TEMPERATURE-INDUCED MORPHOLOGICAL CHANGE IN *CULEX PIPIENS*

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ABSTRACT. The effect of temperature on *Culex pipiens pipiens, Cx. p. quinquefasciatus,* and reciprocal hybrids of the two was investigated by monitoring the DV/D ratios of these stocks maintained at 15.6°C and 23.9°C over 10 generations. Little variation occurred in mean values of the parental subspecies at either temperature. At 23.9°C, the mean ratios for both hybrid lines rose from an intermediate value to a level well above the accepted minimum for *Cx. p. quinquefasciatus* (0.4), and the proportion of individuals identifiable as that subspecies increased 16-fold. At 15.6°C, hybrid mean DV/D ratios decreased to or below the *pipiens* maximum value (0.2), and the proportion of *pipiens* individuals increased 4 to 5 times. The need to monitor closely the status of *Cx. pipiens* hybrid colonies is evident.

INTRODUCTION

Colonies of the subspecies of *Culex pipiens* Linn. in North America, *Cx. p. pipiens* and *Cx. p. quinquefasciatus* Say, remain morphologically stable, but experience has shown that under colonization their hybrids may show an increase in DV/D values, i.e., may become progressively more *quinquefasciatus*-like over time. The DV/D ratio, derived from measurements of the dorsal and ventral arms of the male phallosome, was devised by Sundararaman in 1949 and remains the most reliable morphometric means of distinguishing adult *pipiens* and *quinquefasciatus* (see Barr 1957 for a diagrammatic illustration). Barr (1957) examined some 3,500 specimens from a wide range of locations in the United States and Canada and concluded that *pipiens* is characterized by ratios equal to or less than 0.2, whereas *quinquefasciatus* have values equal to or greater than 0.4. Ratios of 0.21 through 0.39 indicate intergrade (hybrid) specimens.

Barr and Kartman (1951) traced changes in the DV/D ratios of *pipiens/quinquefasciatus* hybrid colonies through four generations which suggested a trend toward higher values. McMillan (1958) noted substantial increases in the mean DV/D ratios of two colonies established in October from material collected in Lawrence, Kansas, an area where morphologically intermediate forms occur in late summer. A 2.4-fold increase in the mean ratio which occurred over approximately 25 generations was seen recently in a colony derived from Chicago, Illinois, *pipiens* and New Orleans, Louisiana, *quinquefasciatus* (D. P. Wilton, unpublished data). The occurrence of such changes in insectary colonies without the introduction of additional *quinquefasciatus* material indicates a selective factor or factors operating within the insectary environment. In the present study, the effect of temperature on expression of the DV/D ratio in North American *Cx. pipiens* was investigated.

MATERIALS AND METHODS

Colonies of *pipiens* and *quinquefasciatus* were established at 23.9 ± 1.1°C from specimens collected in Fort Collins, Colorado, and Houston, Texas, respectively, during October 1981. Hybrid colonies using each of the subspecies as the male parent were subsequently set up, and a subcolony of each of the four lines was placed in each of two similar rearing chambers maintained at 23.9 ± 1.1°C and 15.6 ± 1.1°C, respectively. Fluorescent lights provided a 16-hr daylength in both chambers. The entire life cycle and all life activities of each of the mosquito strains took place at the assigned temperatures and photoperiod. Larvae were reared in screen-covered pans on alfalfa pellets and deactivated yeast, and adults were maintained in plastic screen colony cages (23 × 23 × 30 cm) with continual access to water and 5% sucrose. Blood meals were supplied by restrained chicks. Each generation of adults in each of the colonies was introduced into its own cage to avoid mixing generations and, thus, maximize the rate of any change in DV/D ratios which might occur.

Baseline DV/D values were determined for the P_1 generation of each colony. Subsequent determinations were made for even-numbered filial generations from the F_2 through the F_{10} from all stocks at both temperatures. Measurements were made with an ocular micrometer at 100X on detached terminalia in clove oil as previously described by Jakob et al. (1979). Sample sizes on which these calculations were based ranged from 112 to 240 specimens ($\bar{x}$ = 204).

RESULTS AND DISCUSSION

As shown in Fig. 1, the mean DV/D ratios in the *pipiens* colonies varied little from the baseline (P_1) value of −0.02 over 10 generations. At both temperatures all individual values remained below the generally accepted maximum of 0.2 for *Cx. p. pipiens*. The *quinquefasciatus* colonies showed greater variation. From a baseline value of 0.96, the mean DV/D ratio in the colony at 23.9°C decreased to 0.75 in the F_6 generation, then increased to 1.02 in the F_{10}. At 15.6°C, this subspecies showed an overall decrease from the initial 0.96 value to 0.88 in the F_{10}. Despite the relatively greater variability of the *quinquefasciatus* stocks, no

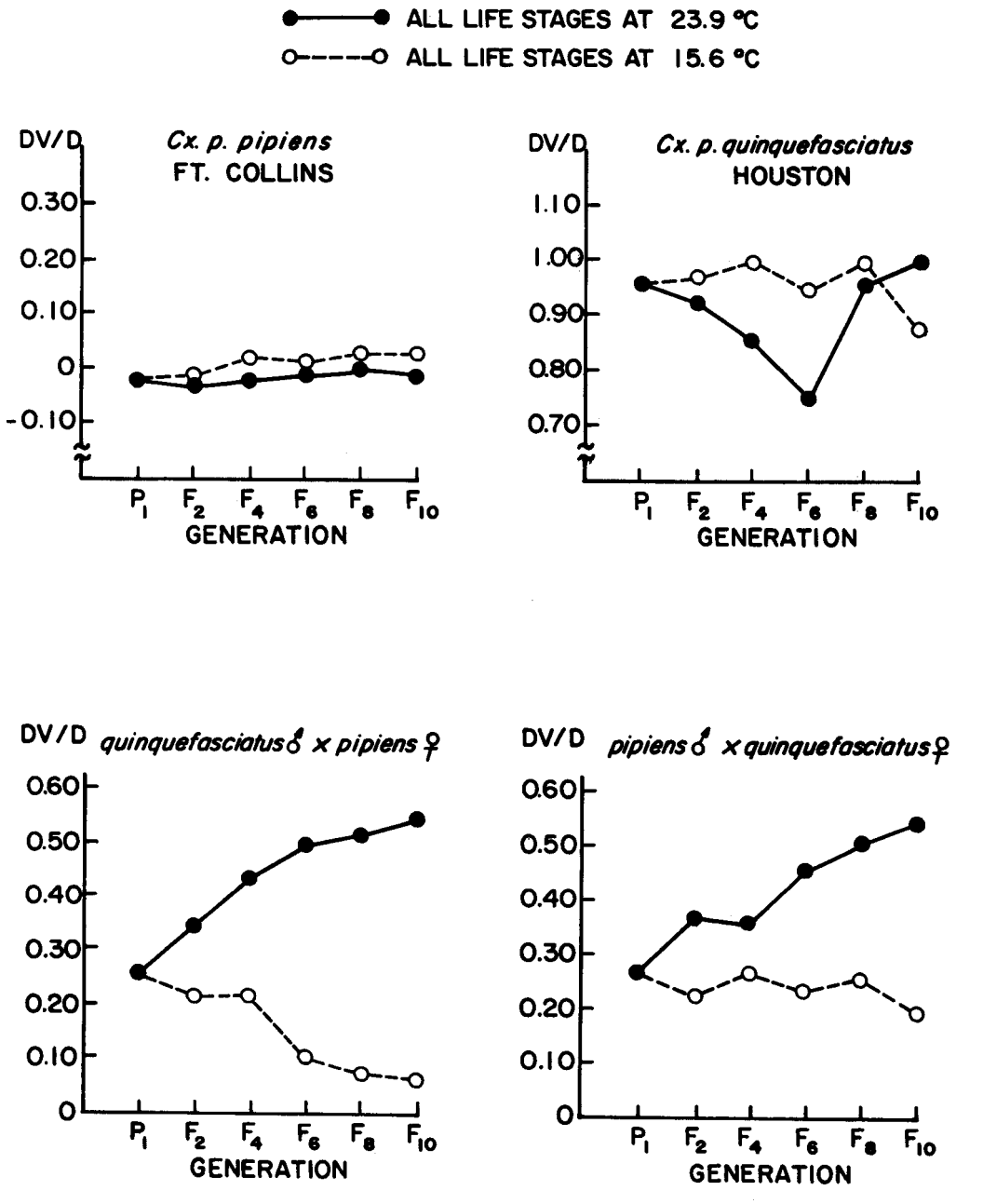

Fig. 1. Mean DV/D ratios of *Cx. p. pipiens* (Ft. Collins, Colorado), *Cx. p. quinquefasciatus* (Houston, Texas), and hybrid colonies.

specimen examined exceeded the accepted 0.4 minimum for *quinquefasciatus*.

Results with the hybrid lines were in marked contrast to those seen with the parent subspecies (Fig. 1). At 23.9°C, the mean DV/D ratio of the male *quinquefasciatus* X female *pipiens* cross had increased from its initial value of 0.26 in the intermediate range to 0.44 within the accepted *quinquefasciatus* range by the F_4. The ratio continued to increase in subsequent generations, reaching 0.55 in the F_{10}. The reciprocal cross (male *pipiens* X female *quinquefas-*

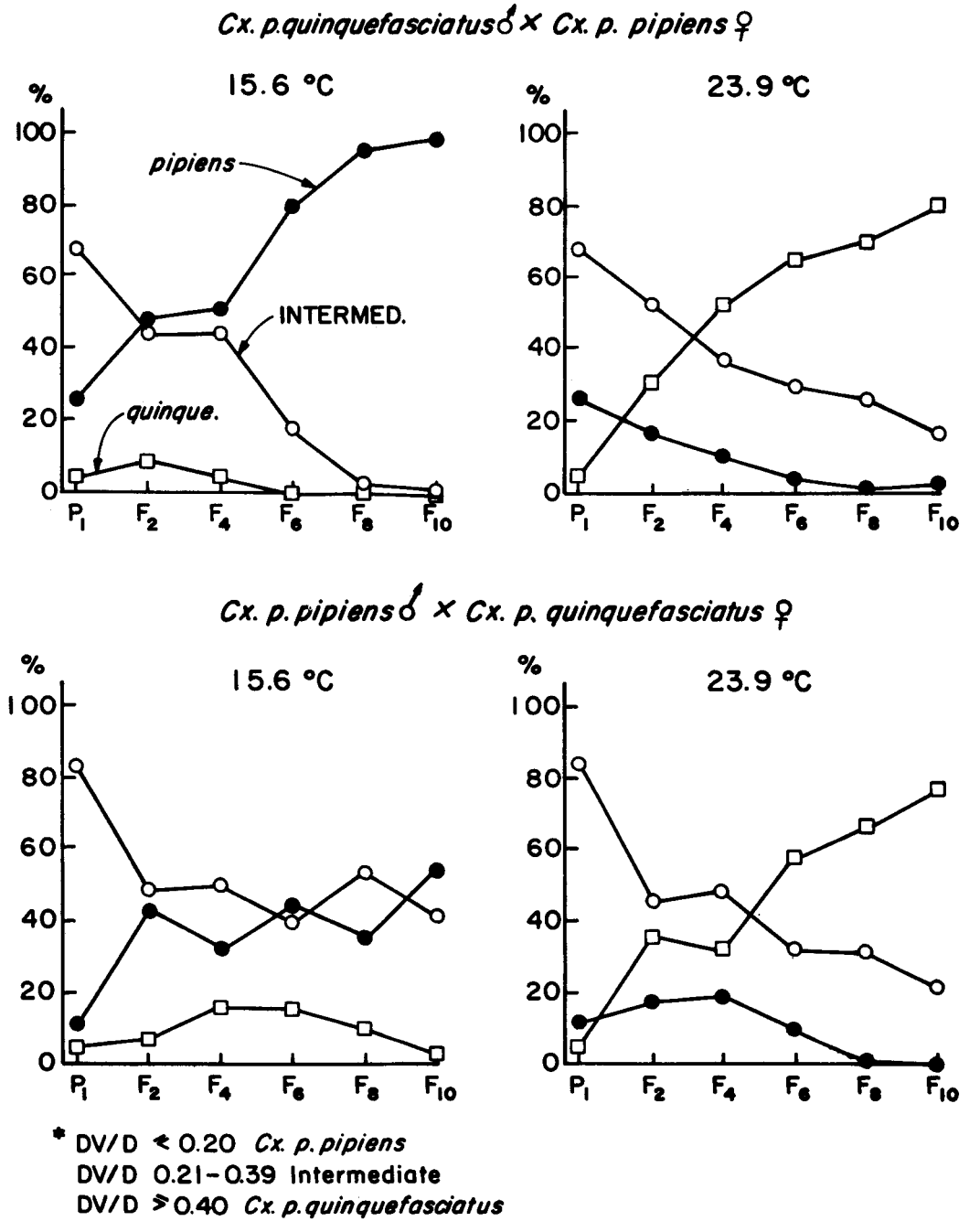

Fig. 2. *Cx. p. quinquefasciatus*/*Cx. p. pipiens* hybrid colonies at 15.6°C and 23.9°C. Percent of *Cx. p. quinquefasciatus*, intermediates, and *Cx. p. pipiens* based on DV/D.*

ciatus) gave similar results at 23.9°C. Its mean DV/D ratio rose from a P_1 value of 0.27 to 0.46 in the F_6 generation and it, too, reached 0.55 in the F_{10}.

At 15.6°C, the opposite trend, i.e., a decrease in mean DV/D ratios, was evident, particularly with the *quinquefasciatus* male X *pipiens* female stock which changed from 0.26 in the P_1 to 0.07, well within the *pipiens* range, in the F_{10}. The decrease over the same generation span with the reciprocal cross was comparatively small (0.27 to 0.20) but highly significant by *t*-test ($P<0.001$).

Of possibly greater significance than the increases and decreases seen in mean DV/D values were the changing proportions of mosquitoes in the hybrid colonies that would, on the basis of DV/D measurements, be identified as intermediates or as one of the parental forms. The magnitude of these changes in proportions and the rapidity with which they occurred are illustrated in Fig. 2. A sample of 234 DV/D determinations indicated that the P_1 generation of the *quinquefasciatus* male X *pipiens* female hybrids consisted of 26.5% *pipiens*, 68.4% intermediates, and 5.1% *quinquefasciatus*. Analysis of 161 F_{10} males showed that, at the higher temperature, these proportions had become 2.5% *pipiens*, 16.8% intermediates, and 80.7% *quinquefasciatus*. A similar sample of 155 specimens from the colony maintained at the lower temperature yielded estimates of 98.7% *pipiens*, 1.3% intermediates, and no *quinquefasciatus* in the F_{10}. Changes in proportion that occurred in the *pipiens* male X *quinquefasciatus* female colony were of lesser magnitude but indicated a similar response to temperature. As estimated from a sample of 235 specimens, this colony was composed initially of 11.5% *pipiens*, 83.4% intermediates, and 4.7% *quinquefasciatus*. These proportions in the F_{10} had become zero, 22.7%, and 77.3%, respectively, at 23.9°C (198 determinations) and 54.8%, 42.1%, and 3.0%, respectively, at 15.6°C (197 determinations.)

The temperature-related shifts in the DV/D ratios of infraspecific hybrids within *Cx. pipiens* demonstrated in this study are somewhat analogous to the temperature responses of a California population of *Drosophila pseudoobscura* discussed by Dobzhansky (1951), in which relative frequencies of chromosomal types undergo cyclic seasonal changes. During the hotter months, selection favors one type; during the cooler spring months, another type has a higher adaptive value. Both types are maintained in the population because of the changing character of the selection pressure. Similarly, the shifts reported here toward *quinquefasciatus* or *pipiens* produced by temperature manipulation could conceivably be reversed by a temperature reversal so long as some morphologically (and, therefore, genetically) intermediate individuals remained in the colonies. It has been shown that *quinquefasciatus* seasonally extends its range into *pipiens* territory in areas such as northern Kansas (McMillan 1958) and northern Utah (Rosay and Nielsen 1974). Since the two forms interbreed freely, the resulting hybrid populations may, under the selective pressure of high temperatures, serve as a significant additional source of the late summer *quinquefasciatus* populations seen in those regions. Investigators who work with *Cx. pipiens* will be well advised to monitor closely the status of their colonies if there is any possibility that they include intermediate specimens.

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