

Male mating costs in a polygynous mosquito with ornaments expressed in both sexes

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Male mate choice in species with conventional sex roles is difficult to explain and has, therefore, been the focus of many recent theoretical models. These models have focused on variance in female quality and, to a lesser extent, male investments/costs associated with mating. In this study, we investigate the costs of courtship and copulation in the polygynous mosquito *Sabethes cyaneus*. In this species, both males and females possess elaborate ornaments. Previous studies suggest that the most likely explanation for the presence of these ornaments is mutual mate choice. Thus, this system provides an excellent model for exploring the evolution of mutual mate choice in polygynous species. We disentangle the costs of courtship and copulation by monitoring male survival in three groups of males: housed alone (group 1); able only to court females (group 2); or able to court and copulate with females (group 3). We show that males incur a cost of courtship and copulation and that courtship intensity is negatively related to male longevity. Our results suggest that courtship and copulation carry additive costs to males. We discuss the implications of these results in the context of current mutual mate choice theory and suggest that courtship costs may be an unappreciated key factor in the evolution of male mate choice.

Keywords: Culicidae; Diptera; mutual mate choice; sex roles; sexual selection

1. INTRODUCTION

Empirical evidence for sexual selection via mutual mate choice is accumulating (for reviews, see Amundsen 2000; Bonduriansky 2001; Kraaijeveld *et al.* 2007). In many reported cases, males and females exhibit 'conventional' sex roles; i.e. multiple mating by males (polygyny) and no paternal care. This is somewhat surprising because such mating systems are expected to be associated with female but not male mate choice. Female mate choice can be understood in terms of (i) high female reproductive investment through both parental care and gamete production (Trivers 1972), (ii) a male-biased operational sex ratio (Emlen & Oring 1977; Clutton-Brock & Parker 1992), and (iii) the fact that the direct and indirect fitness effects of mating on females may depend in part upon which individual male they mate (Andersson 1994; Arnqvist & Rowe 2005). In contrast, because females are essentially a limiting resource for males, it is much more difficult to explain the existence of male mate choice in conventional mating systems (Bonduriansky 2001).

Male mate choice has been largely attributed to partial or complete sex role reversal characterized by high levels of male mating investment (e.g. large/nutritious ejaculates/spermatophores; Rutowski 1982; Sakaluk 1985; Gwynne & Simmons 1990; Hyashi 1993; Savalli & Fox 1998; Fedorka *et al.* 2004; Pauku & Kotiaho 2005), reduced male reproductive rates (e.g. sperm limitation; Friedländer 1991; Gage & Cook 1994; Gage 1998) and/or a reduced male bias in the operational sex ratio (e.g. owing to differential mortality; Fahy 1971; Wearing-Wilde 1996). In many systems, especially in

taxa with negligible parental care by either sex, male mating investment often takes the form of ejaculate production (see references above) and courtship displays (Cordts & Partridge 1996; Mappes *et al.* 1996; Clutton-Brock & Langley 1997; Kotiaho *et al.* 1998). A high male mating investment is often associated with reduced longevity (Williams 1966; Pauku & Kotiaho 2005; Pomiankowski *et al.* 2005; Burton-Chellew *et al.* 2007; Simmons & Kotiaho 2007) and/or a reduced amount of ejaculate remaining for future matings (Parker 1982; Wedell *et al.* 2002). Thus, male mating investment is most often referred to as a 'cost', and will be referred to as such for the remainder of the paper.

The importance of male mating costs owing to ejaculate investment is widely accepted in mutual/male mate choice theory (Dewsbury 1982; Wedell *et al.* 2002; Härdling *et al.* 2008). However, the role that courtship costs may play in driving the evolution of male mate choice has received much less attention (for an exception, see Bonduriansky 2001). In his review of mutual/male mate choice in insects, Bonduriansky (2001) reported ample evidence of male mate choice in systems where there was no evidence of male parental investment, reduced male reproductive rates or reduced male bias in the operational sex ratio. Thus, it seems likely that less obvious costs of mating to males may commonly occur in species with male mate choice. We suggest that male courtship costs may represent such a widespread but 'hidden' male investment in mating.

Regardless of the form they take, male mating costs are central to mutual/male mate choice theory (Dewsbury 1982; Wedell *et al.* 2002; Härdling *et al.* 2008). In this study, we explore the costs to males of courtship and copulation in the sabethine mosquito *Sabethes cyaneus*. This species provides an excellent model system for

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investigating the effects of male mating investment and its implications for mutual mate choice for three reasons. First, males undertake a prolonged and apparently energetic courtship display (Hancock *et al.* 1990a). During courtship, males display the striking paddle-like ornaments on their mid-legs in front of the female. Second, two lines of evidence suggest that mutual mate choice occurs in *S. cyaneus*. Both males and females possess the ornaments and their removal negatively affects mating success in both sexes, although only significantly for males (Hancock *et al.* 1990b). In further support of mutual mate choice, a detailed morphometric analysis of the ornaments by South & Arnqvist (2009) found that both female and male paddles show many of the classic hallmarks of sexually selected traits. Remarkably, although sexual dimorphism in paddle size and shape was very slight, these hallmarks were more pronounced in females. Third, and very surprisingly given the aforementioned observations, female *S. cyaneus* mate only once (i.e. are strictly monandrous; South & Arnqvist 2008), whereas males are likely to mate multiply, a common mating system of mosquitoes (Thornhill & Alcock 1983; Clements 1999; Klowden 1999). Thus, *S. cyaneus* provides a suitable model for investigating the possible factors that may promote mutual mate choice in a polygynous mating system.

In order to disentangle the possible mating costs associated with courtship and copulation, our experimental design involved placing males in one of the three treatment groups: housed alone (group 1); able only to court females (group 2); or able to court and copulate with females (group 3). Mating costs were estimated as reduced longevity. The requirements of the second treatment group were met by manipulating females so that they were unable to copulate (for details, see §2). This design allowed us to disentangle the distinct costs of courtship and copulation to males without the confounding effects that may result from manipulating males. Under all scenarios, we predict that male longevity will decrease in the courtship and copulation treatments relative to when housed alone. However, the pattern of mating cost manifestations may differ depending on the relative costs of courtship and copulation. If males invest heavily in courtship, but not in copulation itself, then we would expect both treatment groups 2 and 3 to suffer similarly decreased longevity. Alternatively, if the costs of copulation and courtship are additive, we expect males in treatment group 2 to experience intermediate longevity and those in treatment group 3 to show the lowest longevity. By offering blood meals to unmanipulated females and allowing them to lay eggs, we also provide the first estimate of lifetime male mating rate in sabethine mosquitoes. Further, we investigate the relationship between longevity and the number of successful copulations in males, as this is central in terms of the fitness effects of decreased lifespan.

2. MATERIAL AND METHODS

(a) Rearing and maintenance

We used a strain of *S. cyaneus* established by R. G. Hancock and W. A. Foster in December 1988 from a sample of mated females collected at the Isla de Maje, Lago Bayano, Panama, Republic of Panama. This colony was maintained at Ohio

State University, USA, at a population size of approximately 200–300 individuals. 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 for the experiments described below 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 a standard ad libitum diet of crushed fish flake food to minimize variance in phenotypic condition across individuals. Pupae were collected in small dishes filled with deionized water (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 included in the experiment were virgins, adults were collected from this terrarium within 24 h of emergence (Becker *et al.* 2003). Males were housed alone and females were housed in groups of 20–30 in terraria with the same dimensions and food conditions as described above.

(b) Experimental design

(i) Male courtship and mating costs

One week post-eclosion, males were assigned to one of the following three treatment groups: housed alone (group 1) ($n = 24$); only allowed to court females (group 2) ($n = 22$); and allowed to court and copulate with females (group 3) ($n = 19$). Males remained alone in the terraria in the first treatment group. In the second treatment group, four manipulated virgin females of various ages who had been made unable to copulate were added to the single male in each male tank. In the third treatment group, four unmanipulated virgin females of various ages were added to the single male in each tank.

Females in the second treatment group were rendered unable to copulate by the placement of a small drop of water-based white acrylic paint over the gonotreme. We then secured the post-genital lobe into the paint to provide a further obstruction to the gonotreme. This procedure was conducted while females were under light CO_2 anaesthesia. A previous pilot study based on 60 manipulated virgin females (six tanks of 10 females) housed with 60 virgin males (10 males per tank) showed that this manipulation was completely effective in preventing sperm transfer over a 14-day period: no copulations were observed, females laid no eggs and the paint was still intact at the conclusion of the pilot study. To ascertain the possible effect of female manipulation on male courtship behaviour, the number of alignments and the total courtship duration were recorded for 1.5 h a day over the 14-day period for both the manipulated female tanks and a control group of 30 unmanipulated virgin females (three tanks of 10 females) housed with virgin males. Female manipulation did not affect the number of male alignments with females ($F_{1,10} = 1.707$, $p = 0.221$) or total courtship duration ($F_{1,10} = 0.247$, $p = 0.630$). The number of alignments with females decreased with time ($F_{13,130} = 4.994$, $p < 0.001$), but this relationship did not differ between the two groups ($F_{13,130} = 0.900$, $p = 0.555$). There was no effect of time ($F_{13,130} = 0.565$, $p = 0.878$) or the interaction with treatment ($F_{13,130} = 0.017$, $p = 1.000$) on total courtship duration.

We note here that although our design standardizes male density (one per tank), it does not control for effects of total mosquito density between treatment group 1 versus 2 and 3

per se (Gaskin *et al.* 2002). Unfortunately, we were unable to control for total density by housing more than one male per replicate in treatment group 1 because males court other males quite eagerly (Hancock *et al.* 1990a, S. South 2006, personal observation). However, we deem a total density effect highly unlikely for several reasons. We provided an ad libitum supply of honey and water on two sponges ($3 \times 3 \times 3 \text{ cm}^3$) and ample perching sites offered by a stick that ran the length of the terrarium (29 cm), the feeding sponges and the netting which formed the top of the terrarium ($29 \times 17.5 \text{ cm}^2$). Furthermore, we have never observed direct competition for resources between or within the sexes nor has it been reported elsewhere. Most importantly, all treatments had very low total densities: the terraria used in this experiment are also used to successfully maintain colonies of up to 60 individuals.

Once a week for the duration of each male's entire lifespan, females were replaced by novel and virgin, manipulated or unmanipulated females, in the second and third treatment groups, respectively. Courtship and mating behaviours were observed during spot checks for 1 h twice per week for all males in the second and third treatment groups. The courtship of *S. cyaneus* consists of six highly stereotyped components that always performed in the following order (for more detail and diagrams, see the original reference; Hancock *et al.* 1990a): (i) alignment, a patrolling male uses his mid-leg to grasp the wing of a female suspended from a stick by her fore- and mid-legs, he then pivots to swing onto the stick, resulting in the male facing the female and grasping the stick with his forelegs, he then begins to wave his free mid-leg in front of the female; (ii) male swing, the male rhythmically swings his free mid-leg and abdomen, if the female is receptive she lowers her abdomen at this stage; (iii) male copulation attempt, the male swings his abdomen towards the female and attempts to grasp her with his gonostyli; (iv) superficial genital coupling, the male is grasping the female with his gonostyli, which are extended away from the gonocoxites creating a gap, the male genitalia are not extended; (v) superficial genital coupling and male sigmoidal wave, the male releases his grip on the female wing and waves with both mid-legs; (vi) full copulation, the male moves his abdomen in a circular motion, the gonostyli are folded against the gonocoxites and male genitalia adpressed to the female. In addition to not lowering her abdomen, a female may also reject a male by kicking with her hind legs (henceforth referred to as female rejection kicking behaviours). Male and female mortality was checked daily and any female housed with a male that died was replaced immediately.

(ii) Male mating rates

The four unmanipulated females removed weekly from each male in the third treatment group were housed together in one-half of a terrarium—as described above but divided with a wire mesh. These females were offered a blood meal (by placing an arm into the holding tank; D.S. or S.S.) twice a week for two weeks or until they fed. Blood-fed females were immediately transferred and housed individually in a terrarium provided with a black plastic container (diameter, 5 cm; height, 4.5 cm) containing water with a 1 cm hole in the lid for oviposition. The subsequent presence of hatched eggs (i.e. larvae) in these containers showed whether females had copulated. The majority of mated females accept a blood meal within two weeks of mating

(S. South 2006, unpublished data), and this procedure thus provided a measure of the minimum number of copulations each male obtained.

(c) Data analysis

We use the following four measures of courtship behaviours throughout the paper: (i) courtship duration, (ii) number of male alignments with females, (iii) number of male copulation attempts, and (iv) number of female rejection kicking behaviours. Males in treatment group 2 were unable to achieve superficial coupling (the final (fourth and fifth) stages of courtship during which no sperm is transferred, see above and Hancock *et al.* 1990a) with manipulated females, presumably because the paint prevented the male from embedding his apical paraprocts into the membranous vaginal area of the female (Hancock *et al.* 1990a). In order for the measure of courtship duration to be the same in both treatment groups 2 and 3, courtship duration was calculated as the time elapsed from male alignment with the female until the pair separated in treatment group 2 and until superficial coupling in treatment group 3. Behaviours reported as 'intensity' represent the totals for the male's entire lifetime divided by the number of spot checks.

We began by investigating whether the treatments in which males could court (2 and 3) differed the mean intensity for each of the four courtship behaviours using *t*-tests. We then compared the relationship between courtship intensity and time in the two treatments by first conducting a principal components analysis (PCA) of the twice-weekly scores of courtship behaviours for all males. For each male, we then regressed our conglomerate measure of courtship intensity (i.e. PC1) on time. Finally, we tested for a difference in slope (β) of this relationship between treatments again using a *t*-test. We investigated differences in survival between the three treatment groups using Cox's proportional hazards regression and likelihood ratio tests (JMP 5.1, SAS Institute, Inc.). Cox's proportional hazards regression provides coefficients that describe the hazard function, which is the rate of mortality for a given time period. This is a robust analysis as it makes no assumptions about the form of the underlying hazard function (Cox 1972). We performed pair-wise post hoc tests of treatments using false discovery rate compensation for multiple tests (Storey 2002). We then tested the pattern of reduction in mean longevity across treatments using polynomial post hoc contrasts (Kirby 1993). We further investigated the effect of courtship on longevity in the two treatment groups showing courtship. To allow this, we first subjected the intensity (lifetime totals over the total number of spot checks made) of the four correlated courtship behaviours to a PCA and then used an analysis of covariance to investigate the relationship between courtship intensity and longevity in the two treatment groups. With the exception of the survival analysis, data were analysed using SYSTAT. Means standard error (s.e.) are presented.

When testing hypotheses in which the sign of the effect was predicted *a priori*, we used directed tests (Rice & Gaines 1994). Directed tests enable detection of patterns that are opposite to predictions while retaining much of the statistical power of one-tailed tests. In all directed tests (denoted p_{dir}), we followed the convention of setting $\gamma/\alpha = 0.8$ (Rice & Gaines 1994).

Table 1. Means and s.e. for the intensity of the four courtship behaviours (lifetime observed behaviour divided by the total number of spot checks) in treatment group two (court only) and three (court and copulate).

behaviour	2—court only (mean \pm s.e.)	3—court and copulate (mean \pm s.e.)	d.f.	<i>t</i>	<i>p</i>
courtship duration (min:s)	06:54 \pm 01:05	07:12 \pm 01:08	39	0.195	0.846
male alignments with females	1.586 \pm 0.229	1.176 \pm 0.135	39	1.484	0.146
copulation attempts	0.690 \pm 0.156	0.654 \pm 0.069	28.8	0.209	0.836
female rejection kicks	0.542 \pm 0.120	0.180 \pm 0.072	33.7	2.587	0.014

3. RESULTS

We found no significant difference between treatment groups 2 and 3 in the mean intensity of male courtship behaviours (lifetime intensity of courtship duration, lifetime intensity of male alignments with females and lifetime intensity of copulation attempts) (table 1). However, there was a significant difference between the treatment group means for lifetime intensity of female rejection kicking behaviour, such that manipulated females of treatment group 2 kicked more frequently (table 1). A PCA of the original four behaviours (totals per spot check, not intensities) resulted in a single PC with an eigenvalue greater than 1 (PC1, $\lambda = 2.592$), which explained 64.80 per cent of the variation in the four original variables. The four courtship behaviours showed approximately equal loadings on this first PC (lifetime intensity of courtship duration, 0.810; lifetime intensity of male alignments with females, 0.866; lifetime intensity of copulation attempts, 0.817; lifetime intensity of female rejection kicking behaviour, 0.719). Although courtship intensity tended to decrease over time in both treatment groups 2 and 3 ($\beta = -0.10 \pm 0.09$ and $\beta = -0.39 \pm 0.24$, respectively), the rate at which this occurred did not differ significantly across treatments ($t = 1.13$, d.f. = 27.3, $p = 0.268$). Note that this is in concordance with the results of the pilot study, which showed no differences between treatment groups in the decline of courtship behaviour intensity over time (§2).

The survival analysis revealed a significant effect of treatment on survival in the predicted direction ($\chi^2 = 7.521$, $p_{\text{dir}} = 0.015$, d.f. = 2) (figure 1). Post hoc tests found no significant difference between treatment groups 1 (males alone) and 2 (court only) ($\chi^2 = 0.841$, $p_{\text{dir}} = 0.224$, d.f. = 1). However, there was a significant difference between treatment groups 2 and 3 (court and copulate) ($\chi^2 = 4.481$, $p_{\text{dir}} = 0.030$, d.f. = 1), as well as between treatment groups 1 and 3 ($\chi^2 = 6.334$, $p_{\text{dir}} = 0.019$, d.f. = 1). Although post hoc comparisons inherently suffer from low statistical power (Quinn & Keough 2002), this analysis would seem to suggest that while copulation carries significant costs to males, courtship does not. However, data on mean longevities are more congruent with additive costs of courtship and copulation: males housed alone experienced the longest lifespan (mean \pm s.e.: 67.17 \pm 6.45 days), those only able to court showed an intermediate lifespan (61.95 \pm 5.38 days) and those able to both court and copulate suffered the shortest lifespan (51.79 \pm 4.14 days). In order to specifically test the pattern of reduction in mean longevity across treatments, we performed polynomial post hoc contrasts (Kirby 1993). Here, additive and distinct costs of courtship and copulation would generate a linear decrease in longevity,

whereas a cost only of copulations (or courtship) would result in a nonlinear decrease. This analysis showed that the first-order polynomial post hoc contrast was significant ($F_{1,62} = 3.69$, $p_{\text{dir}} = 0.037$) while the second was not ($F_{1,62} = 0.13$, $p_{\text{dir}} = 0.449$), which strongly supports the hypothesis that there are distinct and additive costs of courtship and copulation.

In order to investigate the relationship between courtship and longevity using a more powerful analytical strategy, we focused on the direct effects of variation on courtship intensity within treatment groups 2 and 3, the only treatments in which males were able to interact with females. A PCA of the intensity of the four courtship behaviours yielded a single PC with an eigenvalue greater than 1 (PC1, $\lambda = 2.96$), which explained 73.96 per cent of the variation in the four original variables. The four courtship behaviours showed approximately equal loadings on this first PC (lifetime intensity of courtship duration, 0.856; lifetime intensity of male alignments with females, 0.865; lifetime intensity of copulation attempts, 0.881; lifetime intensity of female rejection kicking behaviour, 0.837). We found a significant negative relationship between male courtship intensity and longevity (ANCOVA: PC1; $F_{1,38} = 16.09$, $p_{\text{dir}} < 0.001$, figure 2). There was also a significant effect of allowing copulations on longevity when controlling for courtship intensity (ANCOVA: treatment; $F_{1,38} = 5.70$, $p_{\text{dir}} = 0.014$), again showing a significant difference in survival between these two groups (figure 2). Males able to court and copulate (treatment group 3) experienced reduced longevity compared with males only able to court (treatment group 2). These results fully support the hypothesis that courtship and copulation carry additive costs to males. We note here that the interaction between courtship intensity and treatment was not significant (ANCOVA: $F_{1,37} = 3.6$, $p = 0.066$) and was therefore not included in the inferential model, allowing the treatment effect to be interpreted as the difference in means between the two treatments.

The lifetime number of successful copulations in treatment group 3 (the only treatment in which males had the possibility to both court and copulate with females) varied from zero to seven, with a mean of 3.03 (± 2.16 s.e.). The number of successful copulations achieved was positively correlated with longevity across males ($r = 0.543$, $n = 19$, $p = 0.016$). Copulation rates were not high, with the most successful male copulating with approximately 30 per cent of the females that he was exposed to. Males courted females eagerly, spending on average 24 per cent of each spot check courting females (mean \pm s.e.: 14 \pm 17 min). Female rejection kicking behaviour was observed in 79 per cent of the observed courtships.

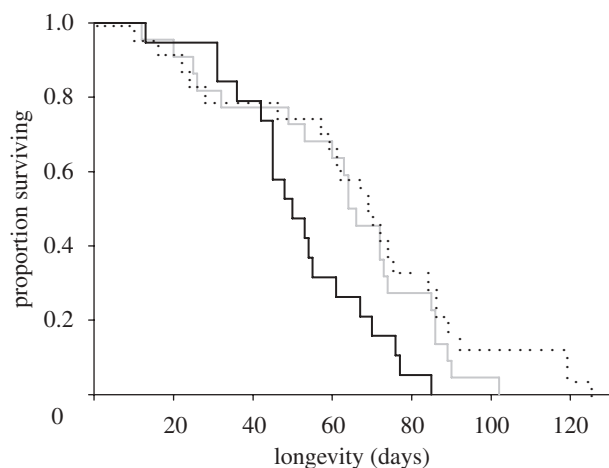


Figure 1. Survival across the three treatment groups. Dotted line, group 1, alone; grey line, group 2, court only; black line, group 3, court and copulate.

4. DISCUSSION

Our results collectively suggest that males suffer additive costs of courtship and copulation. The survival analysis showed a significant effect of treatment on male lifespan, with a significant cost to males of courtship and copulation. The average lifespan of males who were able to both court and copulate was 15 days shorter than males who were housed alone, corresponding to an approximate 25 per cent reduction in lifespan. Although a comparison of overall survival across treatments did not reveal a significant cost of courtship *per se*, our analyses of data on mean longevity did. First, the pattern of longevity reduction across treatments was only consistent with additive costs of courtship and copulation. Second, a closer inspection of variation in courtship investment across individuals showed a negative relationship between courtship intensity and longevity. Reduced longevity should represent a considerable fitness cost to males, as shown by the positive correlation between longevity and the number of copulations obtained by individual males. We note that our study also provides the first evidence that male sabethine mosquitoes are indeed polygynous.

The exact cause for the observed cost of copulation cannot be determined from this study. Production of the ejaculate is very likely to be associated with some costs to males. There are no reports of voluminous spermatophores in mosquitoes, and females of many species, including *S. cyaneus*, appear to gain all the nutrients required for offspring production from blood feeding. However, females of *S. cyaneus* are monandrous (South & Arnqvist 2008), which is probably due to male control via accessory gland substances in the ejaculate as is commonly found in other mosquito species (Craig 1967; for a review, see Klowden 1999). It is possible that these substances and/or sperm are costly to produce and account for our observed cost of copulation. However, it may be, at least in part, due to the fact that it was necessary to exclude the superficial coupling phase of courtship from our measure of total courtship in all treatment groups because males were unable to reach this stage with manipulated females. Indeed, during observed pairings that led to copulations ($n = 17$), males spent a mean of 27 per cent (s.e. = 4.21) of their total courtship time in

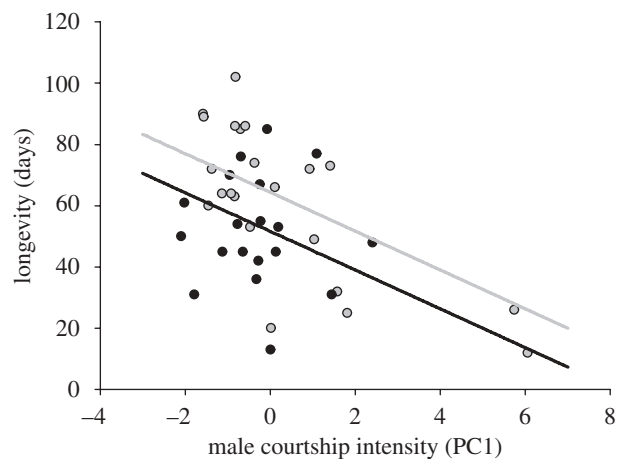


Figure 2. The relationship between male courtship intensity (PC1) and longevity for the two treatments in which male courtship was allowed. Lines represent equal slopes regressions for the two treatments (see text for statistical evaluation). Grey line, group 2, court only; black line, group 3, court and copulate.

the superficial coupling stage. During both superficial coupling and copulation itself, males continue to display their ornamented mid-legs to the females. Thus, it is possible that a portion of the observed cost of copulation seen in comparisons between treatment groups 2 and 3 can be explained by the continued courtship after superficial coupling. Alternatively, although we detected no significant differences in our measures of male courtship behaviour between the treatment groups 2 and 3, there might have been differences that we either did not measure or did not have the power to detect. If a change in behaviour did occur, it would need to culminate in higher costs of courting in treatment group 3 where males could court and copulate in order to lead to our results. In our view, however, it seems unlikely that preventing females from being able to copulate would reduce the costs of courtship to males.

It is likely that the relatively low number of male copulations observed is primarily due to the high rate of rejection by virgin females rather than to male constraints (such as sperm limitation; Friedländer 1991; Gage & Cook 1994; Gage 1998) as males were observed to court females eagerly throughout their lifetime. Our approach provides a somewhat conservative estimate of copulation rate as some mated females may not have accepted a blood meal and produced eggs. It is also possible that the low observed mating rates could be attributed to individuals not encountering a preferred or acceptable mate owing to the experimental design. As adult densities in the wild are unknown, it is difficult to extrapolate our findings to natural male mating rates. However, Hancock *et al.* (1990a) also observed that a low percentage of courtships led to successful copulations (33%), and moreover, found a significant negative relationship between female kicking behaviour and copulation. This suggests that the low mating rates and high levels of female rejection seen here are not an artefact of our experimental design.

The costs of courtship and copulation to males documented here are sizeable and may be large enough to drive male mate choice in this system. The observed

25 per cent reduction in lifespan may actually be a conservative estimate of the costs that a male would face in the wild as we housed individuals at low densities and significantly reduced the energetic costs of searching for food and perch sites. Yet, the magnitude of male mating costs needed to generate male choice is generally unknown and theory in this field makes no quantitative predictions. Further, encounter rates between the sexes are clearly important for the evolution of male mate choice, but male–female encounter rates in the wild have not been estimated in *S. cyaneus*.

The possible male benefits of exhibiting mate choice for females carrying large ornaments in *S. cyaneus* include: mating with more fecund females (female ornament size is positively correlated with body size (South & Arnqvist 2009), which is a trait often associated with fecundity in insects (Bonduriansky 2001)), preferentially courting young and, therefore, also unmated and receptive females (the female ornaments wear with age; S. South 2006, personal observation) and avoidance of courting heterospecific congeners who share a large proportion of the *S. cyaneus* distribution but lack these species-specific ornaments (Lane & Cerqueira 1942). Any benefit to males that may offset the costs associated with choosiness will facilitate the spread of a male mating preference (Servedio & Lande 2006). In a system such as that of *S. cyaneus* where males and females are likely to assess one another using a trait expressed by both sexes, it is probable that males will invest more in courting females who also show a preference for them (Lande *et al.* 2001). This is because positive linkage disequilibrium will develop both between the male preference and trait and between the female preference and trait. However, Servedio & Lande (2006) found that this effect was modest and not necessary to maintain male choosiness.

The importance of male courtship costs has received little attention in mutual/male mate choice theory, which may, in part, be due to the important role of the costs of male signals in maintaining honesty of signals of quality (Zahavi 1975, 1977; Grafen 1990*a,b*; Kotiaho 2000; Getty 2006) through trade-offs with other life-history traits (Williams 1966; Cordts & Partridge 1996; Kotiaho & Simmons 2003; Paukku & Kotiaho 2005; Simmons & Kotiaho 2007). Alternatively, the lack of attention given to courtship costs in mutual mate choice theory may be attributable to the fact that this concept is confounded by its implications for constraints on male choosiness (Parker 1983; Watson *et al.* 1998). If male mate choice is realized in part as increased courtship intensity or duration (Engqvist & Sauer 2001; Servedio & Lande 2006), then the cost of exhibiting a preference for a high-quality female will increase not only because of male–male competition for that female (Servedio & Lande 2006) but also as an intrinsic property of exhibiting choice itself. That is, male choosiness may inherently increase the energetic and time costs of mating.

The above argument thus appears circular, for if males face costs owing to high investment in courtship, then selection would favour males who decrease or stop courting rather than increase their investment. However, Servedio & Lande (2006) found that by allowing choosy males to invest more in courtship, male mate choice was more likely to be maintained. Alternatively, male choice

through increased courtship with preferred females may lead to an overall reduction in the cost of courtship to males: prudent investment into courting a limited number of preferred females may result in a reduced total investment. Kokko & Johnstone (2002) suggested another scenario in which reduced male longevity owing to investment in courtship signals leads to a female-biased operational sex ratio, thereby favouring male mate choice. It is also possible that male choosiness can be maintained even in the face of high competition when males are able to adjust their preference based on the observed competition and their own condition. For example, Candolin & Salesto (2009) found that under intense competition, males in poor condition ceased to exhibit mate choice. A return to the abundant empirical literature showing costs of courtship (see references above), combined with future studies investigating costs to males in species with suspected mutual/male mate choice such as this study, will hopefully lead to advances in mutual/male mate choice theory in species with conventional sex roles.

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