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SEX RATIOS OF MOSQUITOES FROM LONG-TERM CENSUSES OF FLORIDA TREE HOLES

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Abstract

Pupal sexes of the most common mosquito species were determined in the course of biweekly censuses (with replacement) of the contents of 3–7 tree holes from 1980–2003 in Vero Beach, FL. A significant ($P < 0.001$) male bias was detected over this period for the most abundant species, *Aedes triseriatus*. No significant deviation from a 1:1 sex ratio was detected among pupae of *Toxorhynchites rutilus* or *Ae. albopictus*, the latter species occurring in this community only since 1991. Although pupae of *Ae. triseriatus* were recorded during every month of the year, significant male biases were detected only in February–May, August, and December. These results are interpreted in the context of multivoltinism and the previously documented differential sensitivity of male and female eggs of this species to hatching stimuli. Sex-specific responses to hatching stimuli are judged to be present but less pronounced in eggs of *Ae. albopictus*. Male biases in container *Aedes* are likely associated with sexual selection, which may also explain seasonal changes in sex ratios, whereby early males compete to mate with high-fecundity females. The overproduction of *Ae. triseriatus* males may be counterbalanced by increased fitness of females, which are known to predominate in delayed hatches.

Keywords

Aedes albopictus; *Ae. triseriatus*; hatching; male bias; multivoltinism

INTRODUCTION

Imbalances in sex ratios are observed commonly in samples from natural populations of container-inhabiting mosquitoes. Collections of diapausing larvae from the southern USA have detected significant male biases in geographic populations of *Aedes triseriatus* (Say) (Holzapfel and Bradshaw 1981) and *Toxorhynchites rutilus* (Coquillett) (McCrary and Jenner 1965). Adults emerging from predominantly univoltine populations are male biased early in the growing season in the tree-hole mosquitoes *Ae. triseriatus* (Scholl and DeFoliart 1978, Shroyer and Craig 1981, Sinsko and Craig 1981), *Ae. sierrensis* (Ludlow) (Hawley 1985), and *Ae. geniculatus* (Olivier) (Yates 1979). The cumulative numbers of tree-hole *Aedes* throughout the year may show a significant male bias in some years, but a significant female bias has never been reported (Sinsko and Craig, 1981, Shroyer and Craig 1981, Hawley 1985, Yates 1979).

Some early observers attributed such male biases to faster development of male larvae followed by late-season drying of certain tree holes, causing differential mortality to slower-developing female larvae (Yates 1979). However, Shroyer and Craig (1981) demonstrated that male eggs of *Ae. triseriatus* are more sensitive than females to suboptimal hatching stimuli, and sex-dependent differential hatching is probably the most important determinant of the aforementioned seasonal sex-ratio shifts in univoltine *Aedes* species. Among some container

mosquito species including *Ae. triseriatus*, larval density has also been shown to affect sex ratios (Chambers 1985, Frank et al. 1985).

The majority of studies on sex ratios of container mosquitoes has focused to date on 1 or 2 years of samples of univoltine populations. The object of this report is to document sex ratios determined from long-term censuses of the aquatic stages of tree-hole mosquitoes in a subtropical location where larvae and pupae occur year-round. Consideration of multiple species in the present study also facilitates a comparative approach for interpreting sex-ratio patterns. As the 2 most abundant species in this tree-hole community are *Aedes* species, observations are discussed in the context of sex biases determined by differential responses to hatching stimuli.

MATERIALS AND METHODS

The data were derived from a census-replacement program conducted fortnightly between 1978 and 2003 of mosquito larvae and pupae occurring in 3–7 tree holes located in an oak-palm “hammock” at the Florida Medical Entomology Laboratory (FMEL) in Vero Beach. Procedural details, tree hole characteristics, and long-term trends in mosquito productivity and interspecific relationships are described elsewhere (Lounibos 1983, Lounibos et al. 1997, Ellis et al. 2006). Briefly, once every 2 wk, the entire aquatic contents of a small number of known tree holes were extracted by aspiration with a hose or a large syringe. The extracted sample, and 1 rinse with tap water, were immediately transported in individual, plastic bottles to the laboratory, where mosquito larvae and pupae were identified to species, staged, and counted. Within 4 h after extraction, mosquito immatures and particulate matter were reconstituted with their original tree hole fluid and returned to their hole of origin. Numbers of censused holes varied from 3 to 7 because several lost the capacity to hold water during the course of the study and were not promptly replaced (see Ellis et al. 2006 for histories of individual holes).

Beginning in 1980, pupae encountered in censuses were sexed by morphological characteristics of their terminal abdominal segments (Harbach and Knight 1980). Analyses of the 24-year data sets of mosquito pupal sexes assume independence of observations, i.e., that the same pupa would not be sampled twice, because the period between fortnightly censuses exceeded the maximum duration of the pupal period, even during the coldest weather observed in these tree holes (Lounibos 1983, Ellis et al. 2006).

Data analyses were performed by chi-square to detect deviations from an expected 1:1 sex ratio in total, annual, and monthly pupal counts, by species. A product-moment correlation was used to detect possible association between male bias (using chi-square value as a measure of strength) in *Ae. triseriatus* and annual rainfall obtained from a National Oceanic and Atmospheric Administration site 6 km southeast of FMEL. Kendall’s coefficient of concordance (Sokal and Rohlf 1995) was used to compare trends in sex ratios of *Ae. triseriatus* and *Ae. albopictus* (Skuse) in the annual time series.

RESULTS

Total counts

Among the 4 species censused in sufficient numbers, only *Ae. triseriatus* and *Orthopodomyia signifera* (Coquillett) showed significant deviations from a 1:1 sex ratio in total pupae over the duration of the study (Table 1). However, the significant female bias among *Or. signifera* disappeared when results from a single tree hole (No. 5) in 1982 were discounted.

Year-to-year patterns

A significant male bias in *Ae. triseriatus* pupae was detected in 11 out of 24 years, whereas a significant female bias was never observed for this species (Fig. 1). Years with male biases were significantly more likely in the 1st half of the observation period (7 of 12 years; $G_{adj} = 188.0$, $P < 0.001$). No correlation was detected between strength of male bias, as measured by chi-square value (Fig. 1) and annual rainfall near the study site (Pearson's $r = 0.07$, 23 df, $P = 0.74$, with 4 negative chi-square values set to 0 to perform the correlation).

For *Ae. albopictus*, a significant male bias was detected in 2 years and a significant female bias in 1 year (Fig. 1). Although the magnitudes of sex-ratio variation differed between the 2 species, from 1997 through 2003 the annual changes, as measured by chi-square, occurred in the same direction (i.e., positive or negative) for *Ae. albopictus* and *Ae. triseriatus* (cf. Fig. 1 top and bottom). Kendall's test for concordance showed that the similarity of patterns for the 2 species was marginally significant ($W_c = 0.894$, $\chi^2_c = 10.73$, 6 df, $0.05 > P > 0.10$).

Seasonal patterns

Three disjunct periods of significant male bias were detected in the monthly totals for *Ae. triseriatus*, the most extensive between February and May, with shorter periods of male bias in August and December (Fig. 2). Spring was also the most productive period for pupae of this species in tree holes, followed by a secondary abundance peak in August.

For *Ae. albopictus*, a significant male bias was detected in 2 disjunct months, August and December, the latter followed by a month of significant female bias in January (Fig. 2). Pupal production in this species peaked in July and August, followed by a secondary peak at year's end.

DISCUSSION

Various factors have been invoked to explain sex-ratio imbalances among container mosquitoes, including sex-specific responses to critical day lengths (McCrary and Jenner 1965, Sims and Munstermann, 1983), density-dependent feedback mechanisms (Frank et al. 1985), or differential mortality from sex-selective predation (Chambers 1985). Although density dependence and sex-selective predation cannot be ruled out as factors, the present results on annual patterns of sex ratios in the 2 *Aedes* species are best interpreted in the context of sex-dependent responses to hatching stimuli, as previously demonstrated for *Ae. triseriatus* by Shroyer and Craig (1981). We hypothesize that the predominance of male pupae in the spring derives from the emergence of late-stage overwintering larvae, known to be male-biased in this species elsewhere (Holzapfel and Bradshaw 1981), as well as spring hatches of eggs laid late in the preceding year. If hatching stimuli are suboptimal, as would be promoted by low vernal temperatures recorded from FMEL tree holes (e.g., Lounibos 1983, Ellis et al. 2006), males would be more likely to eclose from eggs sooner than females. The breadth of the spring peak in pupal productivity and male bias (Fig. 2A) in *Ae. triseriatus* is probably also related to year-to-year variations in temperature and rainfall, which determine spring conditions suitable for mosquito development.

The 2nd peak in male bias and pupal productivity of *Ae. triseriatus* during August is probably derived largely from the hatching of eggs laid by females that emerged in the spring or early summer. In August, tree-hole temperatures do not retard development, so the period of significant male bias is briefer than in the spring. Although the significant male bias in December is based on smaller numbers of pupae, this period may represent a 3rd, or later, generation of *Ae. triseriatus* adults. Despite the small number of tree holes sampled and the absence of long-term records from other local containers occupied by *Ae. triseriatus* and *Ae.*

albopictus, the length of the current time series supports the contention that observed patterns are robust.

The monthly patterns for *Ae. albopictus* provide less evidence for sex-ratio imbalances, the only detected being male biases in August and December, the latter followed by a female bias in January. Although differential responses of *Ae. albopictus* sexes to hatching stimuli have not been reported, both the December–January pattern (Fig. 2B), and the concordance between *Aedes* spp. of annual changes in proportion of males between 1997 and 2003 (cf. Figs. 1A, 1B), suggest that male eggs of both *Ae. triseriatus* and *Ae. albopictus* hatch before their female counterparts. In laboratory populations of *Ae. aegypti* (L.), a close relative of *Ae. albopictus*, males are known to hatch before females (Elzinga 1961, Wood 1962). Given that eggs of *Ae. albopictus* respond much more quickly to hatching stimuli than those of *Ae. triseriatus* (Lounibos et al. 2001), it is not surprising that the census data more readily show biases and seasonal distortion of sex ratios in the latter species.

Both *Ae. albopictus* and *Ae. aegypti* belong to the subgenus *Stegomyia*, known to be promptly responsive to initial hatching stimuli (Lounibos 1981). By contrast, aedines of other subgenera are more likely to undergo delayed or installment hatching (Lounibos 1981), which has been shown to result in higher fitness for late hatchlings of *Ae. triseriatus* (Livdahl and Koenekoop 1985). If the male-biased sex ratios of *Ae. triseriatus* are indicative of differential mortality of female embryos in nature, then the latter sex may be compensated by the enhanced fitness of delayed hatching, which is known to produce a predominance of females (Livdahl and Koenekoop 1985).

The different annual phenologies of *Ae. triseriatus* and *Ae. albopictus* pupal production (Fig. 2) warrant comment in the context of the asymmetric outcome of larval competition between these species, which is known to favor the invasive *Ae. albopictus* (Livdahl and Willey 1991, Teng and Apperson 2000). Although the different periods of peak productivity of the 2 species might be interpreted in relation to interspecific competition, the 1st 10 years of censuses of *Ae. triseriatus* occurred before *Ae. albopictus* invaded this community (cf. Figs. 2A, 2B), when a spring peak in emergence was already apparent (Lounibos 1985). Also, declines in abundance of *Ae. triseriatus* in 2 regularly censused holes were detected before the arrival of *Ae. albopictus* (Lounibos et al. 1997).

Many experimental studies of *Ae. triseriatus* have used composite performance indices, which presume a 1:1 sex ratio among larvae that are introduced to treatments as age structured (e.g., Livdahl and Sugihara 1984, Edgerly and Livdahl 1992) or uniform-aged cohorts (e.g., Léonard and Juliano 1995, Alto et al. 2005). The current work and previous studies (e.g., Scholl and DeFoliart 1978, Shroyer and Craig 1981, Sinsko and Craig 1981) demonstrate that 1:1 sex ratios are not the norm in natural populations of this species and, thus, performance indices that assume this property may be biased.

This study corroborates others on *Ae. triseriatus* (e.g. Sinsko and Craig 1981, Shroyer and Craig 1981), which showed that the annual sex ratio is male biased, as has also been noted for whole-year studies of the European tree-hole mosquito *Ae. geniculatus* (Yates 1979) and the western tree-hole mosquito *Ae. sierrensis* (Hawley 1985); delayed hatching would also be expected in these 2 species. In the rock pool mosquito *Opifex fuscus* Hutton, a highly male-biased operational sex ratio is associated with strong competition for mates, larger males being more successful in mating (Slooten and Lambert 1983, 1984). A similar process of sexual selection may operate to favor a preponderance of males among tree hole *Aedes*, with a trade-off between overproduction of males and higher fitness of late-hatching females.

Evolutionary theory also predicts seasonal changes in sex ratio associated with monogamy, monogamous bird species being more likely than polyandrous counterparts to favor the

production of males early in the breeding season (Andersson 1994, Andersson et al. 2003, Sheldon 1998). Mosquitoes are generally monogamous (Klowden 1999), so the seasonal patterns favoring early male production in tree-hole *Aedes* species are consistent with sexual selection theory. Pronounced protandry in a univoltine population of *Ae. sierrensis* has been hypothesized to be favored by the high fecundity of early-emerging females (Hawley 1985). Thus, early-emerging males may achieve higher fitness by mating with larger, nulliparous females, which predominate early in the season (Hawley 1985).

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Fig. 1. Deviations from an expected 1:1 sex ratio of (top) *Aedes triseriatus* pupae collected from 1980 to 2003 and (bottom) *Ae. albopictus* pupae collected from 1991 to 2003 in fortnightly censuses of 3–7 tree holes at the Florida Medical Entomology Laboratory. Values of χ^2 above and below the dotted lines exceed the 5% probability level for statistical significance. Numbers above each bar represent total pupae censused annually (median *Ae. triseriatus* = 63 [range 5–239, $n = 24$ year]; median *Ae. albopictus* = 65 [range 23–139, $n = 13$ year]).

Fig. 2. Mean numbers ($\pm$ SE) of pupae per month, separated by sex, of (top) *Ae. triseriatus* (1980–2003) and (bottom) *Ae. albopictus* (1991–2003). Asterisks identify statistically significant (* $P < 0.05$, *** $P < 0.001$) deviations from a 1:1 expected sex ratio for the aggregate data for each month. Absence of asterisks indicates that the χ^2 test for that month was nonsignificant.

Table 1Numbers and sexes of pupae censused during the observation period.¹

Species	No. of males	No. of females	χ^2 for deviation from 1:1
<i>Aedes triseriatus</i>	1,123	820	47.3*
<i>Ae. albopictus</i>	396	373	0.6
<i>Toxorhynchites rutilus</i>	38	55	3.1
<i>Orthopodomyia signifera</i>	37	96	26.2*
<i>Or. signifera</i> (Hole No. 5, 1982, excluded)	37	53	2.8

¹ Observation period was 1980–2003 for all species except *Ae. albopictus* (1991–2003).

* P < 0.001.