Male, but not female, preference for an ornament expressed in both sexes of the polygynous mosquito *Sabethes cyaneus*

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The evolution of female ornaments in species with a female-biased operational sex ratio (OSR) and intense female competition is well understood. In contrast, the adaptive value of female ornaments in species with a male-biased OSR and male competition remains largely unresolved. Mutual mate choice is one proposed explanation for the evolution of ornaments expressed in both males and females, a hypothesis supported by the increasing empirical evidence of mutual mate choice in species with a male-biased OSR. None the less, the evolution of female ornaments remains constrained, as investment in ornaments may detract from any direct benefits being signalled to males and females may fail to reap benefits sufficient to outweigh the costs of signalling. We used phenotypic engineering (i.e. manipulation of ornament size) to ask whether both sexes show a preference for sexually homologous ornaments in the polygynous mosquito, *Sabethes cyaneus*. We found a directional male preference for ornamented females, but no female preference for ornamented males. There was no evidence of assortative mating based on ornament size. We discuss these results within the framework of current sexual selection theory, addressing implications for both the evolution of male mate choice and the evolution of female ornaments.

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Elaborate ornaments are expressed in both males and females in many species, including birds (Amundsen 2000; Kraaijeveld et al. 2007), lizards (Ord & Stuart-Fox 2006), insects (e.g. LeBas et al. 2003) and fish (e.g. (Amundsen & Forsgren 2001; Basolo & Delaney 2001). In a subset of these species, the evolution of female ornaments is understood in terms of what is commonly known as 'sex role reversal' (e.g. in pipefish, Berglund & Rosenqvist 2001). These systems are characterized by high male parental investment and the resulting female-biased operational sex ratio (OSR) which leads to female competition and assumedly male mate choice based on female ornaments (Burley 1977). In socially monogamous species such as the crested auklet, *Aethia cristatella* (Jones & Hunter 1999), the occurrence of ornaments in both males and females has been correspondingly attributed to an equal male and female investment in parental care leading to an approximately equal OSR (Johnstone et al. 1996; Johnstone 1997; Kokko & Johnstone 2002).

Female ornaments are also reported in 'conventional sex role' species with a higher female than male parental investment, resulting in a male-biased OSR and the predicted male competition and

female choice, although they appear rarer than male ornaments in these species (Darwin 1871; Andersson 1994; Clutton-Brock 2009). In such species, female ornaments with homologues in males have traditionally been seen as a nonadaptive manifestation of an intersexual genetic correlation and sexual selection for large ornaments in males (Darwin 1871; Lande 1980, 1987; Lande & Arnold 1985). More recently, the possibility that both female and male ornaments are under direct selection has begun to be explored (reviewed in Amundsen 2000; Kraaijeveld et al. 2007), being suggested to result from mutual mate choice (Huxley 1914), selection for sexual ambiguity in females (Burley 1981; Robertson 1985; Langmore & Bennett 1999) and/or female contest competition (West-Eberhard 1983; Gwynne 1991; Heinsohn et al. 2005; LeBas 2006).

The strength of selection on female ornaments resulting from male mate choice was thought to be weak in species with 'conventional sex roles', as male competition was often portrayed as precluding male choice (Emlen & Oring 1977; LeBas 2006). However, mutual mate choice in such species does not appear to be as rare as first interpreted from the apparent scarcity of female ornaments relative to male ornaments, with an increasing number of empirical studies showing mutual mate choice in species with intense male competition (reviewed in Amundsen 2000; Bonduriansky 2001; Hooper & Miller 2008). In an interesting parallel, 'unexpected' female mate choice for male ornaments has been found in

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'sex role-reversed' species (e.g. Berglund et al. 2005). This highlights the fact that not only will sex roles fall along a continuum of variation depending on relative levels of parental investment, but also that they can only be defined in terms of the relative levels of parental investment and competition, not mate choice (Vincent et al. 1992). The strength of selection for male mate choice is better conceptualized by considering the costs and benefits of choice. The costs of male choice include increased male competition for a limited number of preferred females (Servedio & Lande 2006; Nakahashi 2008), mate assessment risks (Rowe 1994; Watson et al. 1998) and lost future mating opportunities (Deutsch & Reynolds 1995). These costs increase with an increasing OSR (i.e. with an increasing number of males relative to females available to mate; Emlen & Oring 1977). The benefits of male mate choice include direct benefits (such as more fecund females) or indirect benefits (such as higher-quality offspring) with the magnitude of these benefits being determined by variance in female quality (Parker 1983; Owens & Thompson 1994; Servedio & Lande 2006; Nakahashi 2008). Thus, even in species with a male-biased OSR and intense male–male competition, male mate choice can evolve if the resulting benefits outweigh the costs.

Therefore, the relative scarcity of female compared to male ornaments despite male mate choice in species with a male-biased OSR and intense male–male competition cannot be explained solely by a lack of male mate choice. It may rather be because investment in signals is not favoured in females, as they attract sufficient mates to exert choice and mate as many times as is optimal (LeBas 2006; Nakahashi 2008). However, it is now widely accepted that females can also benefit from attracting additional mates, for example through increased rates of egg production and fertility as seen in many insects (Arnqvist & Nilsson 2000). The conditions under which female ornaments can evolve are predicted to be further limited as female investment in signals will detract from their fecundity and therefore the direct benefits to males of choice (Fitzpatrick et al. 1995). These predictions are supported by a bias towards male preferences for nonornamental female traits that are correlated with fecundity (such as body size) in studies of polygynous species (Andersson 1994; Bonduriansky 2001).

The mosquito Sabethes cyaneus (Diptera: Culicidae) provides a unique study system for exploring the evolution of ornaments via mutual mate choice in species with a male-biased OSR and male–male competition under controlled laboratory conditions. Both male and female S. cyaneus possess elaborate iridescent paddle-like ornaments (henceforth referred to as 'paddles') on their midlegs. Male S. cyaneus mate with multiple females (i.e. are polygynous; South et al. 2009), whereas females mate only once (i.e. are strictly monandrous; South & Arnqvist 2008). There is no parental care by either sex. Females face significant risks associated with the acquisition of the blood meal, which is necessary for egg production in this species. These risks include host defence behaviours (e.g. Edman et al. 1984) and even predation by the hosts themselves (e.g. Guinan & Sealy 1986) while searching for and acquiring a blood meal. These host defence and predation risks are heightened when females are engorged and have decreased agility (e.g. Roitberg et al. 2003), in addition to predation by predators attracted to the vertebrate blood carried after feeding (e.g. Roitberg et al. 2003; Jackson et al. 2005). There are no indications that the paddles are used in intra- or intersexual contest competition for access to resources or mates. Contest competition for resources in this species appears absent, possibly because sites for oviposition (plant-held waters), feeding (flowers and primates) and mating (horizontal sticks) are abundant in natural populations. Females do not engage in contests for access to mates: courtship begins when males approach and align with resting females that are perched on the underside of horizontal sticks (Hancock et al. 1990a; also see Supplementary video of male courtship). Male–male contest

competition is evident from the fact that multiple males often attempt to align with females that are already being courted by another male and this can lead to disruption and termination of the courtship (S. H. South, unpublished data). However, males do not engage their paddles during these male–male interactions and courting males are not in a position to assess the intruding males' paddles visually. These behavioural observations of male–male, but not female–female, competition are in line with theory, as there is a predicted male-biased OSR resulting from the higher male than female mating rate, female 'time out' (Kokko & Jennions 2008) for blood feeding and oviposition, and the shorter expected life span of females because of the risks associated with reproduction.

Despite this, the consideration of multiple lines of evidence suggests that both male and female paddles are under selection by mutual mate choice. First, males display the paddles in an elaborate courtship dance which suggests they may be used during female mate choice (Hancock et al. 1990a; see Supplementary video of male courtship). Second, complete removal of the paddles appeared to reduce both male and female mating success, yet flight and oviposition behaviours were unaffected (Hancock et al. 1990b). The negative effect of paddle removal on mating success was statistically significant only in females and thus provided evidence only of male mate choice. These two lines of evidence suggest that both males and females show mating preferences for paddle size. The potential role of mutual mate choice was supported by a morphometric analysis that revealed that both male and female paddles show many of the classic hallmarks of sexually selected traits, including large phenotypic variation in ornament size, positive allometry and a negative relationship between fluctuating asymmetry in ornament size and body size (South & Arnqvist 2009). This combination of a male-biased OSR, male–male competition, no parental care by either sex and extravagant ornaments expressed in both sexes makes S. cyaneus an intriguing challenge to current sexual selection theory.

In this study, we aimed to explore whether ornament size affects the mating success of male and female S. cyaneus. Hancock et al. (1990b) found that the number of females inseminated in tanks where female paddles had been completely removed was lower than that of control tanks, suggesting that female paddles may affect their mating success. However, their experimental design did not control for the possibly confounding effects of the treatment on male and female behaviours (which could be achieved through conducting observations of male and female behaviours after paddle removal) or whether a simple presence or absence of the paddle was required for a successful mating (as opposed to the size of the paddle). Here, we provide a powerful test of male and female paddle preferences using phenotypic engineering (Sinervo & Basolo 1996), that is, experimental manipulation of ornament size, as first applied by Andersson (1982) in his pioneering experiment on mate choice in widowbirds, Euplectes progne. We employed an experimental design that allowed us to test for preferences for ornament size in both sexes simultaneously.

METHODS

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. This colony was maintained at Ohio State University, U.S.A. at a population size of approximately 200–300 individuals. Our colony has been housed at Uppsala University, Sweden since April 2006 at $26 \pm 1\,°C$, 78–82% RH and a 12:12 h light:dark photoperiod, at a population size of at least 400 individuals. Larvae for the experiments described below were reared in plastic trays (21.5×14.5 cm and

5 cm high) 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 × 17.5 cm and 18 cm high). These terraria were large enough to allow males and females to engage in feeding, resting and courtship behaviours as are observed in the wild (Hancock et al. 1990a). During our laboratory experiments we have never noted any divergence from the behaviours reported in the wild and in studies conducted elsewhere (Hancock et al. 1990a, b). An ad libitum supply of honey-soaked sponges and deionized water wicks was provided. Ample perching sites allowing all individuals to perch were provided by a stick that ran the length of the terrarium, reflecting the natural abundance of perching sites in nature (Hancock et al. 1990a). To ensure that all individuals included in the experiment were virgins, we collected adults from this terrarium within 24 h of emergence (Becker et al. 2003). Females were housed in groups of 20–30 in terraria with the same dimensions and food conditions as described above, while males were housed alone in one-half of an otherwise identical terrarium divided by a wire mesh (to prevent males from gaining courtship experience prior to the experiment by courting other males).

Experimental Design

Paddle manipulation and attractiveness

Thirteen days after eclosion, males and females were randomly assigned to one of three treatment groups: paddle intact (group 1); paddle partially removed (group 2); paddle completely removed (group 3). The paddles were partially removed (group 2) by using a pair of fine ocular surgery scissors, and completely removed (group 3) by gently brushing them using a fine paint brush (see Fig. 2 in the Results for illustration of paddles in each treatment group). The paddles in groups 2 and 3 (partially removed and completely removed, respectively) were smaller than the minimum paddle size observed in newly eclosed *S. cyaneus* (South & Arnqvist 2009). However, the partially removed paddles (group 2) were at the lower end of the range of natural paddle size variation observed in older individuals owing to wear (S. H. South, personal observation). Individuals with no paddles at all as represented by group 3 have not been observed in the field (Judd 1996). Such phenotypic engineering of traits beyond the range of natural variation is a commonly used tool in evolutionary ecology, as it allows the testing of the fitness effects of single traits in isolation (Sinervo & Basolo 1996; Travis & Reznick 1998). Phenotypic engineering increases the statistical power, allowing the detection of even weak selection, although results may be biased if the underlying fitness function is nonlinear (Sinervo & Basolo 1996; Travis & Reznick 1998). Regardless of any potential bias, this approach remains informative, as the complete removal of traits (such as the paddles) allows approximation of the fitness of a mutant phenotype lacking the trait which is relevant for understanding the origin and maintenance of the trait in question (Sinervo & Basolo 1996; Travis & Reznick 1998; Maklakov & Arnqvist 2009). All individuals were individually marked for identification during the behavioural trials using a drop of water-based paint on the thorax and handled for the same amount of time (2 min) under light CO_2 anaesthesia. All individuals were then returned to their tanks to recover overnight.

On the 14th day after eclosion, three males and three females from each of the three treatment groups were placed in terraria of the same dimensions and conditions as described above ($N = 261$ males and 261 females; i.e. nine females and nine males in each of 29 tanks). Behavioural observations were conducted for 3.5 h between 1030 and 1400 hours under a combination of incandescent, fluorescent and UV light. These lighting conditions aimed to mimic natural lighting to ensure that individuals would be able to perceive the iridescent colour of the paddles. The behaviours recorded were: (1) male alignment with females on the perching stick (the first stage of courtship), a measure of male preference; and (2) successful copulations, a measure of female preference, as females are able to reject courting males by kicking them with their hindlegs or by simply not lowering their abdomen into a receptive posture. For more details on the courtship behaviour of *S. cyaneus*, see Hancock et al. (1990a) and the Supplementary video. The identity of all interacting males and females was recorded.

Paddle manipulation and female behaviour

To test for the possible confounding effect of a change in female behaviour resulting from the paddle manipulation, we conducted a second experiment. Because females must be perched on the underside of a stick in order for males to be able to align with and court them, we used this as a measure of whether or not females differed in their availability to be courted across the three treatment groups. Three virgin females of each treatment group (treated in exactly the same manner as described above for the main experiment) were added to each of the 11 observation tanks ($N = 99$ females in total). Nine virgin males (completely unmanipulated) were then added to the tank to mimic the main experimental design and control for the possible effects of males on female behaviour. Spot checks were made every 15 min between 1100 and 1415 hours (14 spot checks in total) and the position of each of the females in the tank was recorded.

Statistical Analyses

Because our response variables were not continuous and our experimental treatments were replicated both within and across tanks, we employed generalized linear mixed models (GLMM) to assess the effects of female paddle manipulation and male paddle manipulation (treated as fixed-effect factors, each with three levels) on male and female attractiveness and behaviour. All inferential models included tank number as a random-effects factor and used a penalized quasi likelihood method for model fitting (Schall 1991; Bolker et al. 2009), while link functions and error distributions differed across models (see Results for details). In all cases, the variance component estimates for the interactions between the fixed-effects factors and the random-effects factor (i.e. tank) were nonsignificant and were thus not included in the inferential models to allow an interpretation of the main effects (Engqvist 2005). All other factors were retained in the final inferential models. All analyses were conducted in GENSTAT version 10.2 (VSN International, Hemel Hempstead, U.K.).

RESULTS

Effects on Female Behaviour

The effect of female paddle manipulation on the number of spot checks in which females were perched on the stick was tested in a GLMM using a binomial error distribution with the total number of spot checks performed in an observation period (one observation period per tank) as the binomial denominator and a logit link function. We found that female paddle manipulation had no effect on the proportion of spot checks (number of spot checks out of the 14 made each observation period) in which females were observed perched on the stick ($F_{2,86} = 1.81$, $P = 0.170$). That is, females in different treatment groups did not differ significantly in their availability to be aligned with and courted by males.

Effects on Male Attractiveness

We first explored the possible confounding effect of male paddle manipulation on the number of male alignments with females in a GLMM, using Poisson errors and a log link function, as the number of male alignments with females represents count data. We found a significant effect of male paddle manipulation on the number of male alignments with females ($F_{2,231.6} = 4.32$, $P = 0.014$), with males with unmanipulated paddles making somewhat more alignments (raw data means $\pm$ SE: paddle intact: 8.4 ± 0.8; paddle partially removed: 5.93 ± 0.7; paddle completely removed: 6.29 ± 0.62). We therefore included the number of male alignments with females as a fixed-effects covariate when exploring the effect of male paddle manipulation on male copulation success.

In our experiment, a female preference for large paddles would be manifested as a higher probability that females would accept copulation attempts by males with larger paddles. Here, we defined male attractiveness to females as his ability to translate courtships into successful copulations. To test for the effect of male paddle manipulation (i.e. treatment group) on male attractiveness, we used a GLMM of the number of copulations per male, where the total number of male alignments with females was included as a fixed-effects covariate. We used Poisson errors and a log link function, as the number of copulations per male represents count data. We note that male attractiveness in this model could, in theory, be captured either by a main effect of the male paddle manipulation or by a significant interaction between male paddle manipulation and the number of alignments. However, the interaction between the number of male alignments with females and male paddle manipulation was not significant (Wald $\chi^2_2 = 0.03$, $P = 0.985$) and was therefore excluded from the model estimating main effects (Engqvist 2005). Furthermore, male paddle manipulation had no main effect on the number of copulations (Wald $\chi^2_2 = 1.13$, $P = 0.569$), while there was a significant positive relationship between the number of male alignments with females and male copulation success (Wald $\chi^2_1 = 21.28$, $P < 0.001$). Thus, males that courted females more had a higher copulation success, but male ornamentation did not significantly affect male ability to translate courtships into successful copulations (Fig. 1).

Effects on Female Attractiveness and Copulations

The effect of female paddle manipulation on female attractiveness (defined here as the number of times courted by males) was assessed using a GLMM with Poisson errors and a log link function, as the number of times females were courted by males represents count data. In stark contrast to the lack of effect of male paddle manipulation on male attractiveness, female paddle manipulation significantly affected female attractiveness ($F_{2,231.4} = 56.16$, $P < 0.001$). Females with paddles intact were courted most, those with paddles completely removed least and those with partially removed paddles to an intermediate extent (Fig. 2a).

We further tested whether female paddle manipulation affected the probability of females copulating during the observation period in a GLMM, using binomial errors where copulating or not was the response variable and the binomial denominator was set to one. We found that female paddle manipulation significantly affected the probability of females copulating ($F_{2,204} = 7.11$, $P = 0.001$), with the same pattern of an increasing probability of copulation with increasing paddle size (Fig. 2b) as shown for female attractiveness (number of times courted by males).

The positive effect of female paddle size on the probability of copulating could be mediated by either (1) females with large paddles being courted more often, presumably reflecting male choice, or (2) females with large paddles being more likely to accept male copulation attempts. We therefore assessed this in a GLMM,
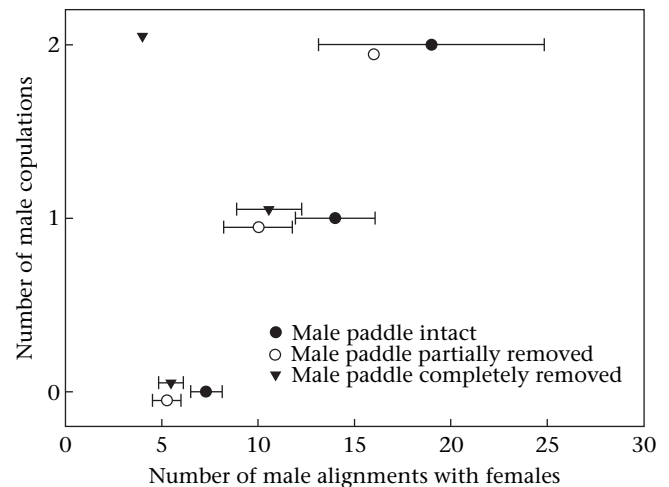
Figure 1. The relationship between the number of copulations by males and the number of male alignments with females for males in treatment groups 1 (paddle intact), 2 (paddle partially removed) and 3 (paddle completely removed). Mean and SEs of raw data are shown.

using binomial errors with copulating or not as the response variable and the number of times a female was courted by males as the binomial denominator. This model thus tests whether female paddle manipulation affects female acceptance rate of male courtships. We found no effect of female paddle manipulation on female acceptance rate ($F_{2,150} = 0.20$, $P = 0.822$).

Thus, collectively, these results show that female paddle manipulation affected female attractiveness (number of times courted by males). Also, because the probability of females copulating was proportional to how much they were courted, females with larger paddles were more likely to mate in our experiment.

Effects on Frequency of Same-sex Courting

We also investigated the effect of male paddle manipulation on their attractiveness to other males (as males will also court perched males) using a GLMM of the number of times males were courted by other males, with Poisson errors and a log link function. This analysis revealed that males also direct their courtship attempts preferentially towards individuals with large paddles in same-sex courtship ($F_{2,57.5} = 35.14$, $P < 0.001$), with the same pattern of increasing attractiveness to other males with increasing paddle size as was seen in females.

Assortative Mating based on Paddle Size

Finally, we explored whether males and females mated assortatively based on paddle size using the following approach. In each tank, there were nine possible pairing types between males and females with regard to the paddle manipulation (i.e. 3×3). For each tank, we first summed the number of copulations observed for each of these nine pairing types. We then conducted a GLMM with the number of observed copulations of a particular type per tank as the dependent variable, female paddle size (three levels), male paddle size (three levels) and their interaction as fixed-effect factors. Poisson errors with a log link function were used as the number of observed copulations represents count data. We found no evidence of assortative mating: there was no significant interaction effect between male and female paddle manipulation on the number of copulations ($F_{4,198} = 0.23$, $P = 0.922$). This model did show that female paddle manipulation affected the number of copulations achieved by females ($F_{2,198} = 3.22$, $P = 0.042$), which concurs with
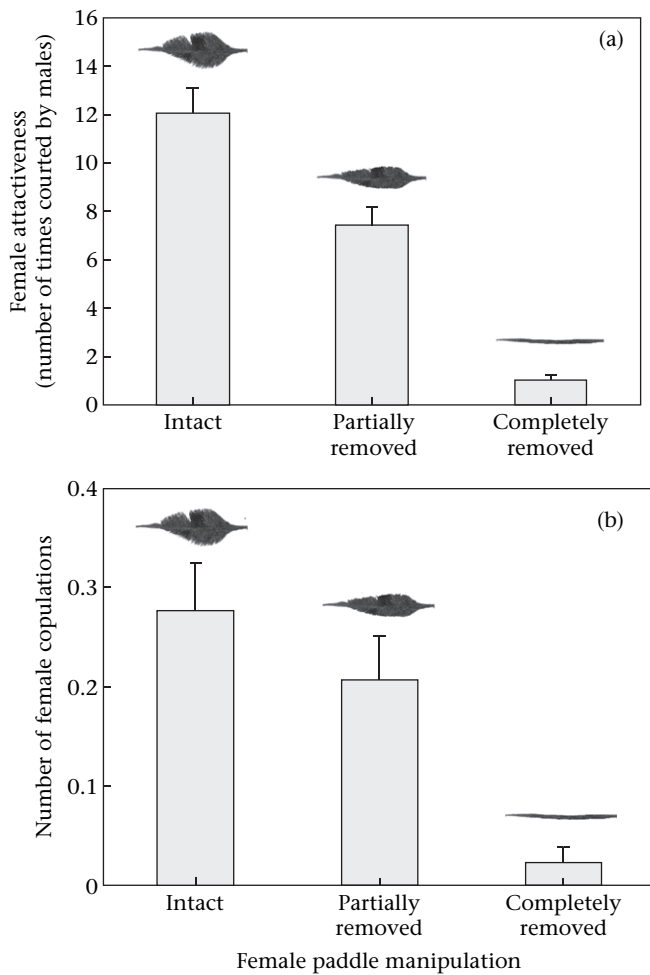
Figure 2. The effect of female paddle manipulation on (a) female attractiveness (number of times courted by males) and (b) the number of copulations by females during the experiment. Mean and SEs of raw data are given. An illustration of a representative paddle from each treatment group is shown above the corresponding bar.

the results of the analysis exploring the effect of female paddle manipulation on female attractiveness at an individual level (see above). The analysis also confirmed that the male paddle manipulation did not affect the number of copulations achieved by males ($F_{2,198} = 0.47$, $P = 0.624$).

DISCUSSION

By employing a phenotypic engineering approach (Sinervo & Basolo 1996), we were able to show that *S. cyaneus* males, but surprisingly not females, prefer mates with large 'paddle' ornaments. These results are in line with the morphometric analyses of the paddles showing that the hallmarks of sexual selection are more pronounced in females than in males (South & Arnqvist 2009). Below, we first discuss our results and then consider some of their implications.

Our results showed a directional male preference for female paddle size, within the phenotypic range examined (i.e. the larger the female paddle, the stronger the male response). Females with intact paddles were not only courted, but also mated, at a higher rate than females with paddles removed, while those with paddles partially removed were courted and mated at an intermediate rate. Males also showed a very similar preference for male paddles during same-sex courtship. In contrast, we found no effect of male

paddles on their attractiveness to females. These effects were not due to changes in male or female behaviour after the experimental treatment. The female paddle manipulation did not affect their perching behaviour (i.e. their availability for courtship by males). There was also no effect of female paddle manipulation on their likelihood of accepting mates; the positive relationship between the amount they were courted and their acceptance rate was the same for all three female treatment groups. Male paddle manipulation affected male behaviour, with males with intact paddles making more alignments. This is most probably a side-effect of the manipulation per se and not a response to their perceived paddle size, as males with partially removed paddles courted somewhat less than those with completely removed paddles. Courtship rate was thus held constant in the relevant statistical analyses. We note that even a failure to control for this effect could not explain our lack of effect of male paddle size on their mating success, as the effect of the paddle manipulation on male behaviour would only have inflated any positive relationship (because of the positive relationship between the amount females were courted and their acceptance rate). Finally, the experimental design used is a powerful design for assessment of assortative mating by ornament size, but we found no such assortment among mates within replicates.

We predicted intense male, but not female, intrasexual competition in *S. cyaneus* owing to the male-biased OSR (see Introduction) and therefore find the lack of female preference for male paddles surprising. Furthermore, males conduct an elaborate courtship display during which the paddles are predominantly displayed and it is thus reasonable to assume that male courtship and/or their paddle size affects their mating success (see Supplementary video). We note that an earlier study (Hancock et al. 1990a) failed to find any significant effects of male courtship behaviours on male mating success. Therefore, although we predict selection via female mate choice on male paddles and courtship, at this stage there is no direct evidence that there is (Hancock et al. 1990a, b). Our and previous experiments may have failed to find female preferences for male courtship and paddles because no-one has independently manipulated these traits, and it is thus possible that a combination of paddle size and some aspect of courtship intensity determines male attractiveness to females.

We feel that the most exciting implication of our study stems from the evidence of a female ornament in a species with a male-biased OSR and intense male–male competition that is under sexual selection via male mate choice. It is difficult to understand why females should invest in an ornament that appears costly because of production costs during ontogeny (Munoz et al. 2008), increased conspicuousness to predators (Godin & McDonough 2003) and impaired ability and energetic costs during flight (Evans 2004; Allen & Levinton 2007). In order for this female signal to evolve by direct selection via male mate choice, there must be benefits to females of signalling that outweigh these costs (Maynard Smith & Harper 2003). Female signals cannot be under selection to secure benefits of additional matings in *S. cyaneus* (Arnqvist & Nilsson 2000), because females are strictly monandrous (South & Arnqvist 2008). Females may benefit from attracting males at a younger age, in more secluded perching sites or from attracting a larger number of courting males which they can then choose between (Bradbury 1981); the fitness consequences of this may be greater under monandry than polyandry (see Shuster & Wade 2003), as postcopulatory sexual selection is precluded (Eberhard 1996; Simmons 2001).

Understanding this system becomes even more complex when one considers the evolution of male choice (see Introduction). South et al. (2009) suggested that the reproductive investment in courtship by *S. cyaneus* resulting in reduced longevity may contribute to the evolution of male preferences by somewhat

decreasing the OSR and, thus, reducing the costs associated with male mate choice (Emlen & Oring 1977). The costs of male mate choice may be further offset in *S. cyaneus* as female paddles may signal direct fecundity benefits, since there are positive relationships between paddle size and body size (South & Arnqvist 2009) and body size and fecundity in many mosquitoes (Packer & Corbet 1989; Briegel 1990a, b; Lounibos et al. 1990; Livdahl & Wiley 1991; Blackmore & Lord 2000; Briegel & Timmermann 2001; Armbruster & Hutchinson 2002). Whether the benefits of choosing a more fecund female outweigh the costs of male mate choice is difficult to quantify. Further studies of *S. cyaneus* are therefore needed that directly test for the relationships between female paddles and possible direct benefits to males.

Male *S. cyaneus* appear to show a directional preference for paddle size in females, which may seem surprising given that investment in ornaments by females will detract from any direct benefits they are signalling (Fitzpatrick et al. 1995). It has been suggested that female signals for direct benefits can evolve when males prefer intermediate signal sizes (Chenoweth et al. 2006). Our results appear incongruous with this prediction. However, because we were unable to increase paddle size beyond the natural range, owing to experimental constraints (i.e. the brittle structure of the paddles), it remains a possibility that the underlying male preference function is stabilizing (Lande 1981; Hall et al. 2000; Nakahashi 2008). Alternatively, male preference for female paddles may indeed be directional. We note that Chenoweth et al. (2006) assumed an exponentially increasing cost function. However, other (e.g. linear) cost functions can result in a correlation between signals of direct benefits and condition (Price et al. 1993). This study joins an increasing number of empirical studies that suggest we need further theoretical exploration of the evolution of directional male preferences for female signals of direct benefits (e.g. LeBas et al. 2003; Siefferman & Hill 2005; Bitton et al. 2008; Doutrelant et al. 2008; Simmons & Emlen 2008; Wright et al. 2008).

The evidence of male, but surprisingly not female, mate choice for an elaborate ornament in a polygynous species provided by this study challenges current theory for the evolution of both preferences and signals via sexual selection. Additional tests of male mate choice for female ornaments originally thought to be the result of a nonadaptive intersexual genetic correlation with ornaments under selection in males (Darwin 1871; Lande 1980, 1987; Lande & Arnold 1985) will contribute to the current reappraisal of sexual selection and the 'sex roles' (Bondurianksy 2009; Long et al. 2009).

Acknowledgments

Supplementary Material

Supplementary material associated with this article is available, in the online version, at doi:10.1016/j.anbehav.2010.12.014.

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