# Life History Correlates of Adult Size in the Malaria Vector *Anopheles darlingi*

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*Adult dry weights of laboratory-reared* Anopheles darlingi *were highly correlated with wing lengths, which were used to estimate size variation in natural populations of this species. Significant differences in mean wing lengths of females trapped at baits were detected among collections in the same week at one site, but not between three sites in Brazil and Bolivia. Relatively higher variability of wing lengths, compared to collections of other* Anopheles *(Nyssorhynchus), and platykurtic size distributions in large, single-night collections suggested that* An. darlingi *females caught at baits emerged from heterogenous larval habitats. No relationship was detected between parous state and the body size of wild-caught females. Adult males and females of laboratory-reared* An. darlingi *did not differ in body size. This absence of sexual size dimorphism is rare among mosquitoes and has not been noted previously in the genus* Anopheles.

Key words: *Anopheles darlingi* - mosquito - adult size - wing lengths - population characteristics - sexual dimorphism

Since publication of the seminal paper by Fish (1985), which described natural variation in adult mosquito body sizes, many workers have noted relationships between mosquito sizes and variables of field populations such as survival (e.g., Hawley 1985, Lounibos et al. 1990), mating success (Yuval et al. 1993), fecundity (e.g., Bradshaw & Holzapfel 1992, Lyimo & Takken 1993), and infection with parasites (Lyimo & Koella 1992). Fish (1985) also suggested that properties of a mosquito population's larval habitat could be deduced from the size distribution of adults trapped in nature. Only a few studies have examined size variation in natural populations of malaria vectors (Lyimo & Koella 1992, Lyimo & Takken 1993, Lounibos 1994), in spite of the awareness that vector body size distribution may affect the capacities of a population or species to transmit disease.

Most, but not all (e.g., Yuval et al. 1993), body size measurements of wild-caught mosquitoes have been made on adult females. For the majority of species of mosquitoes, adult males are smaller than females and eclose earlier, a condition known as protandry (Thornhill & Alcock 1983). Protandry does not occur among some species of the mos-

quito genus *Toxorhynchites* (Corbet & Ali 1987) in which sexual size dimorphism may be absent or slight (Lounibos 1979 and unpublished data). The widespread occurrence of protandry among holometabolous insects is considered to be a reproductive strategy of males in evolutionary response to competition for mates (Wiklund & Fagerstrom 1977, Thornhill & Alcock 1983).

Adult dry weight is, arguably, the most suitable indicator of size (=mass), but this measurement is tedious and often inaccessible for field studies. Many mosquito workers have, instead, measured wing lengths (e.g., Siegel et al. 1992, 1994) or pupal weights (Hawley 1985, Kleckner et al. 1995), which are both highly correlated with adult dry weights. Some early studies (e.g., Christophers 1960, Fish 1985) related mosquito dry weights to the cube of wing lengths in regression analyses, but Siegel et al. (1992) demonstrated that logarithmic data transformations provided superior fits to linear regression models. Siegel et al. (1994) cautioned against the use of wing lengths for predicting adult body weights.

The principal objectives of the present study were to examine the wing length - dry weight relationship for *Anopheles darlingi* Root and to explore associations between body size and life history characteristics of field populations and laboratory samples of this species, the most important vector of human malaria in South America. Untransformed wing lengths were used as indicators but not as predictors of body size. Because

wing lengths may underestimate the extremes of body mass (Siegel et al. 1994), our conclusions about natural size variability in *An. darlingi* may be conservative.

## MATERIALS AND METHODS

Host-seeking *Anopheles* females were collected between 18:00 and 22:00 hr from stationary human baits at sites in Bolivia, Brazil, and Venezuela (Tables I,III). In Capanema (CP) only, *An. darlingi* were collected from the walls of a water buffalo corral. In Porto Velho (PV), *An. darlingi* were captured at human baits on three nights within one week by the same collectors in the same *fazenda*. Live anopheline mosquitoes held overnight in humidified containers were anesthetized and identified to species through a dissecting microscope with morphological keys (Faran & Linthicum 1981, Linthicum 1988). One wing of each specimen was removed and measured, from the base of the alula to the apical wing margin excluding fringes, with an ocular micrometer at 20X. From an arbitrarily chosen subset of *An. darlingi* collected at site PV, reproductive organs were dissected and parity determinations made on ovaries in Christophers' stages I or II by the tracheolar skeining technique of Detinova (1962). Interspecific comparisons of wing lengths of *An. darlingi* to other field-collected *An. (Nyssorhynchus)* species used measurements published previously (Lounibos, 1994). The *An. albitarsis* (*sensu lato*) were identified as *An. marajoara* according to Linthicum (1988).

Gravid *An. darlingi* females collected in Tefé (TE) and Puerto Ayacucho (PA) were allowed to oviposit on wet filter paper in individual vials at 26±1°C in a quarantined insectary in Florida. Forty-eight hours after oviposition, eggs were floated on distilled water in plastic drinking cups. First instars hatching within 24 hr were divided into vessels containing 75 ml of distilled water and initial densities of 25 or 75 larvae. With the first day in these vessels indicated as day one, each cohort received 15 mg daily of finely ground liver powder (ICN Biochemicals), except for day three when no food was provided. Dead larvae and scum were removed daily from rearing containers. Live pupae were held individually until adult emergence in screened, nine-dram vials containing 10 ml of distilled water.

Twenty-four hours after eclosion, adults were killed by freezing, sexed, and one wing was measured as described above. The effect of sex on wing lengths of *An. darlingi* was compared to three other species of *An. (Nyssorhynchus)* reared under identical laboratory conditions. Because larval density can have an effect on adult size (Lounibos 1994),

mosquitoes were segregated by initial rearing density for size comparisons between the sexes.

Adults reared from progeny of collections at site TE were killed by freezing, then placed in individually marked containers in a drying oven at 80°C for 48 hr. Both wings were removed for length measurements and to ensure homogeneity of weighed individuals. Oven-dried adults, maintained in a desiccator over calcium sulfate, were weighed to the nearest 0.001 mg on an electrobalance (Cahn).

Analyses of variance (ANOVA) and regressions were performed on wing length and weight measurements and coefficients of variation by the GLM procedure (SAS Institute 1985). ANOVA was used, in spite of some non-normal wing length distributions, because this statistical procedure is robust to moderate departures from normality and homoscedasticity (Scheffé 1959). Following ANOVAs, means were compared by the *a posteriori* Ryan-Eliot-Gabriel-Welsch multiple range test (REGWQ). The relationship between wing lengths and the numbers of *An. darlingi* having completed one or more gonotrophic cycles was analyzed by logistic regression with the procedure CATMOD (SAS Institute 1985). Calculations and tests for significance of coefficients of variation (CV), skewness, and kurtosis followed Sokal and Braumann (1980) and Sokal and Rohlf (1981).

## RESULTS

*Relationships between dry weight and wing lengths* - Regressions of dry weight (y-variate) versus wing length (x-variate) were significant both for untransformed ($F_{1,118} = 140.25$, P <0.001) and log-log transformed data ($F_{1,118} = 95.62$, P <0.001). However, the regression of untransformed data explained 9% more variance ($R^2 = 0.54$) than the log-transformed data ($R^2 = 0.45$) and, thus, only the former was subjected to further analyses (Fig.). One apparently aberrant wing length measurement of 1.60 mm was detected by the method of Sokal and Rohlf (1981) as an outlier in the regression analysis ($r_s = 4.60$, $r_{.005} = 3.81$, n = 120, one-tailed test). When this outlier was removed, justified by the assumption that abnormal development caused the shortened wing, an added 19% variance was explained by the linear regression model ($F_{1,117} = 317.74$, P <0.001, $R^2 = 0.73$).

*Size variation in natural populations* - Mean sizes of *An. darlingi* from CP, GU and the first night's collection at PV did not differ significantly (Table 1). However, significantly different mean wing lengths were detected among the three, single-night collections of *An. darlingi* made within one week's span at the PV site in Brazil .
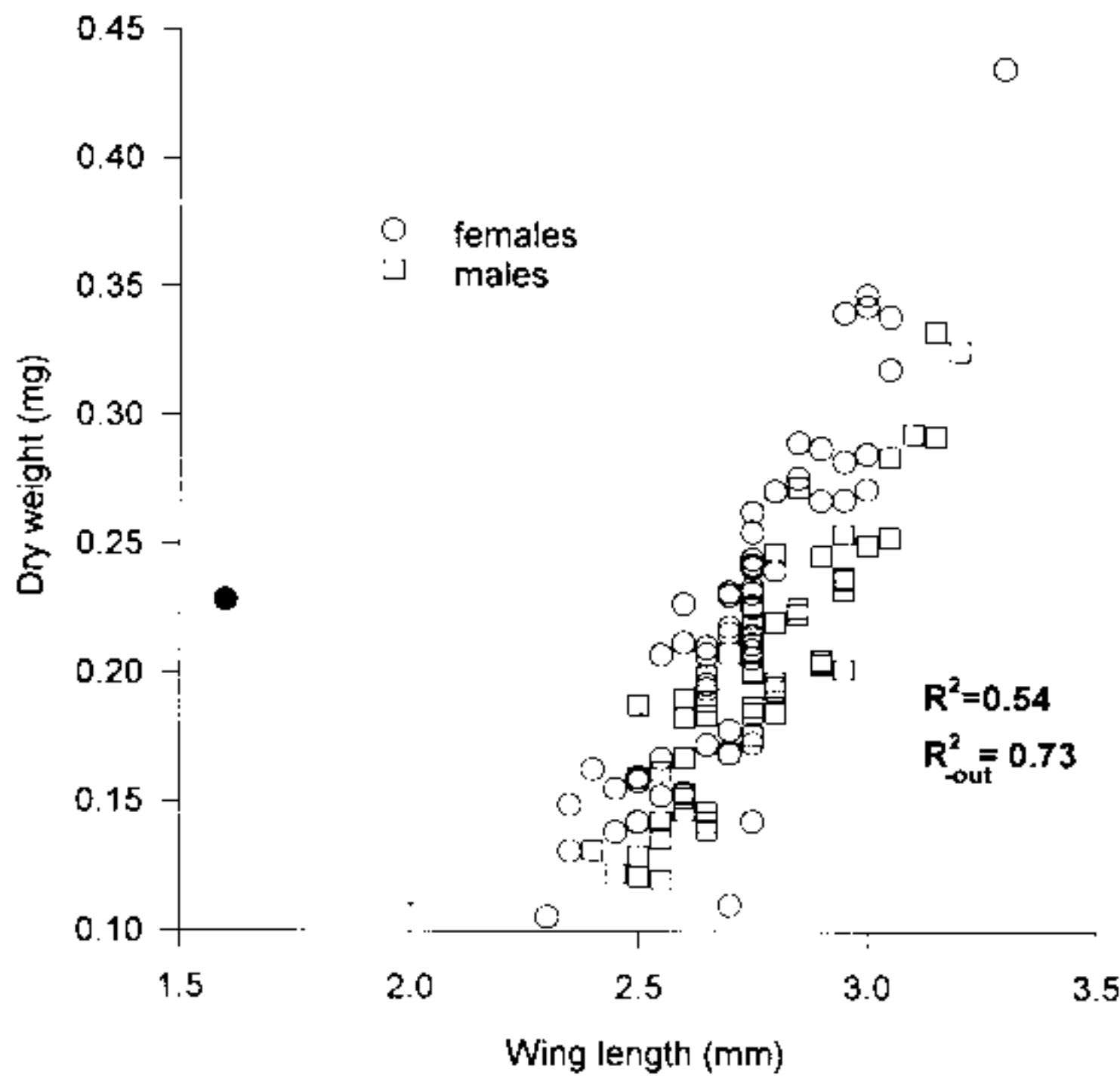
The relationship between wing length and dry weight from 120 *Anopheles darlingi* reared at initial densities of 25 and 75 larvae per container. Coefficients of determination from regressions with ($R^2$) and without the outlier (filled circle) point ($R^2_{-out}$) are provided.

Wing length CVs of mosquitoes from five night collections ranged from 5.03 to 6.70, which variability is homogeneous according to Bartlett's test performed on log-transformed variances (chi-squared = 5.41, P > 0.10). Skewnesses of wing length distributions in single-night collections were consistently negative, but none significantly so (Table I). All five wing length distributions tended to deviate platykurtically from normality, one night's collection showing significantly negative kurtosis (Table I).

Wing length CVs of field-collected *An. darlingi* were compared to CVs of four other species of *An. (Nyssorhynchus)* bait-trapped elsewhere in South America (Lounibos 1994). ANOVA detected significant variation among species ($F_{4,47}$ = 2.77, P = 0.038), with *An. darlingi* recording the largest mean CV (Table II). However, *a posteriori* comparisons indicated no significant differences between species in mean CVs.

Of 170 *An. darlingi* females from PV dissected for parity determinations, 104 (61.2%) were diagnosed as having completed one or more gonotrophic cycles. However, no significant relationship was detected by logistic regression between parous state and wing lengths (d.f. = 1, chi-

TABLE II

Coefficients of variation[a] of wing lengths of *Anopheles (Nyssorhynchus)* females field-collected in South America

| Species | Number collections | Number sites[b] | Mean CV[c] | S.E. |
|---------|--------|--------|--------|------|
| *An. darlingi* | 5 | 3 | 6.10A | 0.29 |
| *An. aquasalis* | 6 | 5 | 5.77A | 0.56 |
| *An. triannulatus* | 10 | 5 | 5.21A | 0.47 |
| *An. albitarsis* (s.l) | 11 | 5 | 4.82A | 0.33 |
| *An. nuneztovari* | 20 | 9 | 4.73A | 0.12 |

a: CVs corrected for sample size
b: specific site information available in Lounibos (1994)
c: means followed by different letters are significantly (P<0.05) different by REGWQ test

TABLE I

Wing lengths and associated statistics of *Anopheles darlingi* females captured at human baits during night collections in Bolivia and Brazil.

| Site[a] | Date collection | Number | Mean[b] wing length (mm) | Coefficient of variation[c] | Skewness | Kurtosis[d] |
|---------|------|--------|--------|--------|--------|--------|
| GU | 8-VIII-90 | 24 | 3.12A | 6.15 | -0.34nt | -0.65nt |
| CP[e] | 20-VIII-91 | 34 | 3.07AB | 5.03 | -0.29nt | -0.40nt |
| PV | 21-VII-94 | 175 | 3.08A | 6.18 | -0.17ns | -0.56ns |
| PV | 26-VII-94 | 236 | 2.99BC | 6.70 | -0.09ns | -0.28ns |
| PV | 27-VII-94 | 220 | 2.96C | 6.43 | -0.05ns | -0.71[f] |

a: sites - GU = Guayamerin, Beni Province, Bolivia (10°51'S, 65°21'W); CP = Capanema, State of Pará, Brazil (1°17'S, 47°34'W); PV = Porto Velho, State of Rondônia, Brazil (8° 49' S, 63° 54'W)
b: means followed by different letters are significantly different (P <0.05) by REGWQ test
c: CV corrected for sample size
d: ns = non-significant, nt = not tested (sample size too small)
e: mosquites trapped at animal bait
f: P< 0.05

TABLE III

Mean wing lengths of *Anopheles darlingi* separated by sex and of three species of the same subgenus reared in standard laboratory conditions

| *Anopheles* sp. | Origin (site)[a] | Init. larval dens. | Males | | | Females | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | No. | Mean w.l. (mm) | S.E. | No. | Mean w.l. (mm) | S.E. | P[b] |
| *darlingi* | TE | 25 | 34 | 2.81 | 0.03 | 36 | 2.77 | 0.03 | 0.377 |
| *darlingi* | PA | 25 | 90 | 2.63 | 0.01 | 73 | 2.62 | 0.01 | 0.581 |
| *aquasalis* | SF | 25 | 71 | 3.11 | 0.02 | 51 | 3.33 | 0.02 | <0.001 |
| *albitarsis* (s.l.) | RS | 25 | 49 | 3.00 | 0.02 | 48 | 3.14 | 0.02 | <0.001 |
| *nuneztovari* | RS | 75 | 60 | 2.86 | 0.02 | 72 | 2.96 | 0.02 | 0.007 |

*a*: TE – Tefe, State of Amazonas, Brazil (3°22'S, 64°43'W); PA – Puerto Ayacucho, State of Amazonas, Venezuela (5°59'N, 67°39'W); SF – Santa Fé, State of Sucre, Venezuela (10°07'N, 64°25'W); RS = Rio Socuavo, State of Zulia, Venezuela (8°54'N, 72°38'W)

*b*: probabilities from unpaired t-test (two-tailed) comparing male and female wing length

squared = 0.63, P = 0.43, likelihood ratio = 17).

*Size dimorphism between sexes* - The regression analyses of wing lengths versus dry weights indicated considerable weight overlap between the sexes of *An. darlingi* (Fig.). Male and female dry weights of *An. darlingi* were not significantly different among individuals reared at the low ($F_{1,68}$ = 3.21, P = 0.08 ) or high density ($F_{1,48}$ = 1.99, P = 0.17), nor were differences observed between wing lengths of males and females of *An. darlingi* from TE or PA (Table III). By contrast, sexual size dimorphism, as indicated by significant wing length differences between males and females, was pronounced among *An. aquasalis*, *An. albitarsis* (s.l.), and *An. nuneztovari* reared in the laboratory under the same conditions (Table III).

## DISCUSSION

In contrast to the results of Siegel et al. (1992), who examined the fits of weight versus wing length regressions for aedine and culicine mosquitoes, untransformed data yielded a superior coefficient of determination than that obtained from the log-log transformation suggested by these authors. Our regression model for *An. darlingi* also explained more variance than coefficients of determination for the weight-wing length relationships of standard laboratory cultures of mosquitoes (Siegel et al. 1994). These authors showed that heterogeneous sources of mosquitoes, such as adults derived from different larval densities, may produce better regression fits than individuals reared in narrowly defined conditions.

Adult *An. darlingi* captured in nature were smaller, on average, than three other species of

the same subgenus (*An. nuneztovari*, *An. aquasalis*, and *An. albitarsis*), but larger than a fourth, *An. triannulatus* (Lounibos 1994). Among these four other species of *Nyssorhynchus*, no significant differences in mean wing lengths were detected among repeated night collections at the same site (Lounibos 1994), in contrast to the significant differences among captures of *An. darlingi* within the same week at PV. Assuming that these short-term changes in local sizes of *An. darlingi* reflect environmental, and not genetic, heterogeneity, the trend toward smaller mosquitoes may be related to a depletion in the quality of the larval habitat or a transition to a different primary production site of the blood-seeking adults.

The mean sizes of *An. darlingi* females captured in the field were markedly larger than individuals reared in laboratory containers (Tables I,III), a discrepancy not observed in field versus laboratory comparisons of *An. aquasalis* or *An. nuneztovari* (Lounibos 1994). The small size of reared *An. darlingi* suggests that our growing conditions for this species were suboptimal.

The wing length CVs of female *An. darlingi* from nature were larger, albeit not significantly so, than reported for the four other *An.* (*Nyssorhynchus*) examined from elsewhere in South America (Lounibos 1994). When these wing length CVs were converted to coefficients of variation for body mass ($CV_{bm}$) by the method of Fish (1985), *An. darlingi* ($CV_{bm}$ = 19.5) fell into the category of moderate size variation when compared against the range of $CV_{bm}$s observed by Fish for North American mosquito species. The slightly higher average $CV_{bm}$ of *An. darlingi*

suggests somewhat greater larval habitat variability for this species than observed for *An. aquasalis* ($CV_{bm}$ = 18.0), *An. triannulatus* ($CV_{bm}$ = 17.1), *An. nuneztovari* ($CV_{bm}$ = 15.0) or *An. albitarsis* ($CV_{bm}$ = 14.9) (Lounibos 1994).

Skewness was consistently negative for all night collections of *An. darlingi*, although not significantly so (Table I). This pattern is consistent with other *An. (Nyssorhynchus)* (see Lounibos 1994) which, like *An. darlingi*, suffer less in nature than many temperate mosquito species from density dependent larval stresses that may produce positively skewed size distributions in natural populations (Fish, 1985) or in laboratory cultures (Bradshaw & Holzapfel 1992) of some adult mosquitoes.

The uniformly negative kurtoses indicate a consistent tendency for body sizes in large collections of wild *An. darlingi* to cluster on either side of the mean value, and this deviation from a normal distribution was statistically significant for one night's collection. The consistent platykurtic trend indicates that more females than expected are larger or smaller than the mean size, which would occur if host-seeking *An. darlingi* emerge from heterogeneous or diverse larval habitats.

Although significant positive correlations between parous rates and body sizes have been demonstrated among diverse populations of temperate mosquitoes (e.g., Hawley 1985, Lounibos et al. 1990, Hu et al. 1993), no significant relationship was detected between wing lengths and parity in the current sample of *An. darlingi*, a characteristic held in common with single-night collections of *An. aquasalis*, *An. nuneztovari*, or *An. triannulatus* (Lounibos 1994). Although we know of no published correlations between body size and parity in a tropical anopheline, Lyimo and Takken (1993) showed that larger females of wild-caught *An. gambiae* (s.l.) were more likely to become gravid than smaller counterparts.

The absence of sexual size dimorphism in *An. darlingi* is the first recognition of this phenomenon in anopheline mosquitoes. The smaller size of most male mosquitoes is typically associated with their faster development because of sex-specific selection for earlier emergence, i.e., protandry (Kleckner et al. 1995). Precise measurements of larval and pupal development times of *An. darlingi* remain to be done to determine whether the lack of size dimorphism in this species is associated with the absence of sex differences in time from hatch to adult eclosion.

## ACKNOWLEDGEMENTS

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