FECUNDITY, PARITY, AND ADULT FEEDING RELATIONSHIPS AMONG *NYSSORHYNCHUS* MALARIA VECTORS FROM VENEZUELA

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Relative to their pre-engorgement weights, nulliparous Anopheles nuneztovari *consumed significantly smaller blood meals than* A. marajoara, A. triannulatus *or* A. aquasalis. *When females were deprived of sugar before blood feeding, only one-quarter of* A. nuneztovari, *but more than two-thirds of* A. marajoara, A. triannulatus *and* A. aquasalis *matured eggs.*

Sugar feeding before blood, or two successive blood meals by sugar-deprived females, increased the proportion of nulliparous A. nuneztovari *which developed eggs, but not significantly so. Nearly all individuals of nulliparous, sugar-fed* A. marajoara, A. triannulatus *and* A. aquasalis *matured eggs after one blood feeding.*

Among A. nuneztovari, A. marajoara *and* A. aquasalis *that matured some eggs in the laboratory, there were no positive correlations between the number of eggs developed and relative blood meal size. However, blood meals larger than the mean size significantly increased the chance that* A. nuneztovari *would develop some eggs. Mean fecundities of gravid* A. nuneztovari *and* A. marajoara *reared in the laboratory were significantly lower than those of the same species captured at human bait in nature. Post-engorgement access to sugar by* A. nuneztovari *(captured at human bait) did not influence fecundity, but significantly enhanced survivorship and the proportion of individuals which retained eggs.*

Release-recapture experiments revealed that relatively small blood meals are typical of A. nuneztovari *only during the first gonotrophic cycle. We suggest that multiple blood feeding, seemingly necessary for most* A. nuneztovari *to develop a first clutch of eggs, may increase the probability of infection with* Plasmodium vivax *where this mosquito species is a primary vector.*

Key words: *Anopheles (Nyssorhynchus)* – Venezuela – mosquito – blood meal size – fecundity – parity – egg development

It is well established that some important vectors of human malaria consume blood two or more times within a gonotrophic cycle. Brengues & Coz (1973) reported that 42% of *Anopheles gambiae* (s. s.) and 63% of *A. funestus* in West Africa needed two or more blood feeds to develop their first egg batch and Gillies (1954) documented this phenomenon among nearly all, newly emerged *A. gambiae* (s. l.) from Tanzania. In the sudanese savanna, an increase in the proportion of *A. gambiae* (s. l.) and *A. funestus* feeding twice per cycle occurred during the dry season (Molineaux & Gramiccia, 1980) when environmental conditions place more stress upon mosquito survival. Among *A. culicifacies* in Pakistan, females may take as many as three blood meals from emergence to first oviposition (Reisen & Aslamkhan, 1976). For most *A. gambiae* (s. s.) and *A. culicifacies*, the first blood feeding occurs before insemination (Gillies, 1954; Brengues & Coz, 1973; Reisen & Aslamkhan, 1976).

The consumption of several blood meals per ovarian cycle is usually attributed to weak or partial feeds (Reisen & Aslamkhan, 1976) caused by disturbances such as defense by the host (Burkot, 1988). The frequency of contact with the host by some mosquito species may also be influenced by larval diet (Grimstad & Haramis, 1984). To our knowledge, scant consideration has been given to the possibility that genetic characteristics may determine the number of feeds per gonotrophic cycle.

The increased frequency of host contact attributable to multiple blood meals may raise the vectorial capacity (VC) of a mosquito population. Of the three determinants of VC in the Ross-MacDonald equation for malaria transmission, host contact is a squared factor, increasing its contribution to VC relative to mosquito density and longevity (Macdonald, 1957). While interrupted feeds do not, *per se*, increase the transmission rate of malaria from man to mosquito (Burkot, 1988), Ponnudarai et al. (1989) reported that a non-infective bloodmeal following an infective one increased the sporozoite load in the salivary glands of *A. stephensi.*

We focus in this study on relationships between the size of the blood meal and egg development, and their implications for multiple blood feeding among four species of the anopheline subgenus *Nyssorhynchus.* Two of these species are important malaria vectors in Venezuela: *A. nuneztovari* in the west (Gabaldon, 1981) and *A. aquasalis* on the coast (Hamon et al., 1970). Both *A. triannulatus* and *A. marajoara* infected with *Plasmodium vivax* have been captured in Brazil (Arruda et al., 1986), and may be secondary vectors of human malaria in various neotropical countries (Faran, 1980; Linthicum, 1988).

MATERIALS AND METHODS

Mosquito collections, identifications and maintenance – Female *Nyssorhynchus* were collected from November 1988 through October 1989 at human bait between 1900 and 2300 h at Guaquitas and El Achote, Tachira State (7.5° N, 71.7° W), Venezuela. A single collection of *Anopheles (Nyssorhynchus) aquasalis* was made at human bait in Santa Fe, Sucre State (10.3° N, 64.4° W), Venezuela in February 1990. Mosquitoes that engorded on the blood of collectors were maintained overnight in humidified, 0.5-litre cups covered with tulle. The following morning, the females were lightly anesthetized with chloroform and identified to species with dichotomous keys (Faran, 1980; Faran & Linthicum, 1981; Linthicum, 1988). Identified females were incubated individually in tulle-covered, 30 ml vials that contained moist filter paper for oviposition. After allowing seven days for oviposition, surviving females were dissected and the number of retained eggs (= stage V follicles of Christophers (1911)) was counted. Fecundity was recorded as the sum of oviposited and retained eggs. As an index of adult size, the length of one wing of the sacrificed female was measured from the base of the costa to the wing margin, excluding fringes.

Embryonated eggs from field-collected females were hatched in distilled water. Larvae were fed a finely ground mixture of 50% yeast, 50% liver powder sprinkled daily on the water surface. The metal rearing trays 25 X 38 X 6 cm were kept covered to prevent evaporation, and the feeding regimen adjusted to maximize growth without fouling the water. At 25-27 °C, pupation occurred 7-10 days after hatch. Adults were allowed to eclose in screened 0.007 or 0.030 m³ cages where they were either deprived of nutrients or had *ad libitum* access to a 10% sucrose solution for 2-3 days. The sugar solution was withdrawn 24 h before blood feeding.

Blood meal weights and fecundities – Blood meal size was determined gravimetrically as the difference between female weights immediately before and after engorgement. Each adult was lightly anesthetized in an individual vial and weighed to the nearest 0.01 mg on a Cahn electrobalance. After a 90-minute recovery period, the female was exposed through tulle to the skin of one of the authors and allowed to feed to repletion. Mosquitoes that did not imbibe blood within five minutes after exposure were not used. Approximately 30 min after engorgement, each female was again lightly narcotized and weighed. Between the initiation of blood-feeding and re-weighing, most females excreted one or two droplets of lightly colored fluid, which consequently was excluded from calculated blood meal weights. For three species of *Nyssorhynchus,* including *A. nunez-tovari,* the average weight of excreted fluid was from 35 to 57% of the average weight of total blood ingested (Perez de Valderrama & Scorza, 1976). These authors did not de-

monstrate significant differences between species in amount of excreted fluid.

In one experiment, sugar-deprived A. nuneztovari were allowed to engorge twice on days two and four after emergence, and blood meal weights were determined gravimetrically after both feeds. These females were dissected 4-5 days after the second feed to record retained eggs.

Since F_1 females were not inseminated, oviposition rarely occurred and fecundities were recorded as the number of stage V follicles in ovaries dissected from females maintained at 25-27 °C for 4-5 days after the blood meal.

Blood meal size and parity — A field experiment, conducted to measure blood meal sizes of A. nuneztovari under semi-natural conditions, was repeated four times in Guaquitas, once in each of the months June, July, September and October. On each occasion, 150 to 200 host-seeking females were captured between 1900 and 2300 h at human bait, but prohibited from imbibing blood. These mosquitoes were maintained without access to sugar at densities of 15-20 per 0.5-1, humidified cup until the following night. On one occasion, females in cups were lightly chloroformed to obtain specific identifications, but this practice was discontinued due to mortality and subsequent reduced blood-feeding.

At 2000 h on the night after capture, approximately one-half of the females were released inside an experimental hut (2.8 X 4.0 X 6.7 m) covered by a curtain-net (Elliot, 1972; Bown et al., 1986) that prevented the escape of exophilic mosquitoes after blood-feeding. Four humans seated in the un-lit hut allowed the released mosquitoes to feed to repletion and disperse. From 2045 h until 2100 h, females resting on the inside of the net or on the walls of the hut were located with flashlights and captured with aspirators. At 2100 h, the collectors were reseated in the hut, and the procedure was repeated with the remaining mosquitoes.

Females in 0.5-1 cups were transported in a cooler to the field station where they were weighed to the nearest 0.01 mg within 3 h after engorgement. Narcotized females were identified to species, and the length of one wing was measured. Ovaries were dissected immediately

and allowed to dry on a microscope slide for subsequent determination of parity by the tracheolar skeining method (Detinova, 1962). Females with ovaries developed beyond stage IIb of Christophers (1911) were excluded from determinations of parity.

Insemination rates — The incidence of insemination among A. nuneztovari collected October 15, 1989 from human bait at Guaquitas was measured by the presence or absence of sperm in dissected spermathecae viewed under a compound microscope.

Data analyses — Two-sample comparisons of means were made with t-tests on unpaired data; an approximate t-tes, t', was used when variances were unequal (Steel & Torrie, 1960). When more than two samples were compared, data were treated by single classification analysis of variance (ANOVA) followed by a multiple means comparison ($\alpha = 0.05$) with Duncan's Multiple Range Test (DMRT) run on the GLM procedure of SAS (1985). No data transformations were used to equalize variances since ANOVA is robust to heteroscedasticity (Scheffe, 1959). Frequencies were compared with G-tests, using Williams' correction (Sokal & Rohlf 1981).

RESULTS

Fecundities of field-collected mosquitoes — Host-seeking A. nuneztovari and A. marajoara caught between November 1988 and early January 1989 at Guaquitas and in June 1989 at El Achote developed, respectively, means of 140.3 and 142.8 eggs (Table I). These values are significantly greater than the 76.2-egg average fecundity of A. triannulatus, which is a smaller species (Table I).

The fecundities of A. nuneztovari from El Achote not given sugar after blood (n = 26) were compared to those of other females from the same collection site and date which had continuous access to a 10% sugar solution (n = 25) after engorgement. The mean fecundities with sugar (128.7 ± 33.9 sd) and without (139.1 ± 18.4 sd) were not significantly different (t' = 1.36, P = 0.18). A high percentage of females developed eggs with post-blood sugar (96.4%) and without (90.3%). However, the proportion of females surviving seven days was significantly greater with sugar (57.8%) than without (31.4%) (G = 4.65, P < 0.05) as was

TABLE I

Number of eggs matured by females[1] caught at human bait between November 1988 and June 1989 at Guaquitas and El Achote, Venezuela

Species	n	Mean[2] wing length in mm (sd)	Mean total eggs developed (sd)
A. nuneztovari	71	2.87(0.15)a	140.3(32.2)c
A. marajoara	30	2.90(0.18)a	142.8(44.5)c
A. triannulatus	10	2.49(0.10)b	76.2(23.0)d
		$F_{(2, 108)} = 29.31$,	$F_{(2, 108)} = 15.36$,
		$P < 0.001$, $R^2 = 0.352$	$P < 0.001$, $R^2 = 0.221$

1: includes only females which matured some egg; no sugar given after blood.
2: means followed by different letters are significantly different according to DMRT after ANOVA;
F: statistics reported at bottom of columns.

TABLE II

Egg development by laboratory-reared, sugar-deprived Nyssorhynchus given one blood meal

| Species	n	Mean[1] wing length in mm (sd)	Mean wt. in mg(sd) before blood	blood meal	% with eggs	No. of eggs eggs (sd) from pos. females
A. nuneztovari	47	2.91(0.19)a	1.43(0.33)a	0.97(0.51)a	25.5	96.3(35.1)ab
A. marajoara	27	2.96(0.16)a	1.42(0.27)a	1.77(0.61)b	66.7	109.2(26.7)ab
A. triannulatus	13	2.51(0.10)b	0.90(0.12)b	1.09(0.41)a	69.2	87.7(13.7)b
A. aquasalis	11	2.99(0.12)a	1.28(0.14)a	2.10(0.75)b	72.7	121.5(29.6)a
		$F_{(3,88)} = 22.35$ $P < 0.001$, $R^2 = 0.432$	$F_{(3,94)} = 13.39$ $P < 0.001$, $R^2 = 0.299$	$F_{(3,94)} = 19.84$ $P < 0.001$, $R^2 = 0.388$		$F_{(3,34)} = 2.54$ $P = 0.069$, $R^2 = 0.150$

1: means followed by different letters are significantly different according to DMRT after ANOVA.
F: statistics reported at bottom of columns.

TABLE III

2 x 2 tests for independence of egg development from relative blood meal weight[1]

Species	pre-blood sugar	G	P
A. nuneztovari	--	3.423	> 0.05
A. marajoara	--	0.071	> 0.5
A. nuneztovari	+	35.84	< 0.001
A. aquasalis	+	0.953	> 0.1
A. nuneztovari[2]	--	9.643	< 0.01

1: presence or absence of mature eggs compared between females with less than vs more than average relative blood meal weight: data from Fig. 1, except for below.
2: females which took blood twice (on days 2 and 4 after emergence). Relative blood meal weight calculated from total blood taken divided by weight before first feed.
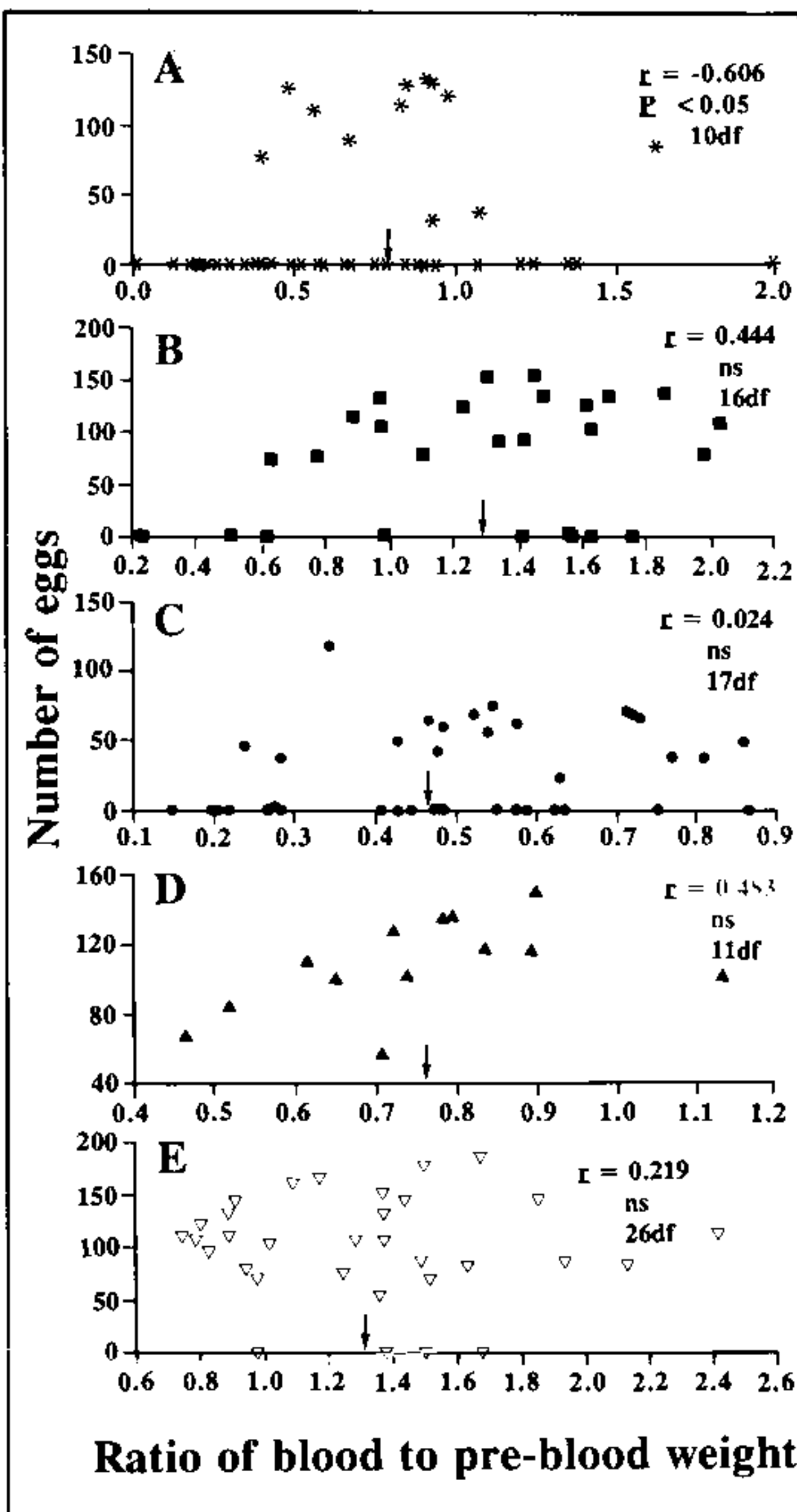
Relationships between number of eggs developed and relative blood meal size. A: sugar-deprived *Anopheles nuneztovari*. B: sugar-deprived *A. marajoara*. C: sugar-fed *A.nuneztovari*. D: sugar-fed *A. marajoara*. E: sugar-fed *A. aquasalis*. Arrows indicate mean relative blood meal sizes. Product-moment *r* calculated only for non-zero y values.

the proportion with retained eggs among gravid females with sugar (80.0%) compared to without (15.4%) (G = 23.18, P < 0.001).

Blood-meal size and fecundity of sugar-deprived F_1 s — Sugar-deprived *A. nuneztovari* consumed an average amount of blood weighing only 54.8% of that taken by comparable-sized *A. marajoara* and 46.2% of that taken by comparable-sized *A. aquasalis* (Table II). Although the mean blood meal weight of *A. triannulatus* was significantly smaller than those of *A. marajoara* and *A. aquasalis* (Table II), the ratios of mean blood meal to mean

pre-blood weights for these three species were 1.8 to 2.4 times that for *A. nuneztovari*. Accordingly, two-thirds or more of the *A. triannulatus, A. marajoara* and *A. aquasalis* developed eggs, but only slightly more than one-quarter of the *A. nuneztovari*. Among the females that developed eggs, the average fecundities of *A. nuneztovari* and *A. marajoara* did not differ, although *A. triannulatus* matured significantly fewer eggs than *A. aquasalis* (Table II). However, compared to field-collected counterparts (Table I), significantly fewer eggs were developed by *A. nuneztovari* (t = 4.31, P < 0.001, 81 df) and *A. marajoara* (t' = 3,26, P = 0.002, ca 46 df), although no difference was detected between field-collected and lab-reared *A. triannulatus* (t = 1.03, P = 0.32, 17 df). Among females that developed some eggs, *A. nuneztovari* exhibited a negative correlation between fecundity and relative blood meal size, but no correlation was evident for *A. marajoara* (Fig. 1A, B). For both species, the probability of a female developing some eggs was independent of whether the relative size of the blood meal was lesser or greater than the mean (Table III).

Two blood meals, administered on days two and four after emergence, did not significantly increase the proportion of *A. nuneztovari* that matured eggs (Table IV) compared to females that consumed blood only once (Table II) (G = 0.74, 0.50 > P > 0.10). The difference in mean fecundities between gravid females that took blood twice (112.0 eggs) and once (96.3 eggs) was not significant (t = 1.27, P = 0.216, 22 df). Weights of first (1.16 mg) and second (0.97 mg) meals taken by *A. nuneztovari* were significantly different (t = 2.19, P = 0.03, 68 df). Among *A. nuneztovari* that fed twice, females consuming more than the mean blood weight (both meals combined) had a significantly higher probability of maturing some eggs (Table III).

Blood-meal size and fecundity of sugar-fed F_1 s — When *A. nuneztovari* females were allowed access to sugar prior to blood, the percentage developing eggs increased to 44.2% (Table V), but this proportion was not significantly different from the 25.5% that developed eggs without sugar (G = 3.48, 0.1 > P > 0.05). However, 100% of the sugar-fed *A. marajoara* and *A. triannulatus* developed eggs (Table V), a proportion in each case significantly higher than obtained by the sugar-deprived individuals

TABLE IV

Egg development of laboratory-reared, sugar-deprived *Anopheles nuneztovari* given two blood meals

N	Mean wing length in mm (sd)	Mean wt. in mg (sd)			% with eggs	Mean no. eggs eggs (sd) from pos. females
		before blood	first meal	second meal		
35	2.99(0.14)	1.39(0.27)	1.16(0.33)	0.97(0.39)	34.3	112.0(24.2)

TABLE V

Egg development by laboratory-reared, sugar-fed *Nyssorhynchus* given one blood meal

Species	n	Mean[1] wing length in mm (sd)	Mean wt. in mg (sd)		% with eggs	No. of eggs (sd) from pos. females
			before blood	blood meal		
A. nuneztovari	43	2.86(0.13)a	1.76(0.39)ab	0.79(0.31)c	44.2	52.7(23.7)b
A. marajoara	13	2.90(0.13)a	2.01(0.42)a	1.48(0.33)b	100.0	106.8(26.9)a
A. triannulatus	5	2.55(0.08)b	1.14(0.17)c	1.04(0.46)c	100.0	73.4(22.8)b
A. aquasalis	32	2.96(0.12)a	1.62(0.30)b	2.06(0.53)a	87.5	115.2(35.2)a
		$F_{(3,85)}$ = 17,24 $P < 0.001$, $R^2 = 0.378$	$F_{(3,90)}$ = 7.96 $P < 0.001$, $R^2 = 0.210$	$F_{(3,90)}$ = 61.18 $P < 0.001$, $R^2 = 0.671$		$F_{(3,61)}$ = 18.31 $P < 0.001$, $R^2 = 0.474$

1: means followed by different letters are significantly different according to DMRT after ANOVA.
F: statistics reported at bottom of columns.

TABLE VI

Anopheles nuneztovari recaptured after release and blood engorgement in an experimental hut at Guaquitas, Venezuela

Date of experiment	Nulliparous recaptures			Parous recaptures		
	n	Mean wing length in mm (sd)	Mean wt. in mg (sd)	n	Mean wing length in mm (sd)	Mean wt. in mg (sd)
30/VI/89	13 ⎫	2.85(0.10)	2.39(0.26)	18 ⎫	2.83(0.15)	2.83(0.57)
8/VIII/89	7 ⎭			2 ⎭		
28/IX/89	37	2.85(0.13)	2.51(0.37)	20	2.89(0.12)	2.92(0.52)
21/X/89	19	2.79(0.11)	2.38(0.41)	32	2.88(0.11)	2.96(0.57)
Totals	76	2.84(0.12)	2.44(0.36)	72	2.87(0.13)	2.91(0.55)

of the respective species (G = 7.23, P < 0.01; G = 23.2, P < 0.001). For *A. aquasalis*, the percentages of females that matured eggs with pre-blood sugar (87.5%) and without (72.7%) were not significantly different (G = 1.20, P > 0.10). Intraspecific comparisons of average fecundities with or without sugar revealed no significant differences except among *A. nuneztovari*, whose mean clutch size dropped to 52.7 eggs per sugar-fed female (t = 4.14, P < 0.001, 29 df). Correlations between fecundity and relative blood meal size were not significant for sugar-fed *A. nuneztovari*, *A. marajoara* or *A. aquasalis* (Fig. 1C-E). Only for *A. nunez-*

tovari, individuals which consumed more than the average relative blood meal size had a significantly greater chance of developing some eggs (Table III). For all four species, the pre-blood weight was significantly higher for sugar-fed than for sugar-deprived females (t-tests, $P < 0.004$ for all). Although the average blood meal weight of all four species was slightly less for sugar-fed individuals, only the value for *A. nuneztovari* was significant (t = 2.01, $P = 0.047$, 77 df). Intraspecific comparisons of wing lenghts for the experimental females in Tables II and V revealed no significant differences.

Blood meal weight and parity — There was considerable variation in the rates of parity and of recapture of engorged mosquitoes among experiment dates. Many of the females anesthetized on July 8, 1989 did not take blood that night, so the few engorged females were pooled with individuals from an experimental replicate one week earlier (Table VI). Parity rates among recaptured, engorded *A. nuneztovari* ranged from 35.1% in late September to 62.7% slightly more than 3 weeks later. Very few engorged individuals of other *Nyssorhynchus (A. marajoara, A. triannulatus* and *A. oswaldoi)* were recovered, so only the data for *A. nuneztovari* were analyzed further.

The common trend for all replicates of the experiment was the significantly higher mean weight of engorged parous (2.91 mg) compared to nulliparous (2.44 mg) *A. nuneztovari* (t = 6.04, $P < 0.001$, 121 df). The mean weight of these nulliparous, engorged females trapped in the field did not differ significantly from the mean weight (2.55 mg) of sugar-fed females engorged in the laboratory (Table V) (t = 1.20, $P = 0.234$). Among the field females, the mean wing lengths of all parous and nulliparous *A. nuneztovari* did not differ significantly (t = 1.47, $P = 0.144$).

Insemination frequency — Sperm was detected in 96.6% of 88 *A. nuneztovari* captured at human bait in Guaquitas the night of October 15, 1989. Among 83 of these individuals suitable for scoring by the tracheolar skeining method, 38.6% were parous. The average wing lenghts of parous (2.85 mm ± 0.12) and nullipa-rous 2.90 ± 0.11 sd) females did not differ significantly (t = 1.74, $P = 0.09$, 82 df).

DISCUSSION

Blood meal size and feeding frequency — Some of the data support the interpretation that most *A. nuneztovari* from western Venezuela must take two or more blood meals to complete their first ovarian cycle. By contrast, nearly all nulliparous females of the related *A. marajoara, A. triannulatus* and *A. aquasalis* matured eggs after one blood feed (Table V).

The apparent requirement of several blood feeds to develop the first egg clutch is associated with smaller meals by *A. nuneztovari*. Nulliparous *A. aquasalis* and *A. marajoara*, species indistinguishable in size from *A. nuneztovari* based on wing lengths, consumed approximately twice as much blood (Tables II, V). These differences in meal sizes, observed in the laboratory in the absence of environmental disturbances such as host defense, appear to be attributable to genetic characteristics of the species or populations. Gillies (1954) reported that small blood meals were a characteristic of the first feed by nulliparous *A. gambiae* (s. l.) from East Africa.

The selective advantage of foraging behavior that apparently requires several, successive excursions, which expend energy and risk predation, to quest blood for egg development is a mystery. Perhaps in western Venezuela and eastern Colombia where *A. nuneztovari* seek hosts primarily late at night (Elliot, 1968) and blood sources are abundant, the risk of mortality during foraging may be low, especially if the smaller blood meal does not impair flight. Perez de Valderrama & Scorza (1981) suggested that small blood meals might facilitate the known exophilic behaviour of *A. nuneztovari* because engorded females could fly from shelters immediately after feeding. However, these authors did not demonstrate significant differences in blood meal size between *A. nuneztovari* and the exophagic *A. darlingi*, possibly because their measurements did not distinguish between parous and nulliparous *A. nuneztovari*.

Because most *A. gambiae* and *A. culicifacies* consume blood before mating, we investigated the possibility that the first blood meal of *A. nuneztovari* might have a prenuptial function independent from the initiation of egg development. However, the high insemination rate (96.6%) of *A. nuneztovari* at a single night's

bait catch, coupled with a relatively low concurrent parity rate (38.6%), suggested that mating occurs before the first blood feed in most individuals of this species. High insemination rates among females trapped at human bait have also been reported for *A. aquasalis* (90.2%) and *A. albitarsis* (= *marajoara*) (97.3%) in Trinidad (Senior White, 1953).

Engorged, parous *A. nuneztovari* weighed significantly more than nulliparous counterparts with similar wing lengths (Table VI). These data indicate that, after laying a first egg batch, *A. nuneztovari* females are no longer constrained to small blood meals. As a corollary, we predict that most parous *A. nuneztovari* should require only one blood meal per egg clutch.

Control of fecundity — The absence of significant, positive correlations between number of eggs developed and relative blood meal size in all laboratory-reared *Nyssorhynchus* examined (Fig. 1) was unexpected. Strong, positive correlations between blood meal weight and fecundity have been demonstrated for laboratory colonies of *A. stephensi* (r = 0.817, 42 df: Reisen & Emory, 1976) and *A. quadrimaculatus* (r = 0.883, 23 df: Hu & Lounibos; unpublished). Although the *Nyssorhynchus* that we tested were uninseminated F_1s from field collections, significant positive correlations between blood meal size and fecundity were obtained from unmated F_1s of field-collected *A. crucians* (r = 0.665, 27 df) and *A. atropos* (r = 0.596, 23 df) (Hu & Lounibos, unpublished). El-Akad & Humphreys (1990) reported no relationship between insemination status and ovarian development in a laboratory colony of *A. pharoensis.*

Working in India, Roy (1940) asserted that insemination was a prerequisite for egg development of *A. subpictus*, but not of the related *A. annularis* or *A. stephensi.* Bates (1949) suggested that *A. superpictus* and *A. maculipennis* (s. l.) from Albania also required sperm to promote egg maturation. Although this phenomenon of "spermathecal stimulation" (Roy, 1940) has not been investigated among *Nyssorhynchus*, our data demonstrate that insemination is not an absolute requirement for egg development among any of the four species herein studied.

Possibly the positive trends in the fecundity versus blood weight relationships for *A. marajoara* and *A. aquasalis* (Figs 1B, D, E) would

become significant with larger sample sizes. For *A. nuneztovari* there is clear evidence that blood meals larger than the mean size increase the probability of egg maturation (Table III). Meanwhile, the significant negative correlation for sugar-deprived *A. nuneztovari* (Fig. 1A) and the decrease in mean fecundity of sugar-fed females of the same species (Table II cf. Table V) are difficult to explain.

We conjecture that the decrease in fecundity of the sugar-fed *A. nuneztovari* may be related to host sensitization and production of immunological factors such as are known to suppress fecundity and survival of other species of mosquitoes (e. g., Reisen & Emory, 1976). Our first experiments on *Nyssorhynchus*, which used one author (LPL) as the predominant blood source, were performed on sugar-deprived *A. nuneztovari*, which matured a mean of 96.3 eggs. The sugar-fed females, which developed a mean of 52.7 eggs, were characterized six months later. During the intervening period the senior author frequently fed wild-caught and lab-reared *A. nuneztovari*, but other species only infrequently, on his blood. Reisen & Emory (1976) reported that *A. stephensi* feeding from a sensitized human matured 59.8 eggs per female compared to 117.0 eggs per female on an immunologically naive host, an approximately two-fold fecundity difference as observed between the two groups of *A. nuneztovari.*

For both *A. nuneztovari* and *A. marajoara*, the average fecundity of females trapped at human bait in the field was significantly greater than that os gravid, laboratory-reared females (Table I cf. Tables I, V). Before imbibing from collectors, the field females had an unknown feeding history. Since mosquito fecundity is affected by the nutritional quality of the host (e. g. Shelton, 1972), the field females may have fed formerly on a nutritionally superior blood source. On the other hand, the clutch size of parous females is usually less than that of nulliparous females for most *Anopheles* (e.g., Detinova, 1962), a pattern not consistent with the discrepancy in fecundities between field and laboratory *A. nuneztovari* and *A. marajoara.* Scorza et al. (1981) observed no significant differences in the sizes of first ($\bar{x}$ = 95.3) and second ($\bar{x}$ = 89.3) egg batches of laboratory-reared *A. nuneztovari.*

The high percentages (90.3% and 96.4%) of field-collected *A. nuneztovari* that matured eggs

after one engorgement may also seem at variance with the hypothesis that nulliparous females need two or more blood meals to develop eggs. However, these females came from a single night's catch at El Achote during the dry season when the incidence of parity may have been high.

Aspects of A. nuneztovari *survival* — The few parity estimates of *A. nuneztovari* samples in this study ranged from 35 to 63%, which are within the spectrum of variation observed in more extensive data on this population (Rubio, unpublished). This broad range in variation occurred in two collections made approximately three weeks apart (Table VI), suggesting rapid turnover in the age structure of host-seeking populations and underscoring the limitations of the parity rate formula (Davidson, 1954) for estimating survivorship. Since ovaries developed beyond stage II cannot be scored by the tracheolar skeining method, we may have underestimated the true parity rates of *A. nuneztovari* by approximately 5% (Charlwood, 1980).

Size variation in female *A. nuneztovari*, as reflected by the constancy of wing lengths and their low standard deviations (Tables I-II, IV-VI), was slight compared to many other species of mosquitoes (Fish, 1985) and showed no seasonal variation (Lounibos & Conn, unpublished). The lack of significant differences in wing lengths between parous and nulliparous samples (Table VI), suggests that, at this level of resolution, there is no evidence for size-dependent survivorship.

The importance of sugar for post-blood meal survival was documented here for *A. nuneztovari*, as it has been for many mosquito species (e. g., Nayar & Sauerman, 1975). The effect of sugar on egg retention is also well known. The absence of an effect of post-blood sugar on fecundity would be expected if clutch size is determined earlier during adult alimentary history.

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