The Journal of Heredity 67:258–266. 1976.

New studies of the alcohol dehydrogenase cline in *D. melanogaster* from Mexico

S. B. Pipkin, E. Franklin-Springer, S. Law, and S. Lubega

THE discovery of extensive protein polymorphisms in *Drosophila pseudoobscura* by Lewontin and Hubby[21], supports the hypothesis that these depend on alleles with individually small selective values[20]. Nevertheless, allozymes, i.e., alternate forms of an enzyme specified by alleles, often exhibit different properties, providing variation on which natural selection may act. For example, the two common alcohol dehydrogenase (ADH) allozymes of *D. melanogaster* differ in specific activity[9,15,27,30], heat stability[10,17,38], catalytic efficiency, substrate specificity, and influence of pH[6,38].

Temperature related clines of *Adh* alleles have been reported from three widely separated parts of the world: Russia[13], the United States[38], and Mexico[28,29]. In cooler regions there is a substantial frequency (0.32 or higher) of Adh^{I}, whereas the frequency of Adh^{II} always increases in the warmer parts of the clines. In Mexico, frequencies of Adh^{II} vary from 0.38 to 0.68 in the Central Highlands above 5000 feet, whereas Adh^{II} has a frequency of 0.90 or more in sea level populations of the Isthmus of Tehauntepec and eastward.

Clines may be generated by a combination of the action of selection and migration[22]. Studies of the 1972 collection of *D. melanogaster* strains in Mexico suggested that gene exchange between highland and lowland flies had indeed occurred. Three coastal strains, Tuxpan, Papantla, and Cardel, situated less than 25 miles from high mountains, displayed frequencies of Adh^{II} ranging from 0.51 to 0.64, similar to those of mountain strains, whereas this allele reached a frequency of 0.95 or higher in other sea level populations[28]. Furthermore, four strains from Coatzocoalcos east to Merida showed signs of isolation in their evolutionary history from those north and west of the Isthmus of Tehauntepec. Individuals homozygous for chromosome 2 of an eastern strain were inviable if the X chromosome and chromosome 3 were replaced by chromosomes from a laboratory strain, although such a replacement was possible with several strains from the central highlands[29]. Evidence of gene exchange among eastern lowland strains included a variation in the interpulse interval of the male courtship song in the Coatzocoalcos population and an excess of heterozygotes for certain aldehyde oxidase alleles at Coatzocoalcos, coupled with an excess of homozygotes at neighboring Villahermosa. A second trip was made in 1974 to obtain flies from different levels in the Sierra Madre Oriental Mountains down to the lowlands including interior as well as coastal sites as far east as Palenque (Figure 1). In this paper it will be shown that parallel clines exist of alleles of alcohol dehydrogenase (*Adh*, chromosome 2, 50.1) and certain alleles of aldehyde oxidase (*Aldox*, 56.6, chromosome 3), but not of esterase-6 (*Est-6*, 36.8, chromosome 3). A cytological analysis of the distribution of autosomal inversions by one of us (E. F.-S.) shows that limited gene exchange has taken place throughout roadside populations between the Sierra Madre Oriental Mountains and Coatzocoalcos. We shall present evidence that the clines of *Adh* and *Aldox* alleles depend both on selection and gene exchange between low and high temperature races of *D. melanogaster*.

Materials and Methods

Collections

Flies were taken by sweeping over garbage dumps and in bathrooms of roadside restaurants, in fruit stores, and markets, indicated by "sw"; and also by sweeping over fallen mango fruits ("sw man"); or by trapping in fruit baited cans ("tr"). All 1974 collections were carried out between July 15 and July 30. Collection sites of 1974 and certain ones of June 1972 are indicated in Figure 1. Abbreviations of collection sites, altitudes in feet, number of founder females, and method of collection are as follows: San Juan de Teotihuacan (S.J.), 7,350, 1 ♀, "tr"; Puebla (Pu), 7,050, 8 ♀, "sw man"; Tehuacan (Teh), 5,410, 38 ♀, "sw"; Orizaba (Oz), 4,070, 2 ♀ "sw"; Oaxaca (Ox), 4,085, 66 ♀, "tr"; Acatlan (At), 5,158, 14 ♀, "sw"; Cordaba (Co), 2,860, 4 ♀ "sw"; Yanga (Ya), 400, 96 ♀, "sw man"; Palenque (Pe) 400, 34 ♀, "tr"; Cosmaloapan (Cos), 213, 1 ♀, "sw"; and the following approximately sea level sites: Tierra Blanca Junction (T B), 23 ♀, "sw"; Aleman (Al), 6 ♀, "sw"; Rio Papaloapan (Rio), 2 ♀, "sw"; Acayucan (Ac), 2 ♀ "sw"; Coatzocoalcos (Cz), 20 ♀, "tr"; Tehauntepec (Tepec), 2 ♀, "sw". Where the species occurred together, as in several lowland populations, *D. simulans* was separated from *D. melanogaster* by removing males of the former in the F_1 generation. In mixed *melanogaster-simulans* popu-

The authors are affiliated with the Department of Zoology, Howard University, Washington, D.C. 20059. Supported by NSF grant DEB 76-01403, NIH grant RR08016, and NIH training grant 3T-01 GM 02196. Thanks are due George P. Pipkin for invaluable aid in the course of the 1974 collecting trip to Mexico.

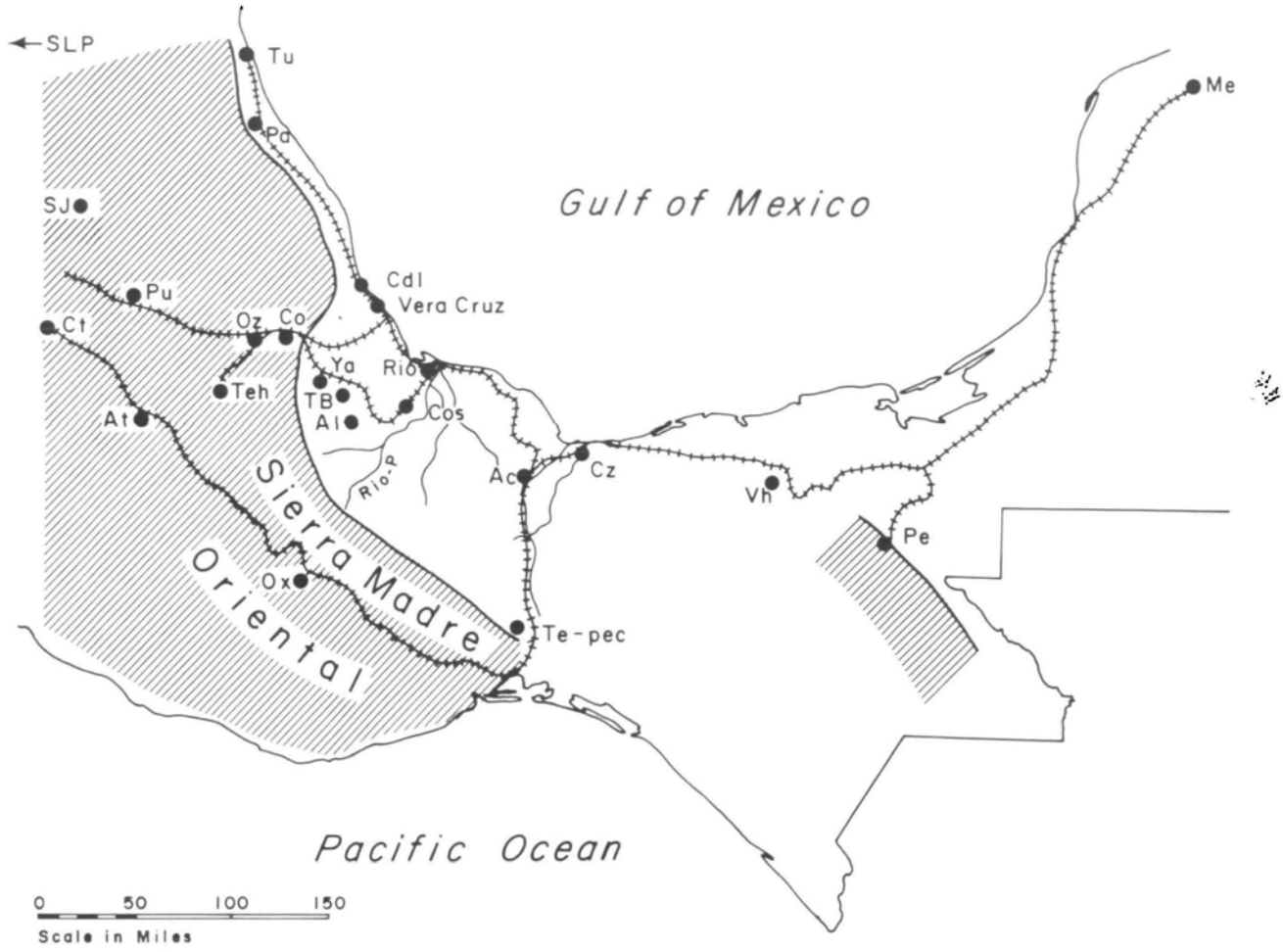

FIGURE 1—Map showing 1972 and 1974 collection sites in Mexico and the only road supplying the collection area.

lations the number of founder *melanogaster* females has been estimated as the total number of females collected less the number of *simulans* males. *D. melanogaster* was not found over fallen mangoes in the countryside of Lake Catemaco or in the park at Villahermosa in spite of the collection of hundreds of other drosophilids. Collection sites and founder numbers of strains collected in 1972 have been listed[28]. For each unselected strain, between 50 and 60 F_1 to F_3 flies descended from founders were assayed singly for ADH, EST-6, and ALDOX. In the 1975 paper by Pipkin et al.[29], an incorrect terminology was unfortunately used for *Est-6* alleles, designated there as *Est-6*^{F} and *Est-6*^{S}, according to migration of corresponding isozymes on noble agar gels. In the present paper *Est-6* alleles are named according to their fast or slow migration on *starch* gels in accordance with the nomenclature of Wright and McIntyre[42]. Our nomenclature for *Adh* alleles follows that of Ursprung and Leone[35]. Our *Adh*^{1} corresponds to *Adh*^{F} (or *Adh*^{4}); our *Adh*^{II} corresponds to *Adh*^{S} or *Adh*^{6} of investigators using starch or polyacrylamide gels at pH 8.6. Our nomenclature for *Aldox* alleles is the same as that used by Pipkin et al.[29]; we do not know which of our alleles corresponds with the two described by Dickinson[7].

Culture method

Unselected strains were maintained in half pint milk bottles on *Drosophila* Instant Medium (Carolina Biological Supply) from the time of collection. The laboratory was kept at 22°–25°C.

Enzyme assays

Single fly crude homogenates were electrophoresed on noble agar gels according to the method of Ursprung and Leone[35]. Histochemical staining of ADH was also done according to these authors except that 2-butanol was used as substrate. ALDOX was stained using the method of Courtright[5]; EST-6, according to Johnson[16].

Cytology

Inversion polymorphism within each strain or strain hybrid was sampled by examining the salivary chromosomes of at least 25 (many more in the case of the Cdl

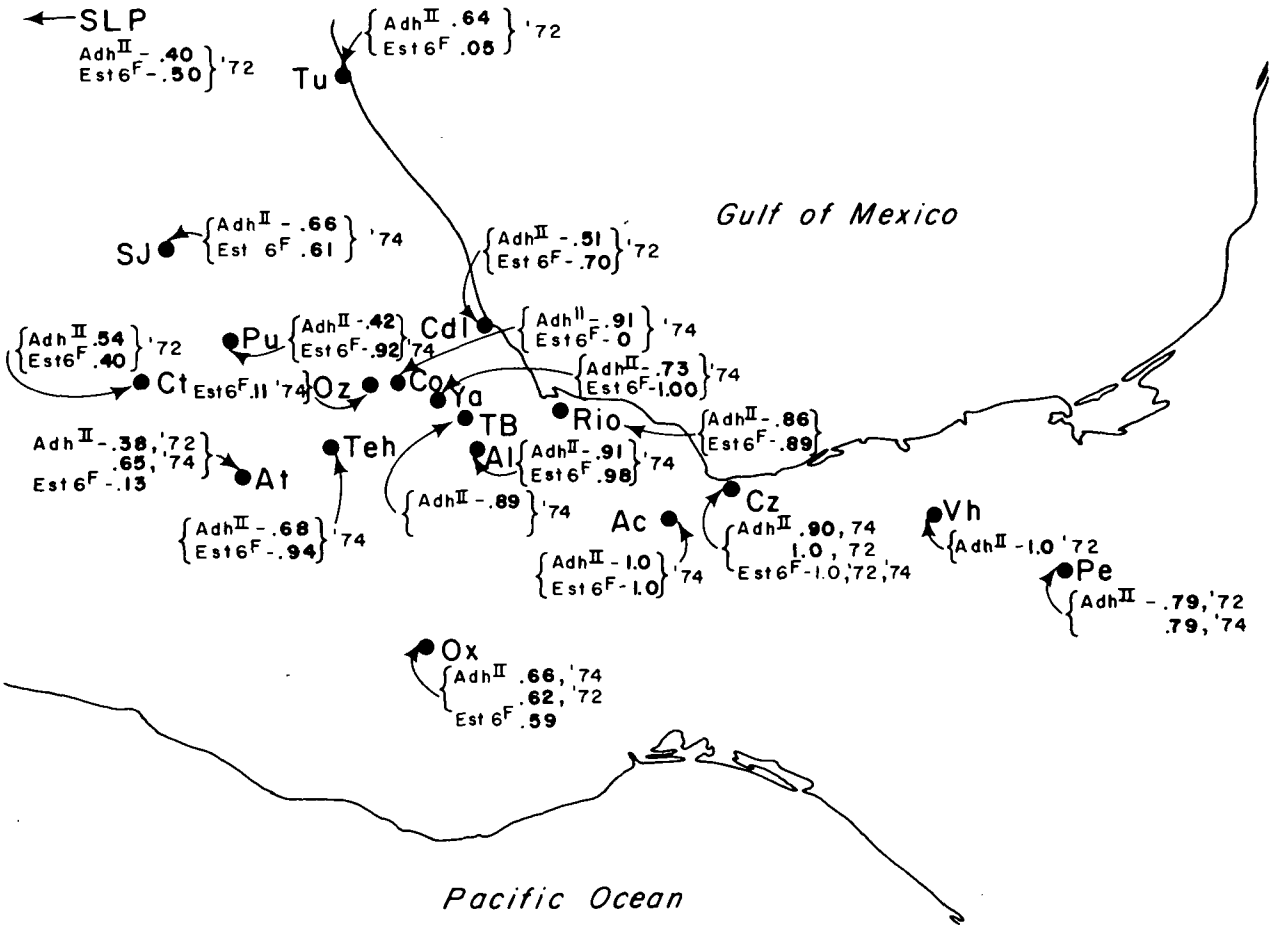

FIGURE 2—Map with frequencies of *Adh*^{II} and *Est-6*^{F} in F_1 to F_3 descendants of founder populations of 1972 and 1974.

and Cz strains) single third-instar larvae, taken from 5 vials containing 2–3 virgin males and an equal number of virgin females, all flies aged 4–5 days before mating. A second set of 10 one male–one female crosses also provided larvae in certain crosses. We did not make isofemale lines of freshly collected material, cross each to a stock with a standard chromosome sequence, and look for inversions in the hybrids, for two reasons: first, many F_1 to F_3 progeny of founders were being used for isozyme studies; and second, we wished to avoid loss of inversions due to allelism of lethals. The process of balancing very large numbers of wild chromosomes from the unselected strains against laboratory chromosomes carrying inversions and dominant markers, which would solve the problem of lethals, was thought too tedious and the possibility existed of losing lines. Although the large number of inversions discovered in this study may have justified this decision, we sacrificed the obvious advantages of the traditional methods of sampling.

Salivary glands of third instar larvae, cultured on *Drosophila* Instant Medium, supplemented by the addition of Fleischman's yeast, were dissected in Insect Ringer's solution[24], placed in a drop of 60 percent aceto-orcein for 2 minutes and then destained with 45 percent acetic acid. Certain preparations were made with Gurr's synthetic orcein in aceto-lacto-orcein; with these, no destaining was necessary. Temporary mounts were made by squashing and sealing with nailpolish. Temporary preparations were frozen and remained good for months. Inversions were photographed using panatomic film in a Zeiss photomicroscope. Photographs will be published elsewhere. Identification of inversion breakpoints was made using Bridges' maps[4] and the photographic maps of Ashburner[2].

Results

The Adh cline

The map shown in Figure 2 indicates that the frequency of *Adh*^{II} ranges from 0.38 at Acatlan to 0.68 at Tehuacan in *Drosophila* strains collected at altitudes above 5000 feet. Descending to the lowlands from

Tehuacan, the Adh^{II} frequency increases rapidly, reaching 0.89 at Tierra Blanca Junction about 75 miles east of Tehuacan. From Acayucan eastward, the frequency of Adh^{II} was 0.90 or higher, except at Palenque on the edge of the Chiapas Mountains, where this allele maintained a frequency of 0.79 in both 1972 and 1974. With four exceptions, the distribution of ADH genotypes was in Hardy-Weinberg equilibrium. At Puebla, a significant excess of heterozygotes was found. At Oaxaca, Aleman, and Rio Papaloapan, there were significant excesses of homozygotes, indicating inbred populations.

Esterase-6 alleles

An obvious absence of clinal variation of *Est-6* alleles characterizes the Mexican populations studied (Figure 2). Johnson and Schaffer[18] also found no cline of *Est-6* alleles in the Eastern United States. Although $Est\text{-}6^{F}$ was by far the predominant allele in many populations taken at all altitudes, it was rare or absent at the mountain stations of Orizaba, Acatlan, and Cordaba in the 1974 collections and at Tuxpan on the coast in the 1972 collection. With two exceptions, EST-6 genotypes were in Hardy-Weinberg equilibrium. At both Oaxaca and Puebla, there were significant excesses of homozygotes and corresponding deficiencies of heterozygotes.

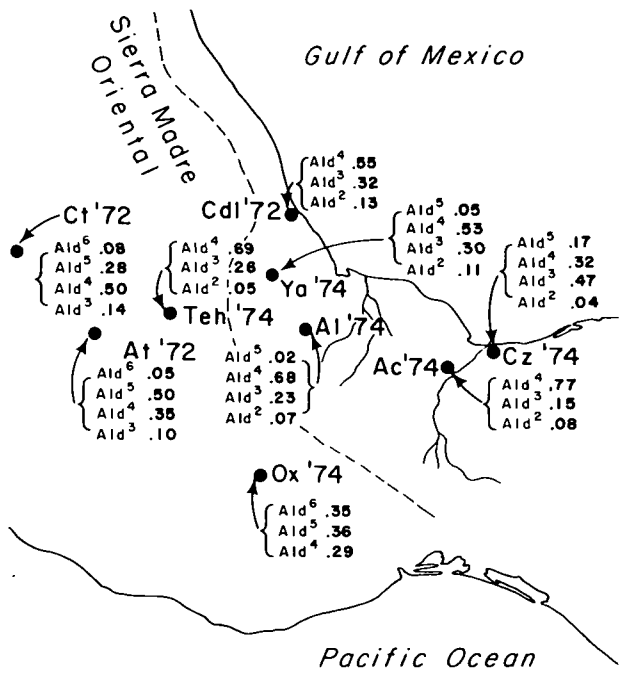

FIGURE 3—Map with frequencies of *Aldox* alleles in F_1 to F_3 descendants of founder populations of 1972 and 1974 collections.

Aldehyde oxidase alleles

Figure 3 shows the frequency of 5 *Aldox* alleles in the region of the *Adh* cline. Distinct differences distinguish mountain and lowland populations examined. The rare Ald^{6} was found exclusively in three of the highland populations. Alleles Ald^{3} and Ald^{5} exhibited

Table I. Breakpoints of 39 distinct autosomal paracentric inversions of which 26 are highly endemic, 2 moderately endemic, and 11 described previously, including 4 inversions reported as cosmopolitan

Inversions	Breakpoints	Location	No. larvae examined	Frequencies, heterozygotes
In 2L (t)†*	*23A–33E*	Cz; Ac/Cz	38; 25	0.053; 0.04
	23A–31C/D–33E	Rio	31	0.032
In (2L) B	*33A–35C/D*	Al/Cdl	30	0.033
In (2L) C	*25F–34B–34F*	Cos/Cdl	36	0.028
In (2R) NS†	*52A–56F*	Ya/Cdl; Ya; Cz/Cos, Cdl/Teh, Ac/Cz; Co/Cdl, Ya/TB	26; 27; 25; 25; 27; 27; 35	0.023; 0.038; 0.04; 0.037; 0.074; 0.057; 0.029
In (2R) A	*49E–56C*	Co/Cdl	27	0.037
In (2R) B	*50B–57A*	Cdl/Cz	32	0.031
In (2R) C	*51A–57C/D*	Al/Cdl	30	0.033
In (2R) D‡	*53B–57 A/B*	Al, Ac/Cz	30; 27	0.033; 0.037
In (2R) E	*53F–57A*	Ac/Cz	27	0.037
In (2R) F	*55F–56F–59 B/C*	Teh/Cz	31	0.032

* = breakpoints of *In 2L(t)* are listed by Mukai and Yamaguchi[26] as *22D–34A*. Our determinations are *23A–33E*. The multiple inversion *23A–31C/D–33E* contains both *2L(t)* and *2LA*, *23A–31C/D*

† = cosmopolitan inversions

‡ = moderately endemic inversions

clinal variation, the former increasing in frequency; the latter decreasing, going from highlands to lowlands. The predominant allele, *Ald*^{4}, accounted for from about ⅓ to ⅔ the total allelic frequency in collections taken from all altitudes, but without clinal variation. Tehuacan, at 5,410 feet, on the eastern slope of the Sierra Madre Mountains, nevertheless displayed particular aldehyde oxidase alleles in frequencies typical in lowland populations. In all 1974 populations, frequencies of ALDOX genotypes did not differ significantly from those expected on the basis of Hardy-Weinberg equilibrium.

Gene exchange in the region of the Adh cline

Gene exchange in 10 populations in the region of the *Adh* cline has been detected by following the distribution of paracentric autosomal inversions. Table I lists 39 distinct inversions, their location in strains or strain hybrids; breakpoints and frequencies of heterozygotes either within a strain or in strain hybrids. Twenty-six "highly endemic" inversions were found either within one strain or in one strain hybrid. Two "moderately endemic" inversions were identified either polymorphic within two

Table I. (*Continued*)

Inversions	Breakpoints	Location	No. larvae examined	Frequencies, heterozygotes
In (3L) P†	*63C–72C*	Al/Cos, Rio/Cos, Rio	25; 27; 31	0.16; 0.037; 0.032
In (3L) A	*63A–67B*	Teh	34	0.029
In (3L) B‡	*63F/64A–67B*	Tb, Yanga	35; 27	0.029; 0.037
In (3L) C	*65B–69F*	Ya/Cdl	26	0.038
In (3L) D	*65E/F–72C*	Teh/Cz	31	0.032
In (3L) E§	*66D–71C*	Co/Cdl	27	0.037
In (3L) F§	*63A–72F*	Rio/Cos; Al/Cos	27; 25	0.037; 0.04
In (3L) G§	*68A–73A/B*	Cos/Ac	30	0.033
In (3L) H	*68F–76D/E*	Co/Cdl, Cdl/Ac	27; 29	0.037; 0.034
In (3L) I	*63F–66A*	Co/Cdl	27	0.037
In (3R) K§	*86E/F–96E/F*	Cos/Cdl; Cdl/TB, Cdl, Cdl/Ac	36; 28; 40; 29	0.028; 0.036; 0.05; 0.034
In (3R) B	*86E/F–98E/F*	TB/Ya, TB/Al	35; 32	0.028; 0.031
In (3R) C	*84F–86D*	Ac/Cdl	29	0.034
In (3R) D	*86F–96A/B*	Cos/Cdl; Cdl; Cdl/Ac	36; 40; 29	0.028; 0.025; 0.034
In (3R) E	*88C–96E*	Cos/Cdl	36	0.028
In (3R) MO§	*98F–93D*	Co/Cz	27	0.074
In (3R) P†	*89C–96A*	Al/Cos, Ya/Cdl; Al; Cos/Ac, Ya/TB Teh/Cz, Al/Cz, Co	25; 26; 30 30; 35 31; 25; 25	0.04; 0.038; 0.033 0.033; 0.028 0.032; 0.04; 0.08
In (3R) G§	*89D–96A/B*	Rio, Cos/Cdl; Co/Cdl	31; 36; 27	0.065; 0.028; 0.037
In (3R) H	*89B–96A*	Cz/Co	27	0.037
In (3R) C208§	*91A/B–96A/B*	Rio/Cz, Cz, TB/Al	26; 38; 32	0.077; 0.026; 0.063
In (3R) I	*90D–96A/B*	Cz/Cdl	32	0.031
In (3R) J	*88F–98E/F*	Co	25	0.04
In (3R) L	*89C–93D–96A*	Ya/Cdl	26	0.038
In (3R) M	*86E/F–89E–95B–96F*	Cos/Cdl	36	0.028
In (3R) N	*86E/F–92E–96F*	Ya/Cdl	26	0.038

§ = previously reported inversions that are not cosmopolitan

different strains or within one strain and also in a strain hybrid distinct from the former, or in two completely different sets of strain hybrids. Eleven inversions were previously described: 5 cosmopolitan inversions and 6 with limited known distribution. The distribution of the two moderately endemic inversions and five of the previously described ones is diagrammed in Figure 4. Clearly there has been gene flow throughout the populations of the study area. The cosmopolitan *In(3R)P* is the most widely distributed, being found polymorphic within Aleman, Yanga, Tierra Blanca, Cordaba, and, according to strain hybrids, probably in Coatzocoalcos and Acayucan. It is the only inversion found both in lowland and also in a mountain population (Cordaba). Of the inversions shown in Figure 4, all but three are widely distributed in the lowlands. Some localization of three inversions is evident: 1) *In2L(t)*, a cosmopolitan, is found at the Rio Papaloapan and east at Coatzocoalcos; 2) the moderately endemic *In(3L)B'* and the cosmopolitan *In(3L)P* were found at two stations west of the Rio Papaloapan. This wide river, which lacked a bridge at the time of our collection, and three others at short distances to the east, were called "Little Amazons" and served as natural barriers to dispersion.

Van Valen and Levins[37] have suggested that the lengths of newly arisen inversions should be distributed normally. To test this hypothesis, distances between breakpoints of the 26 highly endemic inversions were measured in millimeters on the maps of Bridges[4]. These distances, divided by the length of appropriate chromosome arms, were plotted on the abscissa against rankits[31] (Figure 5*A*). A similar graph was made for the 2 moderately endemic and 11 previously described inversions (Figure 5*B*). From the multiple inversion, *In(2L)C*, two single highly endemic inversions, *25F-34B* and *25F-34F* were used in Figure 5*A*. From *In(2R)F*, the two single highly endemic inversions had breakpoints *55F-59B/C* and *56F-59B/C*. The previously described *In(3R)K°* of Kodani was present in both *In(3R)M* and *In(3R)N*. However, a single endemic inversion was also present in *In(3R)M*, i.e., *89E-95B*; and in *In(3R)N*, i.e., *86E/F-92E*. The longer of two possible single inversions within a multiple inversion was arbitrarily chosen for Figure 5*A*. Fig. 5A shows that the fit of observed lengths of highly endemic inversions to the line expected is not perfect, especially at the ends of the distribution. There is an indication of bimodality and a paucity of very short inversions. However, the Kolmogarov-Smirnov test of departure from normality[32] does not provide evidence for rejecting the null hypothesis that the inversion length/arm length ratios are normally distributed. ($D_{max} = 0.1158$; $P > 0.2$; where the 26 ratios were grouped into 9 classes). In Figure 5*B*, the lengths of moderately endemic and previously described inversions clearly diverge from expectation that these are distributed normally.

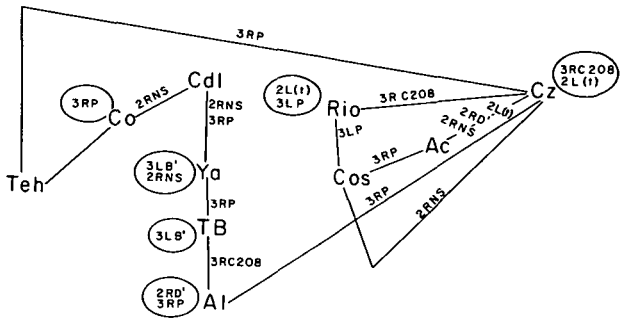

FIGURE 4—Diagram showing distribution of inversions detected at more than one site among 10 strains or strain hybrids located between Tehuacan and Coatzocoalcos.

Discussion

The correlation between high frequencies of *Adh*^{II} of *D. melanogaster* in warm regions of three parts of the world with a higher heat stability of the ADH-II than of the ADH-I enzyme[6,10,17,38] has led to general agreement among these authors that *Adh*^{II} confers a selective advantage in warm climates.

The role of ADH as a detoxifying agent was early recognized[11]. In laboratory experiments, using a defined medium, Ainsley and Kitto[1] showed that whereas living larvae with ADH-I were able to oxidize ethanol alone more efficiently than those with ADH-II, the latter could better oxidize I-propanol. Relative efficiency of Adh-I or ADH-II in oxidizing mixtures of short chain alcohols varied with the mixture. These authors think that polymorphism of ADH depends on changing selective values of the two enzymes in different habitats, according to the alcohol substrates present. On the other hand, McKenzie and Parsons[23] have shown that ethanol resistance is greater in a *D. melanogaster* population breeding within a winery cellar than in a population just outside the cellar but with free access to it. Nevertheless, *Adh* allelic frequencies are similar in cellar and outside populations. Thus, although ADH contributes to alcohol detoxification, ethanol resistance must be multigenic in inheritance. In support of this, J. David (personal communication) had shown that *D. melanogaster* responds to positive selection for ethanol resistance over a number of generations.

The clinal variation of *Adh* alleles of *D. melanogaster* in Mexico appears dependent on natural selection acting to preserve coadapted gene complexes of mountain and lowland races, respectively, and also on gene flow between them. When 9 highland and 13 lowland strains are considered, there is a significant regression of *Adh*^{II} frequency (and consequently of *Adh*^{I} frequency) on extreme minimum temperature ($b = 25.27 \pm 4.42$; $t_s = 5.7176$; for $n\text{-}2 = 20$, $P < 0.01$.) A map showing the locations of the collection stations in the climatic zones is given in Figure 6 (*Atlas Climatologico de Mexico*). The regression of *Adh*^{II} (and thus of *Adh*^{I}) on extreme minimum temperature is not significant among the nine highland strains, considered separately. Our finding a cline of certain aldehyde oxidase alleles paralleling the *Adh* cline, together with the absence of such a cline of alleles of esterase-6, suggests that these *Aldox* alleles may be part of multigenic complexes selected by temperature in highlands and lowlands, respectively. It is noteworthy that both ADH[36] and

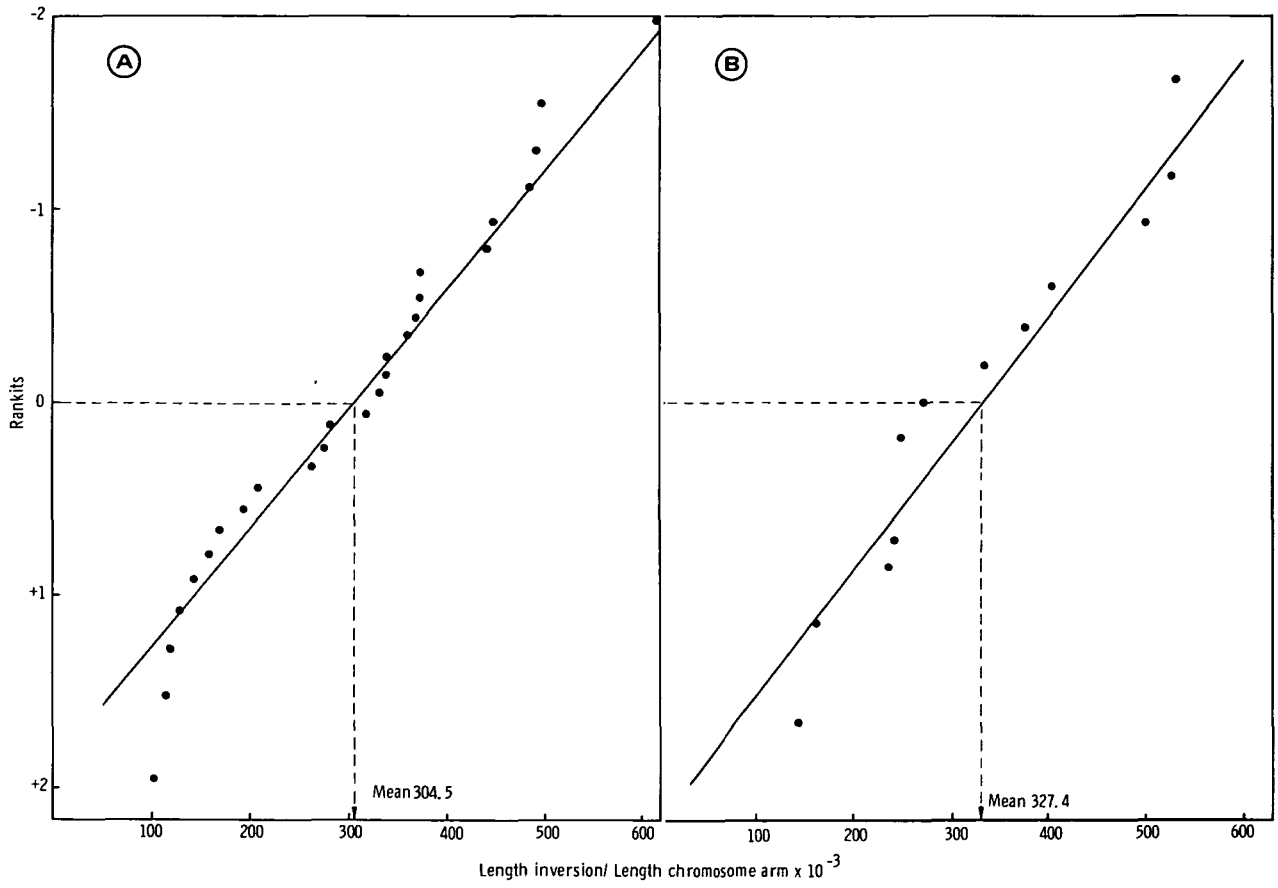

FIGURE 5—Test for normal distribution of inversion lengths in (*A*) 26 "highly endemic" inversions and (*B*) 2 "moderately endemic" plus 11 previously described inversions. The length of each inversion measured on Bridges'[4] maps divided by the length of the appropriate chromosome arm is plotted on the abscissa against rankits[31] on the ordinate. Two previously described inversions were of the same length.

ALDOX[7] are present in appreciable amounts in larval fat bodies where ALDOX may use a substrate provided by the action of ADH. However, the relative amount of ADH is greater than that of ALDOX in this tissue.

Further evidence that temperature races exist in Mexico comes from Gallarda's finding[8] that third instar larvae of four of our Mexican mountain strains survive a cold shock of 10°C for 24 or 48 hours better than four of our lowland strains. Also, Levins[19] observed that *D. melanogaster* imagines collected at Carrizales at 3,000 feet in the mountains of Porto Rico did not survive well enough at 28°C to be tested for thermal acclimation, in contrast to flies from several lowland sites. Timofeef-Ressovsky[34] described temperature races of *D. funebris* in Russia.

Our studies of the distribution of autosomal inversions collected from two sites in the Sierra Madre Oriental Mountains and in eight lowland sites show that gene exchange has occurred throughout the lowlands, but it has been slow or limited. The cosmopolitan *In(3R)P* alone was found both in several lowland strains and in one mountain strain. The period of time over which this gene flow has taken place is unknown. Further evidence that gene flow has been limited is the finding of 26 "highly endemic" inversions, each detected at one site only. Until recently when a number of new inversions were reported in *D. melanogaster*[26,33,41,43] it was thought that this species supports few chromosomal polymorphisms. It is probable that many of our endemic inversions are not newly arisen. We found neither translocations nor X-chromosome inversions, both of which are subject to rapid adverse selection. It is possible that the endemic inversions reflect a limited dispersal of small populations of *D. melanogaster*. Wallace[40] concluded from a study of allelism of lethals that *D. melanogaster* has a relatively limited vagility. Our founder numbers were very small although the species is capable of enormous expansion when food supply is available. In the area and time of year studied, we were sampling small populations. Even several days trapping carried out at certain stations attracted few *D. melanogaster* although other species were collected in greater numbers. The occurrence of four Mexican populations with significant excesses of homozygotes of *Adh* or *Est-6* alleles

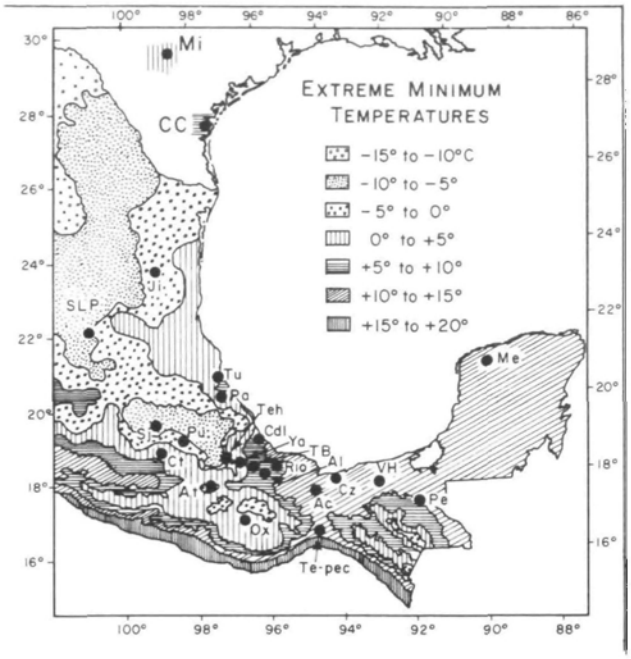

FIGURE 6—Climatological map showing extreme minimum temperature, calculated as an average of the mean minimum temperature of the coldest month over a 10-year period from 1921–1930.

is further evidence of limited dispersal. However, the similarity of the kinds and frequencies of *Aldox* alleles at Tehuacan to those in the lowlands implies that gene exchange has occurred. Our view of the relation of the *Adh* cline to gene flow favors the hypothesis of Hastings and Rohlf[14] that in relatively small populations, gene exchange can lengthen the time during which selection can act on a cline and thus contribute to its maintenance.

The contribution of linkage disequilibrium to the Mexican cline of *Adh* alleles is difficult to assess because 1) inversions were studied in only two highland populations, and 2) we determined frequencies of inversion heterozygotes only. The cosmopolitan inversion, *In2L(t)*, which would be expected to influence recombination involving the *Adh* locus, was found only east of the Rio Papaloapan, 75 miles east of the clinal area. Each of the other two inversions in 2L, i.e., *In(2L)B* and *In(2L)C*, was found only once.

Summary

An altitudinal cline of frequencies of alcohol dehydrogenase alleles occurs in *D. melanogaster* populations of southeastern Mexico. A similar cline of two aldehyde oxidase alleles is present, but frequencies of esterase-6 alleles are not distributed clinally. Collections were made from small dispersed populations. Some gene flow occurred throughout the lowlands according to the distribution of two moderately endemic autosomal inversions and five previously described inversions. The clines are believed dependent on a limited gene flow between temperature races of *D. melanogaster*.

Literature Cited

1. AINSLEY, R. and G.B. KITTO. Selection mechanisms maintaining alcohol dehydrogenase polymorphisms in *D. melanogaster*. *In* Isozymes II. Physiological Function. Academic Press, N.Y. p. 733–742. 1975.

2. ASHBURNER, M. Puffing patterns in *D. melanogaster* and related species. *In* Developmental Studies on Giant Chromosomes. W. Beerman, Ed. Springer-Verlag, Berlin. 1972.

3. ATLAS CLIMATOLOGICO DE MEXICO. Servicio Méterologico de Mexico, 1921–1930.

4. BRIDGES, C.B. Salivary chromosome maps, with a key to the banding of the chromosomes of *D. melanogaster*. *J. Hered.* 29:11–13. 1935.

5. COURTRIGHT, J.B. Polygenic control of aldehyde oxidase in *Drosophila*. *Genetics* 57:25–39. 1967.

6. DAY, T.H., P.C. HILLIER, and B. CLARKE. The properties of genetically polymorphic isozymes of alcohol dehydrogenase of *D. melanogaster*. *Biochem. Genet.* 11:141–153. 1974.

7. DICKINSON, W.J. Aldehyde oxidase in *D. melanogaster*: A system for genetic studies on developmental regulation. *Developmental Biol.* 26:77–86. 1971.

8. GALLARDA, S. Survival after cold temperature shock in Mexican strains of *D. melanogaster* and its relation to alcohol dehydrogenase. M. Sc. Thesis, University of Maryland, College Park, Md. 1975.

9. GIBSON, J. Enzyme variability in *D. melanogaster*. *Nature* 227:959–960. 1970.

10. ———. and R. MIKLOVICH. Modes of variation in alcohol dehydrogenase in *D. melanogaster*. *Experientia* 27: 99–100. 1970.

11. GRELL, E.H., K.B. JACOBSON, and J.B. MURPHY. Alterations of genetic material for analysis of alcohol dehydrogenase isozymes. *In* Multiple Molecular Forms of Enzymes. *Ann. New York Acad. Sci.* 151:441–455. 1968.

12. GROSSMAN, A.I. The distribution of breaks limiting the autosomal inversions in the natural populations of *D. melanogaster*. *Soviet Genet.* 3:31–35. 1967.

13. ———, L.G. Koreneva, and L.E. Ulitskaya. The variability of ADH locus in a natural population of *D. melanogaster*. *Soviet Genet.* 6:91–96. 1970.

14. HASTINGS, A. and F.J. ROHLF. Gene flow: effect in stochastic models of differentiation. *Am. Nat.* 108:701. 1974.

15. HEWITT, N.E., S.B. PIPKIN, N. WILLIAMS, and P.K. CHAKRABARTTY. Variation in ADH activity in class I and class II strains of *Drosophila*. *J. Hered.* 65:141–148. 1974.

16. JOHNSON, F.M. An isozyme analysis of *Drosophila* speciation and development. Ph.D. Thesis. University of Texas, Austin. 1966.

17. ——— and A. POWELL. The alcohol dehydrogenases of *D. melanogaster*: frequency changes associated with heat and cold shock. *Proc. Natl. Acad. Sci.* 71:1783–1784. 1974.

18. ——— and H. SCHAFFER. Isozyme variability in species of the genus *Drosophila*. VII. Genotype-environment relationships in populations of *D. melanogaster* from the eastern United States. *Biochem. Genet.* 10:149–163. 1973.

19. Levins, R. Thermal acclimation and heat resistance in *Drosophila* species. *Am. Nat.* 103:483–499. 1969.

20. LEWONTIN, R.C. The Genetic Basis of Evolutionary Change. Columbia Univ. Press, N.Y. 1974.

21. ——— and J.L. HUBBY. A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *D. pseudoobscura*. *Genetics* 54:595–609. 1966.

22. MAYR, E. Animal Species and Evolution. The Belknap Press of Harvard Univ. Press, Cambridge, Mass. 1963.

23. McKENZIE, J.A. and P.A. PARSONS. Microdifferentiation in a natural population of *D. melanogaster* to alcohol in the environment. *Genetics* 77:385–394. 1974.

24. MILLER, A. The internal anatomy and histology of the imago of *D. melanogaster*. *In* Biology of Drosophila. John Wiley and Sons, Inc. N.Y. 1950.

25. MOURAD, A.M. and G.S. MALLAH. Chromosomal polymorphism in Egyptian populations of *D. melanogaster*. *Evolution* 14:166–170. 1960.

26. MUKAI, T. and O. YAMAGUCHI. The genetic structure of natural populations of *D. melanogaster*. XI. Genetic variability in a local population. *Genetics* 76:339–366. 1974.

27. PIPKIN, S.B. and N. HEWITT. The influence of the X chromosome on specific activity of alcohol dehydrogenase of *Drosophila*. *D.I.S.* 46:66–67. 1971.

28. ———, C. RHODES, and N. WILLIAMS. Influence of temperature on Drosophila alcohol dehydrogenase polymorphism. *J. Hered.* 64:181–185. 1973.

29. ———, J.H. POTTER, S. LUBEGA, and E. SPRINGER. Further studies on alcohol dehydrogenase polymorphism in Mexican strains of *D. melanogaster*. Isozymes IV. *In* Genetics and Evolution. p. 547–560. Academic Press, N.Y. 1975.

30. RASMUSON, B., L.R. NIELSEN, M. RASMUSON, and E. ZEPPEZAUER. Effects of heterozygosity on alcohol dehydrogenase (ADH) activity in *D. melanogaster*. *Hereditas* 56: 313–316. 1967.

31. ROHLF, F.J. and R.R. SOKAL. Statistical Tables. W. H. Freeman & Co. San Francisco. 1969.

32. SOKAL, R.R. and F.J. ROHLF. Biometry. W. H. Freeman & Co. San Francisco. 1969.

33. STALKER, H.D. Chromosome studies in wild populations of *D. melanogaster*. *Genetics* 82:323–347. 1976.

34. TIMOFEEF-RESSOVSKY, N.W. Über geographische temperaturrassen bei *D. funebris*. *Arch. Naturgesch. N.F.* 4: 245–257. 1935.

35. URSPRUNG, H. and J. LEONE. Alcohol dehydrogenase: A polymorphism in *D. melanogaster*. *J. Exp. Zool.* 160: 147–154. 1965.

36. ———, W.H. SOFER, and N. BURROUGHS. Ontogeny and tissue distribution of alcohol dehydrogenase in *D. melanogaster*. *Archiv. für Entwicklungsmechanik der Organismen* 164:201–208. 1970.

37. VAN VALEN, L. and R. LEVINS. The origins of inversion polymorphism. *Am. Nat.* 102:5–24. 1968.

38. VIGUE, C. and F.M. JOHNSON. Isozyme variability in species of the genus *Drosophila*. VI. Frequency-property-environment relationships of allelic alcohol dehydrogenases in *D. melanogaster*. *Biochem. Genet.* 9:213–227. 1973.

39. WALLACE, B. On the dispersal of *Drosophila*. *Am. Nat.* 100:551–564. 1966.

40. ———. Distance and the allelism of lethals in a tropical population of *D. melanogaster*. *Am. Nat.* 100: 565–578. 1966.

41. WATANABE, T.K., O. YAMAGUCHI, and T. MUKAI. The genetic variability of 3rd chromosomes in a local population of *D. melanogaster*. *Genetics* 82:63–82. 1976.

42. WRIGHT, T.R.F. and McINTYRE, R. The genetics of an esterase in *D. melanogaster*. *Genetics* 48:787–801. 1963.

43. YANG, H.Y. and K. KOJIMA. Chromosomal polymorphism and lethal alleles in a southwest Texas population of *D. melanogaster*. *Univ. Tex. Public.* 7213:229–236. 1972.