Turgid female toads give males the slip: a new mechanism of female mate choice in the Anura

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In many anuran species, males vocalize to attract females but will grasp any female that comes within reach and retain their hold unless displaced by a rival male. Thus, female anurans may face strong selection to repel unwanted suitors, but no mechanism is known for doing so. We suggest that a defensive trait (the ability to inflate the body to ward off attack) has been co-opted for this role: by inflating their bodies, females are more difficult for males to grasp and hence, it is easier for another male to displace an already amplexed rival. Inflating a model female cane toad (*Bufo marinus*) strongly reduced a male's ability to maintain amplexus; and females who were experimentally prevented from inflating their bodies experienced no successful takeovers from rival males, in contrast to control females. Thus, the ability of a female cane toad to inflate her body may allow her to manipulate the outcome of male–male competition. This overlooked mechanism of anuran mate choice may reflect a common evolutionary pattern, whereby females co-opt defensive traits for use in sexual selection.

Keywords: Amphibia; anti-predator; co-optation; male–male rivalry; sexual selection

1. INTRODUCTION

In any sexually reproducing species, a female can benefit by mating preferentially with males who provide resources or genetic benefits (Cunningham & Birkhead 1998; Widemo & Saether 1999; Jennions & Petrie 2000). Females assess the fitness of potential mates in many ways: for example, the gaudy plumage of a male bird may indicate that he has good genes, or will produce attractive sons (Andersson 1994). Females also make indirect choices by encouraging male–male competition prior to mating (Wiley & Poston 1996; Friedl & Klump 2005). However, a female's opportunity to choose a particular mate may be constrained by male behaviours: in some taxa, males obtain copulations forcibly (Shine *et al.* 2005; Morris *et al.* 2008), and in others, males are the choosier sex (Cunningham & Birkhead 1998).

Female anurans (frogs and toads) are typically the choosier sex, and discriminate among potential mates

based on male displays and resource defence (Duellman & Trueb 1994). Most male anurans give vocal displays to claim space and attract females (Gerhardt 1994), and females can exercise mate choice by approaching desirable calls or mating sites. The importance of vocal signalling, coupled with the observable nature of mating, has made anurans a classical system for studies of sexual selection, competition and signalling (Andersson 1994; Gerhardt 1994; Greenfield 2005; Wells 2007).

If the only way a female anuran can exercise mate choice is by approaching quality calls or sites, then males can evolve cheating strategies. A female approaching quality territories or males may be intercepted by 'satellite' males, which attempt to force a mating by grasping the female in amplexus and not releasing her (Wells 2007). High densities around suitable breeding sites may render it difficult for a female to approach a preferred male without passing near unwanted rivals. Thus, wrestling matches between males for the primary amplexus position may ensue (Davies & Halliday 1977; Byrne & Roberts 2004). Is a female anuran a passive participant in such male–male conflict, or can she facilitate the conflict and/or actively select a suitor?

Here we suggest a hitherto overlooked mechanism of female mate choice in anurans. Frogs and toads often defend themselves against predators by inflating their body: the increased girth may deter predators by increasing the apparent size of the anuran, and by rendering it too large to ingest (Duellman & Trueb 1994; Williams *et al.* 2000). We suggest that this anti-predator mechanism has been co-opted by selection for female mate choice. Male anurans use their forelimbs to grasp the female firmly in the armpit or groin, and maintain this position until the female oviposits. Rival suitors often dislodge amplexant males (Davies & Halliday 1979; Byrne & Roberts 2004). Female anurans frequently inflate their bodies when amplexed by males (B. Bruning & B. L. Phillips 2007, personal observation)—as they do when grasped by predators (figure 1a). We suggest that a female anuran may be able to facilitate male–male competition (and hence alter her mating outcome) by inflating her body and hence, reducing the ability of a male to cling to her.

To test this possibility, we used cane toads (*Bufo marinus*). Male toads often call in localized groups, and rival males wrestle for mating access (Bowcock *et al.* 2008) as described for *B. bufo* (Davies & Halliday 1978). In bufonids, the amorous attention of multiple males can drown females (Davies & Halliday 1979; Wells 2007), and size mismatches between males and females reduce fertilization success (Davies & Halliday 1977), so both natural and sexual selection may favour female toads able to dislodge unwanted males.

2. MATERIAL AND METHODS

(a) Female inflation pressure

To measure the internal pressure of naturally inflated females, we used a sphygmomanometer with the cuff wrapped around a female's body. During handling, females typically inflated their bodies. The cuff was pressurized until the female was forced to exhale and the pressure following subsequent inhalations was taken as the maximum internal pressure achievable by the toad. We tested 23 toads to determine how female body size affected internal pressure, and hence the appropriate inflation pressure for model toads.

Figure 1. Female cane toads may influence the outcome of male–male rivalry by inflating their bodies when grasped, thus rendering it more difficult for males to retain a hold. (a) Female toads often inflate their body in response to being grasped by a male. (b) In laboratory trials where tenacity of amplexant males was measured with a spring balance, less force was required to pull a male off a female's back if she was inflated than if she was deflated (relaxed). Error bars represent 1 s.e. Filled bars, large females; cross-hatched bars, small females.

(b) Model toads and the measurement of male amplexus strength

We compared the tenacity with which reproductive male toads retained their hold on a female between trials with inflated versus non-inflated females, to determine the effect of inflation on the ease with which males can be displaced by rival males. Because it was difficult to control the inflation pressure of live females, we used dead females as models. We euthanized four females using a lethal injection of sodium pentobarbital, cleared the thoracic cavity and placed a balloon into the cavity. The balloon was connected to a pump and pressure gauge, allowing us to control pressure. We used four female toads with (pre-dissection) masses of 99.9, 103.5, 263.5 and 271 g to measure the amplexus strength of 41 males. The small females (99.9 and 103.5 g) were inflated to 45 mm Hg, and the large females (263 and 271 g) to 65 mm Hg (based on the linear relationship determined previously). We used 15 mm Hg as the relaxed internal pressure.

Male toads (collected from a breeding pond the previous night) were brought into reproductive mode with a single dose of leuporelin acetate, a synthetic gonadotrophin (see Bowcock *et al.* 2008, 2009). We measured the strength of male amplexus by placing a male onto the back of the model, allowing him to clasp and then pulling him off with vertical pressure applied through a spring balance to a belt around the male's shoulders (see Bowcock *et al.* 2009 for details). Males who refused to amplex after three attempts were removed from the experiment. The force required to displace an amplexant male was recorded on the spring balance at the time of release.

(c) Male takeover experiment

To test whether an amplexed toad's ability to inflate her body affects male takeover, we experimentally eliminated the female's inflation ability. Treatment females ($n = 9$) were intubated using a brief

surgical procedure, in which a catheter was placed into the trachea. Control females ($n = 7$) underwent the same surgery, but were not intubated. The tube prevented treatment females from closing their tracheal glottis (essential to keep the body inflated).

Following recovery, each female was placed into a 1×1 m arena with three males (simulating male densities around breeding pools). At dusk, the smallest male was allowed to amplex the female. Thus, competitors were always larger than the amplexant male: across the 16 bins amplexant males averaged 99.3 g (s.d. = 23.3), and rival males averaged 138.4 g (s.d. = 36.9). At 5 min intervals for the first hour, hourly for the next 3 h, and then again at dawn, we recorded whether or not a takeover had occurred. Following this experiment, all females were euthanized with sodium pentobarbital.

3. RESULTS

Internal pressures of live female toads were positively correlated with body mass (pressure = $0.14 \times \text{mass} + 29.06$; $r^2 = 0.22$, $F_{1, 21} = 5.8$, $p = 0.03$). This relationship was used to determine the internal pressure for our amplexus strength experiment. A linear mixed effects regression with model state, and relative size of the male (male weight divided by female weight) as fixed independent variables, and male ID within female ID as the random structure, revealed non-significant interactions between the fixed variables ($t = 0.96$, d.f. = 39, $p = 0.34$), so we removed the interaction term. The resultant analysis revealed effects of relative mass (larger males were stronger: $t = 2.88$, d.f. = 36, $p = 0.027$), and model state (males were more easily detached from inflated models: $t = 9.28$, d.f. = 40, $p < 0.0001$; figure 1b).

A female's inflation ability affected mating outcomes: males amplexing experimentally deflated females were never dislodged by their rivals (none of nine trials), whereas such takeovers were common (in four of seven trials) with control females (Fisher's exact test: $p = 0.019$), and always involved displacement by larger males. These differences occurred despite a similar number of takeover attempts between control (mean: 3.4 attempts) and treatment females (mean: 3.2 attempts; linear model, number of attempts against treatment: $F_{1, 14} = 0.011$, $p = 0.92$).

4. DISCUSSION

Our experiments indicate that inflated female toads are more difficult for males to hold on to, and that the ability of a female to inflate her body facilitates takeovers by larger rival males. Thus, the ability of a female to inflate her body can affect the outcomes of male–male rivalry. In females who were unable to inflate their body, the small male maintained his amplexant position despite takeover attempts by larger rivals. Thus, a female toad's ability to inflate her body can influence the body size of her eventual mate.

Male body size affects female fitness in bufonids because fertilization success is highest when the males and females are similar in size (Davies & Halliday 1977). Because females are the larger sex, they often may benefit from choosing larger-than-average males. Traditionally, female anurans have been thought to select the size of their partner based on the dominant frequency of the male's call (larger males make deeper calls; an honest signal; Gerhardt 1994). Our experiments show that female anurans also may be able to exercise choice despite being grasped by a less suitable

(smaller) male. This mechanism of female mate choice relies upon a capacity (for body inflation) that is a widespread anti-predator adaptation in the Anura (Duellman & Trueb 1994; Williams *et al.* 2000).

Field observations of mating *B. bufo* reveal that larger males often displace smaller (already amplectant) rivals, but smaller males rarely displace larger rivals (Davies & Halliday 1979). Our experiments show that smaller males are able to resist takeover attempts if the female does not inflate her body. Thus, takeovers by larger males may reflect female as well as male tactics.

Given the commonness of male–male competition and defensive inflation among frogs and toads, this mechanism of female mate choice may be widespread. Why has it remained undetected? We suspect that inflation by the female during male–male rivalry has been interpreted as a simple response to being pushed, kicked and occasionally flipped over by amorous suitors. Here we show that such a defensive response can be co-opted by selection for mate choice. The same co-optation may well be common among other animals, because many of the traits that enable a female to repel a predator also allow her to repel unwanted suitors, and hence facilitate mate choice (Arnqvist & Rowe 2005; Stuart-Fox & Whiting 2005). Thus, not only does bodily inflation represent a new mechanism of mate choice in the Anura, but it also points to a potentially general mechanism, whereby defensive traits are co-opted to perform a new role in female mate choice.

All studies were approved by the University of Sydney Animal Ethics Committee (L04/5-2009/1/5072). Surgery and euthanasia were performed by experienced personnel, and the number of animals undergoing experimental procedures was kept to a minimum.

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