Host-feeding patterns of Culex tritaeniorhynchus and Anopheles sinensis (Diptera: Culicidae) in a ricefield agroecosystem.

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Heterogeneity in the host preference of Japanese encephalitis vectors in Chiang Mai, northern Thailand

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Abstract

Experiments, using the capture–mark–release–recapture technique inside large nets, were carried out in Chiang Mai, northern Thailand, to examine heterogeneity in the host preference of Japanese encephalitis (JE) vectors. A significantly higher proportion of the vector species that were initially attracted to a cow fed when released into a net with a cow than when released into a net containing a pig. However, *Culex vishnui* individuals that had been attracted to a pig had a higher feeding rate in a net containing a pig rather than a cow. When mosquitoes were given a choice by being released into a net containing both animals, they exhibited a tendency to feed on the host to which they had originally been attracted. This feeding preference was, however, not shown by the offspring of pig-fed individuals. We have therefore shown evidence of physiological/behavioural conditioning in the host preference of JE vectors rather than genetic variability. Our results suggest that effective control of JE might be achieved by increasing the availability of cows (the dead-end hosts of JE virus) to deflect the vectors from pigs (the amplifying host). The behavioural imprinting which we have found would tend to re-inforce the initial tendency of the vectors to bite cows.

Keywords: Japanese encephalitis, vectors, *Culex tritaeniorhynchus*, *Culex gelidus*, *Culex vishnui*, host preference, pigs, cows, heterogeneity, conditioning, Thailand

Introduction

Although modernization of agricultural activities has resulted in the decline of Japanese encephalitis (JE) in Japan (KAMIMURA, 1998), the disease continues to gain prominence in other Asian countries including Thailand, Malaysia and India (GAJANANA *et al.*, 1997; VYTHILINGAM *et al.*, 1997; MALAINUAL *et al.*, 1998). To control JE, a thorough knowledge of the host preference of the vectors, including any role that genetic variability may have in their host selection, is essential.

The host preference of mosquito vectors may be influenced by a number of factors including host availability and genetically determined factors. For example, the important JE vectors in Asian and South-East Asian countries, *Culex tritaeniorhynchus*, *Cx. vishnui* and *Cx. gelidus*, have been shown to feed mainly on cows in some places and pigs in others depending on host availability (PENNINGTON & PHELPS, 1968; GOULD *et al.*, 1974; REISEN & BOREHAM, 1979; MORI *et al.*, 1983; REUBEN *et al.*, 1992). When the host preference of JE vectors was examined in Thailand with equal availability of hosts, they were shown unequivocally to prefer cows to pigs (MWANDAWIRO *et al.*, 1999).

It has been suggested that genetic variability may also affect the behaviour of vector mosquitoes. For example, the existence of a consistent tendency of some individuals to feed on humans and others to feed on animals has been demonstrated in *Anopheles minimus* in Thailand (NUTSATHAPANA *et al.*, 1986) and *An. balabacensis* in Malaysia (HII, 1985). It has also been reported that genetic heterogeneity influences the feeding preferences of *Aedes aegypti* and *Ae. simpsoni* (MUKWAYA, 1977) and the house-entering behaviour of *Ae. aegypti* in Kenya (TRPIS & HAUSERMANN, 1978). However, no studies have so far been conducted to investigate the existence of genetic variability in the feeding preference of JE vectors.

The present study was carried out to investigate the genetic basis of the host preference of JE vectors by a capture–mark–release–recapture technique using baits (a cow and a pig) that were tethered inside large nets. First, we examined whether individual adult mosquitoes tend to return to the host to which they were previously attracted. Next, we examined whether the offspring showed the same preference as their parent population. Unlike previous studies, the enclosed system used here improved recapture rates and avoided ethical considerations that discourage release of large numbers of potential vectors of human pathogens. It also ensured equal availability of the hosts used in the experiments.

Materials and Methods

Study site

The study was carried out on the Mae Joh University campus, in Chiang Mai, Thailand, where various animals including pigs and cows are kept and the JE vectors are found in abundance. The study site has been described in detail elsewhere (MWANDAWIRO *et al.*, 1999).

Mosquitoes released into the nets containing only one animal

Two large nets were erected 7 m apart. A pig was then tethered in 1 of the nets while a cow was tethered in the other. In the first part of the experiment unfed female mosquitoes were aspirated on the outside walls of the nets as they tried to gain access to the animals between 19:00 and 22:00. They were then kept in 2 separate cages overnight and provided with water. The following day, those that had been attracted to the cow were marked with blue fluorescent dye and released into the net containing the cow. Similarly those that had been attracted to the pig were marked red and released into the net containing the pig (Fig. 1, a). Feeding was permitted between 19:00 and 22:00 after which all the mosquitoes inside the nets were recaptured and sorted out into species, fed and unfed. In the second part of the experiment, mosquitoes collected and marked in the same way were placed with the host to which they had not been attracted: those caught on the cow-net were released in the pig-net and vice-versa (Fig. 1, b).

Mosquitoes released into a net containing both animals

Net collections. About 250 mosquitoes were collected on the outside of a net baited with a cow and a similar number were caught on a pig-baited net. They were marked as above and released into a net containing both animals (Fig. 1, c).

Parents. About 500 engorged females were collected on 2 nights from 19:00 to 22:00 by mouth aspirator in a cowshed and a similar number were collected in a pigsty about 400 m away. They were then maintained in the laboratory (temperature, 24°C; relative humidity, 70%) for 1 week with sugar solution and oviposition dishes placed inside the cages. After oviposition 200 females of various species from the cowshed and 220 from the pigsty were marked blue or red respectively and released on 1 evening into a net containing both animals (Fig. 2, ii).

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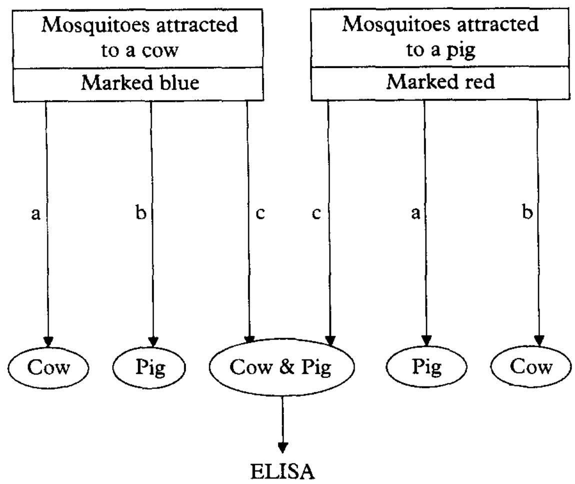

Fig. 1. A flow chart summarizing the experimental procedures whereby unfed mosquitoes which had been attracted to either a cow or a pig were differentially marked and released into nets containing (a) the animal to which they had been attracted, (b) the other animal, (c) both animals, with ELISA used to determine the host choice.

Offspring. Eggs oviposited by parents used in the preceding part of the experiment were reared in the laboratory in separate containers under similar temperature and humidity conditions to the parent populations. Approximately 130 F1 female mosquitoes were marked blue and 110 marked red according to the origin of their parents and released into a net containing both animals (Fig. 2, iii).

In all the experiments, feeding was permitted from 19:00 to 22:00 after which all the mosquitoes were recaptured, identified to species, and then sorted into fed and unfed samples. The abdominal contents of all fed females were smeared on to filter papers for blood meal identification by the direct enzyme-linked immunosorbent assay (ELISA) method of BEIER *et al.* (1988). Two replications were made of each part of the experiment.

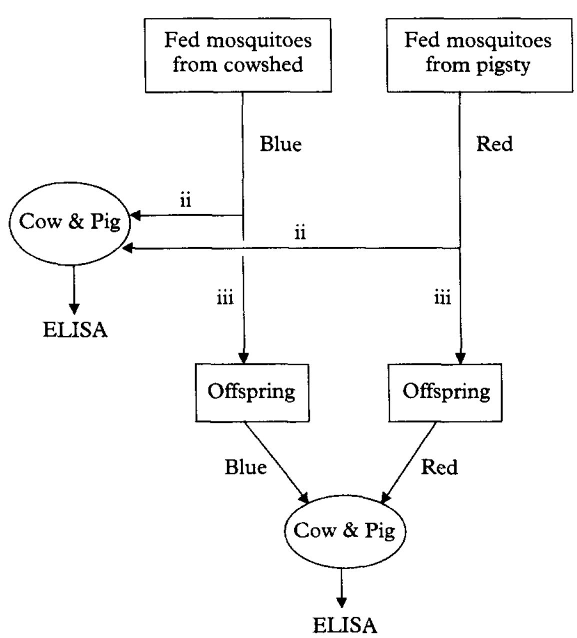

Fig. 2. Flow chart showing experimental design for fed mosquitoes collected from a cowshed and pigsty. They were, after oviposition, (ii) differentially marked and released into a net containing both animals and ELISA was used to test host choice. (iii) The offspring were also differentially marked and released into a net containing both animals after which ELISA was used to test the host choices made. The (ii) and (iii) labelling used here corresponds with that used in the Table.

Data analysis

We used Pearson's χ^2 test to analyse the data for each species and Mantel Haenszel χ^2 test stratified by species for all species combined. In the experiment in which animals were in separate nets, *Ae. vexans* mosquitoes were included for comparison with JE vectors because they were obtained in large numbers. Mosquitoes that obtained blood meals from both hosts (mixed blood meals) were included in the tests for both hosts.

Results

Mosquitoes released into the nets containing only one animal

As shown in Figure 3 there was an overall tendency for mosquitoes that had been previously attracted to the cow to feed more readily when presented with the cow than when presented with the pig. This tendency was significant for the 3 *Culex* species (Fig. 3A–C) but not for *Ae. vexans* (Fig. 3D). Among the mosquitoes initially attracted to the pig, only *Cx. vishnui s.l.* had a significantly higher feeding rate on the pig than the cow (Fig. 3C). The pig-attracted mosquitoes of the other 3 species did not differ significantly in their feeding rates on the 2 animals.

Mosquitoes released into a net containing both animals

Relationship between previous exposure and subsequent feeding. The Table reveals whether individuals that had fed on (or been associated with/exposed to) either pigs or cows tended to preferentially choose the same host, when given a choice of both. Mosquitoes from the collections on the outside of the nets and each of the animal sheds showed associations between their previous and subsequent host choices (see χ^2 values for each mosquito species and highly significant χ^2_{M-H} values from tests stratified by species in the Table). The results therefore show conclusively that JE vectors tend to return to the host to which they had been previously attracted.

Relationship between parent exposure and offspring host preference. A further question was whether the parental choice of a particular host was manifested by the offspring. Overall there was no significant association between the feeding preference of the adults and that of their offspring (see χ^2 and χ^2_{M-H} values in the Table). However, there was a strong tendency of the offspring of mosquitoes from each collection site to choose the cow for feeding (χ^2 compared with null hypothesis of 1:1 = 29·85, $P < 0·001$).

Discussion

All 3 JE vectors, *Cx. tritaeniorhynchus*, *Cx. gelidus* and *Cx. vishnui s.l.*, exhibited a tendency to return to the cow or the pig depending on which host they had previously been attracted to, or had previously fed upon. They seemed to have acquired a feeding behaviour, through contact with 1 of the 2 hosts provided, which made them take a higher proportion of feeds from that host whether provided alone or with an alternative. Host availability can affect the range of hosts to which a particular mosquito species orientates in nature and can produce a feeding pattern determined by repeated contact with a particular host rather than a fixed feeding behaviour (EDMAN *et al.*, 1972). In his experiments with *Drosophila*, JAENIKE (1983) demonstrated that prior exposure of adults to a given breeding substrate enhanced their subsequent preference for it, although their oviposition site preference was not affected by the type of food they had developed on as larvae.

The acquired tendency shown by the 3 JE vectors that made them select hosts depending on previous contact, however, seems to be due to physiological (behavioural) conditioning rather than genetic polymorphism, or differentiation of the population into sibling species with different host preferences, since there is no evidence that

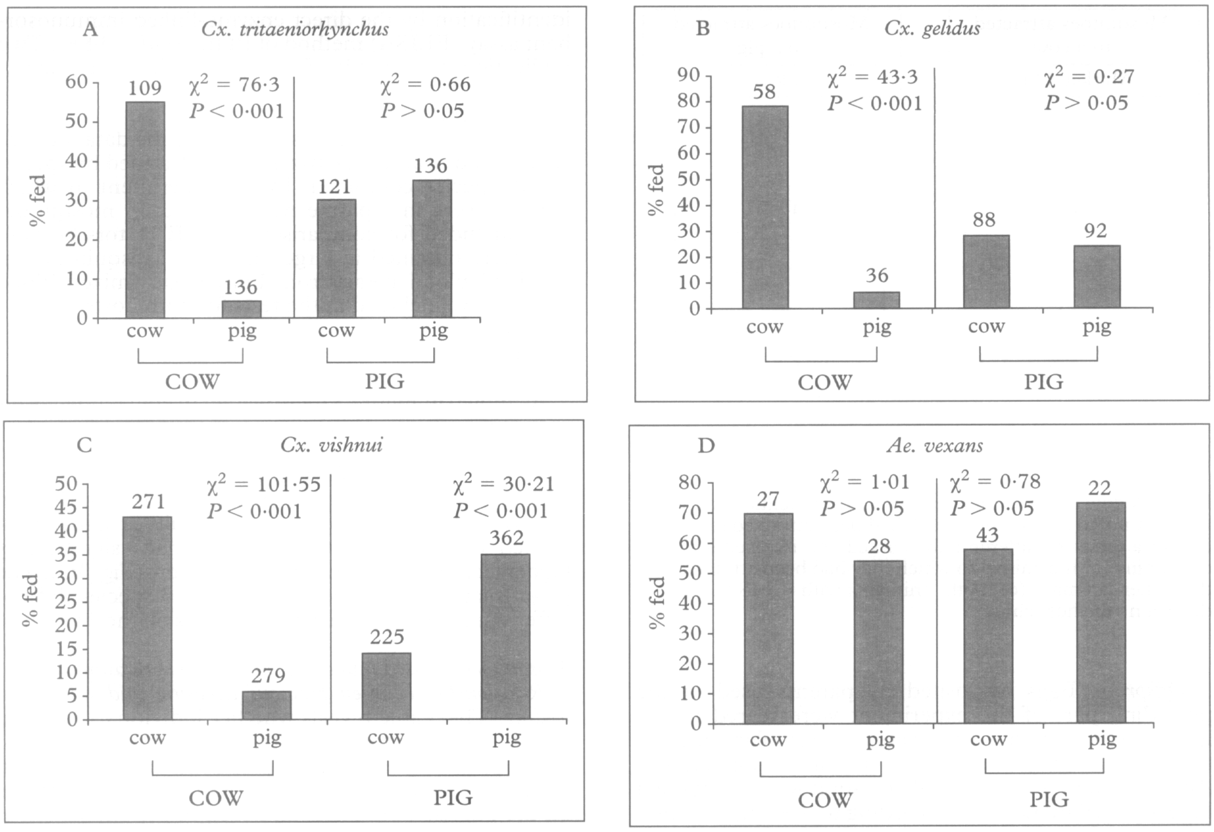

Fig. 3. Results for 4 mosquito species collected from the nets baited with a cow or a pig and then released into a net containing either the animal to which they had been attracted or the other animal. Under every histogram the animals printed in capital letters are those to which the mosquitoes were originally attracted. The animal printed in lower case letters under each column was the occupant of the net into which the batch of mosquitoes were released; the numbers at the tops of the columns are the numbers of mosquitoes (both fed and unfed) recaptured in the net.

the character was passed on to the offspring. RAWLINGS & CURTIS (1982) found no evidence from mark–release–recapture experiments for heterogeneity between individual *An. culicifacies* in their tendency to bite man rather than cattle or to rest indoors rather than to exit after feeding. In contrast, NUTSATHAPANA *et al.* (1986) did find such heterogeneity in *An. minimus*. However, in both studies, the offspring of those that had made different choices were not tested. Although we observed evidence of variability in the feeding behaviour of culicine mosquitoes, its absence in their offspring suggests that it is not genetic in nature but an acquired characteristic. The data seem to support a hypothesis of behavioural imprinting on whichever host happened to be experienced first.

Whilst karyotypic evidence is sometimes enough to detect behavioural polymorphism in mosquitoes (CURTIS & ISHERWOOD, 1984), our results emphasize the need to test both the parent populations and their offspring to assess a genetic hypothesis not based on visible/karyotypic variation. For instance, by conducting mark–release–recapture field experiments using 2 isofemale lines of *Drosophila tripunctata*, JAENIKE (1985) showed that line identity strongly and consistently affected the preferences of males and females for mushrooms or tomatoes. Environmental conditioning was demonstrated by the fact that females showed an augmented preference for the type of food on which they had been kept prior to release. However, the behaviour of F2 flies from reciprocal crosses between the 2 strains demonstrated that genetic influence in food preference was due to autosomal and largely additive genes.

The tendency by our laboratory-reared individuals of the JE vector species to feed more on the cow than on the pig when placed in a single net [Table (iii)] is an indication that naïve JE vectors usually prefer cows to pigs if given a choice. However, the data in the Table (i) and (ii) and Figure 3 show that some of the mosquitoes were attracted to a different host and this choice may be reinforced as a result of experience. Therefore increasing the availability of cattle (where possible) would tend to control the spread of JE by diverting the mosquito vectors from pigs (amplifiers of the virus) to cows (the dead-end hosts of the virus) because the initial tendency of the vector species to feed on the cattle and not the pigs would be reinforced by their probability of biting cattle early in life.

Although the capture–mark–release–recapture approach has been used to test variability in the behaviour of mosquitoes, a common limitation encountered is the low recapture rates (RAWLINGS & CURTIS, 1982; NUTSATHAPANA *et al.*, 1986), and especially for long-distance fliers such as *Cx. tritaeniorhynchus* this could be a major handicap. Furthermore, since the preference shown by a particular mosquito species for one host or another is also likely to be strongly influenced by environmental conditions (GILLIES, 1964), we avoided these shortcomings by releasing mosquitoes inside large enclosed nets where the baits were tethered. Our approach can be used to test the host preference and genetic variability of mosquito vectors, using various combinations of hosts provided under equal conditions, without releasing large numbers of potential vectors of human diseases into the environment.

Table. Number of mosquitoes from different origins fed on a cow, a pig or both when released into a net containing both animals

		Mosquitoes originally attracted to pig, fed on pig or descended from those fed on pig			Mosquitoes originally attracted to cow, fed on cow or descended from those fed on cow				
		Number fed on:			Number fed on:				
Mosquito origin	Species	cow	pig	both	cow	pig	both	χ^2	P
(i) From collection on net baited with cow or pig	*Cx. tritaeniorhynchus*	3	6	2	9	1	1	3·53	0·06
	Cx. gelidus	9	13	3	12	4	0	3·05	0·081
	Cx. vishnui	6	9	3	10	1	0	5·06	0·024
	Total	18	28	8	31	6	1	$\chi^2_{M\text{-}H}$ = 13·80	0·0102
(ii) Fed mosquitoes collected from cowshed or pigsty	*Cx. tritaeniorhynchus*	9	12	1	7	1	0	3·04	0·081
	Cx. gelidus	5	9	2	5	0	0	3·66	0·056
	Cx. vishnui	3	9	1	13	0	1	10·30	0·001
	Total	17	30	4	25	1	1	$\chi^2_{M\text{-}H}$ = 19·60	<0·001
(iii) Offspring of mosquitoes from cowshed or pigsty	*Cx. tritaeniorhynchus*	5	0	1	5	0	1	0·58	0·45
	Cx. gelidus	23	4	3	26	10	3	0·47	0·59
	Cx. vishnui	7	0	1	4	2	0	0·16	0·69
	Total	35	4	5	35	12	4	$\chi^2_{M\text{-}H}$ = 0·93	0·33

Mosquitoes whose origin was associated with the pig were marked red and those whose origin was associated with the cow marked blue. Blood meals were identified by ELISA. See the text and Figures 1 and 2 for the study design.

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