

# Flight tone of field-collected populations of *Anopheles gambiae* and *An. arabiensis* (Diptera: Culicidae)

J. W. WEKESA, W. G. BROGDON, W. A. HAWLEY\* and N. J. BESANSKY†

Centers for Disease Control and Prevention, Division of Parasitic Diseases, Chamblee, U.S.A., and \*Kenya Medical Research Institute, Clinical Research Centre, Nairobi, Kenya.

**Abstract.** Laboratory colonies of the human malaria vectors *Anopheles gambiae* Giles and *An. arabiensis* Patton have distinct flight tones. If flight tone similarly distinguishes natural populations of these sympatric sibling species, it may play a role in reproductive isolation of swarms that are otherwise behaviourally identical. To assess the fidelity of flight tone differences in natural populations, flight tone was measured in the F1 progeny of mosquitoes of both species captured in western Kenya. Flight tone distributions of wild *An. gambiae* and *An. arabiensis* were similar to their laboratory conspecifics. However, interspecies comparisons of flight tone of wild mosquitoes revealed significantly different but overlapping distributions for both sexes. Furthermore, when the effect of body size on flight tone was determined, there was a positive correlation between wing length and flight tone for both sexes of *An. gambiae* and *An. arabiensis*, suggesting that mosquito size is a significant variable affecting flight tone. Although these findings diminish any practical benefit of flight tone as a diagnostic tool in species identification, its potential role in pre-mating species recognition needs further investigation.

**Key words.** Acoustic communication, *Anopheles arabiensis*, *A. gambiae*, cryptic species, flight tone, reproductive isolation.

## Introduction

*Anopheles gambiae* Giles and *An. arabiensis* Patton are the principal vectors of human malaria in Africa (Coluzzi *et al.*, 1979; Service, 1985). These two sibling species are extensively sympatric over most of sub-Saharan Africa (White, 1974). In the laboratory, interspecific matings are frequent and produce fertile female, but sterile male, hybrids (e.g. Coluzzi *et al.*, 1979; Charlwood & Jones, 1980). However, the frequency of natural hybridization is extremely low (estimated at 0.1–0.2%), suggesting that a mating barrier exists in the field but not in the laboratory (Davidson *et al.*, 1970; Coluzzi *et al.*, 1979).

As in most other mosquito species, sexual behaviour in these species is associated with aerial aggregations, known as swarms, that consist mostly of males (Downes, 1969; Charlwood & Jones, 1980; Marchand, 1984). Although swarms form over specific marker sites, the fact that swarming occurs for a brief crepuscular period has made field observations of mating behaviour notoriously difficult. The few published reports on swarming in *An. gambiae* and *An. arabiensis* found no well-defined spatial or temporal differences that could explain their reproductive isolation (Marchand, 1984). Indeed, mixed swarms of the two species were observed.

Laboratory and field observations of swarming males belonging to *An. gambiae* or another sibling species, *An. melas*, demonstrated that males are attracted over a short range by female flight tone (Charlwood & Jones, 1979, 1980). Furthermore, Brogdon (1998) found that both sexes from laboratory colonies of different sibling species in the *An. gambiae* complex were distinguished by flight tone, except for the two allopatric species from coastal West and East Africa. Together, these results suggested that distinct flight

Correspondence: Dr J. W. Wekesa, Division of Parasitic Diseases, Mail Stop F-22, Centers for Disease Control and Prevention, 4770 Buford Highway, Chamblee, GA 30341, U.S.A. Fax: +1 770 488 7794. E-mail: wbw7@cdc.gov

†Current address: Department of Biological Sciences, University of Notre Dame, IN 46556, U.S.A.

tones might be a factor in reproductive isolation within the *An. gambiae* complex in nature. The objectives in this study were to measure flight tone in wild *An. gambiae* and *An. arabiensis*, and to determine whether flight tone differentiates populations or individual specimens of these species.

## Materials and Methods

### *Mosquito collection and maintenance*

The species and laboratory strains used, *An. gambiae* RSP and *An. arabiensis* AHERO, originated from Kenya and were reared at the Centers for Disease Control under standard conditions (e.g. Benedict, 1997). Wild specimens of *An. gambiae* and *An. arabiensis* were collected from Asembo and Ahero in western Kenya as blood-engorged females. They were allowed to oviposit, and then were karyotyped for species identification (Coluzzi & Sabatini, 1968). The eggs were shipped to the Centers for Disease Control in Atlanta and reared to adults. After flight tone measurements, the species identification was confirmed by a polymerase chain reaction assay (Scott *et al.*, 1993).

### *Audio sampling and data collection*

Individual mosquitoes were held in a 300 × 15 mm plexiglass tube, covered at one end by a stretched latex diaphragm. The microphone was placed at the opposite end of the tube to prevent escape of the mosquito. Flight sounds were recorded by a commercial condenser microphone (Radio Shack model 33–1060), amplified and sampled at 5000 Hz using the AUDIOMASTER IV computer software package (Oxxi, Long Beach, CA) according to Brogdon (1994). Soundfiles were converted into plots of sound frequency vs. time (sonograms) using SPECTROGRAM (public domain software by Richard Horne), as described previously (for details and instrumentation set-up see Brogdon, 1994). Three to five harmonics of the wingbeat frequency were observed for all individual mosquitoes, but only the fundamental harmonic was analysed. Recordings were made from at least 100 males and 100 females of *An. gambiae* RSP and *An. arabiensis* AHERO laboratory colonies, and more than 120 male and 150 female progeny of field-collected *An. arabiensis* and *An. gambiae*. All adults used in the experiments were at least 48-h old. To minimize the effect of temperature on wing beat frequency, all experiments were conducted at 24–26°C.

### *Mosquito size*

Wing length was used as an index of body size. This measure has been found to correlate with size and weight in many mosquito species (e.g. Fish, 1985; Packer & Corbet, 1989; Yuval *et al.*, 1993) and has been used previously as an index of size for *An. gambiae sensu lato* (Lyimo & Takken, 1993; Ameneshewa & Service, 1996). After recording of the flight

tone, the left wing was excised and placed on a double-sided sticky Scotch tape affixed to a microscope slide. Wings were measured from the auxiliary incision to the tip, excluding the fringe, using an ocular micrometer mounted on a dissecting microscope.

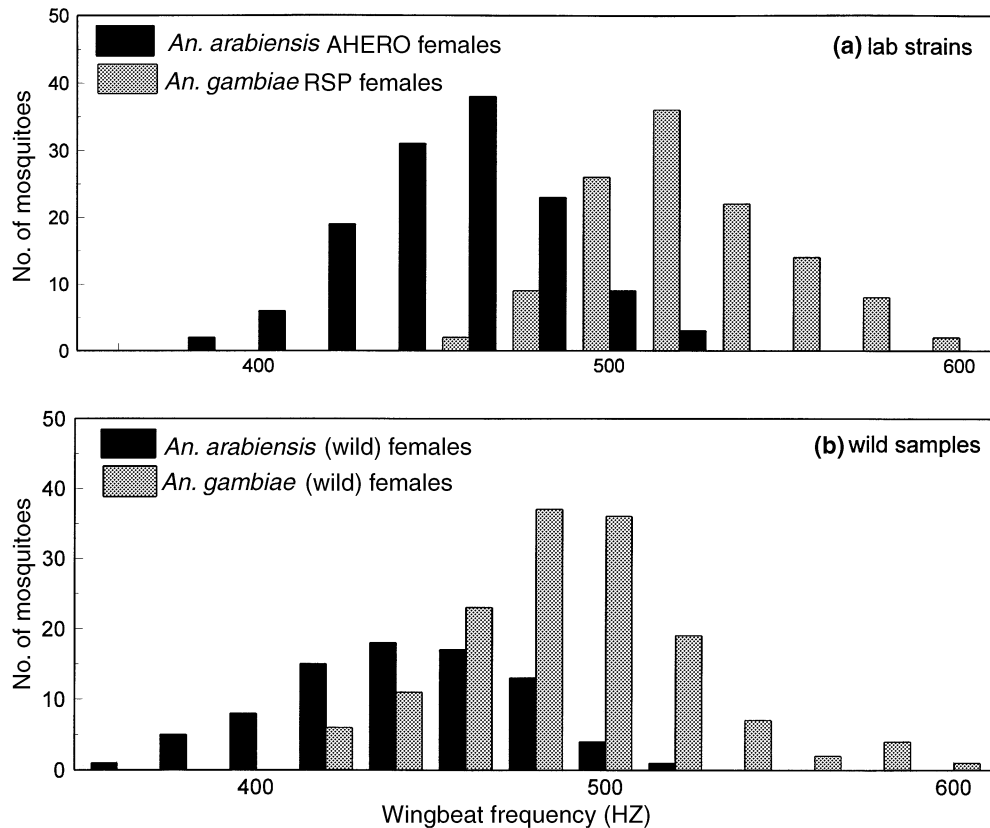
## Results

To test the reproducibility of the flight tone measurements by Brogdon (1998), conspecific comparisons of different laboratory colonies were made (Table 1). The distributions of flight tones measured from females of the *An. gambiae* RSP and G3 (Brogdon, 1998) colonies were similar ( $Z = 0.8$ ,  $P > 0.05$ ). However, the distributions of flight tones from male *An. gambiae* RSP and G3 (Brogdon, 1998) colonies were not ( $Z = 6.74$ ,  $P < 0.05$ ), with the former having a broader range than the latter. Flight tone distributions from both female and male *An. arabiensis* AHERO and ARZAG (Brogdon, 1998) were similar ( $P > 0.05$ ). Although the measurements of this study and the previous one are comparable, in most cases a broader distribution of flight tones was found within a given colony.

Interspecies comparisons of flight tone using colonized mosquitoes revealed overlapping but significantly different distributions for both sexes (Figs 1a, 2a; Table 1). Mean flight tone for female *An. gambiae* RSP was significantly higher than that of female *An. arabiensis* AHERO, with a range between means of 70 Hz ( $F = 17.96$ , d.f. = 1,182,  $P = 0.001$ ). Similarly, mean flight tone for male *An. gambiae* RSP was significantly higher than that of male *An. arabiensis* AHERO, with a range between means of 78 Hz ( $F = 26.27$ , d.f. = 1,187,  $P = 0.0001$ ).

To establish the reproducibility of flight tone measurements made on individual *An. gambiae* RSP mosquitoes, three samples were taken from the same individuals over a 24 h interval. No significant differences were noted among repeated measurements of eighty-two (forty-nine female and thirty-three male) *An. gambiae* RSP mosquitoes ( $F = 2.89$ , d.f. = 2,80,  $P = 0.07$ ).

Flight tone distributions for the progeny of wild *An. arabiensis* females and males were similar to those of laboratory conspecifics (female:  $F = 0.71$ , d.f. = 1,142,  $P = 0.82$ ; male:  $F = 2.16$ , d.f. = 1,118,  $P = 0.07$ ). Similarly, flight tone distributions for wild *An. gambiae* females and males were statistically identical to those of laboratory conspecifics (female:  $F = 3.76$ , d.f. = 1,244,  $P = 0.072$ ; male:  $F = 5.01$ , d.f. = 1,237,  $P = 0.056$ ). However, interspecies comparisons of flight tone of wild mosquitoes revealed overlapping frequency distributions for both sexes, just as they did for laboratory strains (Figs 1 and 2). Although interspecific differences in the flight tone distributions of wild females were significant, with a range between means of 62 Hz ( $F = 23.78$ , d.f. = 1,289,  $P = 0.001$ ), interspecific differences between wild males were only marginally significant ( $F = 6.25$ , d.f. = 1,263,  $P = 0.046$ ). In general, the degree of overlap between flight tone distributions of different species used in this study, whether colonized or wild, exceeded that observed by Brogdon (1998).



**Fig. 1.** Comparison of wingbeat frequency for the laboratory and field samples of *An. gambiae* and *An. arabiensis* females.

**Table 1.** Mean flight tone frequencies recorded from colonized and field-derived *An. gambiae* and *An. arabiensis* males and females.

Sex	Species: Source <sup>1</sup>	Mean $\pm$ SE (Hz)	Range (Hz)	Reference
Female	<i>An. gambiae</i> :			
	RSP	529 $\pm$ 27	460–600	This study
	G3	533 $\pm$ 21	460–580	Brogdon, 1998
	Field	501 $\pm$ 23	420–600	This study
	<i>An. arabiensis</i> :			
	AHERO	459 $\pm$ 16	380–520	This study
Males	ARZAG & AHERO	435 $\pm$ 18	400–460	Brogdon, 1998
	Field	443 $\pm$ 17	360–520	This study
	<i>An. gambiae</i> :			
	RSP	817 $\pm$ 19	700–900	This study
	G3	789 $\pm$ 16	750–820	Brogdon, 1998
	Field	760 $\pm$ 36	660–900	This study
	<i>An. arabiensis</i> :			
	AHERO	738 $\pm$ 28	600–820	This study
	ARZAG & AHERO	703 $\pm$ 34	610–740	Brogdon, 1998
	Field	719 $\pm$ 21	580–800	This study

<sup>1</sup>Geographic origin of laboratory strains: *An. gambiae* RSP (ReSistant to Permethrin), Kenya; *An. gambiae* G3, The Gambia; *An. arabiensis* AHERO, Ahero, Kenya; *An. arabiensis* ARZAG (ARabiensis ZAGtouli), Zagtoui, Burkina Faso.

After flight sound recording, the left wing was clipped from each of 677 *An. gambiae* RSP (362 females, 315 males). Of the *An. gambiae* RSP females measured, the mean wing length was 3.61 mm (SE = 0.067) with a range of 2.62–5.17 mm. Similarly, the mean wing length of male *An. gambiae* RSP

was 3.51 mm (SE = 0.036) with a range of 2.38–5.01 mm. The wing length of female (mean = 3.56 mm, SE = 0.024, range 2.51–5.11 mm) and male (mean = 3.48 mm, SE = 0.018, range 2.40–4.83 mm) *An. arabiensis* AHERO was not significantly different from that of *An. gambiae* RSP ( $P <$

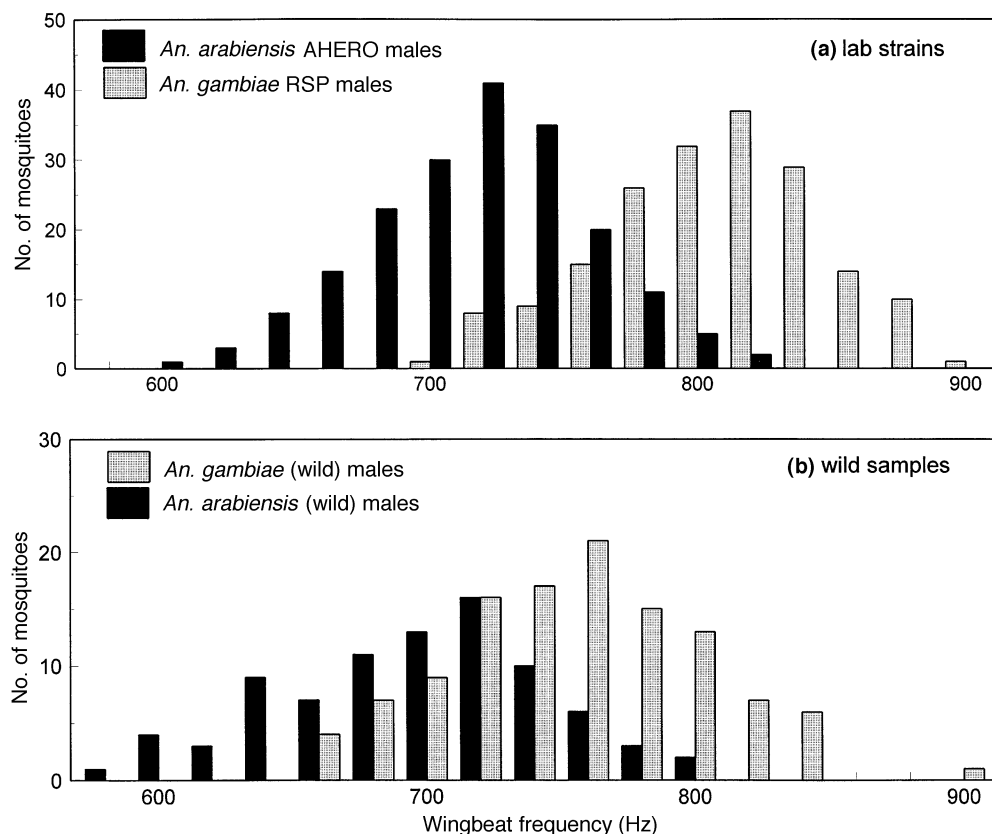


Fig. 2. Comparison of wingbeat frequency for the laboratory and field samples of *An. gambiae* and *An. arabiensis* males.

0.05). For both sexes, flight tone was positively correlated with wing length (female:  $r = 0.47$ , d.f. = 361,  $P < 0.01$ ; male:  $r = 0.62$ , d.f. = 314,  $P = 0.001$ ; Fig. 3a). Similar results were observed for ninety *An. arabiensis* AHERO (female:  $r = 0.46$ , d.f. = 45,  $P < 0.05$ ; male:  $r = 0.37$ , d.f. = 43,  $P < 0.05$ ; Fig. 3b).

Because wingbeat frequency was shown to be size-dependent, flight tone measurements and wing length measurements made from both sexes of *An. gambiae* RSP and *An. arabiensis* AHERO were subjected to analysis of covariance. This analysis revealed that the distributions of flight tone for both sexes of *An. gambiae* RSP were statistically higher than the corresponding sex of *An. arabiensis* AHERO (female:  $F = 13.49$ , d.f. = 1,381,  $P = 0.001$ , male;  $F = 17.86$ , d.f. = 1,374,  $P = 0.0001$ ).

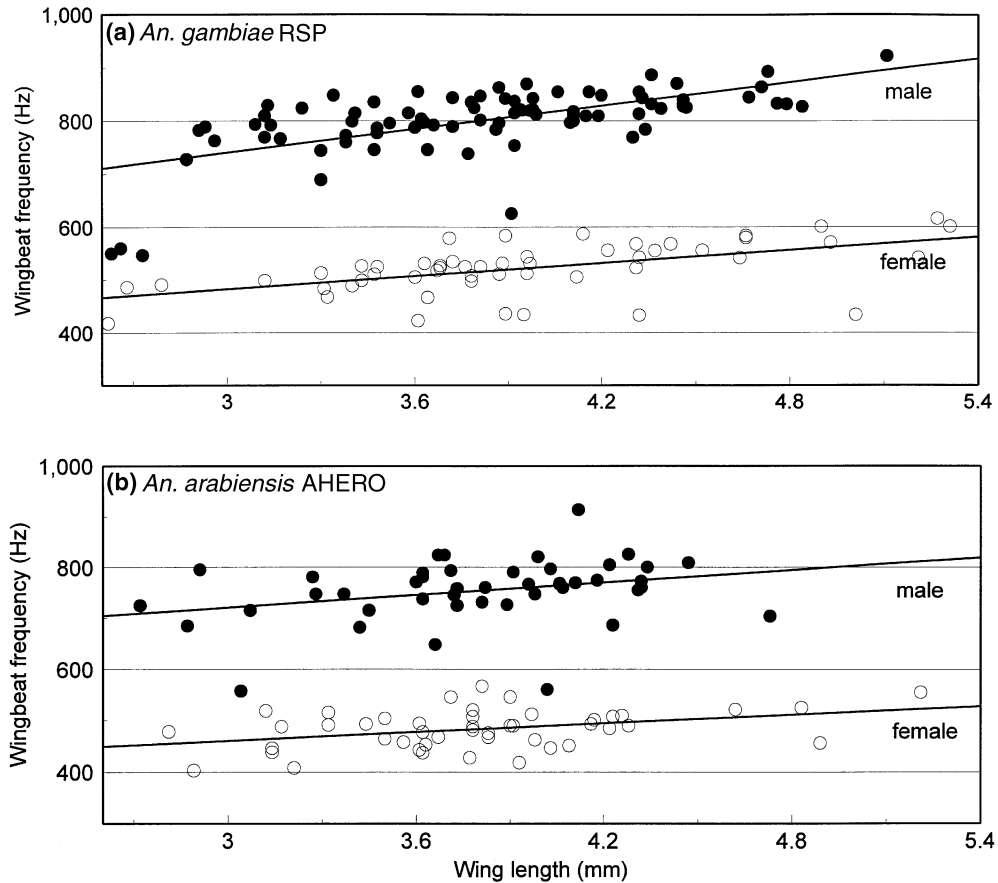
## Discussion

Unlike many higher flies, including some drosophilid and tephritid species, that produce sound by wing vibration during paired courtship and mating duets on a stationary substrate (Bennet-Clark & Ewing, 1970; Henry, 1994; Stewart, 1997), many lower flies, including mosquitoes, produce distinctive sound during flight, and their mating often occurs in swarms (Downes, 1969; Ewing, 1977). Swarms probably serve as

efficient assemblages that bring the sexes together and, at short distances, flight tone may play a significant role in species discrimination (Downes, 1969; Sullivan, 1981). The plumose antennae of males among the lower flies is a conspicuous and elaborate auditory (and olfactory) organ, sensitive to the flight tone of females (Downes, 1969). As females lack plumose antennae, it has been suggested that only males are capable of discriminating the species of the incoming females (Clements, 1963; and references therein). Based on this model, Roth (1948) and Ikeshoji *et al.* (1985) were successful in trapping male *Aedes aegypti* using synthesized sound played in the field at a frequency characteristic of females.

Brogdon (1998) discovered that different flight tones characterize laboratory colonies of sympatric members of the *An. gambiae* complex. Because mosquitoes in this complex mate in swarms, these differences were interpreted to reflect possible premating species-isolating mechanisms. Also, because only small percentages of each of the measured species showed flight tone overlap, Brogdon (1998) suggested that single mosquito flight tone observation was potentially useful for species and swarm identification.

Recordings in this study from the offspring of wild specimens of *An. gambiae* and *An. arabiensis* from Kenya showed that the mean flight tones measured for each sex were in accord with Brogdon's (1998) previous measurements, and between-species comparisons showed that the means were significantly



**Fig. 3.** Effect of body size on the flight tone of *An. gambiae* and *An. arabiensis*. The correlation was significant among both (a) *An. gambiae* (males,  $r = 0.62$ ,  $P < 0.001$ ; female,  $r = 0.47$ ,  $P < 0.01$ ), and (b) *An. arabiensis* (males,  $r = 0.37$ ,  $P < 0.05$ ; female,  $r = 0.46$ ,  $P < 0.05$ ) species.

different. However, the distributions of flight tones from these offspring of wild specimens showed much more extensive overlap between species than had previously been reported for colonies (see Figs 1 and 2). These results also revealed that wingbeat frequency was correlated significantly with the size of the mosquito, with smaller mosquitoes of both species having significantly lower wingbeat frequencies than larger individuals. The extensive overlap of flight tone frequencies between these two species, the dependence of flight tone on body size, and the requirement for live specimens diminish the advantage of flight tone as a diagnostic tool in species identification of individual mosquitoes. Nevertheless, the observation that the offspring of wild caught specimens of *An. gambiae* and *An. arabiensis* have the same mean flight tones as was observed in laboratory colonies supports Brogdon's (1998) speculation, based on laboratory colonies, that these flight tone differences may contribute to species isolation. Furthermore, the observation that flight tone frequencies of female *An. gambiae* and *An. arabiensis* were much more differentiated than their male counterparts is consistent with the suggestion that it is the males who use this information to discriminate among females.

### Acknowledgements

The authors thank J. M. Vulule (Kenya Medical Research Institute) for mosquito eggs from the field, and Boaz Yuval, Frank Collins and Tovi Lehmann for their critical suggestions on earlier versions of the manuscript. This work was supported in part by a research grant to N.J.B. from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). During this investigation, J.W.W. was an American Society for Microbiology Postdoctoral Fellow.

### References

- Amenesheva, B. & Service, M.W. (1996) The relationship between female body size and survival rate of the malaria vector *Anopheles arabiensis* in Ethiopia. *Medical and Veterinary Entomology*, **10**, 170–172.
- Benedict, M.Q. (1997) Care and maintenance of anopheline mosquito colonies. *Molecular Biology of Insect Disease Vectors: a Methods Manual* (ed. by J. M. Crampton, C. B. Beard and C. Louis), pp. 3–20. Chapman & Hall, London.

- Bennet-Clark, H.C. & Ewing, A.W. (1970) The love song of the fruit fly. *Scientific American*, **223**, 84–92.
- Brogdon, W.G. (1994) Measurement of flight tone differences between female *Aedes aegypti* and *A. albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*, **31**, 700–703.
- Brogdon, W.G. (1998) Measurement of flight tone differences between members of the *Anopheles gambiae* species complex. *Journal of Medical Entomology*, **35**, in press.
- Charlwood, J.D. & Jones, M.D.R. (1979) Mating in the mosquito, *Anopheles gambiae* s.l. I. Close range and contact behaviour. *Physiological Entomology*, **4**, 111–120.
- Charlwood, J.D. & Jones, M.D.R. (1980) Mating in the mosquito, *Anopheles gambiae* s.l. II. Swarming behaviour. *Physiological Entomology*, **5**, 315–320.
- Clements, A.N. (1963) *The Physiology of Mosquitoes*. Pergamon Press, Oxford.
- Coluzzi, M. & Sabatini, A. (1968) Cytogenetic observations on species A and B of the *Anopheles gambiae* species complex. *Parasitologia*, **10**, 155–165.
- Coluzzi, M., Sabatini, A., Petrarca, V. & Di Deco, M.A. (1979) Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **73**, 483–497.
- Davidson, G., Odetoynbo, J.A., Colussa, B. & Coz, J. (1970) A field attempt to assess the mating competitiveness of sterile males produced by crossing two members of the *Anopheles gambiae* complex. *Bulletin of the World Health Organization*, **42**, 55–67.
- Downes, J.A. (1969) The swarming and mating flight of Diptera. *Annual Review of Entomology*, **14**, 271–298.
- Ewing, A.W. (1977) Communication in diptera. *How Animals Communicate* (ed. by T. A. Sebeok), pp. 403–417. Indiana University Press, Bloomington, U.S.A.
- Fish, D. (1985) An analysis of adult size variation within natural mosquito populations. *Ecology of Mosquitoes* (ed. by L. P. Lounibos, J. R. Rey and J. H. Frank), pp. 419–430. Florida Medical Entomology Laboratory, Vero Beach, U.S.A.
- Henry, C.S. (1994) Singing and cryptic speciation in insects. *Trends in Ecology and Evolution*, **9**, 388–392.
- Ikeshoji, T., Sakakibara, M. & Reisen, W.K. (1985) Removal sampling of male mosquitoes from field populations by sound-trapping. *Japanese Journal of Sanitation and Zoology*, **36**, 197–203.
- Lyimo, E.O. & Takken, W. (1993) Effects of adult body size on fecundity and pre-gravid rate of *Anopheles gambiae* females in Tanzania. *Medical and Veterinary Entomology*, **7**, 328–332.
- Marchand, R.P. (1984) Field observations on swarming and mating in *Anopheles gambiae* mosquitoes in Tanzania. *Netherlands Journal of Zoology*, **34**, 367–387.
- Packer, M.J. & Cobert, P.S. (1989) Size variation and reproductive success of female *Aedes punctator* (Diptera: Culicidae). *Ecological Entomology*, **14**, 297–309.
- Roth, L.M. (1948) A study of mosquito behaviour. An experimental laboratory study of the sexual behaviour of *Aedes aegypti* (L.). *American Midland Naturalist*, **40**, 265–352.
- Scott, J.A., Brogdon, W.G. & Collins, F.H. (1993) Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene*, **49**, 520–529.
- Service, M.W. (1985) *Anopheles gambiae*: Africa's principal malaria vector, 1902–84. *Bulletin of Entomological Society of America*, **31**, 8–12.
- Stewart, K.W. (1997) Vibrational communication in insects: epitome in the language of stoneflies? *American Entomologist*, **43**, 81–91.
- Sullivan, R.T. (1981) Insect swarming and mating. *Florida Entomologist*, **64**, 44–65.
- White, G.B. (1974) *Anopheles gambiae* complex and disease transmission in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **68**, 278–298.
- Yuval, B., Wekesa, J.W. & Washino, R.K. (1993) Effect of body size on swarming behavior and mating success of male *Anopheles freeborni* (Diptera: Culicidae). *Journal of Insect Behavior*, **6**, 333–342.

Accepted 10 March 1998