# POPULATION GENETICS OF *CHIRONOMUS STIGMATERUS* SAY (DIPTERA : CHIRONOMIDAE)

## I. CYTOGENETIC VARIABILITY IN POPULATIONS OF THE SOUTH-WESTERN UNITED STATES

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SUMMARY

*Chironomus stigmaterus* Say (Diptera : Chironomidae) exhibits genetic divergence between a chromosomally polymorphic form in Texas and New Mexico and a virtually monomorphic form restricted to California. Twenty-eight rearrangements have been recorded in the species but only eight were observed in California while 21 occurred in Texas and New Mexico. The majority of these rearrangements were found only in single heterozygotes but 10 inversions formed polymorphisms sufficiently stable to become established in populations throughout Texas and New Mexico. Inversion frequencies were high in populations of western Texas and eastern New Mexico but became lower in peripheral populations, suggesting a clinal reduction to monomorphism in California. However, no populations were found in Arizona to prove distribution continuity.

When these data are considered with those of a companion electrophoretic study (Hilburn, 1979), it appears the observed dichotomy in rearrangement heterozygosities is a manifestation of divergence in the total genetic compositions of the two taxa. The data support the hypothesis that, in the south-western United States, *C. stigmaterus* is represented by two subspecies having different levels of chromosomal heterozygosity, different enzyme alleles, and, possibly, partial reproductive isolation.

## 1. INTRODUCTION

CHIRONOMUS STIGMATERUS Say (Diptera : Chironomidae) is a frequent component of the midge fauna in standing water throughout most of the south-western United States. The species is morphologically uniform over this entire region (James Sublette, pers. comm.). However, cytological investigations have indicated an underlying genetic complexity suggesting, at least, intraspecific divergence between forms in California and in Texas and New Mexico.

The salivary gland polytene chromosomes of *C. stigmaterus* were first described by Martin and Wülker (1974) who found only two inversions in material from California while seven inversions occurred at localities in Texas and New Mexico. Hilburn (1974) made a more extensive examination of chromosomal variation in populations from Texas and New Mexico confirming these authors' suggestion of considerable chromosomal heterogeneity in populations of this area. These data suggested genetic differentiation between a chromosomally uniform race in California and a more polymorphic form in the centre of the species range.

While the occurrence of chromosomal heterogeneity in a species of insects is not unusual, the dichotomous pattern of inversion heterozygosities described in *C. stigmaterus* has not been observed in other species occurring in the south-western United States (cf. *Drosophila pseudoobscura*, reviewed by Dobzhansky, 1970; and *Glyptotendipes barbipes*, Martin and Porter, 1973; Hilburn and Atchley, 1976) except where such a pattern was accompanied by other evidence of intraspecific divergence (*e.g. Drosophila mojavensis*, Mettler, 1963; Zuoros, 1973; Richardson, *et al.*, 1977). Since fixation of inverted chromosomal sequences often has accompanied speciation in the genus *Chironomus* (Keyl, 1962), *C. stigmaterus* seemed to offer an ideal opportunity to examine the population genetics of a species undergoing the early stages of divergence.

In this paper the genetic variability in populations of *C. stigmaterus* from the south-western United States is examined as a measure of the degree of divergence between the two taxa, and answers to the following questions are sought: (1) Are the two cytologically different taxa geographically isolated? (2) What is the nature of chromosomal variability in any intervening contact zone, *i.e.* intergradation, parapatry, *etc.*? (3) Do the differences in chromosomal heterogeneity reflect intraspecific divergence as measured by other methods of genetic analysis?

## 2. Materials and methods

In order to examine the cytogenetic variability of *C. stigmaterus* in the south-western United States, an extensive collecting trip was made through the states of Texas, New Mexico, Arizona, Nevada, California, Colorado, Kansas, and Oklahoma during the summer of 1975. As many possible chironomid localities as could be located were sampled. During this survey, no *C. stigmaterus* were found in the northern portion of the study area, suggesting that if a zone of contact existed between the two cytogenetic taxa it probably was in southern Arizona or Mexico. Therefore, in 1976 an intensive collecting effort was made between Lordsburg, New Mexico, and Palm Springs, California. In addition, an attempt was made to locate *C. stigmaterus* at more localities in northern California and in eastern Texas.

Localities with large populations of *C. stigmaterus* are listed in table 1 and shown in fig. 1. Included in table 1 are the date each site was sampled and the number of individuals of each sex examined cytologically. For completeness, the most important data reported in Hilburn (1974) are repeated here.

All samples were taken as larvae using a dip net. Larvae were removed from the sediment and washed in clear water. Collections made in 1973 and 1975 were then dried on paper towelling and placed into 3 : 1 ethanol : acetic acid fixative. Larvae taken in 1976 were divided into groups of 20 after being washed and dried and then placed in small plastic vials and stored in liquid nitrogen to be used for both cytogenetic and electrophoretic analyses (Hilburn, 1979).

Salivary gland squashes were prepared using lactic-propionic orcein stain. The identities of the polytene banding sequences were determined by comparison with previously published photographs of known chromosomal sequences in *C. stigmaterus* (Hilburn, 1974; Martin and Wülker, 1974). Gametic frequencies were calculated for all sequences observed (tables 3-4), and it is these gametic frequencies that are used in the statistical analyses.

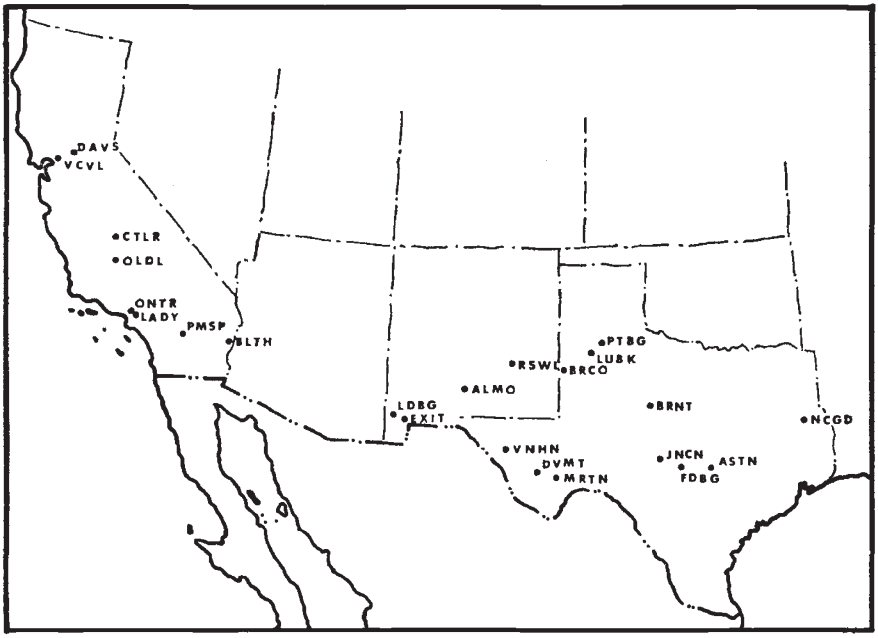


FIG. 1.—Collection sites for *C. stigmaterus* in the south-western United States. Locality codes refer to table 1.

## 3. RESULTS

The karyotype of *C. stigmaterus* is composed of three pairs of metacentric and a pair of acrocentric chromosomes. In the salivary glands the chromosomal homologues are tightly synapsed, making it easy to recognise rearrangements. A thick band of centromeric heterochromatin divides each metacentric chromosome into two easily discernible arms. Using the nomenclature introduced by Keyl (1962) to standardise the naming of sequences in species of the genus *Chironomus*, the following are the most commonly encountered sequences in *C. stigmaterus:* chromosome I: arms $A_2$ and $B_1$; chromosome II: arms $C_1$ and $D_1$; chromosome III: arms $E_1$ and $F_1$ in females, arms $E_1$ and $F_1/F_2$ in males; chromosome IV: arm $G_1$. For convenience, this combination of arm sequences will be referred to as the standard karyotype.

Although rearrangements were found in all arms of the karyotype of *C. stigmaterus*, most were observed only in single heterozygotes in one sample. With the exceptions of $A_3$, which was a deletion, and $G_7$, a duplication, observed rearrangements were paracentric inversions. Figs. 2-5 present the most common sequences of each arm and the extent of the less frequent rearrangements. Localities at which the more common rearrangements were found are listed in tables 2-4; those for rearrangements found in a single sample are presented in the legends to the figures.

The eight populations in California were almost all monomorphic for the standard karyotype thus confirming the previous studies. Only nine of the 1088 larvae examined bore rearrangements, excluding $F_2$ which is

TABLE 1

*Collection localities of* C. stigmaterus *from the south-western United States with locality codes referred to in article, dates of collection, and number of larvae of each sex examined cytologically*

I. California
   A. Davis, Campus sewer ponds south of the Primate Research Center, University of California, Davis: (DAVS 76) 26/VI/1976; 117F., 3M.
   B. Vacaville, Municipal sewer ponds: (VCVL 75) 23/VII/1975; 75F., 74M.
   C. Cutler, Municipal sewer ponds: (CTLR 76) 24/VI/1976; 97F., 13M.
   D. Oildale, Municipal sewer ponds: (OLDL 76) 24/VI/1976; 85F., 5M.
   E. Ontario, Municipal sewer ponds: (ONTR 76) 23/VI/1976; 70F., 19M.
   F. Cerritos, Stock tank at Hilarides Dairy: (LADY 75) 22/VII/1975; 53F., 47M.
   G. Palm Springs, Water hazards on the municipal golf course: (PMSP 75) 25/VII/1975; 100F., 100M. (PMSP 76) 22/VI/1976; 93F., 56M.
   H. Blythe, Municipal sewer ponds: (BLTH 76) 21/VI/1976; 70F., 14M.

II. New Mexico
   A. Lordsburg, Overflow pool from the municipal sewer: (LDBG 75) 17/VII/1975; 100F., 100M.
   B. 7·4 km west of the Quincy exit on I-10, Stock tank: (EXIT 76) 24/VII/1976; 81F., 70M.
   C. Alamogordo, Sewer playa of Holloman Air Force Base: (ALMO 73) 24/V/1973; 66F., 33M. (ALMO 75) 3/VII/1975; 64F., 28M. (ALMO 76) 21/VII/1976; 92F., 14M.
   D. Roswell, Bitter Lakes National Wildlife Refuge: (RSWL 73) 12/V/1973; 44F., 22M. (RSWL 75) 3/VII/1975; 58F., 38M. (RSWL 76) 30/VII/1976; 62F., 27M.

III. Texas
   A. Bronco, Windmill overflow pond 4·7 km west of town: (BRCO 73) 15/VIII/1971; 5F., 1M. (Collected previously by Dr William R. Atchley.)
   B. Van Horn, Municipal sewer ponds: (VNHN 75) 5/VIII/1975; 115F., 85M.
   C. Davis Mountains, Water tank north of Highway 505, 4·5 km west of the intersection with Highway 166: (DVMT 76) 4/VIII/1976; 67F., 84M.
   D. Marathon, Municipal sewer pond: (MRTN 75) 5/VIII/1975; 101F., 99M. (MRTN 76) 5/VIII/1976; 65F., 24M.
   E. Lubbock, 4th Street sewer ponds: (LUBK 73) 8/VI/1973; 62F., 46M. (LUBK 75) 12/VIII/1975; 83F., 51M. (LUBK 76) 11/IX/1976; 72F., 2M.
   F. Petersburg, Municipal sewer ponds: (PTBG 73) 21/VII/1973; 76F., 32M. (PTBG 76) 28/VII/1976; 75F., 2M.
   G. Bronte, Municipal sewer ponds: (BRNT 73) 18/VII/1973; 64F., 26M.
   H. Junction, Municipal sewer pond: (JNCN 73) 16/VII/1973; 36F., 15M.
   I. Fredericksburg, Municipal sewer ponds: (FDBG 76) 9/VIII/1976; 68F., 22M.
   J. Austin, Brackenridge Research Station, University of Texas, Austin: (ASTN 75) 6/VIII/1975; 96F., 44M.
   K. Nacogdoches, Municipal sewer ponds: (NCGD 76) 11/VIII/1976; 135F., 21M.

heterozygous in all males. The six inversions from these larvae were all observed as heterozygotes from the Palm Springs population.

Samples from Texas and New Mexico contained 20 inversions, of which eight were found in more than one sample. In no population from this area were fewer than four rearrangements assorting, again without including the sex-linked $F_2$. Statistical analyses were performed to examine the quantitative characteristics of temporal and geographic variation in the polymorphisms of these sequences.

The three major sequences of arm A, the two of arm C, and the six of arm G were tested for differences in frequency between the sexes. A chi-square contingency table (Sokal and Rohlf, 1969) was used to test the hypothesis that the frequencies of the chromosomal genotypes were

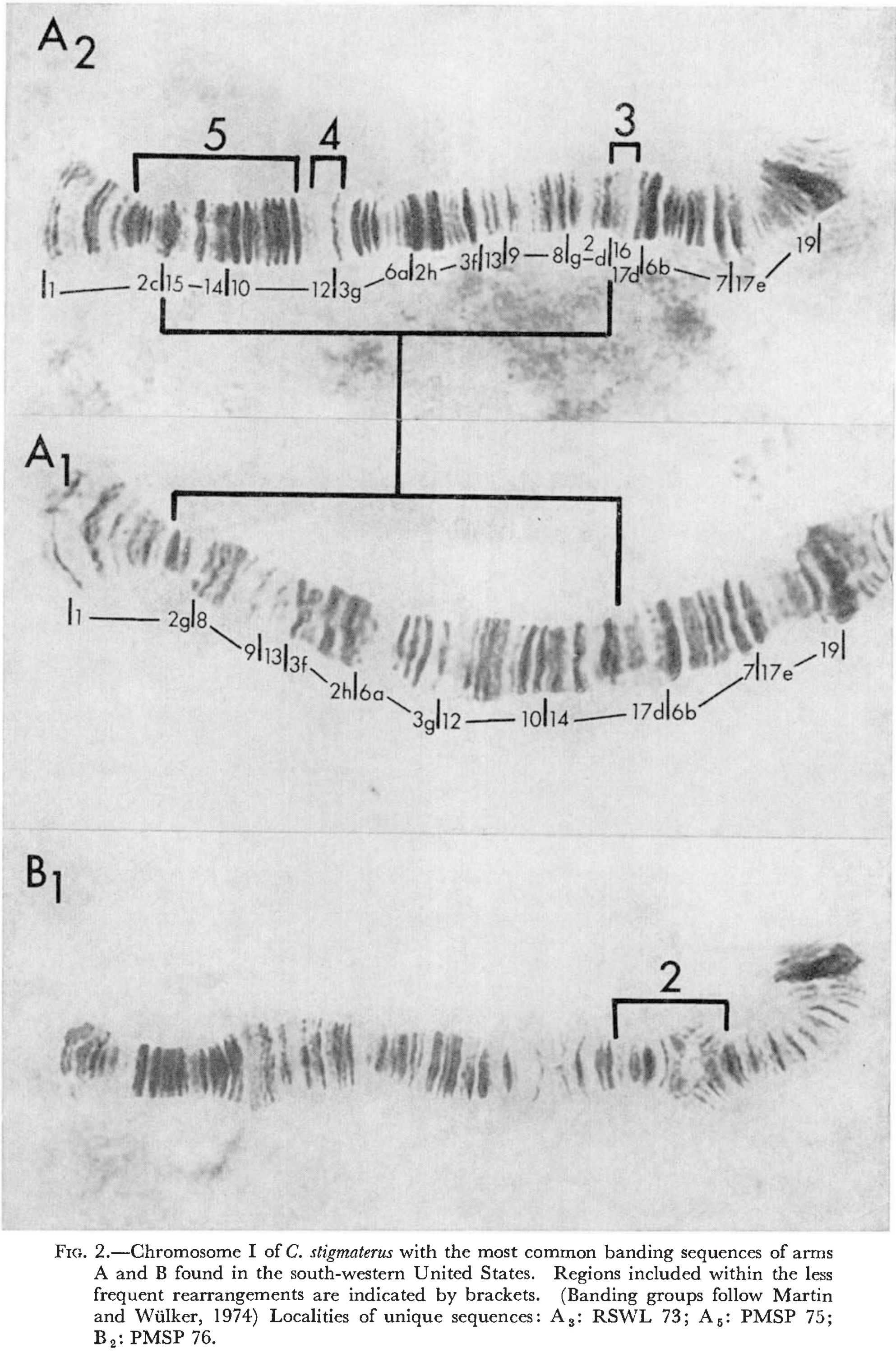


FIG. 2.—Chromosome I of *C. stigmaterus* with the most common banding sequences of arms A and B found in the south-western United States. Regions included within the less frequent rearrangements are indicated by brackets. (Banding groups follow Martin and Wülker, 1974) Localities of unique sequences: $A_3$: RSWL 73; $A_5$: PMSP 75; $B_2$: PMSP 76.

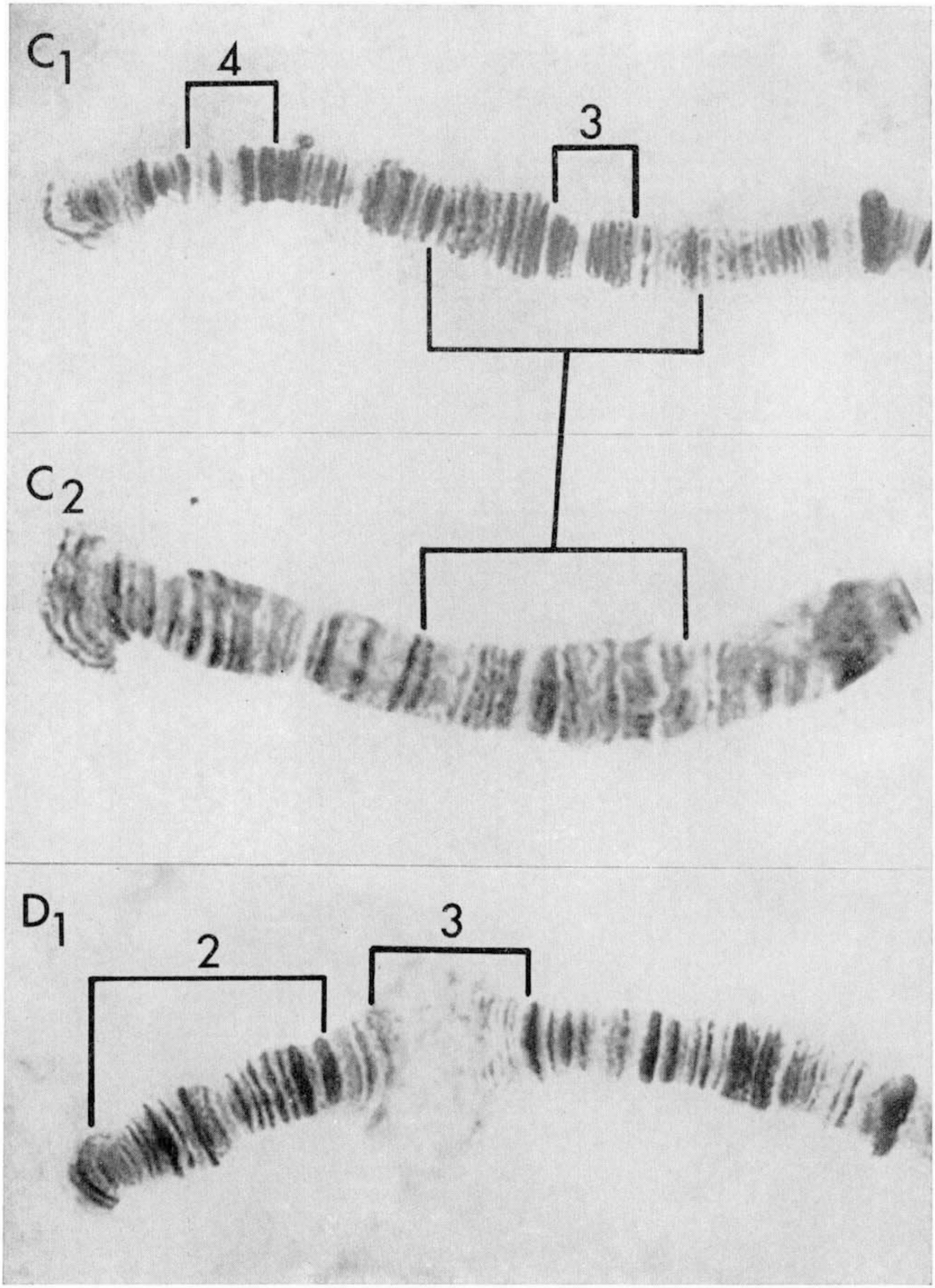


FIG. 3.—Chromosome II of *C. stigmaterus* with the most common banding sequences of arms C and D found in the south-western United States. Localities of unique sequences: $C_3$: PMSP 73; $C_4$: PMSP 76; $D_2$: ASTN 75; $D_3$: PMSP 75.

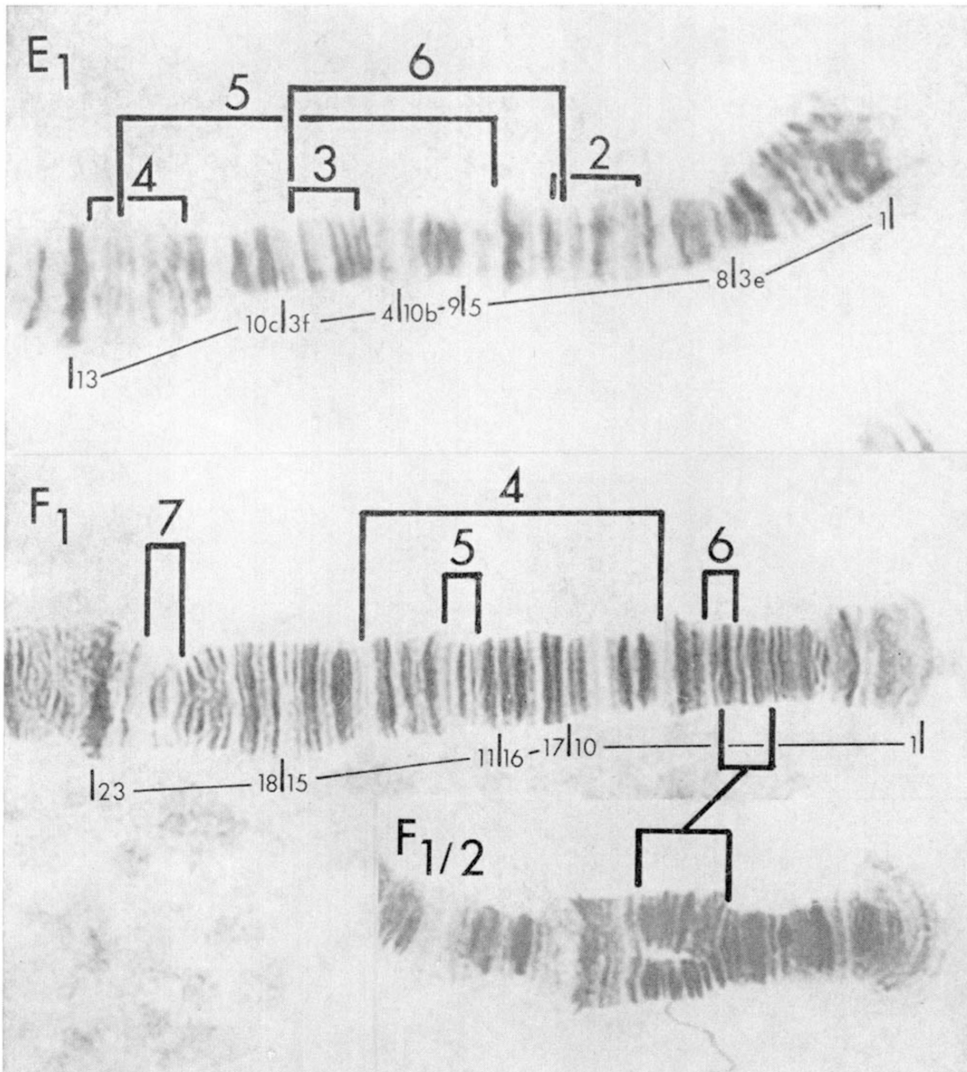


FIG. 4.—Chromosome III of *C. stigmaterus* with the most common banding sequences of arms E and F found in the south-western United States. Localities of unique sequences: $E_2$: LUBK 73; $E_3$: ALMO 73; $E_4$: BRCO 73; $E_5$: NCGD 76; $E_6$: NCGD 76; $F_4$: ALMO 75; $F_5$: MRTN 75; $F_6$: ALMO 76; $F_7$: PMSP 76.

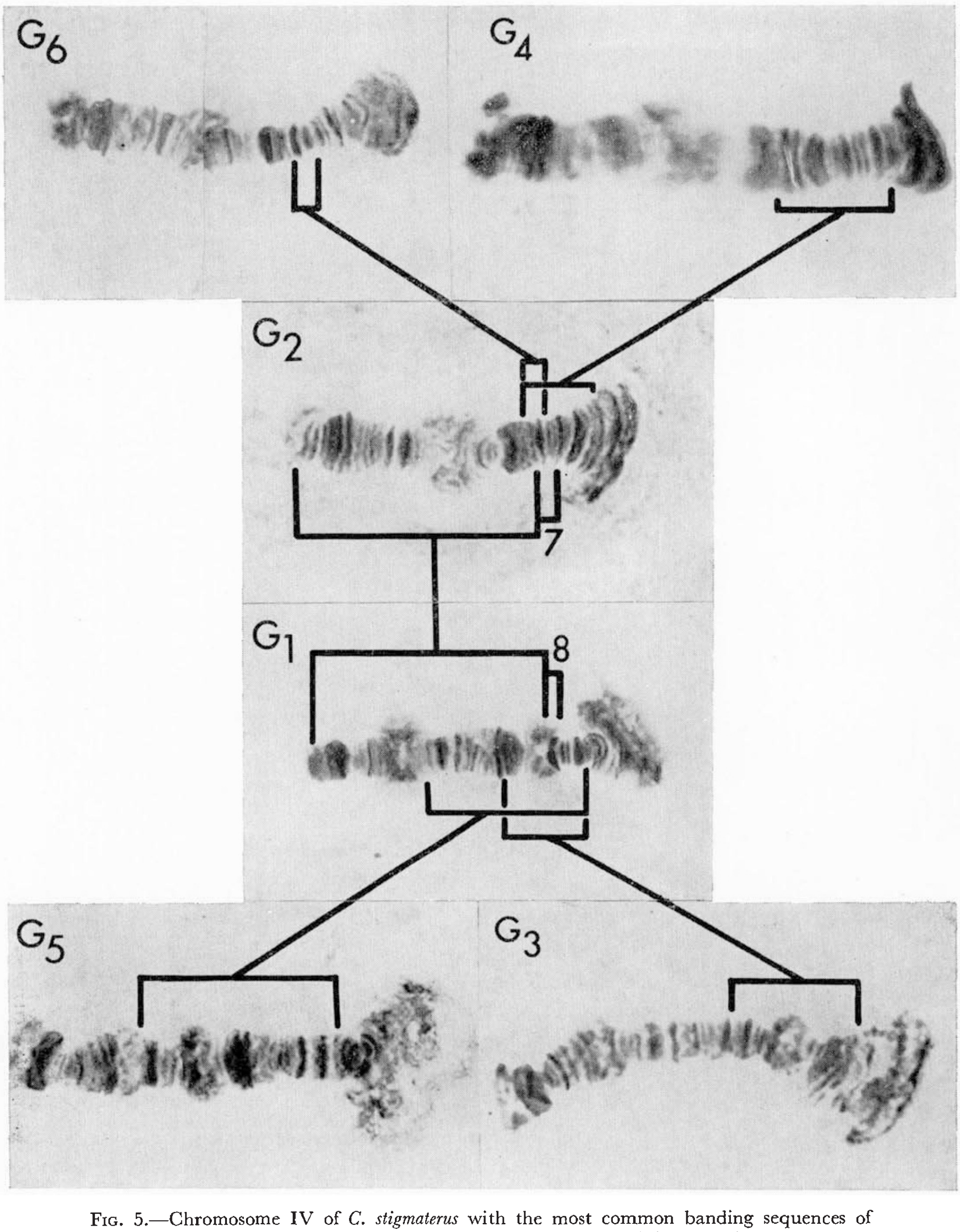


FIG. 5.—Chromosome IV of *C. stigmaterus* with the most common banding sequences of arm G found in the south-western United States. Localities of unique sequences: $G_7$: Portales, New Mexico 73; $G_8$: MRTN 76.

TABLE 2

*Frequencies of the common banding sequences of arm A of* C. stigmaterus. *All other sequences had frequencies less than 0·01*

| Locality | Year | N | $A_1$ | $A_2$ | $A_4$ |
|---|---|---|---|---|---|
| Davis | 1976 | 120 | — | 1·000 | — |
| Vacaville | 1975 | 146 | — | 1·000 | — |
| Cutler | 1976 | 110 | — | 1·000 | — |
| Oildale | 1976 | 90 | — | 1·000 | — |
| Ontario | 1976 | 89 | — | 1·000 | — |
| Hilarides Dairy | 1975 | 100 | — | 1·000 | — |
| Palm Springs | 1975 | 200 | — | 0·997 | — |
| | 1976 | 150 | — | 1·000 | — |
| Blythe | 1976 | 84 | — | 1·000 | — |
| Lordsburg | 1975 | 200 | 0·295 | 0·705 | — |
| Quincy Exit | 1976 | 150 | 0·290 | 0·710 | — |
| Alamogordo | 1973 | 99 | 0·419 | 0·581 | — |
| | 1975 | 91 | 0·385 | 0·615 | — |
| | 1976 | 90 | 0·311 | 0·689 | — |
| Roswell | 1973 | 66 | 0·508 | 0·485 | — |
| | 1975 | 96 | 0·703 | 0·297 | — |
| | 1976 | 89 | 0·702 | 0·298 | — |
| Van Horn | 1975 | 200 | 0·298 | 0·673 | 0·029 |
| Davis Mountains | 1976 | 151 | 0·272 | 0·728 | — |
| Marathon | 1975 | 200 | 0·178 | 0·820 | 0·002 |
| | 1976 | 88 | 0·176 | 0·824 | — |
| Lubbock | 1973 | 108 | 0·046 | 0·954 | — |
| | 1975 | 134 | 0·045 | 0·937 | 0·019 |
| | 1976 | 74 | 0·020 | 0·980 | — |
| Petersburg | 1973 | 108 | 0·028 | 0·968 | 0·004 |
| | 1976 | 94 | 0·149 | 0·840 | 0·011 |
| Bronte | 1973 | 90 | 0·189 | 0·811 | — |
| Junction | 1973 | 54 | 0·128 | 0·872 | — |
| Fredericksburg | 1976 | 90 | 0·044 | 0·956 | — |
| Austin | 1975 | 139 | 0·831 | 0·169 | — |
| Nacogdoches | 1976 | 156 | 0·388 | 0·612 | — |

independent of the sex of the larva bearing the combination. In all populations, the genotypes at these three arms were independent of the sex of the larva. On the other hand, the sequence $F_2$ is sex-linked. All females examined were homozygous for $F_1$, all males were $F_1/F_2$ heterozygotes. No homozygous $F_2$ individuals were observed. All subsequent statistical tests were performed with the data for the two sexes pooled.

The 23 samples from Texas and New Mexico were tested to determine if the sequences occurred in Hardy–Weinberg equilibrium, using a chi-square goodness of fit test. The only statistically significant result ($P < 0{\cdot}001$) was obtained for the sequences of arm C in the 1973 sample from Bronte, Texas. Seventy-one individuals were homozygous for $C_1$ and 66 were expected, while there were 11 heterozygotes compared to an expected number of 23.

The localities at which samples were taken more than once were tested using the chi-square contingency table to determine if the number of individuals having a particular genotype was independent of the year of collection. At Alamogordo the genotypes of both arms C and G differed at the $P < 0{\cdot}001$ level of significance. Examination of tables 3 and 4 reveals that the frequency of $C_1$ was greatly reduced between 1973 and 1975, then

TABLE 3

*Frequencies of the common banding sequences of arm C of* C. stigmaterus. *All other sequences had frequencies less than 0·01*

| Locality | Year | N | $C_1$ | $C_2$ |
|---|---|---|---|---|
| Davis | 1976 | 120 | 1·000 | — |
| Vacaville | 1975 | 146 | 1·000 | — |
| Cutler | 1976 | 110 | 1·000 | — |
| Oildale | 1976 | 90 | 1·000 | — |
| Ontario | 1976 | 89 | 1·000 | — |
| Hilarides Dairy | 1975 | 100 | 1·000 | — |
| Palm Springs | 1975 | 200 | 0·997 | — |
| | 1976 | 150 | 0·997 | — |
| Blythe | 1976 | 200 | 1·000 | — |
| Lordsburg | 1975 | 200 | 0·992 | 0·008 |
| Quincy Exit | 1976 | 150 | 1·000 | — |
| Alamogordo | 1973 | 99 | 0·419 | 0·581 |
| | 1975 | 91 | 0·269 | 0·731 |
| | 1976 | 90 | 0·572 | 0·428 |
| Roswell | 1973 | 66 | 0·417 | 0·583 |
| | 1975 | 96 | 0·328 | 0·672 |
| | 1976 | 89 | 0·360 | 0·640 |
| Van Horn | 1975 | 200 | 0·880 | 0·120 |
| Davis Mountains | 1976 | 151 | 0·785 | 0·215 |
| Marathon | 1975 | 200 | 0·650 | 0·350 |
| | 1976 | 88 | 0·591 | 0·409 |
| Lubbock | 1973 | 108 | 0·977 | 0·023 |
| | 1975 | 134 | 0·963 | 0·037 |
| | 1976 | 74 | 0·912 | 0·088 |
| Petersburg | 1973 | 108 | 0·963 | 0·037 |
| | 1976 | 94 | 0·883 | 0·117 |
| Bronte | 1973 | 90 | 0·939 | 0·061 |
| Junction | 1973 | 54 | 0·931 | 0·069 |
| Fredericksburg | 1976 | 90 | 0·833 | 0·167 |
| Austin | 1975 | 139 | 0·896 | 0·104 |
| Nacogdoches | 1976 | 156 | 0·994 | 0·006 |

increased in 1976 to a level greater than that in 1973; while the frequencies of $G_1$, $G_2$, and $G_6$ increased at the expense of $G_3$ and $G_4$. Roswell and Petersburg were significantly heterogeneous for the genotypes of arm A ($P<0{\cdot}001$), both showing an increase in the frequency of $A_1$. At Roswell, this change in frequency occurred between 1973 and 1975 with no significant alteration between 1975 and 1976.

In addition to the differences between the monomorphic populations of California and the polymorphic ones of Texas and New Mexico, there was a significant geographic heterogeneity of the frequencies of the inversions in arms A, C, and G among the populations of the eastern region ($P<0{\cdot}001$) as judged by use of a variance test for the homogeneity of the binomial distribution (Snedecor and Cochran, 1967). The frequency of $A_1$ was high at Alamogordo, Roswell, and Austin and generally greater in the south than in the north. $C_2$ was most frequent at Alamogordo and Roswell becoming less common by concentric steps until it virtually disappeared on both the eastern and western peripheries. $G_1$, $G_2$, and $G_6$ occurred in all populations in the two states while $G_3$ and $G_4$ had high frequencies in samples from western sites and disappeared in the east and $G_5$ was absent in the west but encountered frequently in east Texas.

TABLE 4

*Frequencies of the common banding sequences of arm G of* C. stigmaterus. *All other sequences had frequencies less than 0·01*

| Locality | Year | N | $G_1$ | $G_2$ | $G_3$ | $G_4$ | $G_5$ | $G_6$ |
|---|---|---|---|---|---|---|---|---|
| Davis | 1976 | 120 | 1·000 | — | — | — | — | — |
| Vacaville | 1975 | 146 | 1·000 | — | — | — | — | — |
| Cutler | 1976 | 110 | 1·000 | — | — | — | — | — |
| Oildale | 1976 | 90 | 1·000 | — | — | — | — | — |
| Ontario | 1976 | 89 | 1·000 | — | — | — | — | — |
| Hilarides Dairy | 1975 | 100 | 1·000 | — | — | — | — | — |
| Palm Springs | 1975 | 200 | 1·000 | — | — | — | — | — |
| | 1976 | 150 | 1·000 | — | — | — | — | — |
| Blythe | 1976 | 84 | 1·000 | — | — | — | — | — |
| Lordsburg | 1975 | 200 | 0·827 | 0·170 | — | — | — | 0·003 |
| Quincy Exit | 1976 | 150 | 0·877 | 0·123 | — | — | — | — |
| Alamogordo | 1973 | 99 | 0·046 | 0·409 | 0·010 | 0·535 | — | — |
| | 1975 | 91 | 0·016 | 0·429 | 0·044 | 0·511 | — | — |
| | 1976 | 90 | 0·122 | 0·472 | 0·033 | 0·350 | — | 0·023 |
| Roswell | 1973 | 66 | 0·402 | 0·136 | 0·439 | 0·023 | — | — |
| | 1975 | 96 | 0·375 | 0·167 | 0·417 | 0·042 | — | — |
| | 1976 | 89 | 0·331 | 0·124 | 0·511 | 0·028 | — | 0·006 |
| Van Horn | 1975 | 200 | 0·455 | 0·423 | 0·027 | 0·027 | — | 0·068 |
| Davis Mountains | 1976 | 151 | 0·593 | 0·384 | — | 0·006 | — | 0·017 |
| Marathon | 1975 | 200 | 0·180 | 0·563 | 0·093 | 0·040 | 0·009 | 0·115 |
| | 1976 | 88 | 0·215 | 0·579 | 0·084 | 0·027 | 0·016 | 0·074 |
| Lubbock | 1973 | 108 | 0·185 | 0·514 | 0·009 | 0·065 | 0·093 | 0·134 |
| | 1975 | 134 | 0·119 | 0·556 | — | 0·153 | 0·071 | 0·101 |
| | 1976 | 74 | 0·142 | 0·554 | — | 0·128 | 0·061 | 0·115 |
| Petersburg | 1973 | 108 | 0·153 | 0·523 | — | 0·153 | 0·088 | 0·083 |
| | 1976 | 94 | 0·090 | 0·580 | 0·005 | 0·144 | 0·096 | 0·085 |
| Bronte | 1973 | 90 | 0·161 | 0·374 | — | 0·017 | 0·300 | 0·128 |
| Junction | 1973 | 54 | 0·412 | 0·284 | — | — | 0·157 | 0·147 |
| Fredericksburg | 1976 | 90 | 0·661 | 0·183 | — | — | 0·156 | — |
| Austin | 1975 | 139 | 0·309 | 0·500 | — | — | 0·176 | 0·015 |
| Nacogdoches | 1976 | 156 | 0·615 | 0·202 | — | — | 0·183 | — |

To consider simultaneously the frequency data for the inversions in all arms, in order to visualise the relationships between all populations in a multidimensional sense, a matrix of average distances was generated for the 31 temporal and geographic samples. These distances were then clustered using the unweighted pair-group and arithmetic averages (UPGMA) method of cluster analysis (Sneath and Sokal, 1973). The resulting phenogram is presented as fig. 6. Two major clusters were noted: one containing all the California populations and those samples taken along the southern periphery of Texas and New Mexico, the other consisting of the more central and northern Texas and New Mexico localities. Within the former cluster, similarities exist in the frequencies of the standard sequences, but the California populations were separable from those in the east using differences between the two taxa in the frequencies of the inversions in populations of the latter region. In the second cluster, Lubbock, Petersburg, and Marathon were similar due to the presence of $A_4$ in these populations and the resemblances in the frequencies of the sequences of arm G. Temporal samples at a locality had distances less than 0·04 except at Alamogordo, Roswell, and Petersburg where greater distance values reflect the significant

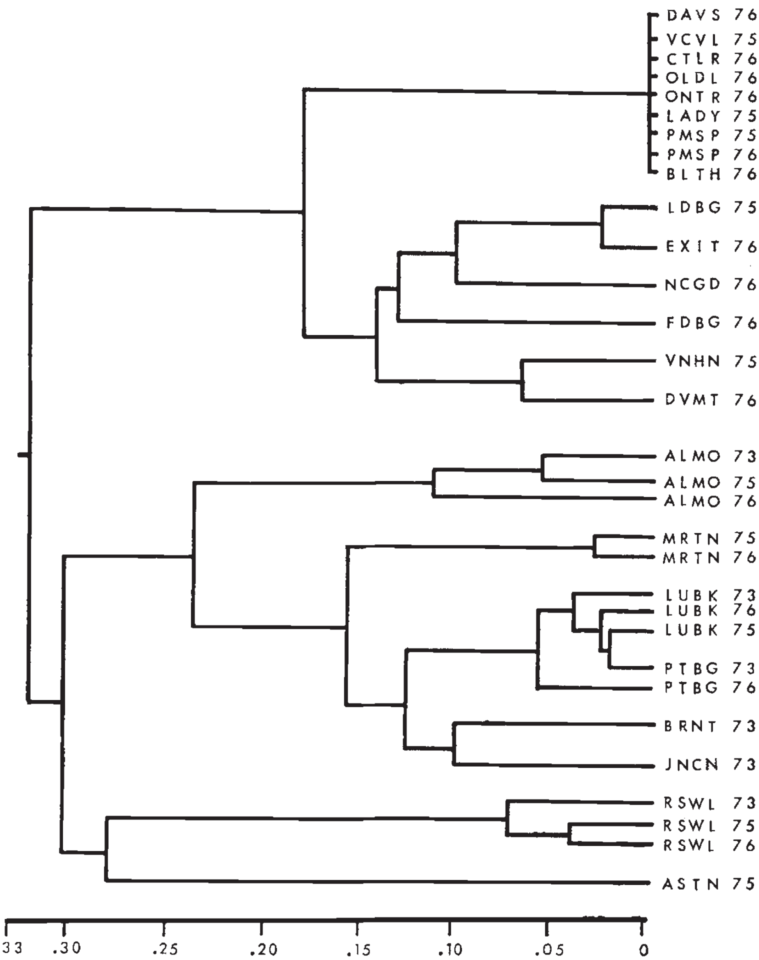


FIG. 6.—UPGMA cluster analysis of the average distance matrix for 31 geographic and temporal samples of *C. stigmaterus* from the south-western United States. Locality codes refer to table 1.

differences in the frequencies of the inversions observed between the collection dates.

## 4. DISCUSSION

A total of 28 rearrangements have been recorded for *C. stigmaterus* with at least one found in each arm. As was previously reported (Martin and Wülker, 1974), samples from California are characterised by low levels of chromosomal heterogeneity. Six inversions were observed in the samples from Palm Springs, but occurred only as one or two heterozygotes each. Thus, even at this locality, polymorphisms were being maintained at a low frequency. The majority of the rearrangements were found in samples from Texas and New Mexico. Only nine of these were observed in more than a single heterozygous larva from a single population. However, every population in these two states had between three and nine inversions present in stable polymorphisms. The stability of these polymorphisms is reflected in the absence of statistically significant heterogeneity in the frequencies of the inversions. Only one population exhibited departures from Hardy–Weinberg equilibrium and only three had significant changes in inversion frequency with time.

These data suggest that there may well be a genetic dichotomy between the California and Texas populations. However, the pattern in the distributions of the inversions in *C. stigmaterus* superficially resembles the well studied cases of *Drosophila robusta* (Carson, 1958) and *D. willistoni* (Da Cunha *et al.*, 1950) in having high chromosomal heterogeneity in central populations and low variability at peripheral localities. From a central group of populations including Alamogordo and Roswell in New Mexico and Marathon in Texas, which had both numbers of inversions and high frequencies of inverted sequences, heterozygosity values decreased clinically in all directions. At Lordsburg and Quincy Exit on the western border of New Mexico, only two inversions were found in more than one heterozygous larva, and at Nacogdoches, in east Texas, only three were present.

Similarities between the inversion frequencies of the California populations and those of the peripheral localities of Texas and New Mexico, reflected by their clustering together in the phenogram, make it difficult to use these chromosomal data as evidence for intraspecific divergence. Extrapolation to the west of the pattern in the distributions of the observed inversions indicates that one need not postulate restricted gene flow between the two taxa to account for the absence of inversions in California. As in *Drosophila*, the differences in chromosomal composition may result from ecological gradients which restrict the distributions of the inversions without completely barring gene flow. Thus the dichotomy in inversion heterozygosities in *C. stigmaterus* may not be a manifestation of either geographic separation or genetic divergence.

In this regard, collection data may be deceptive, also. Extensive collecting in Arizona did not yield a single collection of *C. stigmaterus* between Lordsburg, New Mexico, and Blythe, California, suggesting that the two segments of the range of this species are allopatric. However, this fact does not preclude the possibility that a zone of contact may exist outside the survey area, nor does it guarantee that migration does not occur between the two geographic regions.

In conjunction with the cytogenetic analyses performed on material collected in 1976, electrophoresis was used to obtain a more critical estimate of genetic variability in *C. stigmaterus* in the south-west (Hilburn, 1979). Of 23 loci examined, five had very different allele frequencies in the two cytogenetic taxa, clearly suggesting substantial genic differentiation.

Data obtained from the sample obtained at Palm Springs, California, is interesting in determining the amount of divergence which has occurred between the two taxa. At the five loci having marked intertaxa differences alleles were present in this population which otherwise were observed only in material from Texas and New Mexico. Moreover, the frequencies of the alleles at these loci more closely resembled the frequencies of the alleles in the estern taxon than those in other California populations. Despite these genic similarities, none of the inversions observed in material from Texas and New Mexico occurred at Palm Springs.

These data suggest that genetic divergence has occurred between the taxa, but apparently not to the extent necessary to prevent gene flow. Introgression has occurred in southern California, but the total genetic backgrounds of the two taxa are sufficiently different to make establishment of polymorphisms of migrant inversions disadvantageous and, thereby, to maintain the dichotomy in the inversion heterozygosities which distinguishes them.

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## 5. References

CARSON, H. L. 1958. The population genetics of *Drosophila robusta*. *Advan. Genet.*, *9*, 1-40.

DA CUNHA, A. B., BURLA, H., AND DOBZHANSKY, TH. 1950. Adaptive chromosomal polymorphism in *Drosophila willistoni*. *Evolution*, *4*, 212-235.

DOBZHANSKY, TH. 1970. *Genetics of the Evolutionary Process*, 505 pp. Columbia University Press, New York.

HILBURN, L. R. 1974. Studies concerned with the cytogenetic and morphometric variation in *Glyptotendipes barbipes* (Staeger) and *Chironomus stigmaterus* Say (Diptera : Chironomidae), 76 pp. M.S. thesis. Texas Tech. University.

HILBURN, L. R. 1979. Population genetics of *Chironomus stigmaterus* Say (Diptera : Chironomidae): II. Enzyme variability in populations of the southwestern United States. (Submitted to *Evolution*).

HILBURN, L. R., AND ATCHLEY, W. R. 1976. Studies on inversion polymorphisms in *Glyptotendipes barbipes* (Staeger) (Diptera : Chironomidae). *J. Kans. Ent. Soc.*, *49*, 419-428.

KEYL, H. -G. 1962. Chromosomenevolution bei *Chironomus*. II. Chromosomenumbauten und phylogenetische Beziehungen der Arten. *Chromosoma* (Berlin), *13*, 464-514.

MARTIN, J., AND PORTER, D. L. 1973. The salivary gland chromosomes of *Glyptotendipes barbipes* (Staeger) (Diptera : Chironomidae): description of inversions and comparison of Nearctic and Palearctic karyotypes. *Stud. Nat. Sci.* (Portales, N. M.), *1* (6), 1-25.

MARTIN, J., AND WÜLKER, W. 1974. A review of the genus *Chironomus* (Diptera : Chironomidae). VIII. The cytology of *Chironomus stigmaterus* Say. *Stud. Natur. Sci.* (Portales, N. M.), *1* (11), 1-18.

METTLER, L. E. 1963. *Drosophila mojavensis baja*, a new form of the mulleri complex. *Drosophila Inform. Serv.*, *38*, 57-58.

RICHARDSON, R. H., SMOUSE, P. E., AND RICHARDSON, M. E. 1977. Patterns of molecular variation. II. Associations of electrophoretic mobility and larval substrate within species of the *Drosophila mulleri* complex. *Genetics*, *85*, 141-154.

SNEATH, P. H. A., AND SOKAL, R. R. 1973. *Numerical Taxonomy*, 573 pp. W. H. Freeman and Company, San Francisco.

SNEDECOR, G. W., AND COCHRAN, W. G. 1967. *Statistical Methods*, 6th ed., 593 pp. The Iowa State University Press, Ames.

SOKAL, R. R., AND ROHLF, F. J. 1969. *Biometry*, 776 pp. W. H. Freeman and Company, San Francisco.

ZOUROS, E. 1973. Genic differentiation associated with the early stages of speciation in the *mulleri* subgroup of Drosophila. *Evolution*, *27*, 601-621.