Physiological Entomology (1977) 2, 133–145

Genetic control of feeding preferences in the mosquitoes *Aedes (Stegomyia) simpsoni* and *aegypti*

L. G. MUKWAYA Department of Entomology, East African Virus Research Institute, Entebbe, Uganda

ABSTRACT. The biting rate of a non-anthropophilic (Bwayise) population of *Aedes simpsoni* was found to be approximately 0.3 mosquitoes per catcher per hour, whereas that of an anthropophilic (Bwamba) population was approximately 101 per catcher per hour. Population density indices, as determined by the number of pupae per wet plant axil, were 0.70 in Bwayise and 1.00 in Bwamba. The big difference in anthropophilic behaviour between these populations was therefore unlikely to be derived from this small population difference. Larval density was higher at Bwamba than at Bwayise, but isolation or crowding of the larvae in the laboratory did not affect the biting behaviour of adult *Ae. simpsoni.* Laboratory studies also failed to confirm field observations that temperature might play a part in determining anthropophily and non-anthropophily in this species. In choice-chamber landing tests, using a rat and a human hand, *Ae.simpsoni* females derived from wild larvae and reared in the laboratory showed that 83% of the Bwamba strain landed on man, whereas only 38% of the Bwayise strain did so. In *Aedes aegypti,* 71% of a long-established laboratory strain (Ilobi) landed on man, whereas 47% of a relatively non-anthropophilic wild (Kampala) strain did so. These preferences persisted in culture. Selective breeding increased the preference for the rodent significantly in the Kampala strain of *Ae.aegypti,* but had no significant effect on the Ilobi strain. Cross-breeding showed that the F_1 and F_2 hybrids between the anthropophilic and non-anthropophilic strains were intermediate in their preference between the parental pure bred strains; the reciprocal crosses were not significantly different from each other. The behaviour of the backcross progenies, at least in *Ae.aegypti,* appeared to indicate that the genotype of the male parent might be the main determining factor.

Introduction

Earlier field studies in Uganda (Gillett, 1951, 1955) showed that *Aedes simpsoni* Theo. occurs in anthropophilic and non-anthropophilic biting behavioural forms, and that *Aedes aegypti* L. from Bwamba (Haddow, 1945) is non-anthropophilic. Later, McClelland & Weitz (1963) found, by means of precipitin tests, a preponderence of rodent feeds among blood-fed resting *Ae.aegypti* mosquitoes at a site near Entebbe, suggesting that this population also might be non-anthropophilic. This observation was confirmed by Mukwaya (1974a), who carried out human-baited catches and found that although larval population densities were high very few adults came to bite man. Mukwaya (1974b) further found that the Uganda populations of *Ae.aegypti* are

Correspondence: Dr L. G. Mukwaya, Department of Entomology, East African Virus Research Institute, P.O. Box 49, Entebbe, Uganda.

exophilic, and (Mukwaya, 1974a) that the non-anthropophilic form of *Aedes simpsoni* feeds preferentially on rodents.

The behavioural correlates of anthropophilism and non-anthropophilism are not well understood in *Ae.simpsoni,* or any other mosquito. Earlier workers attributed the behavioural difference largely to differences in density (Gibbins, 1942; Haddow *et al.*, 1951; Lumsden & Buxton, 1951). However, Gillett (1951, 1955), after extensive field studies, came to the conclusion that population density alone cannot account for the biting behavioural differences in *Ae.simpsoni.* Further observations on *Ae.aegypti* (Mukwaya, unpublished) support this conclusion, since the density of the weakly anthropophilic Kampala population of *Ae.aegypti* is comparable to that of the strongly anthropophilic Mombasa population. Gillett (1951) suggested that temperature or altitude could play a part in determining the behavioural differences, directly or indirectly. The distribution of the biting behavioural forms of *Ae.simpsoni* in East Africa (Mukwaya, 1974a) and also of *Ae.aegypti* seems to confirm Gillett's hypothesis. The anthropophilic forms of *Ae. simpsoni* and *Ae.aegypti* are generally, though not invariably, associated with the lower parts of mountains and with dry areas. Experimental evidence for this hypothesis is still lacking, however.

Gillett (1955) further suggested alternatively that it seemed reasonable to regard the anthropophilic and non-anthropophilic forms of *Ae.simpsoni* as separate races, although they display no morphological differences. Such genetic variation in behaviour could have been brought about by isolation, since plants suitable for the breeding of *Ae.simpsoni* are often separated by many miles of unsuitable country, and the species could be split up into many demes.

If the behavioural biting differences between populations of *Ae.simpsoni* and of *Ae.aegypti* are genetic in origin, it is possible that they are due to an uneven distribution of gene frequencies maintained by partial or complete genetic, geographical or behavioural barriers to gene flow.

Some authors, commenting on these behavioural differences in mosquitoes, have suggested genetic differences in host preference (McClelland & Weitz, 1963; Gillett, 1969), but to date there has been no experimental support for this assumption. The techniques of classical genetics depend upon the crossing of two animals which differ in some character, and then looking for segregation in their descendants. Extension of such techniques to the study of mosquito biting behaviour is complicated by the labour required to detect segregating units of behaviour in the absence of reliable morphological marker genes in the different behavioural forms of *Ae.simpsoni* and *Ae.aegypti.*

The main purpose of this paper is to determine the relative importance of genetic and environmental effects on feeding preference in mosquitoes, with special reference to *Ae.simpsoni* and *Ae.aegypti.*

Materials and Methods

Experimental mosquitoes, their host relationships and behaviour

The strains of *Ae.simpsoni* used in this study were from two localities in Uganda: Bwamba and Bwayise. The Bwamba strain is known to bite man readily (Gillett, 1951, 1955; Mukwaya, 1974a), whereas the Bwayise (Buganda) strain is zoophilic (Mukwaya, 1974a). Experiments on *Ae.simpsoni* were supplemented with experiments on behaviourally similar strains of *Ae.aegypti,* since *Ae. simpsoni* is difficult to breed in captivity. The Ilobi strain of *Ae.aegypti* which orginated from Nigeria and has been maintained in this laboratory for over 15 years, was one strain used (it was originally endophagic, endophilic and anthropophilic in nature; Kerr, 1933), the other was a local strain (type *formosus*) from Kampala which is exophilic, exophagic and weakly anthropophilic (Mukwaya, 1974b).

Determination of population density

The probability of catching a biting adult should vary with the absolute density of the adult population. This is difficult to determine in areas where *Ae.simpsoni* is non-anthropophilic. Gillett (1951, 1955) indirectly determined density by taking the percentage of water-containing plant axils which contained larval mosquitoes, but did not take into consideration the number of larvae per plant axil. The young larval stages of *Ae.simpsoni*, particularly in Bwamba, are preyed upon by larval *Eretmapodites* spp. and the survival to the adult stages is therefore lower.

The best estimate for the adult population size in a given area was therefore taken as the number of pupae per wet axil. The knowledge of the number of leaf axils available for colonization in a given area is then important if meaningful comparisons between man-biting and non-man-biting populations are to be made. This is difficult to determine, but it was assumed that by making observations in plantations of comparable size, the number of axils available for colonization would be nearly the same and the mean larval or pupal density per axil could then serve as an index for population size comparisons.

The larvae and pupae from randomly selected plant axils were removed by means of pipettes, placed in a dish and recorded, and then taken to the laboratory to be reared to adults for identification. Comparisons were then made between the size of the populations as indicated by pupal density (no. pupae per occupied axil) and the biting rate indicated by the number biting per human catcher per unit time.

Laboratory technique for differentiating between anthropophilic and non-anthropophilic strains of Ae.simpsoni

Gouck (1972) has developed an olfactometer for testing host preference in mosquitoes, but it is not reliable for species which are exophilic, such as *Ae.simpsoni* (Mukwaya, 1972, 1974b). The technique described by Mukwaya (1974a) for the preferences of *Ae.simpsoni* for different rodents, seems to overcome most of the handicaps, and was used here. In this technique two hosts are compared in a cage (90 x 45 x 45 cm, made of cotton mosquito netting) containing fifty adult female mosquitoes 4–5 days old. In this case two mammal hosts were used, a rat (*Arvicanthis niloticus*) known to be fed upon (Mukwaya, 1974a), and a human hand. The rat was anaesthetized and both the human hand and rat simultaneously introduced into the cage, each being placed at opposite ends of it. The rat was placed (with forceps) on a rack at the same height as the human hand.

The number of landings observed on each host per minute was recorded by two observers. The time chosen for testing (15.00 hours) corresponded to one of the peaks of biting activity in nature. The same cage population was tested on four occasions at 2-day intervals. Mosquitoes dying or escaping were replaced by mosquitoes of the same age and from the same stock. The positions of the hosts in the cage were alternated for each test. These two hosts have comparable surface areas. Different human individuals attract mosquitoes differently, and in order to reduce this possible source of error both hosts were changed for each trial during the testing period.

Method of analysis

Assuming that every mosquito in a group under test has an equal chance of landing on a particular host, the behaviour of the strain under study can be determined by the total number of observed landings in a given time.

The experiments involved a count of the number of landings which the two hosts (a human hand and a rodent) received from a batch of fifty mosquitoes of either of the two strains during a 3-min period on each of four alternating days.

Statistical tests such as *t*-test or χ^2 should not be used in this, or other similar cases, because there is no easy way of telling whether the significant difference detected in the analysis is due to differences in the strain (irrespective of all the other factors such as host, time and trial) or due to differences in the experiments themselves, rather than in the strains used. For this reason, all *P*s quoted in the results are calculated from analyses of variance.

Results

The comparison of larval population densities with the biting behavioural densities of Ae. simpsoni at Bwamba and Bwayise

The results show (Table 1) that the proportion of wet axils colonized by *Ae.simpsoni* was similar in the two localities: in each case

TABLE 1. Larval and pupal surveys of *Ae.simpsoni* at the two sample localities (Bwayise and Bwamba)

Locality	No. of water-containing axils sampled	% with larvae or pupae	% with fourth instar larvae or pupae
Buganda			
Bwayise 1	80	100	34
Bwayise 2	79	100	38
Bwayise 3	118	78	21
Bwayise 4	113	70	52
Bwayise 5	119	98	56
Kajansi	75	100	41
Bwamba			
Kirumya 1	121	87	36
Kirumya 2	112	97	37
Nyahuka	69	97	59

TABLE 2. Comparison of population density index (see text) and larval density index with the adult biting rate of *Ae.simpsoni* at two localities

Sample locality	Pupae/axil	Larvae/axil	Biting adults/catcher/h
Buganda			
Bwayise 1	0.64	5.95	0.17
Bwayise 2	0.82	6.92	0.17
Bwayise 3	0.25	2.60	0.33
Bwayise 4	0.77	3.35	0.50
Bwayise 5	1.00	2.55	0.50
Kajansi	0.72	5.93	0.00
Mean	0.70	4.55	0.28*
Bwamba			
Kirumya 1	1.21	8.60	37.70
Kirumya 2	0.82	9.18	231.00
Nyahuka	0.97	3.93	35.00
Mean	1.00	7.24	101.23†

* Six catchers for each sample.

† Two or three catchers for each sample.

at least 70% being occupied by larvae, and 20% with pupae. The results for the population size index (as determined by the number of larvae or pupae in an equal number of axils randomly sampled in two plantations of comparable size) are given in Table 2. The results indicate that the larval population size index was about twice as high at Bwamba as at Bwayise, but the pupal population size index was only slightly higher.

The effect of larval isolation and crowding on host preference by Ae.simpsoni

As far as the individual *Ae.simpsoni* larva is concerned the habitat is the single axil and it is irrelevant to it how many other axils are populated. Larval density within each such sub-population, i.e. axil, as a possible determinant of biting behaviour has never been investigated in *Ae.simpsoni* or any other mosquito. However, larval density has profound effects on many aspects of life in other insects, for example determining morphology, type of reproduction, and food plant in species which have a cycle of alternate hosts.

Laboratory studies were therefore carried out to test whether the larval density affected the biting behaviour of the adult *Ae.simpsoni.* Larvae, reared from adult female mosquitoes collected from the non-anthropophilic population at Bwayise, were kept either individually in 7 x 2 cm tubes or crowded in dishes (each 24 cm in diameter) at about a 100 larvae in each dish; the same temperature was used in each case. The resultant adults were fed on sugar for 2 days, and on the third or fourth post-emergent day tested for host preference between a human hand and the rat, *Arvicanthis niloticus* (see Methods).

The distribution of landings between man and rat was, respectively, 36% and 64% (n = 113) in the isolated culture, and 38% and 62% (n = 94) in the crowded culture, these differences being not statistically significant.

The effect of rearing temperature on host preference in Ae.simpsoni

The observed distribution of anthropophily in *Ae.simpsoni* populations seemed to suggest that higher temperatures favour anthropophily and lower ones non-anthropophily (Mukwaya, 1974a). Larvae reared from *Ae.simpsoni* collected at Bwayise were kept at 23–24°C and 27–28°C, the average annual temperatures for Bwayise and Bwamba, respectively. The resulting adults were kept and tested at the same temperatures. The results showed that at rearing temperature of 23–24°C, respectively 36% and 64% (n = 146) of landings were on man and *A.niloticus,* and at 27–28°C, 36% and 64% (n = 114). Again, this difference was not statistically significant.

TABLE 3. Results of laboratory host-preference tests between man and rat on anthropophilic (Bwamba) and non-anthropophilic (Bwayise) strains of *Ae.simpsoni*

Trials	Bwamba									Bwayise								
	Total number of landings	Percentage of landings								Total number of landings	Percentage of landings							
		Man				Rat					Man				Rat			
		1st min	2nd min	3rd min	Total	1st min	2nd min	3rd min	Total		1st min	2nd min	3rd min	Total	1st min	2nd min	3rd min	Total
1	7	43	57	0	100	0	0	0	0	13	39	0	8	46	31	23	0	54
2	34	50	12	15	77	12	12	0	24	34	21	6	0	27	56	12	6	74
3	19	47	32	11	90	5	5	0	10	32	31	6	0	38	50	9	3	62
4	27	48	7	11	66	19	11	4	34	35	29	11	0	40	60	0	0	60
Mean	22	47	27	9	83	9	7	1	17	29	30	6	2	38	49	11	2	63

TABLE 4. Results of laboratory host-preference tests between man and rat on the Ilobi and Kampala strains of *Ae.aegypti*

Trials	Ilobi									Kampala								
	Total number of landings	Percentage of landings								Total number of landings	Percentage of landings							
		Man				Rat					Man				Rat			
		1st min	2nd min	3rd min	Total	1st min	2nd min	3rd min	Total		1st min	2nd min	3rd min	Total	1st min	2nd min	3rd min	Total
1	27	52	19	4	75	22	4	0	26	40	35	10	5	50	30	18	3	51
2	37	41	16	8	65	22	8	8	35	28	14	18	4	36	39	14	11	64
3	28	46	21	11	78	14	7	0	21	19	26	26	5	57	11	16	16	43
4	40	38	20	8	66	23	10	3	36	18	33	0	11	44	44	11	0	55
Mean	33	44	19	8	71	20	7	3	30	26	27	14	6	47	31	15	8	53

Laboratory measurement of host preferences in anthropophilic (Bwamba) and non-anthropophilic (Bwayise) strains of Ae.simpsoni

Host preference differences between these anthropophilic and non-anthropophilic strains were tested, as above, against rat and man (see Methods). The proportion of landings by the Bwamba strain of *Ae.simpsoni* was 83% on a human hand and 17% on the rat (Table 3). In all four trials, the difference was of this order. In the case of the Bwayise strain, however, the proportions of landings were 38% and 63%, respectively. In each case these differences were significant ($P<0.01$). The number of landings on each host decreased gradually with time; the landing rate in the two strains was the same regardless of host but the preferences remained unaltered.

Laboratory measurement of host preferences in the semi-anthropophilic wild (Kampala) and anthropophilic laboratory (Ilobi) strains of Ae.aegypti

Preferences for man or rat were tested as with the *Ae.simpsoni* strains. The results are presented in Table 4. The proportions of landings by the Ilobi strain of *Ae.aegypti* were 71% on the human hand and 30% on the rat, all the four trials showing the same trend. In the Kampala strain, the results were 47% on man, 53% on rat. The difference in the proportion of mosquitoes on the two hosts was significantly different. As in *Ae.simpsoni*, landings declined after the first minute, regardless of strain or type of host, but unlike *Ae.simpsoni* there was a significant difference in the rate of landing ($P<0.05$) between the two strains, the rate being higher in the Ilobi strain.

Persistence of the preference in culture

The results obtained in the previous section indicate that the feeding differences observed in the field in *Ae.simpsoni* and *Ae.aegypti* populations persist under controlled conditions in the laboratory. Both strains of *Ae. simpsoni* and the Kampala strain of *Ae.aegypti* were therefore reared for further generations in the laboratory and tested again for host preference. The long-standing Ilobi laboratory strain of *Ae.aegypti* was then compared with the fourth generation of the Kampala strain. For *Ae.simpsoni*, only one generation was reared in the laboratory because of the technical difficulties of mating this mosquito in captivity. For these laboratory-bred Bwamba *Ae.simpsoni*, the proportion of landings received on the human hand was 81% and on the rat 19% ($n = 98$), for the Bwayise *Ae.simpsoni*, 38% and 62% ($n = 149$), respectively. The two strains remained significantly different ($P<0.01$) with respect to host preference. There was no significant difference in either strain between adults derived from wild larvae and those obtained from F_1 laboratory-reared material. Likewise, the two strains of *Ae.aegypti* remained significantly different with respect to host preference, and there were no significant differences between the F_4 laboratory-reared Kampala *Ae.aegypti* and adults derived from wild larvae.

Response to selection in the Ilobi and Kampala strains of Ae.aegypti

An attempt was made to increase the rat-preference in the Kampala strain of *Ae.aegypti* and the man-preference in the Ilobi strain. After emergence, adult females and males were put separately into cages and fed on sugar. A moist plug of cotton wool was put on top of each cage to keep the inside of the cage humid. Fifty 4–5-day-old females were then put into the cage used for preference testing (Mukwaya, 1974a). A small opening was made in this through which a sucking tube could be inserted for removing mosquitoes.

The two hosts (human hand and rat) were simultaneously introduced into the cage and the mosquito allowed to land and feed. From the Kampala strain, mosquitoes feeding on the rat were selected and from the Ilobi strain those feeding on the human hand. Successive groups of fifty were tested until a sufficient number was obtained. The fed mosquitoes were divided into groups of ten to fifteen mosquitoes and each group given one male from the same stock of eggs. As it was not possible to assess the preference of male mosquitoes, unselected males had to be used. The mosquitoes were allowed to mate for about 36–48 h during which time they were offered sugar-water. On the third day they were fed on a guinea-pig; those that engorged were individually placed in 7 x 2 cm tubes with netting-covered open ends and furnished with moist filter paper. They were fed every other day. As the eggs were laid on the moist paper, the females were transferred to new tubes.

In the first instance, about 200 eggs were removed from each group and reared separately. At the same time unselected Kampala or Ilobi parental strains were also tested as controls. The rest of the eggs of each group were kept aside for further selective breeding. The remaining eggs of the group which responded most to selection were set in tap water, reared as before, and the adults carried into the second generation. The other groups were discarded. The whole process was then repeated in the next generation. No selection was exercised on the fourth generation of the Ilobi strain or the third generation of the Kampala strain, owing to insufficient eggs, but all other generations were selected. Seven generations of the Ilobi strain and eight of the Kampala strain were selected and reared in this manner.

The Kampala strain of *Ae.aegypti* initially showed no marked host preference in laboratory conditions. The results of selecting it for rat-preference are shown in Fig. 1, from which it appears that as early as the first generation the mosquitoes were already deviating from the controls by approximately 9% (38% of landings were made on the human hand as compared to 46% in the control). This response to selection was not, however, statistically significant. By F_2 there was more response to selection: the proportion landing on the rat was 68% against the control figure of 53%, a deviation that was just significant ($P < 0.05$). In the third generation, selection pressure was relaxed because the mosquitoes laid too few eggs for selection, but when selection was resumed in F_4 and F_5 the rat-preference was maintained ($P < 0.01$). Continued selection to the eighth generation did not further increase the tendency to land on the rat.

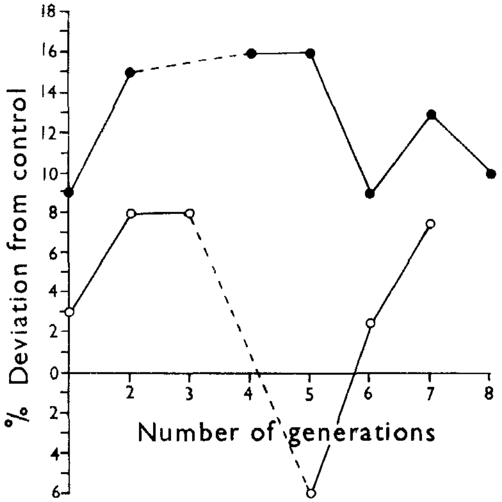

FIG. 1. Selection for rodent preference in the Kampala strain, and man preference in the Ilobi strain of *Ae.aegypti*. The proportion of the selected strain feeding on rat in the Kampala strain or man in the Ilobi strain is expressed as a percentage of the contemporary, unselected controls doing so. Dotted lines refer to generations where selection was discontinued. Open circles refer to Ilobi strain; filled circles to Kampala strain.

The results for selection for man-preference in the Ilobi strain of *Ae.aegypti* are also shown in Fig. 1. In the F_1 adults only a slight response to selection appeared (71% of landings on the human hand as compared to 69% in the control). By F_2 a further response appeared (74% of the landings were on the human hand compared with 66% in the controls), and was maintained through F_3 but in the fourth generation no selection took place, and by F_5 preference was less than in the controls. After selection was resumed, preference for the human hand built back up again to the F_3 level. None of these preferences were statistically significant, however.

Host preference by F_1 and F_2 progenies obtained by crossing the anthropophilic and non-anthropophilic strains of Ae.aegypti and Ae.simpsoni

Since selection increased rat-preference in the Kampala strain of *Ae.aegypti* but failed to increase man-preference in the Ilobi strain, it was considered desirable to analyse the behaviour of their hybrid F_1 and F_2 progenies. The two unselected strains of *Ae.simpsoni* were similarly cross-bred and their hybrid F_1 and F_2 progenies tested.

The results shown in Figs. 2 and 3 indicate that the F_1 and F_2 progenies in both *Ae.simpsoni* and *Ae.aegypti* were intermediate in their selection of hosts; but in the By♀ × Bw♂ and Ka♀ × Il♂ progenies there was a tendency to prefer man more than in the reciprocal crosses. As expected, the difference in preference of the parent strains (Bw *v.* By in *Ae.simpsoni,*

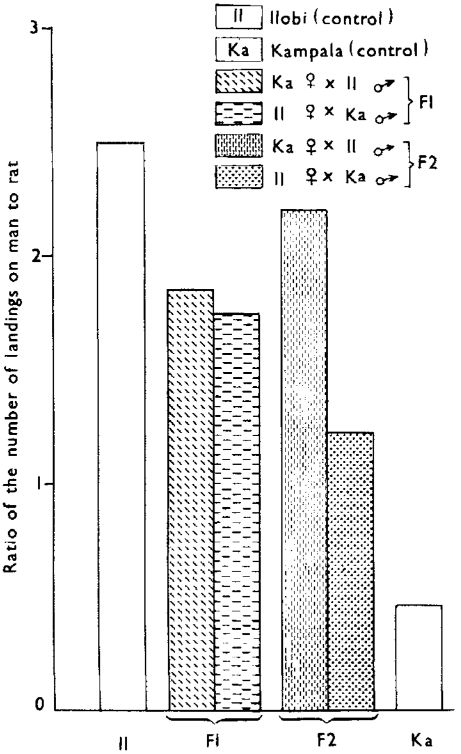

FIG. 2. Host preference in the F_1 and F_2 hybrids obtained by crossing the Kampala and Ilobi strains of *Ae.aegypti.*

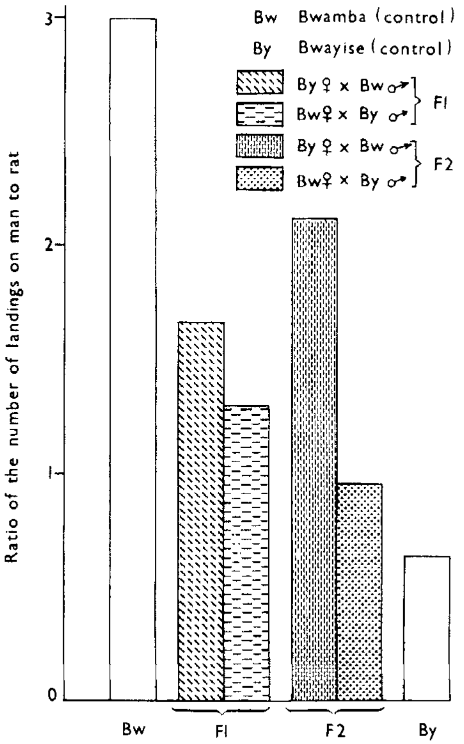

FIG. 3. Host preference in the F_1 and F_2 hybrids obtained by crossing Bwayise and Bwamba strains of *Ae.simpsoni.*

and Il *v.* Ka in *Ae.aegypti*) were significant ($P<0.01$).

For the F_1 *Ae.simpsoni* (Fig. 3) the Bw♀ x By♂ cross was significantly different from the Bw pure bred strain ($P<0.01$), but the reciprocal By♀ x Bw♂ cross was not. On the other hand, both Bw♀ x By♂ crosses were statistically different from the pure bred By ($P<0.01$). In the F_2, the two reciprocal crosses were significantly different from each other ($P<0.01$), but whereas the By♀ x Bw♂ cross showed a significant preference for man, the reciprocal (Bw♀ x By♂) did not.

In *Ae.aegypti* (Figs. 2 and 4) neither of the F_1 crosses was significantly different from each other or from the Il parent, but were both different from the Ka parent ($P<0.01$). In the F_2, as in *Ae.simpsoni,* the two reciprocal crosses were significantly different from each other ($P<0.01$), but not from the parent strain whose behaviour they most nearly approached.

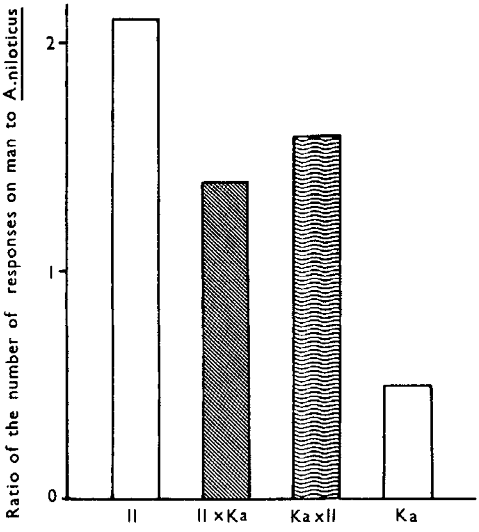

FIG. 4. Host preferences in F_1 hybrids between the man-preferring Ilobi and the non-man-preferring Kampala strains of *Ae.aegypti,* tested in Gouck's olfactometer. Il, parent Ilobi; Ka, parent Kampala strain Il X Ka, F_1♀ X ♂ cross; Ka X Il, F_1♀ X ♂ cross.

TABLE 5. Host-preference in the back-cross progenies of *Ae.aegypti* (see text), tested in Gouck's (1972) olfactometer

Type of cross	No. of trials	Total no. responding mosquitoes	% responding to man	% responding to rat	Total % response*
Il♀ × (Ka♀ × Il♂)♂	8	591	68	32	25
Il♀ × (Il♀ × Ka♂)♂	10	1053	64	36	24
Ka♀ × (Ka♀ × Il♂)♂	14	736	39	61	13
Ka♀ × (Il♀ × Ka♂)♂	10	1101	63	37	34
Il (control)	10	1244	68	32	32
Ka (control)	12	744	33	67	15

* No. responding to man and rat as % of no. in chamber.

The behaviour of the back-cross progenies

In order to determine the mode of inheritence, the F_1 individuals were back-crossed to the parental strains. Because of the large number of crosses to be tested, Gouck's (1972) type of olfactometer was used. This works well for *Ae.aegypti*, but *Ae.simpsoni* responds very poorly in it (Mukwaya, 1974b). Hence, only strains of *Ae.aegypti* were used.

The parental strains of Ilobi and Kampala were tested as controls each time the progenies of a particular back-cross were tested. Four hundred mosquitoes (4–5 days old) were used for each test as before. The results, shown in Table 5, indicate that there was no significant difference between the back-crosses Il♀ × (Ka♀ × Il♂)♂ and Il♀ × (Il♀ × Ka♂)♂ both in host preference and response rate. The progeny of the back-crosses of the male hybrids to Ka females, however, behaved quite differently. The hybrid (Ka♀ × Il♂) males back-crossed to females, produced a generation in which the progenies preferred a rat to man ($P<0.01$), whereas the hybrid (Il♀ × Ka♂) males back-crossed to Ka females produced a generation in which the progenies preferred man ($P<0.01$). The two back-cross progenies were also significantly different with respect to their ability to respond in Gouck's olfactometer.

The relationship between landing-response rate and host preference in Ae.aegypti

It was observed that the landing rate of the Ilobi strain of *Ae.aegypti* was significantly higher than that of the Kampala strain. This observation confirmed the earlier finding (Mukwaya, 1974b) in Gouck's olfactometer that Ilobi *Ae.aegypti* had a higher response rate than the wild Kampala strain.

This difference in landing responsiveness to host surface occurs only when there is also an intraspecific variation in host preference as well as in indoor/outdoor feeding behaviour: a higher landing rate for anthropophilic/indoor strains and a lower landing rate for zoophilic/outdoor strains (see Mukwaya, 1974b). This relationship was examined in the two available strains of *Ae.aegypti* (Ilobi and Kampala), which were crossed, and the two F_1 reciprocal crosses tested in a Gouck's olfactometer, both for landing response rates and for host preferences.

The results are similar to those shown in Figs. 2 and 3 in that the F_1 hybrids are intermediate in host preference between the two parental strains, with man-preference being partially dominant to rat-preference. There was no significant difference between the two reciprocal crosses. Fig. 5 shows the response rates of the same mosquitoes and indicates that the inheritance of this response has very similar characteristics to that of the host preference response (Fig. 4).

Discussion

The mechanisms and factors regulating feeding preferences have been less studied in haematophagous than in phytophagous insects. Part of the reason is probably that techniques for the laboratory study of host preference, in mosquitoes at least, are not well developed. The fear has mainly been that generalization from laboratory experiments to nature could

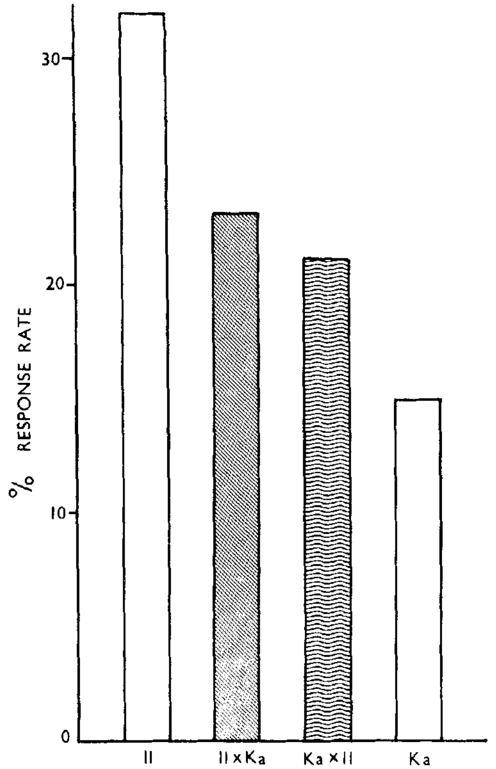

FIG. 5. The landing response rates of the F_1 hybrids between the Kampala and Ilobi strains of *Ae.aegypti*, tested in Gouck's olfactometer. For abbreviations see Fig. 4.

be hazardous. Further, host preference has usually been considered to be environmentally controlled, mainly by the availability of hosts, in which case the preference would disappear if the environments were kept uniform.

There have been, however, some attempts to test feeding preference in the laboratory (Corradetti, 1937; Rice & Barber, 1937; Van Thiel, 1939; Hu & Yu, 1936), although in some of these studies the surface area of the hosts was not taken into consideration. Perhaps the first detailed study on host preference in mosquitoes was by Gillies (1964), but his technique is expensive and laborious and, like Gouck's (1972), might not work for exophilic species. Moreover, since it involves killing the fed mosquitoes for precipitin testing in order to find the source of blood meal, information such as effect of age or differences in the landing rates and other behavioural interactions would be difficult to observe. The technique used in the present studies is more convenient and cheaper, and more of some kinds of information can be derived from it. The behaviour of the mosquitoes under laboratory conditions was found to be comparable to the behaviour in the field even though a whole animal (a rat plus exhalent breath) was compared with just a human hand.

In some of the present experiments replicates were significantly different. This variation could be due to some heterogeneity in the behaviour of the insects or in the conditions of the experiment. It seems, however, that this sort of variation is to be expected in behavioural experiments, because of changes in central nervous excitability (Dethier *et al.*, 1965; Dethier, 1968) and spontaneous activity (Roeder, 1955).

The adult population density (as indicated by the number of pupae per wet axil) was only slightly higher in Bwamba than in Bwayise (assuming comparable densities of wet axils/hectare), whereas the difference between the biting rates on man at the two localities approached two orders of magnitude, and was thus very unlikely to have been due to population difference alone. The present laboratory observations confirm the initial supposition that this difference was largely due to an innate behavioural difference in the two local strains.

Although the distribution of these behavioural forms of *Ae.simpsoni* in East Africa had seemed to indicate that higher temperatures might favour anthropophily, and lower temperatures non-anthropophily (Gillett, 1951; Mukwaya, 1974a), the results do not support that hypothesis: differences persisted under identical temperature conditions of culture. Another hypothesis, often advanced by field workers, is differential availability of hosts. However, rodents are more abundant at Bwamba (where the mosquitoes are anthropophilic), both in numbers and in species composition, than at Bwayise (Mahaffy, 1942; Mukwaya, unpublished). Conversely, the human population is higher in Bwayise (where the mosquitoes are zoophilic). Therefore, this hypothesis seems not to be the explanation here.

Some parental influence which may be either genetic or cytoplasmic must be suspected, especially in view of the heritability of the traits reported here. That the differences could also be due to factors resulting from earlier environmental influence on the larvae is ruled out by the persistence of the differences in laboratory culture. The results of the selection experiments in the Kampala strain of *Ae.aegypti* show that it is possible to select for rat-preference in only a few generations. The positive response to selection, and the fact that inter-strain hybrids were intermediate in host preference between their parental strains, confirms the existence of genetic control of these behavioural differences.

As the F_1 hybrids from the reciprocal crosses in both *Ae.simpsoni* and *Ae.aegypti* were found to be identical, a maternally inherited cytoplasmic component is unlikely to be involved. The finding in *Ae.aegypti* that response to selection occurred within a few generations suggests that relatively few genes may be involved. The partial loss of the response after selection was relaxed, and the fall in the response after a few generations might have been caused by co-adapted gene complexes controlling host preference before selection, perhaps coupled with genetic homeostasis. Gillies (1964) found a similar phenomenon while selecting for calf preference in a strain of *A.gambiae*.

If host preference were determined by a single dominant gene, the behaviour should be transmitted unchanged to the first generation (F_1) hybrids. This was not the case. Thus the gene appears to be partially dominant. On the other hand, if one of the characters were controlled by a single non-dominant gene, the crossing of an F_1 individual with a parental strain should give two distinct classes of backcross offsprings, one like the parent and one like the F_1 individual. This also did not occur.

If both hybrids are genetically identical with respect to host preference, the difference between their progenies may be due to differential segregation as a consequence of sex linkage. Table 6 summarizes this hypo-

TABLE 6. Hypothesized inheritance of host preference in *Aedes aegypti*. M is a dominant allele known to cause maleness. The locus which controls host preference is assumed to be closely linked to M; a dominant, or perhaps partly dominant allele A causes enhanced response to the human host stimulus.

Type of cross		Genotype	Behaviour	Progeny genotypes		
				Female	Behaviour	(Males)
P_1	Illobi females	$\frac{A\,m}{A\,m}$	Anthropophilic			
P_1	Ilobi males	$\frac{A\,m}{A\,M}$				
P_1	Kampala females	$\frac{+\,m}{+\,m}$	Non-anthropophilic			
P_1	Kampala males	$\frac{+\,m}{+\,M}$				
F_1	Il♀ X Ka♂	$\frac{A\,m}{A\,m} \times \frac{+\,m}{+\,M}$		$\frac{A\,m}{+\,m}$	Anthropophilic	$\frac{A\,m}{+\,M}$
F_1	Ka♀ X Il♂	$\frac{+\,m}{+\,m} \times \frac{A\,m}{A\,M}$		$\frac{+\,m}{A\,m}$	Anthropophilic	$\frac{+\,m}{A\,M}$
B_1	Il♀ X (Ka♀ X Il♂)♂	$\frac{A\,m}{A\,m} \times \frac{+\,m}{A\,M}$		$\frac{A\,m}{+\,m}$	Anthropophilic	$\frac{A\,m}{A\,M}$
B_1	Il♀ X (Il♀ X Ka♂)♂	$\frac{A\,m}{A\,m} \times \frac{A\,m}{+\,M}$		$\frac{A\,m}{A\,m}$	Anthropophilic	$\frac{A\,m}{+\,M}$
B_1	Ka♀ X (Ka♀ X Il♂)♂	$\frac{+\,m}{+\,m} \times \frac{+\,m}{A\,M}$		$\frac{+\,m}{+\,m}$	Non-anthropophilic	$\frac{+\,m}{A\,M}$
B_1	Ka♀ X (Il♀ X Ka♂)♂	$\frac{+\,m}{+\,m} \times \frac{A\,m}{+\,M}$		$\frac{+\,m}{A\,m}$	Anthropophilic	$\frac{+\,m}{+\,M}$

thesized method of inheritance. According to the hypothesis, the character Ka would be under the control of recessive genes (+/+) whereas the character Il would be under the control of dominant or partly dominant genes (A/A). In *Ae.aegypti* males are M/m (heterogametic) and females are m/m (McClelland, 1962a, b). In the hybrids Il♀ x Ka♂, the factor for Ka is introduced paternally and will consequently show linkage with the sex differential segment M/m in further transmission. Conversely, in the reciprocal cross Ka♀ x Il♂ where Ka is maternally introduced, transmission of the Ka factor to the back-cross progeny follows the opposite trend, since the sex-determining segment M/m shows linkage with the factor Il.

This hypothesis appears the most satisfactory explanation of the mode of inheritance of host preference in these species of *Aedes.* In any case, whatever the mode of inheritance, this study has provided clear evidence that host preference in *Aedes* mosquitoes is genetically determined.

Mukwaya (1974b) observed that differences in landing response rates of the endophilic and exophilic strains of *Ae.aegypti* persisted in laboratory culture and suggested that intrinsic factors could be involved. The fact that inter-strain hybrids have been found intermediate in landing response rate between their parental strains confirms the existence of genetic control. The mode of inheritance appears to be similar to that of host preference. Hence, in species which have intra-specific variation in outdoor and indoor biting behaviour, a change in one type of behaviour would most likely lead to a change in the other. The introduction of residual insecticides could thus lead to increases in the zoophilic or anthropophilic element in various populations of outdoor or indoor feeding mosquitoes.

The question arises, as with phytophagous insects (Dethier, 1953, 1966), as to what sensory modalities are involved in these feeding preferences. This question has not yet been answered for *Aedes.* A host must present a series of stimuli to which the mosquito may respond positively by landing and feeding, or negatively by not doing so. It seems likely that the stimuli producing these alternative responses will be mainly chemical and visual. Our observations favour chemical stimuli as being the more important (Mukwaya, 1976). Since different human hands or different rats presumably offer very similar visual stimuli, the involvement of visual cues in the expression of the preferences observed seems unlikely. On the other hand, the rat and human hand presumably produce different spectra of volatile chemical compounds. Lacher (1967) has shown electrophysiologically that some chemical compounds excite whereas others inhibit the receptor cells on the antennae of *Ae. aegypti,* and it seems likely that these differences in sensory excitation or inhibition are what the various genetic strains make differential behavioural responses to.

Acknowledgments

I wish to express my sincere gratitude to Professor C. H. Fraser Rowell for guidance and stimulating discussions during the course of this work, and for his critical reading of the manuscript. Many thanks go to Dr T. E. Rowell for her advice and encouragement during the initial stages of these investigations. My thanks are further due to Mr S. K. Lwanga for advice on the statistics. I would like to thank Dr M. C. Williams, formerly Director of East African Virus Research Institute, for his interest in this work and for providing research facilities, and the East African Community for financial assistance. I am also grateful to Professor Vincent G. Dethier for critically reading the manuscript. This work forms part of a Ph.D. thesis approved by the University of East Africa at Makerere University College under the supervision of Professor C. H. Fraser Rowell and Dr R. J. Olembo, to whom I am indebted.

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Accepted 11 January 1977