HYBRIDIZATION EXPERIMENTS WITH *CULEX PIPIENS* AND *C. QUINQUEFASCIATUS* (DIPTERA, CULICIDAE)[1]

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It has been shown that *Culex pipiens* and *C. quinquefasciatus* will freely interbreed in the laboratory, that fertile hybrids are obtained from either cross, and that hybrid populations can be maintained with undiminished vigor for a number of generations (reviewed by Barr and Kartman, 1951, and Mattingly, *et al.,* 1951). Furthermore, the presence in nature of specimens similar to laboratory hybrids has been demonstrated (Sundararaman, 1949). Evaluating these phenomena by means of Mayr's (1942, 120) species concept, Farid (1949) and Sundararaman (1949) have both recommended that *pipiens* and *quinquefasciatus* be treated as sub-species.

One question that immediately comes to mind when the above facts are considered concerns the fate of hybrids when exposed to populations of the parents. Sundararaman (1949) speculated that hybrid stocks of *pipiens-quinquefasciatus* may revert to parent types through backcrossing.

The study reported in this paper was made to investigate this possibility. While the work was in progress, a paper describing a similar investigation by Barr and Kartman (1951) was published. However, since the present study utilized completely different parent stocks it was thought worthwhile to complete the work.

The *pipiens* adults used were reared from a sample of the Cairo population collected as mature larvae and pupae from a cement basin in a mosque (coll. 257, June and July 1951, elevation approximately sea level). The biological and morphological characteristics of the Egyptian Cairo population have been described in detail by Knight and Abdel Malek (1951), who suggested as a result of their study that it should be treated as an urban biotype of *pipiens* and be given the noncommittal name of form "molestus."

The *quinquefasciatus* adults were part of a colony established from a larval collection made at Asmara, Eritrea in a small cement basin (coll. 236, 29 June 1951, elevation approximately 7,500 feet). Nothing definite is known of the biological characteristics of this population except that it will feed on infant laboratory mice and rats but not on man, pigeon, and toad; and that it will copulate in a space as small as 177 cubic inches.

Two morphological characters, one adult and one larval, were used to evaluate the effects of hybridization. The adult character used was the DV/D ratio of the male genitalia. In this ratio, which was developed by Sundararaman (1949), DV is the distance between the outer apical margins of the dorsal and ventral arms (measured for each side and averaged), and D is the distance between the outer apical margins of the dorsal arms. Standard procedures were used to prepare male genitalia for determination of the DV/D ratio. However, in contrast to the method employed by Sundararaman (1949), each cover slip was supported by the use of strips of thin plastic. No difficulty is experienced in determining the DV/D ratio for pure stocks of *pipiens* or *quinquefasciatus*. However, the measuring of the hybrid specimens is rather difficult because of the profound changes which take place in the shape of the distal portion of the dorsal arms.

The larval character used was the num-

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ber of branches possessed by the subdorsal hair on abdominal segment III (hair 4 of Belkin, 1950). This character was suggested as a means of separating the larvae of *pipiens* and *quinquefasciatus* by Howard, Dyar, and Knab (1915, 228). The determination of the branching of this hair was made from the larval skins. However, it can be accomplished from the living larva without too much difficulty. This hair is paired and in each case both members of the pair were examined. The subdorsal hair of segment IV may also be used but is slightly less trustworthy than on segment III because it exhibits more variability.

Larvae were fed from hatching until the fourth day with a dilute aqueous suspension of Mead's brewer's yeast powder (0.1 gram per egg raft). On the fourth day, larvae were counted and placed in 500 cc. of water in large crystallizing dishes. Lyphilized horse serum was dissolved in 500 cc. of water at the rate of 0.008 gram per larva. Brewer's yeast powder was again added, this time at the rate of 0.002 gram per larva. The resulting mixture was then added to the dish. If more than 100 larvae were present in a dish, it was found advisable to add a third volume of 500 cc. of water. Each dish was then covered with a glass plate and not touched further until pupation began. This procedure was worked out to avoid the great amount of contamination which so often resulted when the standard foods such as ground dog biscuit, rabbit-food pellets, or dried bread were used. Also, it retarded the otherwise rapid formation of a surface film.

Pupae were isolated in cotton-stoppered two ounce bottles. Upon emergence the adults were released in the desired combinations in laboratory mouse jars (possessing a volume of 375 cubic inches) which were closed with stockinette sleeves held in place by rubber bands. Oviposition dishes and cotton wicks soaked in table sugar solution were available at all times. Beginning on approximately the fourth day following the release of adults in the jars, infant white mice or rats were taped onto small cards and placed in the jars overnight. No records were kept as to the percentage of females taking blood from these sources, but it was estimated to be about 25–30 percent. Egg rafts were collected daily and isolated onto water in china soup bowls.

Not all of the females of this population of *quinquefasciatus* successfully copulated in the laboratory. In one case, 10 females were dissected 48 hours after 12 males and 12 females were confined in a glass cylinder with a volume of 177 cubic inches, and examination of the spermathecae disclosed that only six of the 10 females were fertilized. On another occasion, females from a small colony in a mouse jar (volume of 375 cubic inches) were dissected on the eleventh day following the mixing of the sexes and only five of them were fertilized. On the other hand, approximately 100 percent fertilization occurred within 96 hours in the *pipiens* population (in volume of 177 cubic inches).

The parent generation of *pipiens* and the F_1 generation of *quinquefasciatus* were used for the straight crosses and for the first backcrosses. The second backcrosses were made to the parent generation of *pipiens* (accomplished by collecting more larvae from the same breeding site) and to the F_2 generation of *quinquefasciatus*. The use of the F_1 generation of *quinquefasciatus* for the straight crosses was necessitated by the small number of individuals in the parent generation.

The straight crosses were made in both directions. The first backcrosses were made in all eight possible combinations. The second backcrosses were made in only the following four combinations: P ♂ × PPq ♀, PPq ♂ × q ♀, QPq ♂ × q ♀, and Q ♂ × QPq ♀ (males are capitalized and placed before females). Neither the straight crosses nor the backcrosses were carried beyond the first generation.

Care was taken in the selection of samples for examination to represent as many egg rafts from each separate population as possible. The actual number of egg rafts from each population ranged from five to 17.

RESULTS. As stated previously, the effects of crossing and backcrossing were determined for two morphological characters, one adult (DV/D ratio) and one larval (number of branches of hair 4 of abdominal segment III). These two characters are the only reliable morphological diagnostic characters presently known for the separation of *pipiens* and *quinquefasciatus,* and the latter character is only diagnostic when used on a percentage basis.

DV/D RATIO. The DV/D ratios for the parent, straight-crossed, and backcrossed stocks are graphically presented in Figure 1. In each case, the solid line indicates the observed range of variation, the cross line the mean, and the dash line the mean plus and minus three standard deviations. The data below these lines present the number of examples measured from each population (N), the means and their standard errors (M), and the standard deviations and their standard errors (S.D.).

It can be seen that both the observed and theoretical ranges of the DV/D ratio were distinct in the two parent stocks. The hybrids obtained by crossing the parent stocks in both directions were intermediate. Similarly, the populations resulting from the backcrossing of the hybrid strains were in each case intermediate between the hybrid and the parent. This shift towards the parent is more pronounced with *pipiens* than it is with *quinquefasciatus.*

Of interest is the fact that, in each case, the observed range of variation is positively skewed. Also of interest, is the fact that this range is considerably greater in *quinquefasciatus* and in the backcrosses to *quinquefasciatus* than in the case of *pipiens.* No explanation for these two peculiarities can be advanced at present.

HAIR 4 OF AB-III. The percentage occurrence of singleness (unbranched condition) of hair 4 of the third larval abdominal segment is illustrated in Figure 2. In this figure each square represents 25 larvae from a single egg raft. In general, this hair is double (occasionally single or triple) in *pipiens* and single (occasionally double) in *quinquefasciatus.* Individual pure-line specimens cannot be accurately identified by this character but if a sample of 10 or more larvae from a single collection are examined a determination can be safely made.

The effect of hybridization upon hair 4 of the third abdominal segment is similar to that upon DV/D in that the hybrids are intermediate, but differs somewhat in that an overlap with *pipiens* occurs. Backcrossing the hybrid line to *pipiens* results in an almost complete approach to *pipiens,* but with more variability occurring than in pure-line stock.

Backcrossing to *quinquefasciatus* results in a rather typical production of intermediates. As with the DV/D ratio, it appears that a third backcross is needed in the case of *quinquefasciatus* to return the stock to a condition resembling the pure line.

MISCELLANEOUS. In connection with the above two characters, no significant differences could be detected between the two combinations of straight crosses, the eight combinations of first backcrosses, and the four combinations of second backcrosses that were made. Consequently, the data were combined for each of these sets of combinations for use in Figures 1 and 2.

It has been shown by Knight and Abdel Malek (1951) that an average of 1.1 percent of *pipiens* females in the Cairo area are capable of laying eggs without a prior bloodmeal (autogeny). Autogeny has never been demonstrated for *quinquefasciatus.* In view of these facts, it is of interest to note that in the hybridization experiments reported in this paper one autogenous fertile egg raft was obtained. The egg raft resulted from a first backcross of hybrid males to female *pipiens* (Pq ♂ × p ♀). Unfortunately, the larvae resulting from this egg raft did not survive to maturity.

DISCUSSION. It is apparent from the study reported by Barr and Kartman (1951) and from this one that hybrid stocks of *pipiens-quinquefasciatus* will revert in two to three generations to the

Fig.1 Comparison of DV/D for C. pipiens, C. quinquefasciatus and hybrid populations

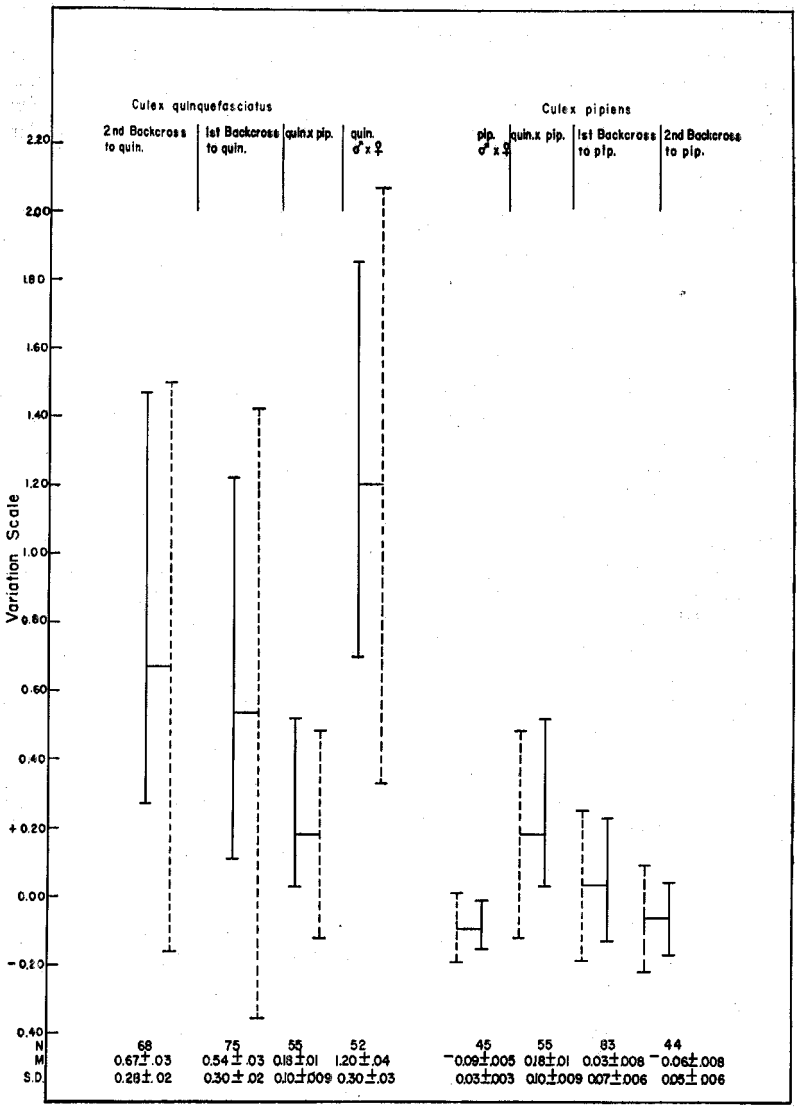

FIG 1. Comparison of DV/D for *C. pipiens, C. quinquefasciatus* and hybrid populations. The solid line indicates the observed range of variation, the cross line the mean, and the dash line the mean plus and minus three standard deviations. The data below these lines present the number of examples measured from each population (N), the means and their standard errors (M), and the standard deviations and their standard errors (S.D.).

parent type if backcrossing is allowed to take place. In this study it was found that reversion to the parent type occurs approximately one generation faster with *pipiens* than it does with *quinquefasciatus*.

On the basis of the data presented here, it is believed possible to determine the parentage of field-collected material by securing egg rafts, rearing them in isolation, and then determining for each raft the DV/D ratio for 10 or more males and the number of branches possessed by hair 4 of abdominal segment III for 25 or more larvae. However, for the most complete accuracy, this procedure should be preceded by a determination of the range and mean for these characters for known pure-line stocks from the general geographic area. Evidence of the importance of this procedure is the fact that Sundararaman (1949) found DV/D for *pipiens* (United States) to be 0.0 to 0.5, and Barr and Kartman (1951) found it to be 0.0 to 0.2. In contrast to this, all *pipiens* examined from Cairo had the DV/D ratio falling in a range of —0.15 to —0.01.

Fig. 2. Hair 4 of third segment of larva of C. pipiens, C. quinquefasciatus and hybrid populations

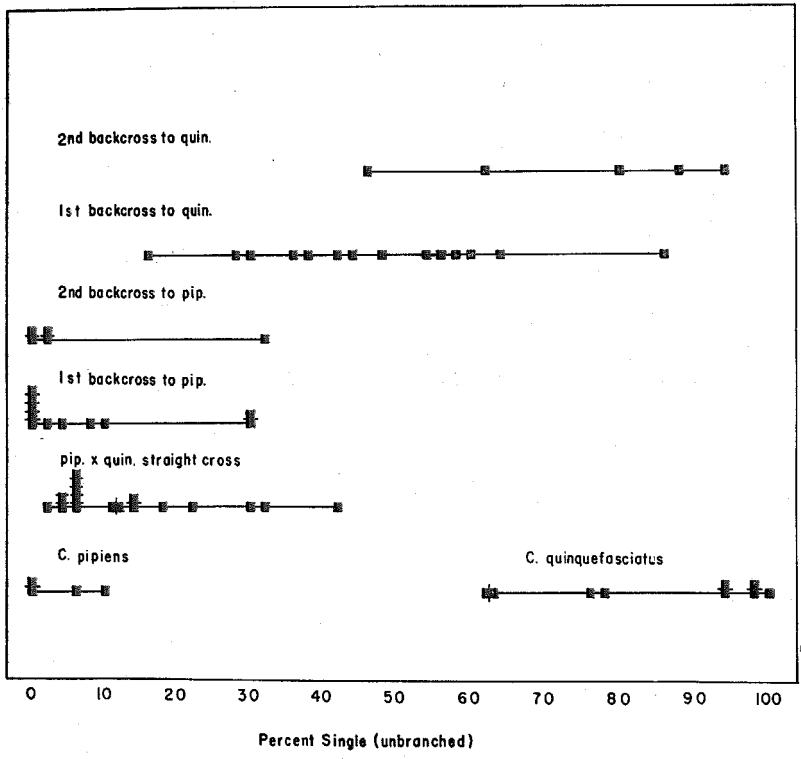

FIG. 2. Hair 4 of third abdominal segment of larva of *C. pipiens, C. quinquefasciatus* and hybrid populations. In this figure each square represents 25 larvae from a single egg raft. Since hair 4 is paired, each square represents the examination of 50 hairs. The position of each square represents the percentage of these 50 hairs that are single.

However, in all three cases the range of variation was distinct from that of *quinquefasciatus*.

A word of caution in connection with the above discussion must be added. Because only a portion of the female *quinquefasciatus* in this colony ever became fertilized, presumably due in part to the small size of the container, an unnatural selective force was in action which may well have decisively colored the results. It is possible that if the adults in the hybridization experiments had been caged in a space sufficiently large to have permitted 100 percent fertilization of the *quinquefasciatus* females, that altogether different results might have occurred. Any future experiments should attempt to remove this possible selective force.

It is a pleasure to express my indebtedness to Mr. Harry Hoogstraal, U. S. Naval Medical Research Unit No. 3, Cairo, Egypt, for the Eritrean *quinquefasciatus* colony. Mr. Sobha Gaber, technician, painstakingly attended the mosquito rearing throughout this project and deserves much credit for his excellent work.

SUMMARY. This study was made to determine the effect of backcrossing upon hybrid stocks of *Culex pipiens* × *Culex quinquefasciatus*. The *pipiens* stock used came from Cairo, Egypt, and the *quinquefasciatus* colony from Asmara, Eritrea. The first backcross was accomplished in all possible combinations. The second backcross was made in four combinations. Two morphological characters, one adult and one larval, were used to evaluate the effects of hybridization. The results indicate that if backcrossing is allowed to take place hybrid stocks of *pipiens* × *quinquefasciatus* will revert in two to three generations to the parent type. This reversion to the parent type occurs approximately one generation faster with *pipiens* than it does with *quinquefasciatus*.

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