HERITABILITY OF WING-BEAT FREQUENCY IN *ANOPHELES QUADRIMACULATUS*

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ABSTRACT. The repeatability of male wing-beat frequency measurements of *Anopheles quadrimaculatus* was determined by using mosquitoes allowed free flight in a confined space. Heritability of the wing-beat frequency trait was estimated for a laboratory and a wild-strain population of *An. quadrimaculatus* by using free-flight measurement with a parent–offspring regression of offspring on dams. Repeatability was 0.75 for free flight. Wing-beat frequency rose for the 1st day after adult emergence and then became steady. Female heritability of wing-beat frequency was 21.6% for colony and 24.0% for wild-strain mosquitoes. Male heritability was 57.2% for colony and 53.7% for wild-strain mosquitoes. Male heritability was significantly different from 0 when probabilities were combined across both populations.

KEY WORDS   Mosquito flight, heritability, *Aedes quadrimaculatus*

INTRODUCTION

Mosquitoes of the *Anopheles quadrimaculatus* species complex are major nuisance pests in many rural and agricultural areas in the southeastern USA, especially in areas where rice cultivation produces large expanses of water that provide habitat for mosquito larvae. This complex is composed of at least 5 sibling species (Reinert et al. 1997), at present morphologically indistinguishable, but reproductively incompatible (Seawright et al. 1991). Forced pairings between sibling species give rise to semisterile female offspring and either sterile or unviable male offspring (Kaiser et al. 1988). Although 2 or more species of *An. quadrimaculatus* complex often are found sympatrically, Lanzaro et al. (1988) found no evidence of hybridization between species and concluded that a premating isolation barrier exists.

If the mating isolation barrier Lanzaro et al. (1988) postulated is removed, natural hybridization between the species would result in reduced mosquito populations through hybrid sterility. The nature of the mating barrier is not known, if it exists at all, but Caprio et al. (2001) noted that the mean wing-beat frequency of male mosquitoes differed among 3 of the sibling species. They hypothesized that male wing-beat frequency may be a criterion for female acceptance of mating partners, that is, they will accept only partners whose wing-beat frequencies fall within a specific range.

To explore this possibility, the initial step is to determine if additive genetic variance exists for the trait, that is, is it a heritable trait subject to selection? Heritability can be described in a broad sense or a narrow sense. Broad-sense heritability is the proportion of the total phenotypic variance of a trait that is attributed to the total genetic variance, that is, it is the degree to which a trait is genetically determined. Narrow-sense heritability is the degree to which a trait is transmitted from parents to offspring, expressed as the ratio of additive genetic variance to phenotypic variance (Roff 1997). Narrow-sense heritability relates the reliability of the phenotypic value as a guide to the breeding value (Falconer 1989), or how much the phenotype of the parents affects the phenotype of the offspring. Narrow-sense heritability is the subset of broad sense heritability that is subject to selection.

Whether a mate-attractive trait such as wing-beat frequency can be heritable is debated. Hedrick (1994) reviewed the history of this debate and noted Maynard Smith's objections based on Fisher's fundamental theorem of natural selection (the rate of increase in fitness under directional selection is equal to the genetic variance in fitness). Maynard Smith interpreted this to mean that those traits closely related to fitness will have little additive genetic variance and, therefore, low heritabilities. This is true only for single loci, and no evidence is available that wing-beat frequency is controlled at a single locus. In fact, evidence has accumulated in the life history literature for the maintenance of large amounts of genetic variance in traits related to fitness (Charlesworth 1987). Hedrick (1994) demonstrated that mate-attractive traits can be heritable. Before determining the heritability of call length in field crickets, she first proposed 3 criteria that should be met before the trait could be considered mate attractive. These criteria are that males must show phenotypic variation with respect to the trait; expression of the trait must be repeatable, that is, remain the same over the life span of the individual, or at least during the period of mate selection; and the trait must be used by females as a specific criterion for mate choice.

Of these criteria, we know only that phenotypic variation in wing-beat frequency exists in males of *An. quadrimaculatus* complex populations (Caprio et al.2001), but the repeatability of this trait was not demonstrated.

The objectives of this experiment were to determine the repeatability of wing-beat frequency mea-

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surement and the heritability of the wing-beat frequency trait in a population of colony and a population of wild mosquitoes.

MATERIALS AND METHODS

Two strains of mosquitoes were used, wild-caught *Anopheles quadrimaculatus* Say, type species of the complex, and a colony of Orlando *An. quadrimaculatus* complex mosquitoes that had been outcrossed with wild mosquitoes. The wild strain was collected in Noxubee County, Mississippi, from a site at which 3 different species of the *An. quadrimaculatus* species complex had been collected. Colony mosquitoes were housed in screen cages for 5 days after eclosion as adults to allow mating to take place. They were supplied with cotton balls soaked in a 10% solution of sugar water. Females were then removed and allowed to take a blood meal from laboratory mice. Females that fed were held for 1 day to allow for digestion to take place and then were measured for wing-beat frequency as described below. For oviposition, females were enclosed in plastic cylinders partially filled with tap water and lined with filter paper. Netting was placed over the open end of the cylinders and held in place with plastic caps whose centers had been removed. Broods were reared in separate 50-ml cups and thinned to 20 larvae after the 2nd stage was reached. Emerging adults of both sexes were held for 1 complete day before being measured for wing-beat frequency, because wing-beat frequency is lower in newly eclosed males (Roth 1948). We attempted to measured 3–5 individuals of each sex from each brood. Broods containing less than 2 members of 1 sex were discarded for that sex. Regression lines were computed for each sex separately in colony and wild-strain mosquitoes. Wild-strain mosquitoes were captured and allowed a blood meal, but were otherwise treated similarly to the colony mosquitoes.

Wing-beat frequency: We placed each mosquito in a 22-ml glass scintillation vial and held the vial between a light source and a T-sinn light sensor (Radio Shack, Fort Worth, TX). Mosquitoes were induced into flight and the sensor recorded the fluctuations of light caused by the moving wings onto audiotape (Moore et al. 1986). The audio recording was digitized with a personal computer recorder soundcard and software and converted into a wing-beat frequency via fast Fourier transform with the software Mathcad (Mathsoft 1991). Wing-beat frequency was measured in units of beats per second.

Repeatability: We used mosquitoes from a laboratory colony of *An. quadrimaculatus,* established in Orlando, FL (Orl A), that had been outcrossed with mosquitoes of a wild strain several generations before these experiments. Upon eclosion as adults, 9 males were measured for wing-beat frequency. Males were maintained on sugar solution and measured each day for 4 days. The repeatability (r) of

a quantitative trait is estimated by making repeated measures on individuals and then calculating the ratio of the between-individual variance ($V_{between}$) to the sum of both the between-individual variance and within-individual variance (V_{within}): $r = V_{between}/(V_{between} + V_{within})$, with the range of r being from 0 to 1 (Falconer 1989). Repeatability was calculated as (Hedrick 1994):

(variation in wing-beat frequency among days measured)

$\div$ (variation among days

+ variation among mosquitoes).

Heritability (statistics): Falconer (1989) described several methods for estimating heritability, among them regression of offspring on parents, half-sibling designs, and full-sibling designs. Because of the reluctance of wild *An. quadrimaculatus* to breed in captivity, a regression of sons on dams and daughters on dams was used to pool data previously collected by Caprio (unpublished) from wild-caught females with new data collected from a laboratory strain, which allowed comparison of the heritability estimates of 2 populations of the same species. Another benefit from comparison of 2 sets is to determine if a change in heritability has occurred in the colony strain. Utilization of wild-caught females of unknown age increases the phenotypic variation of the parents, and heritability estimate therefore may be lower than had measurement of all female parents at the same age been possible.

Heritability was calculated by a linear regression of mean wing-beat frequency of offspring on their corresponding dams. From each brood, 2–5 offspring of each sex were selected at random and their average wing-beat frequencies were measured. Separate regression lines were calculated for each sex within wild and colony populations. Because of the unequal variances of female and male wing-beat frequency, a correction factor was established by dividing the variance of the dams by that of the sons (Falconer 1989). Regression coefficients and their standard errors were multiplied by the correction factor. No correction factor was necessary for the regression of daughters on dams.

RESULTS AND DISCUSSION

Mean wing-beat frequency increased for the 1st day after adult emergence; therefore, the repeatability measurement was calculated only with measurements made after 1 day (Fig. 1). Repeatability of the wing-beat frequency trait in males was 0.76 (Fig. 1). Measured across all ages, repeatability was 0.25.

The heritability of wing-beat frequency varied between the colony and field-collected mosquitoes and between females and males (Tables 1–3 and Fig. 2), and the correction factor for unequal vari-
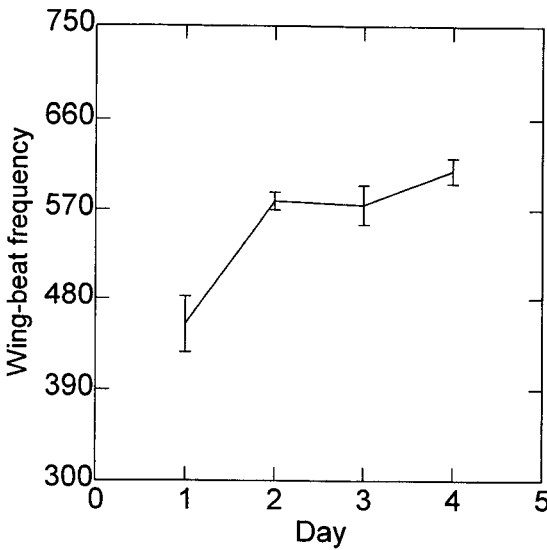
Fig. 1. Wing-beat frequency of male *Anopheles quad-rimaculatus* measured in scintillation vials over a 4-day period. Repeatability $(r)_{all\ days} = 0.25$, repeatability $(r)_{after\ day\ 1} = 0.76$.

Table 2. Heritabilities (%) of wing-beat frequency in *Anopheles quadrimaculatus* estimated from parent–offspring regression.[1]

	Male	Female
Colony	57.2 ± 33.9	21.6 ± 28.6
Wild	53.7 ± 28.1	24.0 ± 15.6

[1] Numbers after mean values represent standard errors of the mean.

ance in males and dams was very similar among populations. Heritability estimates were high for colony and wild males, although confidence intervals also were large (Table 2). The regression of wild males on dams was significant at the $\alpha = 0.10$ level and the regression of colony males on dams yielded a *P* value just greater than the $\alpha = 0.10$ level. However, combination of these probabilities (Sokal and Rohlf 1995) suggests that heritability is significantly different from 0 at the $\alpha = 0.05$ level in males ($\chi^2 = 10.14$, df = 4), but not in females ($\chi^2 = 5.63$, df = 4).

Heritability calculated by a correlation of female siblings on male siblings was very high (Table 3). However, Falconer (1989) warned that calculating heritability with full siblings has weak points, and maintains that such an estimate only can be used as an upper boundary to heritability. A disadvantage of the full-sibling correlation is the factor of shared environment. All siblings in each brood were reared in a single cup and were growing under the same conditions of food availability, bacterial contamination, and so on.

The large standard errors for heritability of wing-beat frequency in the regression of offspring on dams were due to variance in the data, probably for

several reasons. The repeatability of the trait was not extremely high. A more natural method of measurement (e.g., longer free flight) might reduce this variance. Another source of unexplained variation may be due to age or condition of dams. As mosquitoes age their wings, become frayed and their wing-beat frequencies may change. No effort was made to test colony females of a specific age, and it was impossible to estimate the age of wild-caught females. Age may have varied from very young to individuals that had overwintered. Although a half-sibling design would have eliminated many of these problems, we could not reliably force-mate males with multiple females, a necessity for this technique.

Colony males were not expected to have a heritability as high as that of wild males. The colony is small, and although it has been recently outcrossed with wild mosquitoes, a generation had elapsed from that time to the time of this study. The normal assumption would be that a smaller population would retain lower variation for all genetic traits. One possible explanation for the similar heritability is that selection for this trait in the cages is very low. Females are in constant contact with all of the males in a cage and colony females already were demonstrated to be less selective than wild females. Colony males, under conditions of relaxed selection, are expected to have similar or greater heritability values than wild males if the trait was used in mate recognition.

In general, the heritability of traits directly related to fitness, and hence under strong selection, are lower than traits under weak selection (Falconer 1989). Analysis of the data presented here suggest that the heritability of the wing-beat trait is lower in females than in males, indicating that expression of that trait may be under stronger selection in females. Mate location by males is a potential trait under selection in this case. Caprio et al. (2001) found that mean male wing-beat frequency was significantly different among 3 species of the *An.*

Table 1. Regression coefficients with standard errors and adjustment factors of offspring on dams for the wing-beat frequency trait in *Anopheles quadrimaculatus*.

	Male	P[1]	n	Female	P	n
Colony	(0.403 ± 0.239) × 0.71	0.101	33	0.108 ± 0.143	0.456	43
Wild	(0.344 ± 0.180) × 0.78	0.062	53	0.120 ± 0.078	0.131	59

[1] Probability that the regression coefficient is equal to or less than 0.

Table 3. Upper limits of heritability based on correlation of male and female siblings with respect to the wing-beat frequency trait in *Anopheles quadrimaculatus*.

	t^1	h^2
Colony	0.401	≤ 80.1
Wild	0.390	≤ 78.0

[1] Correlation statistic.

quadrimaculatus species complex (*Anopheles quadrimaculatus* Say, *An. smaragdinus* Reinert, and *An. maverlius* Reinert), whereas mean female wing-beat frequency was not different. The differences in male wing-beat frequencies suggested the possibility that this trait was utilized by females in mate acceptance, but under such conditions, one would expect the heritability of the trait to be low. Alternatively, wing-beat tone in males may have diverged because of genetic drift among the reproductively isolated sibling species. Female wing-beat tone may not have diverged between species because it is under selection (as evidenced by the low heritability). In other anopheline species, males form mating swarms (Charlwood and Jones 1980, Marchand 1984), but we have been unable to locate such swarms for *An. quadrimaculatus*, nor are there reports of such in the literature. If wing-beat tone is an important criteria in mate attractiveness in *An. quadrimaculatus*, we suggest that the expression of this trait in females likely is the target of selection, and that this trait has been conserved among the 3 sibling species studied by Caprio et al. (2001). This is not likely to be an example of sexual selection because mate choice in that case is primarily by females and males are in a scramble competition to gain access to female reproductive capacity (Partridge 1994). Perhaps the trait allows males to locate females, after which other behaviors govern mating acceptance.

Two of Hedrick's (1994) criteria for mate-attrac-

tive traits were met by *An. quadrimaculatus* in this study. Wing-beat frequency is repeatable. The repeatability of wing-beat frequency is probably higher under natural conditions of sustained flight than those under which it was measured in the laboratory. Wing-beat frequency in males also is variable and heritable, whereas the heritability estimate was approximately one half of that of males in females. Whether this trait is used by conspecifics as a specific criterion for mate choice remains unknown.

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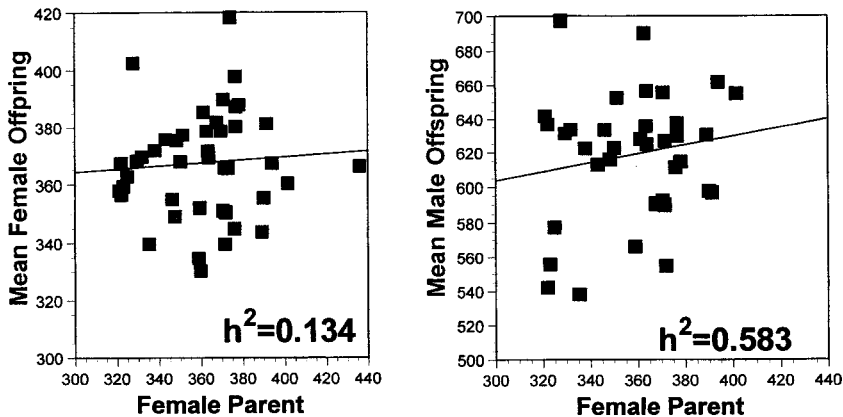

Fig. 2. Means of male and female mean brood wing-beat frequency compared to the wild female parent wing-beat frequency.

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