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Inbred Strains of Culex Mosquitoes

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Many studies are in progress using mosquitoes as laboratory animals, some of the most prominent of which are studies of various insecticides and the resistance of mosquito strains to them. The results of these experiments have varied widely for a number of reasons. One important source of the varying results can probably be found in the inherent genetic differences in the stocks used.

The genetic experiments using Culex mosquitoes in this laboratory have indicated that wild-caught strains of Culex pipiens and C. quinquefasciatus vary widely in many respects. Ordinary laboratory-bred strains also show a wide range of expression of phenotypic characteristics. In the course of our experiments it has been necessary to develop inbred strains of these two species, with the ultimate aim of as high a degree of homozygosity as possible.

One stock of C. quinquefasciatus maintained in this laboratory was originally obtained from Don W. Micks at Galveston, Texas, and had been maintained by him for several generations as a laboratory stock. A wild-caught strain of C. pipiens, from Champaign, Ill., is at present in use.

These stocks have been carefully inbred according to the plan described below. All stocks were begun from single egg rafts. The larvae from each raft were raised separately, and the pupae transferred to a new cage. Males and females from the same egg raft were allowed to mate, the egg rafts were collected, and the larvae raised in separate pans. From this F_1 , one egg raft was selected, the adults were allowed to emerge and mate, and F₂ rafts collected. Thus each generation is the result of brother-and-sister mating, and each succeeding generation $(F_1, F_2, F_3,$ F_4 , etc.) is derived from a single egg raft, raised in a new cage. There is no backcrossing and no outcrossing, even to members of the same original stock, and thus the inbreeding is as strict as possible in animals with bisexual reproduction.

The C. quinquefasciatus stock is at present in the \mathbf{F}_{20} generation, and the C. pipiens stock in the \mathbf{F}_{12} . Following the method given by Wright (1) the pipiens stock in the F_{12} is calculated to be more than 95%homozygous, and the quinquefasciatus in the F_{20} should be about 99% homozygous. Sixteen generations of brother-sister matings will result in about 98% homozygosity. By way of contrast, one technique commonly used in mass cultures of these mosquitoes has been to isolate all F_1 rafts, then isolate the F_2 , and so forth. This method is little more than random mating and, assuming that other factors (mutation, selection, etc.) are negligible, the population should be just as heterozygous in the nth generation as it was in the first. It is therefore possible that many laboratory colonies, as well as wild-caught strains, have considerably different genetic backgrounds. Other characteristics of these stocks, defined in terms of the biological properties which they possess are as follows:

C. pipiens

a) Stenogamic: Mating and egg deposition take place in cages $30 \times 30 \times 30$ cm. Although most strains of C. pipiens (not molestus or autogenicus) are eurygamic, we have never experienced any difficulty in getting this strain to mate in cages of this size. If, of course, eurygamy is defined on the basis of mating on the wing rather than on the basis of cage size, the term is a matter of definition. This strain has never been observed to mate at rest, but will mate in flight. It is a striking coincidence that of several strains of C. pipiens which have been collected in the vicinity of Champaign, none has ever been found which would not breed in cages of this size if other rearing conditions were right.

b) Anautogenous: A blood meal is required before egg deposition. As far as we know, we have never had an autogenous egg raft. Repeated isolations of males and females, supplied with water, moistened prunes, raisins, apple slices, or sugar solutions, have never resulted in a single egg raft. On the other hand, egg deposition has always been associated with a previous blood meal.

c) Ornithophily: Although this strain will bite man-infrequently, and only when no bird blood is available---its definite preference is for birds. We normally use pigeons, but the strain has also fed on chickens. Individual females seem to differ in their biting habits. Some will avidly feed on the pigeon no matter at what time it is introduced into the cage; others prefer to feed in the evening.

d) Modified heterodynamy: Heterodynamy is a term which has been applied to those strains which undergo a diapause, or temporary cessation of reproductive function, especially during the winter months. Few laboratories have succeeded in rearing C. pipiens on a year-round basis, owing principally to this factor. Our strain seems to possess the genetic basis for this character, but its manifestation is modified, we believe, by the use of continuous light in the insectary during the winter months. Without light 24 hr a day, the females will (1) not feed, (2) refuse to lay eggs, or (3) lay infertile eggs.

In continuous electric light and favorable laboratory conditions, fertilization and the deposition of fertile eggs do take place, although at a noticeably slower rate than in the summer. The reduced fertility is also marked in comparison with the behavior of C. quinquefasciatus, which in our stocks, at least, does not seem to possess this characteristic.

Culex quinquefasciatus

Three stocks of this species are currently maintained. One is the Galveston, Texas, stock described above. A second originated from a single egg raft from Bakersfield, Calif., obtained through the courtesy of R. E. Bellamy and Lewis W. Isaak. The third was obtained from Albert Miller at Tulane University. As far as we can tell, these three stocks are exactly alike in their biological characteristics. All breed readily in cages $30 \times 30 \times 30$ cm and are therefore stenogamic. All three require a blood meal before egg deposition, and are hence anautogenous. They feed avidly on both pigeons and chickens, at almost any time of the day, as well as at night. The Galveston strain will bite man reluctantly; the other two have not been tested in this regard. As noted above, none of these strains shows any evidence of a seasonal pause in reproductive activity.

The stocks maintained in this laboratory, therefore, might be of use in various types of experiments in which stabilization of the genetic background of the strain is desirable.

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Comments and Communications

Vitality of the Aged

C. H. FORSYTH has presented a note (SCIENCE, 115, 251 [1952]) indicating that in contrast with the conclusions of his earlier study (SCIENCE, 70, 85 [1929]) mortality of the aged now seems to be improving. He concludes that, from 1890 on, mortality increased and reached a maximum between 1910 and 1920 and, since then, has been improving (i.e., rates have been decreasing).

Forsyth's data are for seven selected states and do not extend beyond 1940. Actual analysis for the entire United States is now possible for the last two decades. During the 1940s there was a rather astounding improvement; this will be definitely indicated when the official life tables based on the 1950 census and vital statistics for 1949-51 are available.

Table 1 gives death rates comparable to Forsyth's, based on official life tables from 1900 through 1940, with the 1948 data developed from official abridged tables. The fact that there are different areas covered in the various years can be taken into account since data for all areas are available for 1929-31 and can be compared. The table shows no evidence of significant changes in mortality of the aged from 1900 through 1930. The low rate for 1919-21 is not especially significant because, as has been pointed out in many places, this was related to the high influenza mortality of 1918, which removed rather prematurely the somewhat impaired lives. Differences in rates in the years before 1930 appear to be due mainly to statistical fluctuations; it should be recognized in any event that mortality rates for ages 70 and over, especially in small population groups such as Forsyth dealt with, are not too reliable because of the relatively small numbers involved, not to mention errors in reporting.

Since 1930, there seems to have been a definite improvement for all categories, and especially for women. The mortality rates for 1948 are well below those for the early part of the century and apparently by much greater amounts than can be attributed to random fluctuations. Data for years between 1940 and 1948 have also been analyzed and show the same general trend.

The earlier Forsyth article draws some conclusions that, in retrospect, seem rather surprising. Forsyth then concluded that "the whole picture, from our earliest records in 1890 to the present time, points constantly and inevitably to a future declining average length of life until the American adult wakes up to the fact that the odds are at present heavily against his living as long as his father or grandfather." Further, he states that the decline in longevity at advanced ages "already dominates and the average length of life—or at least the expectation from age ten—is already going down."

Table 2 shows the opposite to be true. The expectation at age 20 has risen steadily and significantly throughout the entire period for both men and women, and the increase over the past half-century amounts to about 15% for men and 20% for women. The expectations for men aged 50 and 70, and for women aged 70 remained more or less constant over the first 30 years of this century, but since 1930 have risen definitely and significantly. The expectation for women aged 50 increased slowly during the first 30 years of the century and more rapidly thereafter.

This analysis corroborates Forsyth's conclusion that mortality among the aged has improved since about 1915—in fact, later data strengthen his conclusion. However, the statistical evidence indicates that there was no significant worsening of mortality among the