

Evidence of Monandry in a Mosquito (*Sabethes cyaneus*) with Elaborate Ornaments in Both Sexes

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Abstract Despite the benefits of multiple mating to females many mosquitoes appear to be monandrous. Members of the mosquito tribe Sabethini are unique among the mosquitoes for they possess iridescent scales and elaborate ornaments in both sexes. Additionally, this tribe boasts the only reported cases of courtship display within the mosquitoes. Due to these singular traits and behaviors, we predicted that members of this tribe have a different mating system with relatively high female mating rate. We tested this prediction in the ornamented mosquito *Sabethes cyaneus*. Contrary to our prediction, however, females were monandrous throughout their lifetime and multiple gonotrophic cycles. We discuss the possible implications of monandry on the evolution of sexually homologous ornaments, with particular consideration of mutual mate choice.

Keywords Mating rate · mating system · polyandry · signal · sexual selection · Diptera

Introduction

Mosquitoes in the tribe Sabethini (Diptera: Culicidae) possess a suit of characteristics that make them unique among the mosquitoes: diurnal activity, a greater reliance on vision (Shannon 1931; Haddow and Corbet 1961; Hancock et al. 1990a), and mating on a substrate (Hancock et al. 1990a). Most notable are the colorful iridescent scales that cover much of the body of these mosquitoes (Judd 1996). This coloration is complemented with elaborate paddle-like ornaments on the legs of some species (henceforth referred to simply as ‘paddles’) (Harbach 1991). In those species with the most elaborate paddles both males and females express these ornaments and the degree of sexual dimorphism is relatively limited, although there are some species in which the paddles are relatively small in males and absent or reduced in females (R. Harbach, personal communication). The lack of pronounced sexual dimorphism in

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those species with the most elaborate paddles may suggest these structures are not the result of sexual selection. Yet, three facts points to a central role for sexual selection. First, when the paddles are experimentally removed from females there is a reduced female mating success in *Sabethes cyaneus*, yet flight and oviposition behaviors remain unaffected (Hancock et al. 1990b). Interestingly, male ability to mate successfully is unaffected by the removal of male paddles. Second, complex male courtship behaviors, during which the mid-legs are prominently displayed in front of the female, have been described in four species of sabethines (Okazawa et al. 1986; Hancock et al. 1990a; Philips et al. 1996; Zsemlye et al. 2005). Third, there is large variation in paddle number, shape and coloration in closely related sympatric sabethine species (Harbach and Petersen 1992; Judd 1996), and rapid divergence of courtship traits is a hallmark of sexual selection (Coyne and Orr 2004).

Female mating rate is of central importance for sexual selection. The opportunity for sexual selection is a product of variance in fitness, such that sexual selection will potentially be very strong when some individuals in a population obtain the majority of matings (Crow 1958; Payne 1979; Wade and Arnold 1980; Crow 1991). Because the opportunity for sexual selection may be higher when both sexes mate multiply, as a result of the added contribution of post-mating sexual selection (Arnqvist and Nilsson 2000), we predict that female sabethine mosquitoes are likely to mate multiply (i.e. be polyandrous). This is in line with other sexually dimorphic insect taxa in which both males and females tend to mate multiply (Thornhill and Alcock 1983). However, this prediction contrasts with what is know about other mosquito mating systems as the majority of female mosquitoes tend to mate only once in their lifetime (i.e. be monandrous) (e.g. Gillies 1956; Goma 1963; Jones 1973; Mahmood and Reisen 1980; Reisen et al. 1984; Baimai and Green 1987; Yuval and Fritz 1994). We note, however, that none of the mosquito tribes where female mating rate has been quantified possess elaborate ornaments or other potentially sexually selected characteristics.

In this study, we aim to test the prediction that ornamented female mosquitoes mate multiply in *S. cyaneus*, a species with elaborate paddles in both sexes (Fig. 1) and a complex courtship display (Hancock et al. 1990a, b). Briefly, females were first mated with sterilized males (by irradiation) and were then provided with the opportunity to remate with new, virgin fertile males for the remainder of their lifetime. Thus, presence of an increase in the number of viable eggs after the introduction of fertile males would provide conclusive evidence for female remating. Females may regain sexual receptivity after several gonotrophic cycles (Williams and Berger 1980; Young and Downe 1982). Hence, we offered females blood meals regularly throughout their lifetime to explore female remating patterns over multiple gonotrophic cycles. In order to investigate whether copulation duration affected female propensity to remate due to

Fig. 1 Male (left) and female (right) *Sabethes cyaneus*. Note the lack of striking sexual dimorphism in the paddle-like ornaments on the mid-legs.



insufficient transfer of sperm during short copulations (Gwadz and Craig 1970), we also recorded the duration of all copulations.

Methods

Rearing and Maintenance

We used a strain of *S. cyaneus* established by R. G. Hancock and W. A. Foster in December 1988 from multiple individuals collected at the Isla de Maje, Lago Bayano, Panama, Republic of Panama. This colony was maintained at Ohio State University, U.S.A.. Our colony has been housed at Uppsala University, Sweden since April 2006 at $26\pm 1^\circ\text{C}$, 78–82% RH and a 12L:12D photoperiod, at a population size of approximately 400 individuals. Larvae were reared in plastic trays ($21.5\times 14.5\times 5$ cm) filled to 2.5 cm with deionized water which was changed weekly. They were fed an ad libitum diet of crushed fish flake food. Pupae were collected in small dishes (diameter, 8 cm; height, 2.5 cm) and these were placed in terraria ($29\times 17.5\times 18$ cm). An ad libitum supply of honey-soaked sponges and deionized water wicks was provided. To ensure that all individuals used were virgins, adults were collected from this terrarium within 24 h of emergence (Becker et al. 2003) and segregated into male and female housing terraria, with the same dimensions and food conditions. Densities were between 20 and 30 adults per terrarium.

Experimental Design

Virgin males were exposed to 60 gray of radiation from a caesium-137 source, 7–8 days post-emergence. A pilot study showed that this was the minimal dose that caused near complete sterility among males. Although no changes in male courtship or mating behaviors were observed during the pilot study, we chose to use a minimal dose as this would minimize any possible hidden harmful side-effects of the irradiation procedure. The irradiated virgin males and virgin females were then placed together in the terraria at densities of 30 individuals per terrarium. The duration of all copulations were recorded. Copulation duration was defined following the guidelines Hancock et al. (1990a); the time from the male ‘genital shift’ into full copulation from the superficial coupling stage up until the time when the pair split. Pairs which mated were gently removed from the terrarium and irradiated males that mated were discarded. Mated females ($n=36$) were isolated in separate terraria, which were identical in terms of dimensions and food conditions to the standard housing terraria. On day 14–15 post-emergence, mated females were offered a replete human blood meal by placing an arm (S.S.) into each terrarium. Mated females were continuously provided with a black plastic cup (diameter, 5 cm; height, 4.5 cm) with a 1 cm hole in the lid for oviposition and these cups were checked for eggs daily. A replete blood meal was thereafter offered every 7 days for the remainder of each female’s life. If she did not accept on the 7th day, a blood meal was offered again on the 8th, 9th and 10th days, if she did not feed by the 10th day blood meals were ceased to be offered until the next 7 day period (from the 7th day).

For each female, three virgin non-irradiated males were added to the tank on the day that eggs were first observed. This time delay following the matings to the sterile males allowed us to verify that the initial matings had been successful by checking that females produced only infertile eggs during this time. Every time a new blood meal was offered (i.e. every 7 days), the three males were replaced with a new triplet of novel virgin males in order to give the female ample opportunity to mate with fertile and vigorous males, and the number of eggs were counted. During this time (approximately 4–6 h a day), we also intermittently observed any reproductive behavior in the tanks. Eggs were stored for 2 months and checked for hatching every 7–10 days. For any given female, we would interpret an increase in the proportion of eggs that hatched from the second egg clutch onwards as evidence that a second mating to a non-irradiated male had occurred.

A control set of females ($N=14$) mated to non-irradiated males only was also established to provide a comparison of hatching rates during the first gonotrophic cycle (first 2 weeks after blood feeding) thus providing a measure of the effectiveness of the radiation treatment. Females and their offspring were housed in identical conditions to the experimental females. These control females were offered one blood meal only. Eggs were collected and hatching rates recorded.

Data Analysis

Data were analyzed using SYSTAT®. All data on the proportion of eggs hatched was arcsine transformed before use in statistical tests, means, confidence intervals, and medians are given for non-transformed data. The difference in overall hatching rate of control females and the experimental females during the first 2 weeks after blood feeding was tested with a non-parametric Mann–Whitney U -test. To test for an increase in egg hatching rate, a regression of the proportion of eggs hatched over time was performed separately for each female. The mean of the regression coefficients across all females (i.e. the average slope of the regression lines) was tested with a t -test of the null hypothesis that the average slope equaled zero. All reported t -tests are two-tailed.

Results

Two out of the 36 mated females did not lay any eggs and were thus excluded from the analyses presented here. Females accepted a mean of 10 (SE=0.609) blood meals over a mean lifespan of 111 days (SE=6.6) days (Table 1). Virgin males courted the once-mated females vigorously throughout the females' lifespan but females invariably rejected these males by kicking courting males with their rear legs

Table 1 Basic Reproductive Parameters for the Experimental Females ($N=34$)

Variable	Mean	Minimum	Maximum	95% confidence interval
Longevity (days)	111.333	33.000	171.000	13.363
Number of blood meals offered	13.412	2.000	21.000	1.752
Number of blood meals accepted	10.235	2.000	15.000	1.239
Proportion of blood meals accepted	0.792	0.500	1.000	0.053
Total number of eggs laid	495.000	11.000	976.000	93.094

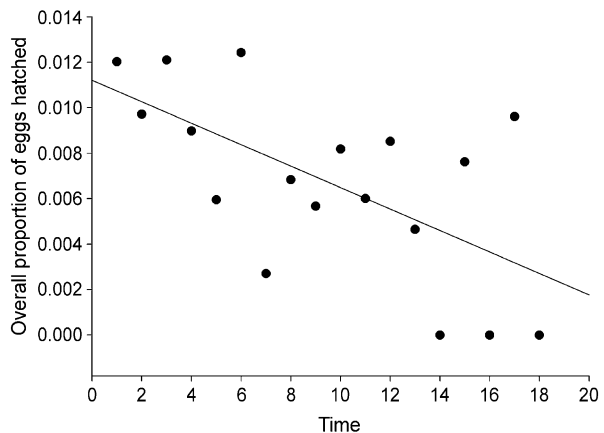
or by simply not adopting an acceptance posture (i.e. not lowering the abdomen and thereby making it impossible for the male to couple). The proportion of eggs that hatched across all clutches ranged from 0.000–0.021 (mean \pm 95% C.I.: 0.006 \pm 0.002; median: 0.004). This is significantly lower than the proportion of eggs that hatched in the control set of females when considering the first gonotrophic cycle only (mean \pm 95% C.I. [median]: Experimental females, 0.012 \pm 0.008 [0.000]; Control females, 0.884 \pm 0.048 [0.886]; Mann–Whitney $U=374.0$, $P<0.001$). We first assessed the association between egg hatching and time within individual females. The slope of the relationship between time and egg hatching rate was not significantly different from zero (separate regressions; $P>0.05$) for 31 of the 34 females. For all three females with a significant slope ($P<0.05$), the trend was negative such that egg hatching rate decreased over time (Fig. 2). More importantly, the mean slope across all 34 females was $\beta=-0.001$ (SE=0.001) and this was not significantly different from zero ($t_{32}=-1.34$, $P=0.19$). In summary, a few hatched eggs were observed from the first egg count onwards and the relationship between the proportion of eggs hatched and time was not significantly different from zero or even negative, both within and across all females. The fact that some females laid eggs that hatched is likely due to incomplete sterilization of the initial male, as hatched eggs were observed in the first egg clutch which could only have been fertilized by the initial sterile male. Thus, we found no evidence for remating among *S. cyaneus* females, despite the long duration of our experiment and continual access to virgin and vigorously courting males.

Mean copulation duration was 0.84 (SE=0.03) min. However, it was not possible to examine the relationship between copulation duration and female propensity to remate as we found no evidence for female remating.

Discussion

Our results provide compelling evidence that females of *S. cyaneus* are monandrous. We note that the experimental design used assumes that the proportion of offspring sired by a second male (P_2) would be greater than zero, and therefore that remating would leave an imprint in subsequent egg hatching rates. There is solid

Fig. 2 The overall proportion of eggs hatched (pooled over all females) over time (per 7–10 day egg count interval increments). Line represents overall regression line and is included only to visualize main trend in data (see text for statistical evaluation).



support for this assumption: Second male paternity values (P_2) in other Culicid mosquitoes are invariably non-zero (*Aedes aegypti*, $P_2=0.15$ (George 1967); *Anopheles gambiae*, $P_2=0.02$ (Bryan 1968); *Culex pipiens*, $P_2=0.11-1.00$ (dependent on remating interval) (Bullini et al. 1976)) and zero P_2 values are extremely rare, if at all, existent among insects in general (see Simmons 2001). The fact that a very small proportion of the eggs laid actually hatched (less than 1%) shows that our irradiation treatment caused near complete sterility among males. This result is not indicative of remating among females, because hatched eggs were observed from the first day of egg laying (before females were given the opportunity to remate) and because hatching rates showed no increase over time.

Within the widely monandrous mosquitoes, female mating rate appears to be under male control via accessory gland substances (Craig 1967; for a review see Klowden 1999). This may have created a conflict of interest over mating rate between males and females (Arnqvist and Andrés 2006). Females can certainly benefit from mating multiply through direct benefits (Arnqvist and Nilsson 2000), such as more or higher quality sperm (Thornhill and Alcock 1983; Arnqvist 1989; Siva-Jothy 2000), resources (e.g. Martens and Rehfeldt 1989), nuptial gifts (Wedell 1997; Wiklund et al. 2001), protection from male harassment (e.g. Rowe 1992) and potentially also through indirect genetic benefits (Jennions and Petrie 2000). On the other hand, there is limited empirical evidence that females may sometimes benefit from monandry. Females may benefit from monandry, for example, if polyandry decreases initial egg production and lifespan is limited (Välimäki et al. 2006), if male ejaculates are toxic (Kemp and Rutowski 2004) or if remating is associated with injuries inflicted by males during mating (Crudgington and Siva-Jothy 2000).

In our view, the most important implications of this study stem from the fact that monandry would seem to limit the strength of sexual selection, especially among females. Yet, both sexes carry ornaments in our study species. These ornaments are likely to be the result of mutual mate choice (Huxley 1914), as one would not expect sexually monomorphic expression of such an extreme character to merely be the result of a genetic correlation between the sexes (Darwin 1871; Lande 1980; Lande and Arnold 1985; Lande 1987). Mutual mate choice for sexually homologous characters has been shown empirically in socially monogamous birds in which both sexes invest a large amount in reproduction through parental care (Jones and Hunter 1993; Kraaijeveld et al. 2004). However, there is no parental care in mosquitoes, and, although it was not directly tested for here, male *S. cyaneus* most likely mate multiply. This is the case in other mosquitoes (Thornhill and Alcock 1983; Clements 1999; Klowden 1999) and is supported by the observed attempts at remating by males during our experiment. Although female monandry precludes post-mating sexual selection among males (Eberhard 1996; Simmons 2001), the opportunity for pre-mating sexual selection among males may actually be higher under monandry than under polyandry when males mate multiply (see Shuster and Wade 2003).

Understanding the evolution of sexually homologous ornaments through mutual mate choice in polygynous species without paternal care, such as *S. cyaneus*, is challenging, because males would be expected to mate indiscriminately and females would thus not be selected to invest in ornaments. This theoretical conundrum can not be ignored in light of the growing empirical evidence of male mate choice in polygynous species (for reviews, see Amundsen 2000; Bonduriansky 2001). Further,

the recent model by Servedio and Lande (2006) predicts that mutual mate choice can indeed be maintained if males can offset the cost of increased male–male competition when courting preferred females. They suggest that the benefits of choosiness to males may include securing matings with females with high fitness. Nonetheless, mating and/or reproduction clearly has to carry significant costs for males in order for male choosiness to evolve. Although we need to accumulate more information on the economics of reproduction in *S. cyaneus*, our study shows that it provides a promising new model system for the study of the evolution of sexually homologous ornaments in polygynous taxa.

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