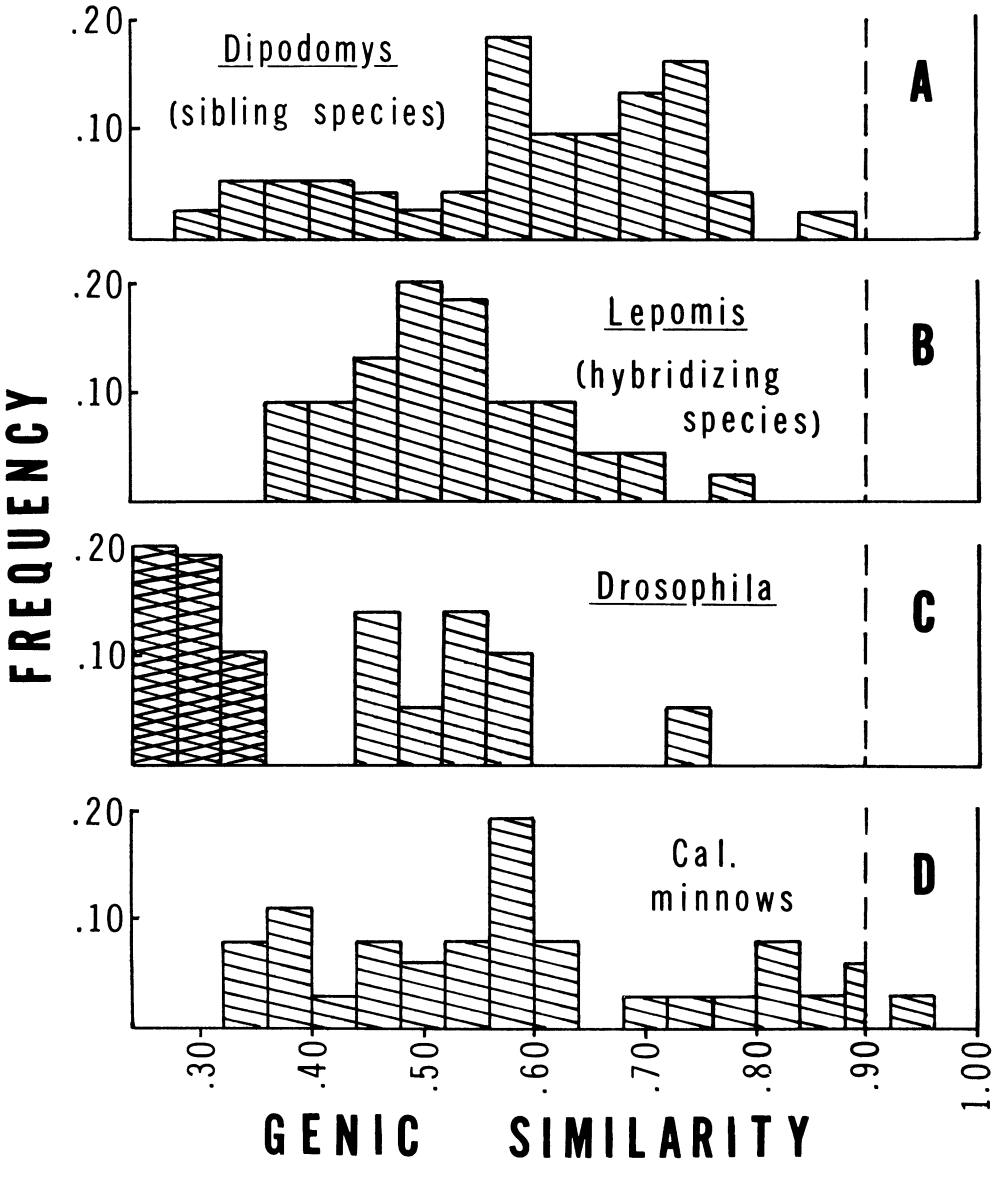


FIG. 2. Percentages of pair-wise species comparisons within a given range of genic similarities in the following species groups: A) species of the kangaroo rats, *Dipodomys* (after Johnson and Selander, 1971); B) hybridizing species of the sunfish, *Lepomis* (after Avise and Smith, 1974*b*); C) sibling (hatched bars) and nonsibling species (crosshatched bars) of the *Drosophila willistoni* group (after Ayala et al., 1974); and D) California minnows belonging to nine genera (Avise and Ayala, in preparation). The coefficients of similarity are calculated according to Rogers' (1972) method in A and B, and according to Nei's (1972) method in C and D.

from the Sacramento-San Joaquin river system, and *ardesiaca* from the Pajaro-Salinas system. Miller (1945) reexamined material from the Pajaro-Salinas system and argued that *ardesiaca* (synonymous with *harengus*) warranted only subspecific separation from *exilicauda*. Hopkirk (1973) described an additional subspecies

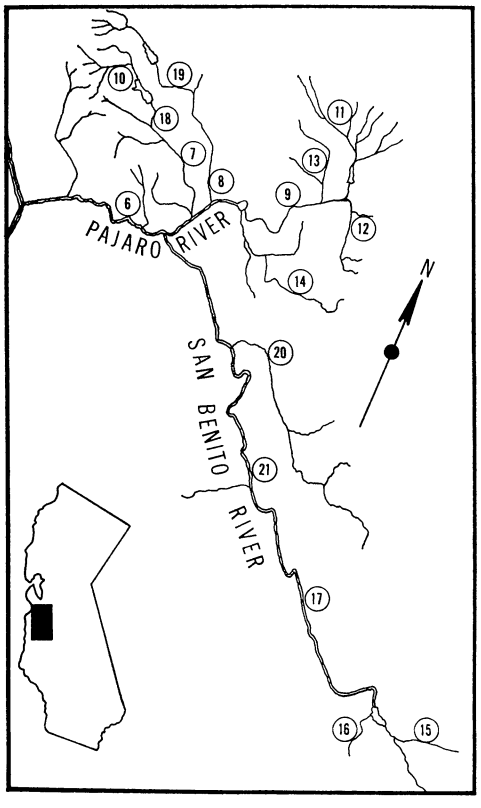


FIG. 3. Sample localities for collections of *Lavinia* and *Hesperoleucus* within the Pajaro drainage. Locality numbers as in Table 1.

of *Lavinia*, *L. e. chi*, inhabiting Clear Lake. We have examined populations of each subspecies (*L. e. exilicauda*, sample 2; *L. e. harengus*, samples 6–9, 18–21; *L. e. chi*, sample 1). As discussed below, these forms are nearly indistinguishable in allelic composition at 24 loci, and hence the biochemical data are compatible with the conclusion that the populations are conspecific.

*Roach populations.* *Hesperoleucus* populations are native to the Sacramento-San Joaquin drainage system and the coastal streams formerly connected with it. Snyder (1913) described six species, largely on the basis of differences in fin ray counts and on the geographic isolation of the populations in different drainages. Murphy (1948) concluded that the morphological differences were minor, and suggested retaining only subspecific designation for the forms. We have examined populations of *H. symmetricus venustus* (samples 4, 5) and *H. s. subditus* (samples 10–21), and found them to be closely similar, showing only minor allele frequency differences at a few loci.

*Laboratory techniques.* The horizontal starch gel electrophoretic procedures employed in this study are described by Ayala et al. (1972). A total of 12 enzymes encoded by 19 loci plus general proteins encoded by 5 loci were consistently scored. Table 2 lists the proteins assayed, and the buffer systems and tissues used.

The histochemical assays for each of the enzymes are described by Ayala et al. (1972), with the following exceptions or changes: *6-phosphogluconate dehydrogenase-1*: 10 mg. NBT, 10 mg. TPN, 200 mg. $MgCl_2$, and 80 mg. phosphogluconic acid in 100 ml. 0.05 M Tris-HCl, pH 8.0. After one hour add 5 mg. PMS. *Phosphoglucose isomerase-1, 2*: 20 mg. NBT, 10 mg. TPN, 200 mg. $MgCl_2$, 20 mg. fructose-6-phosphate, 25 mg. EDTA, and 80 units glucose-6-phosphate dehydrogenase in 100 ml. 0.1 M Tris-HCl, pH 7.1. After one hour add 5 mg. PMS. *Esterase-1, 2, 3*: 60 mg. fast blue RR and 1.5 ml. $\alpha$-napthyl proprionate in 100 ml. 0.1 M phosphate buffer, pH 6.5. *Glutamate oxalate transaminase-1, 2*: 150 mg. fast blue BB, 10 mg. pyroxidol 5'phosphate, 200 mg. aspartic acid, and 100 mg. $\alpha$-ketoglutaric acid in 100 ml. 0.05 M Tris-HCl, pH 8.0. *Lactate dehydrogenase-1, 2*: 20 mg. NBT, 25 mg. NAD, and 10 ml. 1 M DL-lactate, pH 7.0, in 100 ml. 0.5 M Tris-HCl, pH 7.1. After one hour add 5 mg. PMS.

All gels were made with Electro-starch (Otto Hiller, Madison, Wisconsin) at 12.5% concentration. Two buffer systems (Table 2) were found to provide adequate resolution for all enzymes: (A), electrode buffer: 300mM boric acid and 60mM NaOH, pH 8.1; gel buffer: 76mM Tris and 5mM citric acid, pH 8.65. (C), electrode buffer: 135mM Tris, 45mM citric

TABLE 1. *Sample number, sample size, locality, morphology and frequency of the* Pt-$2^F$ *allele in populations of* Lavinia *and* Hesperoleucus *from California. Sample numbers 6–21 correspond to those in Figure 1.*

| Locality | Sample number | Morphological appearance* | Number of individuals sampled | Number of loci sampled | Freq. *Pt-2*$^F$ ±95% conf. limit |
|---|---|---|---|---|---|
| Clear Lake, Lake County, north of Kelseyville | 1 | H | 18 | 24 | 1.00 |
| Bass Lake, Madera County, east of Merced | 2 | H | 48 | 24 | 1.00 |
| Coyote Creek, Santa Clara County, SE of San Jose | 3 | H | 26 | 12 | 0.92±0.07 |
| Russian River, Mendocino County, east of Hopland | 4 | R | 32 | 24 | 0.00 |
| Bear Creek, Lake County, east of Clearlake Oaks | 5 | R | 38 | 19 | 0.00 |
| Pajaro River System, San Benito, Santa Clara, Monterey Counties | | | | | |
| Pajaro River | 6 | H | 69 | 12 | 0.64±0.08 |
| Uvas Creek | 7 | H | 21 | 21 | 0.40±0.15 |
| Llagas Creek | 8 | H | 29 | 21 | 0.71±0.12 |
| Pacheco Creek | 9 | H | 68 | 12 | 0.62±0.08 |
| Uvas Creek | 10 | R | 22 | 21 | 0.04±0.06 |
| Pacheco Creek, North Fork | 11 | R | 19 | 12 | 0.05±0.07 |
| Pacheco Creek, South Fork | 12 | R | 10 | 12 | 0.00 |
| Cedar Creek | 13 | R | 20 | 12 | 0.00 |
| Dos Picachos Creek | 14 | R | 17 | 12 | 0.00 |
| Clear Creek | 15 | R | 12 | 12 | 0.00 |
| Laguna Creek | 16 | R | 11 | 12 | 0.00 |
| San Benito River | 17 | R | 15 | 12 | 0.00 |
| Uvas Creek | 18 | H, R, H×R | 126 | 21 | 0.51±0.06 |
| Llagas Creek | 19 | H, R, H×R | 37 | 21 | 0.34±0.11 |
| Tres Pinos Creek | 20 | H, R, H×R | 50 | 12 | 0.38±0.10 |
| San Benito River | 21 | H, R | 37 | 12 | 0.46±0.11 |

* H = hitch; R = roach; H × R = possible hybrids; morphological types based on overall appearance and counts of anal and dorsal rays.

acid, 1.3mM EDTA, pH 7.0; gel buffer: 9mM Tris, 3mM citric acid, 0.08mM EDTA, pH 7.0. These buffers are the same as in Ayala et al. (1972), except that EDTA has been added to the (C) buffers.

### ALLOZYME ANALYSIS

Many of the proteins assayed in this study are now routinely scored in populations of many vertebrates, including fish. The zymogram patterns of proteins of *Hesperoleucus* and *Lavinia* are similar to those described in characid fish (Avise and Selander, 1972), sunfish (Avise and Smith, 1974*a*, *b*), salmonids (Utter et al., 1974), and pupfish (Turner, 1974), and will be discussed in detail elsewhere. We have found most of these proteins to band with exceptional clarity not only in *Hesperoleucus* and *Lavinia*, but also in many other minnows native to California (Avise and Ayala, in prep.).

Less than 24 loci were scored in some populations listed in Table 1. In the populations where all loci were examined, heterozygosity estimates (mean percent of individuals heterozygous per locus, $\overline{H}$) are very similar to the average heterozygosity value (5.8%) calculated by Selander and Kaufman (1973) for all vertebrates previously studied. The observed heterozygosity in Clear Lake hitch (sample 1) is 4.9 ± 2.5%; most of the heterozygosity is due to segregation of two alleles at each of four loci (*Pgi-1*, *Est-1*, *Pgm-1*, and *Ipo-1*). The Bass Lake hitch (sample 2) show $\overline{H}$ = 5.7 ± 2.8%, again largely due to polymorphism at four loci (*Ldh-1*, *Pgi-2*, *Pgm-1*, and *Ipo-1*), two of which are different from the polymorphic loci in Clear Lake.

Mean heterozygosity in the roach sample where all 24 loci were examined (sample 4) is 6.8 ± 2.6%. The following loci were polymorphic: *Ldh-1*, *Ldh-2*, *Gpd-1*,

TABLE 2. *Proteins assayed, tissues examined, and buffer systems used in a study of* Lavinia *and* Hesperoleucus.

| Protein | Symbol | Tissue[1] | | Best buffer[2] |
|---|---|---|---|---|
| | | Liver | Skeletal muscle | |
| *6-Phosphogluconate dehydrogenase* | *Pgd-1* | ++ | — | C |
| *Triosephosphate isomerase* | *Tpi-1* | + | ++ | C |
| *Lactate dehydrogenase-1* | *Ldh-1* | + | ++ | C |
| *Lactate dehydrogenase-2* | *Ldh-2* | + | ++ | C |
| *Malate dehydrogenase-1* | *Mdh-1* | ++ | ++ | C |
| *Malate dehydrogenase-2* | *Mdh-2* | ++ | ++ | C |
| *Malate dehydrogenase-3* | *Mdh-3* | — | ++ | C |
| *Isocitrate dehydrogenase-2* | *Idh-2* | — | ++ | C |
| *α-Glycerophosphate dehydrogenase-1* | *Gpd-1* | ++ | ++ | C |
| *Phosphoglucose isomerase-1* | *Pgi-1* | ++ | + | C |
| *Phosphoglucose isomerase-2* | *Pgi-2* | + | ++ | C |
| *Phosphoglucomutase-1* | *Pgm-1* | ++ | ++ | C |
| *Indophenol oxidase-1* | *Ipo-1* | ++ | + | A |
| *Esterase-1* | *Est-1* | ++ | + | A |
| *Esterase-2* | *Est-2* | ++ | ++ | A |
| *Esterase-3* | *Est-3* | ++ | + | A |
| *Glutamate oxalate transaminase-1* | *Got-1* | + | — | A |
| *Glutamate oxalate transaminase-2* | *Got-2* | ++ | + | A |
| *Alcohol dehydrogenase-1* | *Adh-1* | ++ | — | C |
| *Protein-0* | *Pt-0* | — | ++ | A |
| *Protein-1* | *Pt-1* | — | ++ | A or C |
| *Protein-2* | *Pt-2* | — | ++ | A or C |
| *Protein-3* | *Pt-3* | — | ++ | A or C |
| *Protein-4* | *Pt-4* | — | ++ | A or C |

[1] ++, strong activity, easy to score; +, weak activity, difficult to score; —, no activity.
[2] See Materials and Methods.

*Pgi-2*, *Ipo-1*, and *Est-2*, while one or two specimens were heterozygous for *Tpi-1*, *Pgm-1*, *Mdh-2*, and *Got-2*.

To quantify the genetic divergence between *Hesperoleucus* and *Lavinia*, we have employed the identity statistic developed by Nei (1972). The normalized genetic identity of genes between two populations at the $j$ locus is defined as

$$I_j = (\Sigma x_i y_i)/\sqrt{\Sigma x_i^2 \cdot \Sigma y_i^2},$$

where $x_i$ and $y_i$ are the frequencies of the $i$ allele in populations $X$ and $Y$, respectively. $I$ values may range from 0 (when populations share no alleles) to 1 (when all alleles are present in identical frequencies). The distribution of $I$ over loci in the comparison between *Hesperoleucus* (sample 4) and *Lavinia* (sample 2) is presented in Figure 4. At 23 of 24 loci, $I$ values are $\geqslant 0.85$, while at a single locus, $I = 0$. The normalized identity of genes between $X$ and $Y$ when all loci are considered is defined as $I = J_{xy}/\sqrt{J_x J_y}$, where $J_x$, $J_y$, and $J_{xy}$ are the arithmetic means over all loci of $\Sigma x_i^2$, $\Sigma y_i^2$, and $\Sigma x_i y_i$, respectively. In the comparison between *Hesperoleucus* and *Lavinia*, $I = 0.948 \pm 0.042$. This high level of biochemical similarity is unusual in interspecies comparisons.

Nei has defined genetic distance as $D = -\ln I$. $D$ estimates the accumulated number of codon substitutions per locus since the time of divergence of two populations. Between *Hesperoleucus* and *Lavinia*, $D = 0.053$. Based on our sample of 24 loci, populations of hitch and roach show only about one codon substitution per 20 loci.

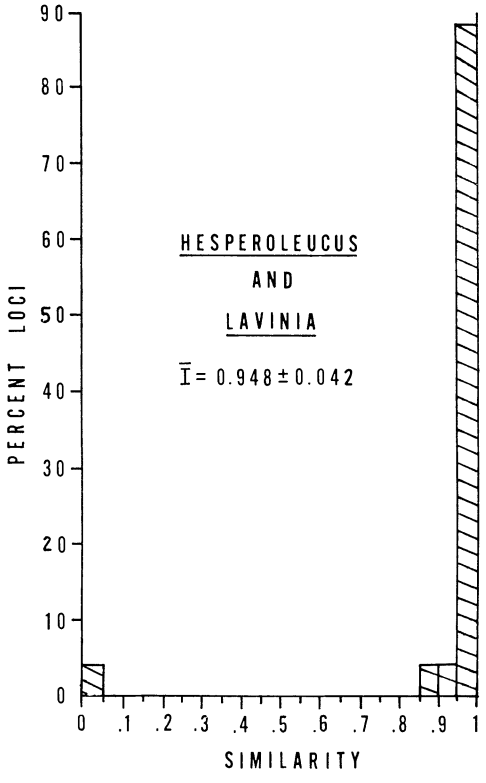


FIG. 4. Percent loci within a given range of genic similarity in the comparison between *Hesperoleucus* (sample 4) and *Lavinia* (sample 2). The total number of loci studied is 24.

## SPECIFIC STATUS OF *HESPEROLEUCUS* AND *LAVINIA*

The close genic similarity between hitch and roach populations is consistent with the notion that they are conspecific. Nonetheless, several lines of evidence indicate that despite their biochemical affinity, *Hesperoleucus* and *Lavinia* represent good biological species.

*Biochemical evidence.* Individuals collected outside the Pajaro River system may be correctly assigned as hitch or roach with greater than 99% accuracy on the basis of genotypes at a single locus, *Pt-2* (Table 1). *Lavinia* populations are monomorphic or nearly so for the $Pt\text{-}1^F$ allele, and *Hesperoleucus* populations are monomorphic for $Pt\text{-}2^S$. The Coyote Creek population of hitch is polymorphic, but 99.4% of the individuals carry the $Pt\text{-}2^F$ allele either in homozygous or heterozygous condition. However, within the Pajaro system, populations with hitch phenotypes (6–9, Table 1) as well as populations of mixed hitch and roach morphologies (18–21, Table 1), are polymorphic for $Pt\text{-}2^F$ and $Pt\text{-}2^S$ (Table 1; see also Figure 5).

Hitch and roach morphologies are both present in collections 18–21. These populations are also highly polymorphic for the *F* and *S* alleles of *Pt-2*. However, the allelic frequencies are nonrandomly associated ($P < 0.005$) with the numbers of dorsal

TABLE 3. *Nonrandom association of dorsal ray counts with allele frequencies at the* Pt-2 *locus in four collections with hitch and roach morphs. Collections numbered according to Table 1.*

| Population number | Contingency $G$ | d.f. | $P$ |
|---|---|---|---|
| 18 | 178.7 | 1 | $< .005$ |
| 19 | 16.5 | 1 | $< .005$ |
| 20 | 18.7 | 1 | $< .005$ |
| 21 | 43.8 | 1 | $< .005$ |

*Example: Population 18*

| Number of dorsal rays | Number of *Pt-2* alleles | |
|---|---|---|
| | *F* | *S* |
| $\leqslant 9$ | 8 | 46 |
| $\geqslant 10$ | 121 | 77 |

TABLE 4. *Nonrandom association of anal ray counts with allele frequencies at the* Pt-2 *locus in four collections with hitch and roach morphs. Collections numbered according to Table 1.*

| Population number | Contingency $G$ | d.f. | $P$ |
|---|---|---|---|
| 18 | 37.8 | 2 | $< .005$ |
| 19 | 31.2 | 2 | $< .005$ |
| 20 | 37.2 | 2 | $< .005$ |
| 21 | 38.7 | 2 | $< .005$ |

*Example: Population 18*

| Number of anal rays | Number of *Pt-2* alleles | |
|---|---|---|
| | *F* | *S* |
| $\leqslant 8$ | 4 | 28 |
| 9–10 | 19 | 35 |
| $\geqslant 11$ | 106 | 60 |

TABLE 5. *Observed and expected genotype proportions at the* Pt-2 *locus in four populations with hitch and roach morphs. Populations are numbered according to Table 1.*

| Population number | Observed | (Expected*) | Genotypes | Chi square | $P$ |
|---|---|---|---|---|---|
| | *F/F* | *F/S* | *S/S* | | |
| 18 | 39(32.9) | 51(63.2) | 36(29.9) | 4.74 | $.05 > P > .025$ |
| 19 | 4(4.1) | 17(16.8) | 16(16.1) | 0.01 | $.975 > P > .90$ |
| 20 | 12(7.1) | 14(23.8) | 24(19.1) | 8.67 | $P < .005$ |
| 21 | 12(7.7) | 10(18.6) | 15(10.7) | 8.16 | $P < .005$ |

* Calculated using Levene's (1949) formula for small samples, on the assumption of random mating equilibrium.

rays and anal rays characteristic of hitch and roach morphologies (see Tables 3 and 4). Two distinct populations exist in each collection, one characterized by high numbers of anal and dorsal rays, high $Pt\text{-}2^F$ frequency, and general hitch morphology; the other characterized by low numbers of anal and dorsal rays, low frequency of $Pt\text{-}2^F$, and general roach morphology.

The occurrence of two distinct populations within each of the collections 18–21 is further confirmed by examination of the distribution of genotypic frequencies at the *Pt-2* locus. Three out of four populations have significantly fewer heterozygotes than would be expected on the assumption of Hardy-Weinberg equilibrium for random mating populations (Table 5). This deficiency is not likely to be due to selection against heterozygotes, since populations monomorphic for the hitch phenotype in the Pajaro system do not deviate significantly from the expected Hardy-Weinberg frequencies, despite their high level of polymorphism at the *Pt-2* locus (see populations 6–9 in Table 1). The deficiency of heterozygotes is also unlikely to be due to close inbreeding. Both hitch and roach are abundant in the Pajaro system, and even short sections of stream contain minimally hundreds of individuals. Furthermore, in both hitch and roach, spawning is a mass affair, each ripe female closely followed by one to five males who simultaneously fertilize the eggs immediately after release, with no territoriality or fighting (Moyle, 1974).

*Morphological evidence.* The morphological differences between hitch and roach are considerable. They involve a suite of apparently independent traits, and led to the original placement of hitch and roach in separate genera. Throughout most of its range, *L. exilicauda* may be distinguished from *H. symmetricus* by its more narrow caudal peduncle (hence the name *exilicauda*, slender tail; see Fig. 6), higher number of dorsal rays (10–13 *versus* 7–10 in *Hesperoleucus*; see Table 6), higher number of anal rays (10–14 *versus* 6–9 in *Hesperoleucus*; see Table 6), pharyngeal tooth formula (usually 0,5–5,0 *versus* 0,5–4,0 in *Hesperoleucus*; Scoppettone, 1974) and number of gill rakers (17–26 *versus* 7–11 in *Hesperoleucus* in the Pajaro River;

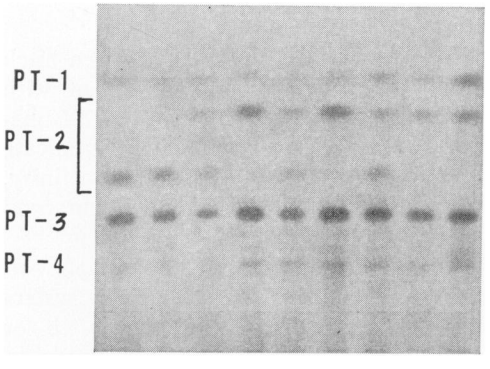


FIG. 5. Muscle proteins encoded by four genetic loci in a collection from a hybrid population of hitch and roach. Genotypes of *Pt-2* from left to right: 2 $Pt\text{-}2^S/Pt\text{-}2^S$; $Pt\text{-}2^F/Pt\text{-}2^S$; $Pt\text{-}2^F/Pt\text{-}2^F$; $Pt\text{-}2^F/Pt\text{-}2^S$; $Pt\text{-}2^F/Pt\text{-}2^F$; $Pt\text{-}2^F/Pt\text{-}2^S$; $2Pt\text{-}2^F/Pt\text{-}2^F$. *Pt-1*, *Pt-3*, and *Pt-4* are monomorphic.

TABLE 6. *Fin ray counts and allele frequencies at the* Pt-2 *locus in various collections of hitch and roach.*

| Populations | Mean±S.E. Anal rays | | Mean±S.E. Dorsal rays | | Mean±S.E. Total rays | | Freq. $Pt\text{-}2^F$ | |
|---|---|---|---|---|---|---|---|---|
| Roach: | | | | | | | | |
| Allopatric (outside Pajaro drainage)[1] | 7.62±0.11 | ** | 8.00±0.00 | ** | 15.62±0.11 | NS | 0.000 | ** |
| Allopatric (within Pajaro drainage) | 6.33±0.04 | ** | 8.10±0.03 | * | 15.43±0.06 | ** | 0.018 | ** |
| Sympatric (within Pajaro drainage) | 7.56±0.11 | | 8.21±0.05 | | 15.79±0.13 | | 0.078 | |
| Hitch: | | | | | | | | |
| Allopatric (outside Pajaro drainage) | 11.97±0.11 | ** | 10.76±0.09 | ** | 22.73±0.14 | ** | 0.953 | ** |
| Allopatric (within Pajaro drainage) | 11.57±0.04 | ** | 10.28±0.03 | ** | 21.85±0.07 | ** | 0.619 | NS |
| Sympatric (within Pajaro drainage) | 11.07±0.05 | | 10.14±0.03 | | 21.21±0.06 | | 0.628 | |
| Possible $F_1$ hybrids (within Pajaro drainage) | 9.52±0.15 | | 9.30±0.10 | | 18.82±0.18 | | 0.135 | |

** $P < .01$, one-tailed $t$-test.
* $.05 > P > .01$, one-tailed $t$-test.
[1] Sample from Bear Creek, subspecies *venustus*.

Miller, 1945). In addition, the body length/width ratio is usually greater in *Lavinia*, and body color is more silvery. Hitch may grow to over 35 cm total length, while adult roach are usually less than 10 cm. Morphological differences of this magnitude are not normally encountered between geographic races within other species of fishes. Unfortunately, the genetic basis of heritabilities of these traits are not known.

The size differences between adult hitch and roach may contribute to sexual isolation of the forms. In Clear Lake, most female hitch do not enter the streams for spawning until they are two years old and over 25 cm long (Murphy, 1948). For females of that size, crossing with male roach is unlikely. Male hitch, which mature as early as their first year, are usually 8 cm or larger (Kimsey, 1960; Moyle, 1974), and probably cross only infrequently with the smaller roach.

*Ecological evidence.* Throughout northern and central California *Lavinia* and *Hesperoleucus* are found in the same drainages. Hitch characteristically predominate in warm lakes and slow moving rivers at low elevations, while roach are most abundant in small, intermittent streams at moderate elevations. In many drainages there is little or no overlap in distribution. Within the Pajaro River system, hitch have been collected alone at 22 sites, roach at 20 sites, and both together at only 15 sites, evincing strong spatial segregation (Smith, in prep.). Even within short sections of stream (50–250 m) there is a striking association of hitch with deeper pools,

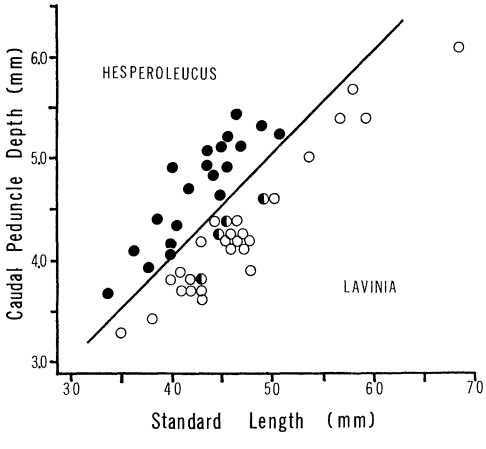


FIG. 6. Caudal peduncle depth *versus* standard length for roach (●), hitch (○), and presumed $F_1$ hybrids (◐) from site 20, Tres Pinos Creek.

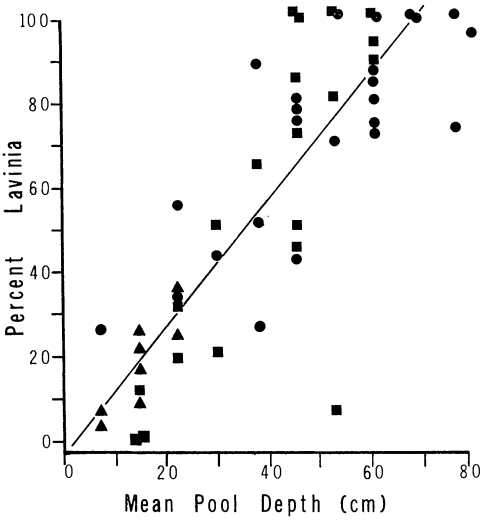


FIG. 7. Percent hitch of total hitch plus roach population *versus* mean depth of pool at the following localities: 18, Uvas Creek (●); 19, Llagus Creek (■), and 20, Tres Pinos Creek (▲). Only fish less than 70 mm standard length were used in analysis.

evincing behavioral habitat selection (Fig. 7). This association holds even for hitch and roach of comparable size (< 70 mm). As hitch continue to grow beyond this length, they become increasingly associated with deeper pools (Smith, in prep.).

The normal spatial segregation of hitch and roach insures that few interspecific matings will take place. Even where the forms are locally sympatric, strong ecological preferences strengthen reproductive isolating barriers through habitat isolation.

*Evidence of reproductive isolation.* In many drainages in California, morphologically intermediate forms between *Lavinia* and *Hesperoleucus* are rare or nonexistent. The complete absence of heterozygotes at the *Pt-2* locus in our limited collections outside the Pajaro system also indicates that hybridization is rare. However, in some collections within the Pajaro system (18–20, Table 1), up to 20% of specimens are difficult to classify as hitch or roach because morphological traits are intermediate to various degrees. Within these collections, as well as in other collections of morphologically "pure" hitch from the Pajaro (6–9, Table 1), up to nearly 50% of all specimens are heterozygotes at the *Pt-2* locus.

Miller (1945) argued that more than 50% of individuals from certain localities within the Pajaro "appear to have mixed blood" between *Hesperoleucus* and *Lavinia*, including perhaps some backcross individuals. From our collections within the Pajaro, 3% (23 out of 725) of all fish are considered probable $F_1$ hybrids on the basis of overall morphology. Assuming intermediate morphological traits, $F_1$ hybrids should show an average of 9.3 dorsal rays and 9.4 anal rays (Miller, 1945). Our presumed $F_1$ hybrids show ray counts of 9.3 and 9.5, respectively (Table 6).

Since populations of morphologically "pure" hitch from the Pajaro drainage are polymorphic for $Pt\text{-}2^F$ and $Pt\text{-}2^S$, the presence of heterozygotes at this locus does not necessarily demonstrate hybrid status. If the $Pt\text{-}2^S$ alleles now present in the hitch populations were originally derived through hybridization with roach, populations of hitch and roach within the Pajaro should show convergence in traits compared to populations outside the Pajaro. Unfortunately this is a weak prediction, since it assumes that in the absence of hybridization, means of morphological traits would not vary between drainages and furthermore that the only cause of convergence would be through gene exchange and not convergent selection pressures. Within the Pajaro, fin ray counts in hitch are significantly lower (show convergence to *Hesperoleucus*), but there is no good evidence for increase in fin ray counts for roach (see Table 6). In the absence of other evidence, it is not possible to decide whether the $Pt\text{-}2^S$ alleles in hitch populations within the Pajaro are derived from hybridization with roach. Locally allopatric roach within the Pajaro show little or no effects of hybridization or introgression with hitch, either in morphology or allele frequency at *Pt-2*. Also, another population of hitch from a different drainage

system where hybridization is not known to occur (locality 3, Table 1) has $Pt\text{-}2^S$ in low frequency. Thus a reasonable alternative explanation is that the polymorphism is present in hitch independently of any gene exchange with roach.

Three populations within the Pajaro (18–20, Table 1) contain $F_1$ hybrids between hitch and roach. There is also evidence of introgression beyond the $F_1$. Several specimens not classified as $F_1$ hybrids nonetheless were difficult to assign as hitch or roach because of somewhat intermediate morphological appearance. Moreover, excluding the presumed $F_1$ hybrids from consideration, in the populations where they occur sympatrically, hitch and roach converge in numbers of anal rays, dorsal rays, and total rays, and roach also show a significant increase in frequency of $Pt\text{-}2^F$ (see Table 6). Most individuals (87%) in these four sympatric populations still appear to be morphologically "pure" hitch and roach, 8% strictly intermediate in morphology and thus probable $F_1$ hybrids, and only 5% may represent backcross or $F_2$ and beyond generations.

In summary, hitch and roach are quite similar biochemically, and yet show considerable differentiation in morphological and ecological attributes. Populations of hitch and roach usually do not come into geographic contact, but where they do they still retain their identities for the most part. Therefore, *L. exilicauda* and *H. symmetricus* must be considered separate species, although they are able to hybridize and there is evidence of some introgression in sympatric populations.

## Discussion

Reports of very high biochemical similarity between species are unusual. Several hypotheses may be considered to explain the close genetic affinity of *Lavinia exilicauda* and *Hesperoleucus symmetricus*.

1) Close genetic similarity may be due to sampling accidents. Only a small number of loci are assayed in this or similar surveys, and the errors involved in estimating similarities may be large. In addition to *Hesperoleucus* and *Lavinia*, seven other species of California minnows have been assayed at 24 loci (Figure 2). The mean genetic similarity between the nine species is 0.59, and the variance 0.0310. Assume that all species of California minnows are equally similar, and that the observed variance is due to sampling errors. If the errors are normally distributed, we would expect that 4% of all species comparisons would yield $I \geq 0.90$. We have observed one comparison out of 36, or 2.7%, with this close similarity.

However, there is no *a priori* biological reason to suppose that all species of a group have achieved the same degree of genetic differentiation from each other. Evolution is a gradual process, involving changes in gene frequency largely mediated by natural selection. The degree of genetic differentiation between a pair of species need not converge to some mean value, but is likely to be due to a variety of factors, including time since divergence, population size, and environmental parameters. Observed biochemical similarities between a large variety of vertebrate and invertebrate species do not appear randomly distributed, but are almost invariably correlated with postulated relationships based on independently derived data from morphology, physiology, ecology, and zoogeography (review in Avise, 1974). Genic relationships between the California minnows also correlate well with their probable evolutionary relationships (Avise and Ayala, in prep.). Biochemical similarities appear to reflect properties of biological and evolutionary significance.

2) Close genetic similarity derives from inability to detect real differences. Because of the degeneracy of the genetic code, many nucleotide substitutions do not alter the amino acid sequence of polypeptides. Many amino acid substitutions do not alter net charge and hence are not detected electrophoretically. Finally, there is a finite number of distinguishable band mobilities on a gel, and some species may appear to

share alleles when they do not. These biases tend to overestimate the amount of genetic similarity between taxa.

However, the same biases apply to all studies of genetic similarity based on electrophoretic data. Yet interspecific comparisons generally show much less genetic similarity than what we have found between *Lavinia* and *Hesperoleucus*. Relative to previous reports, the degree of genetic differentiation between hitch and roach is typical for comparisons between populations of one species.

3) Close genic similarity may reflect extensive hybridization. Sufficient gene exchange between hitch and roach would tend to homogenize their allele frequencies. However, in our collections outside the Pajaro drainage, hybridization has not had a significant effect in altering frequencies of either biochemical or morphological traits. Within the Pajaro, hitch and roach hybridize but the extent of gene exchange is not sufficient to cause their merging into a single gene pool. The degree of hybridization between hitch and roach within the Pajaro is not rare among fishes. Many species of fish engage in considerable hybridization, and yet largely retain their identities throughout their ranges (Hubbs, 1955).

Nor is hybridization among species of fish necessarily indicative of close genic similarity. Eleven species of sunfish (genus *Lepomis*) are among the most renowned fish hybridizers, but they are completely distinct in allelic composition at an average of 47% of their loci (Avise and Smith, 1974). Moreover, other species of California minnows which occasionally hybridize, such as *Orthodon microlepidotus* with *Gila bicolor* (La Rivers, 1962) and *Richardsonius egregius* with *Gila bicolor* (Hopkirk and Behnke, 1966) are not genetically very similar ($I = 0.60$ and 0.64, respectively; Avise and Ayala, in prep.).

4) Close genic similarity is due to convergent selection pressures. This hypothesis postulates that natural selection causes convergence to similar allelic states at most loci in separate lineages. This is most unlikely. Hitch and roach are morphologically and ecologically quite distinct and most certainly experience different selective pressures. Furthermore, the many examples of analogous but non-homologous traits (that is, those exhibiting similar functions but different genetic basis) make it clear that there are many genetically different ways to respond to a common environmental challenge.

5) The close genetic similarity between *Hesperoleucus* and *Lavinia* reflects their recent speciation; there has been little time for accumulation of genetic change. Zoogeographic data are compatible with a relatively recent speciation between hitch and roach. Miller (1959) describes seven major drainage basins west of the continental divide in North America, each characterized by its own endemic fish fauna. The Sacramento-San Joaquin is the largest of these basins in California, and has maintained an effective isolation from surrounding drainages, as evidenced by the fact that 75% of its fish species are endemic. The Central Valley through which the Sacramento and San Joaquin Rivers flow was apparently formed more or less as it is today during the late Pliocene (Poland and Evanson, 1966; Wahrhaftig and Birman, 1965). Ancestors of modern day fishes probably invaded the Central Valley from the ancient Columbia River system to the north, when the Sierra Nevada mountains had been eroded to low hills. New mountain building at the beginning of the Quaternary separated these drainages again. Hitch and roach are endemic to the Sacramento-San Joaquin system; they most likely evolved from a common ancestor which entered the Central Valley before it was cut off by mountain building. The isolation of the Sacramento-San Joaquin and Columbia drainages thus places a probable upper limit on the time of speciation of hitch and roach at about 2–4 million years.

The Sacramento-San Joaquin basin includes at least seven distinct drainage re-

gions which have been mutually isolated to varying degrees (Moyle, 1974). Roach inhabit six of these regions: the Central Valley proper, Goose Lake, North coastal streams, Pit River, Clear Lake, and the Pajaro-Salinas system. Their widespread distribution is probably a function of their ecological preferences for small shallow streams coupled with a common means of interdrainage transfer—headwater capture (Murphy, 1948). Hitch are less widely distributed, being native to three of the regions: the Central Valley proper, Clear Lake, and the Pajaro-Salinas system.

Hitch and roach are thus common to two distinct drainage regions besides the Central Valley in central California. Since parallel speciation events are unlikely, hitch and roach must have split from a common ancestor before invading these other drainages. The Pajaro-Salinas system appears to have had two separate connections to the Sacramento-San Joaquin during the middle and late Pleistocene, and is now completely isolated. Although Clear Lake is now connected to the Sacramento River through Cache Creek, the steep gradient and deep valley of this connection make it presently impassable for most quiet water fish. Hopkirk (1973) strongly argues that Clear Lake itself is a small center of endemism in fishes, indicating effective isolation from the Sacramento for a considerable period of time. Therefore, most transfer of fish between these regions may have occurred in middle and early Pleistocene times, before tectonic activity raised the coast ranges and increased the gradient of Cache Creek.

It appears from these considerations that a lower estimate of time of speciation for hitch and roach may be as recent as late Pleistocene, or, if transfer of fish to Clear Lake preceded the elevation of the Clear Lake Valley, middle Pleistocene. In the absence of a fossil record for hitch and roach, further discussion of possible times of speciation of hitch and roach on the basis of geographic considerations becomes highly speculative.

Nei (1971) has developed a formula which estimates absolute divergence time of two populations using genetic distances. The formula is $t = D/2cn_t\lambda a$, where $t$ is the time since isolation of two populations, $c$ is the proportion of amino acid substitutions which are electrophoretically detectable, $n_t$ is the average number of amino acids per protein, and $\lambda a$ is the rate of amino acid substitutions per polypeptide per site per year. Before applying this formula, we note several reservations. Estimates of divergence times depend heavily upon several assumptions about protein structure and evolution. Estimates of $c$ range from about 0.25 to 0.88 (Bernstein et al., 1973), $n_t$ varies considerably among proteins, and estimates of $\lambda a$ vary 1500 fold from the fast evolving fibrinopeptides to some slowly evolving histones (Dayhoff, 1972). Since the product of these parameters appears in the denominator, even small changes in any of them may greatly alter $t$. Furthermore, the standard error of $D$ ( $= (1 - I/In_s)^{1/2}$, where $n_s$ is the number of proteins examined (Nei, 1971) between *Hesperoleucus* and *Lavinia* is large, $D = 0.053 \pm 0.48$.

With these reservations in mind, we may use the following figures to obtain a very crude approximation of possible time of divergence for *Hesperoleucus* and *Lavinia*: $\lambda a = 2.1 \times 10^{-9}$ (see Nei, 1971); $c = 0.30$; $n_t = 818$ (from 90,000/110, where 90,000 is the approximate average molecular weight of enzymes examined in this study, and 110 is the average molecular weight of an amino acid). These values yield $t =$ 51,400 years. Using the same estimates for $\lambda a$, $c$, and $n_t$, and $D$'s which differ nonsignificantly from 0.053, values of $t$ may range from a low of 0 years to a high of 145,000 years. Using other estimates of $c$, $n_t$, and $\lambda a$ would of course greatly alter divergence times. For example, with more conservative numbers for $c$, $n_t$, and $\lambda a$ (0.25, 360, and $2 \times 10^{-10}$, respectively), $t$ equals up to 4,140,000 years.

Levels of biochemical similarity $\geq 0.90$ between species have been occasionally

found in other animals. They are in fact typical for subterranean rodents of the genera *Spalax* and *Thomomys* (Nevo and Shaw, 1972; Nevo et al., 1974) where speciation has apparently occurred through recent and extensive remodeling of karyotypes with little time for genic divergence. Speciation times for five species of *Thomomys* were estimated from genic evidence at 121,000 ± 79,000 years, and these estimates are consistent with the fossil record (Nevo et al., 1974). Genic similarities between rodent species are generally much lower (see example in Figure 2). Avise et al. (1974) report similarities $\geq$ 0.90 between several described species of *Peromyscus*, most of which are restricted to islands in the Gulf of California, but their specific status is subject to question.

Close genic similarity has also been reported between several species of Death Valley pupfish which may have undergone rapid post-Pleistocene evolution (Turner, 1974). These pupfish exhibit considerable morphological and ecological differentiation with little genic change. Turner (1974) suggests that an invariant portion of the genome encodes a strongly coadapted, homeostatic, metabolic core of enzymes, and a variant portion encodes certain morphological and physiological characters which are responsive to environmental selection pressures. Since most of the pupfish occupy disjunct habitats, are allopatric, and are interfertile in laboratory crosses, the specific status of some species may be questioned. But their close genic similarity appears to be due, in any case, to recent separation.

*Genetic differentiation and speciation.* Mayr (1963) has stressed that species are not characterized by a given number of counted gene differences, but rather by the totality of physiological and developmental interactions leading ultimately to patterns of reproductive isolation which determine species status. Nevertheless, Mayr (1963), Dobzhansky (1959), and others have argued that reproductive isolation normally requires major genetic changes. This has stimulated interest in quantifying the amount of genic differentiation involved in speciation (Hubby and Throckmorton, 1965, 1968; Selander et al., 1969; Ayala et al., 1970, 1974; Hedgecock and Ayala, 1974; Nevo et al., 1974).

It is unlikely that all speciation events will involve the same amount of genetic change. Even when new species arise according to the general model of geographic speciation, the amount of genetic change involved may vary from one case to another. It is indeed possible that, at least occasionally, significant adaptive divergence eventually leading to reproductive isolation may be forged out of relatively few genetic changes. This appears to be the case with *Hesperoleucus* and *Lavinia*. Conversely, considerable allelic differentiation may occasionally occur between conspecific populations without leading to reproductive isolation and speciation. Hitch and roach exhibit fewer genetic differences than do many conspecific populations of other organisms.

One reason genetic divergence between hitch and roach is low may be their recent speciation. This raises the question of how much of the genetic differentiation observed between species preceded their reproductive isolation, and how much took place after speciation was completed. The question, "how much genetic differentiation accompanies the process of speciation?" can be most appropriately answered by studying groups of populations at the stage when reproductive isolation, and thus speciation, is being completed. Not surprisingly, such studies are rare. A well documented study of the various stages of the speciation process involves the *Drosophila willistoni* group of species (Ayala et al., 1974). In that group, between 20 and 25 electrophoretically detectable allelic substitutions per 100 loci have taken place before the completion of the speciation process. Only a fraction of that amount of genetic change seems to have occurred in the speciation of *Lavinia* and *Hesperoleucus*.

## Summary

We have studied allelic variation at 24 loci coding for soluble proteins in two presumed species of California minnows—*Hesperoleucus symmetricus* and *Lavinia exilicauda*. Our estimates indicate that they differ at one electrophoretically detectable codon substitution for every 20 loci. This small amount of genetic divergence is unusual in interspecies comparisons. Nonetheless, there is evidence that *Hesperoleucus* and *Lavinia* are different species. They are considerably different in morphological and ecological attributes, and exhibit strong prezygotic isolating barriers. Their distinctness is largely retained also where they are sympatric.

The close genic similarity between *Hesperoleucus* and *Lavinia* is due in part to their recent separation from a common ancestor, perhaps during the middle or late Pleistocene. In any case, significant adaptive differentiation leading to speciation has occurred within the context of relatively few genic changes.

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