

# SELECTIONS FOR BLOOD-FEEDING EFFICIENCY IN COLONIZED *Aedes aegypti*

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**ABSTRACT.** Laboratory-reared *Aedes aegypti* were selected for blood-feeding efficiency. Feeding speed and host irritating properties were used as selection criteria. Mice and man respectively served as mosquito hosts. Bidirectional selections during 14 generations did not result in changes in feeding speed in either selection line. It was concluded that the heritability of variations in feeding speed was either very low or absent in these mosquitoes. This implied that the bimodality of the distribution of the variations in feeding speed ob-

served had to be attributed to phenotypical factors. Selections by the repeated test method for 4 generations did not result in changes in the host irritating properties of the mosquitoes, nor were these properties found to be a stable characteristic of the individual mosquito. It consequently appeared that differences in host irritating properties were not genetically determined either. Variations in host-seeking behavior were found to be affected by multiple factor inheritance.

## INTRODUCTION

The capability of a mosquito to serve as a vector of infectious agents is, amongst others, directly related to its blood-feeding abilities. Differences in blood-feeding properties may therefore lead to differing vectorial capacities of mosquito populations (e.g. Mellink 1980). As it has recently been shown that mosquito taxa feed differently (Service 1971, Do Si Hien 1976, Magnarelli 1979) and that intraspecific divergences occur as well (Mellink 1980), further research on variations of the blood-feeding process of mosquitoes

is inviting. The present report deals with the possible genetical foundation of the variability in this respect. As such it is a sequel to the work of Gillett (1967) on this subject.

In accordance with the recommendations of Spielman and Kitzmiller (1967) concerning the study of insect genetics, selection procedures were applied at the outset of the study. A laboratory strain of *Aedes aegypti* (L.) was used. It was assumed that selections for blood-feeding efficiency offered the most promising approach (cf. Gillett 1967). Feeding speed and host irritating properties were here

considered to constitute the most important components. The selections for feeding speed were carried out on mice, those for host irritation on a human volunteer who was highly hypersensitive to bites.

## MATERIALS AND METHODS

The wild type stock colony of *Ae. aegypti* used was obtained from the Royal Tropical Institute at Amsterdam in 1969. It has since continuously been maintained by mass culture in our laboratory. Culture conditions were based on the procedures described by Morlan et al. (1963) and were well-defined and strictly standardized (see also Mellink 1980). When tested for the 1st time the mosquitoes were 1 week old and had no blood feeding history. At 1 day prior to testing, sugar feeding of the mosquitoes was discontinued to ensure a satisfactory biting avidity. The mice were of common laboratory stock. A single human volunteer was employed throughout.

**SELECTION AND TESTING APPARATUS.** The testing and selection apparatus for the experiments on mice (selections for feeding speed) consisted of a small plastic cylinder (diam 2.5 cm, 1 cm high) without a bottom. It was sideways connected with a plastic tube (diam 1 cm) provided with a plastic sluicing barrier for the introduction of the mosquitoes. The open end of the cylinder was applied to the shaven abdominal skin of a mouse. The mice were sedated by pentobarbital-sodium injections, the anaesthesia lasting for periods of up to 2 hr. None of the mice was employed more than once within a fortnight. In pilot studies no effects of either the anaesthetizing procedures or repeated exposures of mice to bites were observed. Mosquito behavior was viewed from above with the aid of a stereomicroscope. All observations took place in a climatized insectary during daytime.

In the experiments on the human volunteer (selections for host irritating properties) the testing chamber consisted of a wide-mouthed plastic bottle which

was removed immediately after the commencement of mosquito biting. The inside of both lower arms served as an attraction and blood source. In pilot studies no relationship between the location of the bites on the arms and the measurements used were noted. Mosquito behavior was observed from aside at close proximity with the naked eye. All observations took place under ambient laboratory conditions during the hours around noon.

**SELECTION AND TESTING PROCEDURES.** In both series the mosquitoes were released individually in the testing apparatus. Subsequently the durations of the periods of blood source searching in the skin (penetration time,  $P_t$ ) and of actual blood intake until spontaneous withdrawal (feeding time,  $F_t$ ) were recorded. Abortive skin penetrations were neglected and mosquitoes not feeding to repletion (i.e. to stage 5 on the scale of Piltit and Jones 1972) or feeding on other fluids than blood were eliminated. All of these occurrences were very rare. Furthermore, in the experiments on man the duration of the period between the start of skin penetration and the onset of itching (safety time,  $S_t$ ; Gillett 1967) and the wheal surface of the immediate (type I) cutaneous response at its maximum (i.e. ca 10 min after the bite) were determined to be used as measurements of host irritation. The bites on each arm of the volunteer had to be spaced at intervals of 20 min to avoid inaccuracies in discerning the separate reactions.

Although selection for feeding speed in nature, if present, would presumably apply to the whole of the feeding process (cf. Gillett 1967), the contribution of the penetration period to its length was judged relatively insignificant, and it was decided to select for blood engorgement speed only. Bidirectional selections based on testing once per generation were executed discerning fast ( $F_t < 120$  sec) and slow ( $F_t > 240$  sec) feeders. For each generation approx. 40 mosquitoes per selection line were tested, utilizing only those exhibiting a distinct biting drive (Cle-

ments 1963) on exposure to the observer's hand and starting skin penetration within 3 min after being released in the testing apparatus. The testing sessions were executed on a single day for each generation. The selection procedures were continued for 14 generations.

For the selections on host irritating properties a bidirectional selection procedure was initiated discerning strongly and slightly irritating ( $S_t <$  and  $S_t >$  respectively) mosquitoes. In this case the repeated selection method was applied to cull inconsistencies in the expression of either the relevant mosquito properties or host reactivity (cf. Rutledge et al. 1975; Gerold 1975). For each generation 20 mosquitoes were chosen by randomization, and 10 were applied evenly spaced on each lower arm, as described above. The most and least irritating 8 of these were retained and retested after 7 days. The 4 respectively most and least irritating of these were tested again after another week. Their offspring constituted the next generation. These procedures resulted in a selection pressure of 80% per generation. Each testing session was executed on a single day. The selection

procedures had to be disrupted after the 5th generation.

**MAINTENANCE OF THE SELECTION LINES.** The mosquitoes selected were either pooled in a medium-sized netting cage (experiments on mice) or retained separately in the testing container (experiments on man). Subsequent culture procedures were the same as applied for the stock colony.

**ASSESSMENT OF THE RESULTS.** All variables measured except for wheel size were found to be lognormally distributed as has been reported elsewhere (Gillett 1967, Mellink 1980) and were accordingly transformed before being subjected to statistical analysis. For the wheel area the square root transformation was applied. The statistical procedures employed are reported under the heading Results.

## RESULTS

**SELECTIONS FOR FEEDING SPEED.** The average selection pressure obtained in the experiments concerned with feeding speed remained virtually constant in both lines and fluctuated around 75% (fast feeders) and 85% (slow feeders) from the

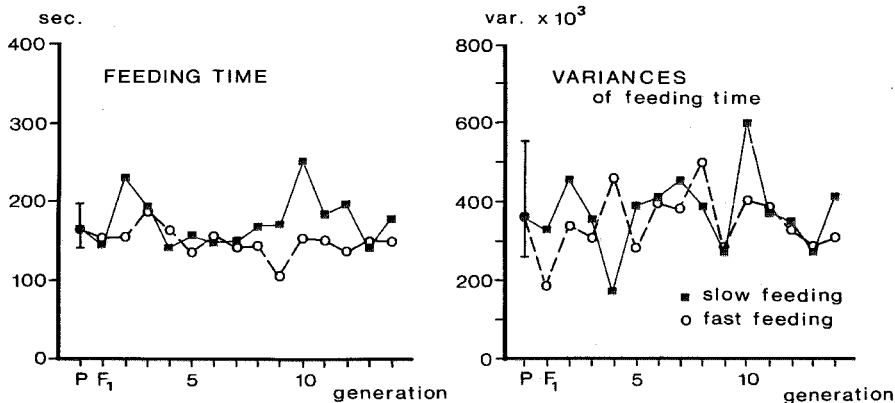


Fig. 1. Mean length of the feeding periods, and their variances during 14 generations of single test selection for fast and slow feeding. For clarity the 95% confidence intervals are only shown for the parent generation, those of the other generations not being essentially different.

parent generation onwards. No changes were observed in the geometric means and variances of the feeding times over the 14 generations (Fig. 1). Neither were divergences of significance found from the equivalent values of the stock population which were concurrently determined at intervals. Nor did a linear regression analysis on the differentials of the means between the 2 lines for each generation reveal any trend ( $r = +0.33$ ,  $0.10 < P_D < 0.20$ ). Similar results were met concerning the penetration times.

During aforementioned selections it was observed that gradually fewer mosquitoes had to be eliminated in the successive testing sessions because of not starting skin penetration within 3 min. In both lines the percentages of mosquitoes discarded dropped from ca 40 to 10. As the interval between being introduced in the testing apparatus and the commencement of penetration, designated the alighting time (cf. Mellink 1980), was measured as well, a further analysis of this phenomenon could be carried out. This revealed a persistent decrease of the geometric means of the alighting times and a simultaneous increase in their variances over the generations in both lines. At least the means differed significantly from those of the parent generation from the 7th and 9th generations respectively onwards ( $P_D < 0.05$ , Student's t-test). The mosquitoes seemed, however, to have retained their preference for humans over rodents all through as did the stock population. No indications for a changed host-seeking behavior could be observed in the stock colony during the same period.

**SELECTION FOR HOST IRRITATING PROPERTIES.** Although a bidirectional selection for host irritating properties over many generations was intended, the experiment had to be discontinued at an early stage. The line being selected for slightly irritating showed an extremely low reproductivity and was subsequently lost after the 2nd generation. As the results of this line were not essentially different from those of the other selection

line, but possibly affected by a diminished vigor of the mosquitoes, these will not be taken into account forthwith.

In the mosquitoes selected for strongly irritating properties no differences of significance from the parent generation were observed in the means and variances of the transformed variables determined for any of the generations tested ( $P_D > 0.10$ , Student's t-test and F-test respectively), with the exception of the mean wheel size in the  $F_3$  generation ( $P_D < 0.05$ ). Neither were divergences of significance found from the equivalent values of the stock population which were concurrently determined at the  $F_3$  and  $F_4$  generations. The results obtained for the most pertinent measurements, safety time and wheel size, are presented in Fig. 2.

Moreover, the values obtained from each of the individual mosquitoes at the respective biting sessions were not found to correlate for any of the measurements used (all  $P_D > 0.10$ ).

In the remaining selection line difficulties also arose in maintaining the line, but they only occurred after the 5th generation. It appeared unlikely that this was due to host factors as the stock colony mosquitoes showed a normal reproductivity when fed on the human volunteer at that stage. In view of the results so far it was therefore decided to discontinue the experiment.

## DISCUSSION

In his paper on the heritability of blood feeding properties of mosquitoes Gillett (1967) postulated that 15 generations of laboratory culture had been sufficient to change the feeding speed characteristics of a colony of *Ae. aegypti*. In his view the absence of host retaliatory action had favored mosquito properties associated with slow feeding to become more prominent in the laboratory. If this assumption is accepted, such should pre-eminently have been the case in our colony which was maintained under the prescribed conditions for a much larger number of generations. This is, however,

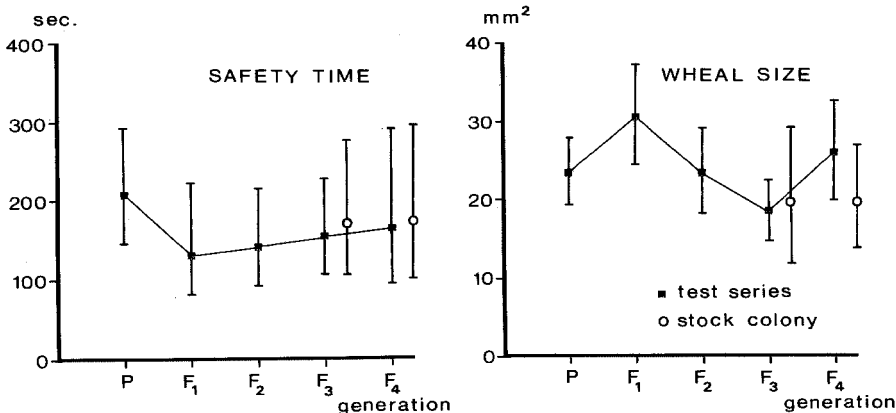


Fig. 2. Mean lengths of the safety periods and mean wheal sizes during 4 generations of repeated test selection for highly irritating and 95% confidence intervals of the means. Equivalent values for the stock colony at the F<sub>3</sub> and F<sub>4</sub> generations are included for comparison. Each point 20 feeding mosquitoes.

contradicted by the lack of a demonstrable genetic gain of 14 generations of bidirectional selections for feeding speed at relatively high selection pressures. By consequence, the heritability of feeding speed differences was either very low or absent in our strain.

It may of course be argued that the original heterozygosity of our strain in this respect was lost during the long-lasting laboratory maintenance. However, as the routine culture procedures probably excluded selections for feeding speed such seems rather unlikely. Moreover, as Spielman and Kitzmiller (1967) have pointed out, loss of heterozygosity is by no means a necessary by-product of laboratory culture of long duration, even in the presence of relevant selective influences. Such is further illustrated by the apparent heterozygosity of our strain in respect of the responsiveness of the mosquitoes to mice in the testing apparatus.

The gradualness of the increase in this responsiveness upon selection and the concomitant tendency of the matching variances to augment, moreover, imply polygenicity. Such is in agreement with

the view expressed by Mattingly (1967) that even elementary behavior in mosquitoes is usually determined by co-adapted gene complexes. As host preferences seemed unaffected, similar behavioral properties were possibly involved as studied by Schoenig (1965 quoted by Rai 1967), Gerold (1970) and Rutledge et al. (1975).

It can be concluded that genotypical differences in feeding speed were lacking in the founder population of our strains as well. As the findings of Gillett (1967) are presumably reducible to intraspecific and/or environmental effects, no experimental evidence exists at present for a heritability of feeding speed differences in mosquitoes.

The negative results of the selections allow all the values obtained with regard to penetration or feeding speed in the course of the experiments on mice to be taken together. Upon joint assessment of the respective data the variations in penetration speed appeared to be lognormally distributed as expected, but those concerning feeding speed indicated a bimodal logarithmic distribution, which

was not demonstrable on the base of small numbers of observations (Fig. 3). This distribution was not essentially different from that found by a large number of observations recorded on man (cf. Mellink 1980). A similar bimodality was reported by O'Rourke (1956) and seemed to be present in the material from previous measurements on rodents (Mellink 1980). The figures of Gillett (1967) would also more or less fit in. Two different ways of blood feeding determined by phenotypical factors apparently exist in mosquitoes. These are unlikely to correspond with a partitioning in pool and vessel feeders (cf. Mellink 1980).

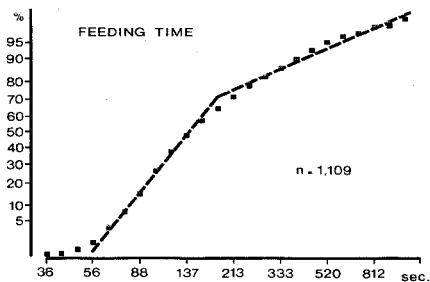


Fig. 3. Cumulative frequency distribution of the duration of the feeding periods from all experiments on mice together. Abscissa: in scale; ordinate: probability scale. Trend lines eye-fitted.

It can be surmised that avoidance of host retaliatory action is accomplished by the minimizing of host irritation as well and that the relevant mosquito properties are subject to genetic variation (Gillett 1967). An increase in host irritation was not observed on pertinent selections, but only 4 generations could effectively be studied. The absence of correlations between the safety times or wheal sizes of the successive bites of the individual mosquitoes in these experiments, however, also rather agrees with non-heritability of the related mosquito properties than otherwise. The problems encountered in

propagating the selection line in the  $F_4$  generation probably implicate a high degree of inbreeding (cf. Craig and Hickey 1967). Homozygosity leading to divergence from a heterozygous parent generation is to be expected in that case, but this did not materialize demonstrably. Differences in host irritating properties were unlikely to be genetically determined in our strain as a consequence.

As safety and penetration times were previously found to be highly correlated (Mellink 1980) it was implicitly selected for penetration speed in the latter experiments as well. The foregoing results therefore suggest that differences in penetration speed were not genetically determined in our strain either.

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