Male terrestrial salamanders demonstrate sequential mate choice based on female gravidity and size

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In many vertebrate mating systems, mate choice evolves when signalling via visual, chemical or auditory traits is an energetically costly process. Selection may favour individuals that can discriminate among potential mates and invest in signalling to mates with particular characteristics. Most commonly, females with costly gametes are thought to be the more selective sex; however, runaway sexual selection can produce elaborate male ornaments and behaviours that are similarly costly to produce, which can lead to male mate choice. In this study, we used behavioural trials to experimentally test male mate choice in a terrestrial salamander, *Plethodon shermani*. We investigated whether males altered the proportion of time they spent performing a potentially costly courtship display, ‘foot dancing’, in the presence of females. Specifically, we explored male mate choice in two experiments: (1) measuring how males modified the time they invested in courtship based solely on female reproductive value, and (2) determining whether males varied the amount of time they invested in courting females of varying sizes but similar reproductive value. In the first experiment, we quantified the duration of male courtship displays when males were paired with females of differing levels of fecundity (nongravid, weakly gravid and strongly gravid). Males displayed longest for females of high reproductive value (strongly gravid females) and less for weakly gravid and nongravid females. In the second experiment, we showed that males paired sequentially with different-sized females of similar reproductive values displayed significantly more often to larger females (i.e. male effort positively correlated with female size). In conclusion, male *P. shermani* are one of the few vertebrates known to modify their display behaviour based on female mate quality, and visual signs, such as size, are likely to provide honest indicators of fitness.

Keywords:

courtship behaviour

fecundity

male mate choice

Plethodon shermani

red-legged salamander

The coevolution of elaborate male ornaments and female perception is a common phenomenon in reproductive biology, leading to the evolution of diverse male displays in many modalities including visual, auditory and chemical signals (Andersson, 1994). In line with the breadth of displays that have evolved, a hallmark of such ornament/perception systems is rapid coevolution (Wilburn & Swanson, 2015). Early quantitative genetic models by Lande (1981) well describe how runaway sexual selection may drive the evolution of increasingly more elaborate male ornaments and female preferences; the extremes of these traits are often bounded by energetic or ecological constraints that impose negative natural selection (Kokko et al., 2006). Anisogamy between sexes generally results in females having the more costly gametes, and hence mate choice has traditionally been documented in a female-centric manner (Andersson, 1994). However, costly male sexual traits within polygynous species may drive the evolution of male mate choice. For example, in *Drosophila* spp., male seminal fluid contains numerous proteins that have strong effects on female remating and ova maturation, but males strategically allocate the release of these energetically expensive proteins based on perceived sperm competition (Wigby et al., 2009). Similarly, in the house mouse, *Mus musculus*, males are less likely to mate with a female if a male perceives that she has recently mated (and sperm competition risk is greater) (Ramm & Stockley, 2014). And in African buffalo, *Syncerus caffer*, horn size in both males and females is negatively correlated with parasite load, but also positively correlated with female lactation, such that males may select mates based on inferred fitness (Ezenwa & Jolles, 2008). While these studies highlight the frequency of male mate choice in diverse taxa, they are among a relatively small number of documented examples (Roff, 2015). In the current study, we explore the influence of male mate choice on a potentially costly display behaviour using a salamander model.

Models predict that male mate choice will evolve in species that meet three criteria: (1) the number of mates available exceeds a male's capacity to mate, (2) females are variable in their reproductive quality and (3) the benefits of choosing females exceeds the cost of assessing them (Edward & Chapman, 2011). Among other factors, limitations on male mating capacity can come from energetically costly or prolonged courtship displays, and this is observed in many species of birds, insects, fish and amphibians (Bouduriansky, 2001). Among amphibians, plethodontid salamanders have some of the most complex and prolonged courtships, which involve visual, tactile and olfactory signals (Arnold, 1977). In particular, one of the more elaborate courtship displays is that of the male red-legged salamander, *Plethodon shermani* (Houck & Arnold, 2003). Typically, after a male *P. shermani* orients towards a female, he signals to the female by performing a visual display termed 'foot dancing', which involves the male raising and lowering his legs while his trunk rests on the ground and remains still (Arnold, 1976; Organ, 1958). Males can choose to foot-dance for a female for multiple hours, although foot-dancing displays are intermittent and punctuated by short breaks (Eddy et al., 2012). It is hypothesized that females respond to the foot-dancing signal by entering the next stage of courtship, since courtship success is positively correlated with male foot-dancing effort in this species (Eddy et al., 2012). Given a successful foot-dancing display, the male and female will next perform 'tail-straddling walk': during this stage, the female will straddle the male's tail while moving forward in tandem with him (Arnold, 1976; Noble, 1929; Stebbins, 1949). While the pair is in tail-straddling walk, the male typically delivers proteinaceous courtship pheromones to the female by bringing his mental gland in direct contact with the female's nares ('slapping'; Arnold, 1972). A successful tail-straddling walk stage leads to the deposition of a spermatophore (an apical sperm mass on a gelatinous base) and transfer of the sperm mass to the

female's cloaca. The overall duration of courtships observed in the laboratory vary greatly between pairs, ranging from 17 min to 4.5 h, with a mean length of 2 h (Eddy et al., 2012). The extensive duration of the courtship in this species and the amount of movement it requires may be energetically costly to males, and thus, males may benefit from greater selectivity in potential mate assessment. In addition, female mate choice is also likely to be important in this system because the female may be assessing male foot-dancing effort and probably choose to complete courtship more quickly with males with particular characteristics. Unlike amphibians that have evolved amplexus, female *P. shermani* are able to terminate courtship at any point (Arnold, 1976; Houck & Arnold, 2003).

Plethodon shermani meet all of the aforementioned criteria for male mate choice to evolve. In addition to the protracted mating display that may be costly, this species lives at high densities often greater than 2.5 salamanders/m² (Connette & Semlitsch, 2013). Second, female salamanders are of variable reproductive quality: as with most salamanders, larger females have higher fecundity among the *Plethodon* and thus have more reproductive value to males (Kaplan & Salthe, 1979). In addition to the variation in body size, female *Plethodon* may only come into reproductive condition every other year (Highton, 1962; Sayler, 1966). The multimonth period that a female must spend defending her eggs prevents her from foraging extensively (Petranka, 1998). Therefore, at the beginning of the mating season, some large females will not have any reproductive value to a male (nongravid), while others will only have a few or poorly developed ova (weakly gravid), and still others will have a high reproductive value to a male (strongly gravid). Female reproductive state also varies later in the season because females are only estimated to produce sufficient yolk in their eggs every other year, and thus, some individuals never develop mature ova within a reproductive season (Petranka, 1998). This variation in

gravidity could place additional evolutionary pressure on males to be selective in investing time and energy into courting particular females. Finally, males must be able to assess females, and the benefit of choosing females with high reproductive value should be greater than the cost of assessing female quality if male mate choice is to evolve. In many oviparous species, the most common indicator of a female's fitness is her size, which is often correlated with fecundity (Servidio & Lande, 2006). In several salamander species, males distinguish among conspecifics by the visual cue of size (Verrell, 1985, 1986). Some species can also detect differences between gravid and nongravid females through olfaction (Dantzer & Jaeger, 2007; Marco et al., 1998). This size assessment has not been documented for *P. shermani*; however, *P. shermani* males can identify the sex and species of an individual based on substrate-borne chemicals (Palmer & Houck, 2005). Hence, finding and assessing mates is unlikely to be highly costly for *P. shermani* males during the breeding season.

We hypothesize that foot dancing represents an example of a costly male ornament whose evolution is balanced by positive sexual selection (increased male mating success) and negative natural selection (expensive with regard to both time and energy), and therefore, males should selectively allocate foot-dancing effort based on female quality. In the present study, we tested for male mate choice in *P. shermani* by experimentally staging courtship trials and measuring the length of time that males invested in foot dancing when presented with females of varying gravidity (nongravid, weakly gravid or strongly gravid). We chose foot dancing as a proxy for male investment as it is easily quantified and is directly correlated with courtship success (Eddy et al., 2012). We also investigated whether males used cues other than gravidity (such as body size) to assess female reproductive condition by quantifying foot-dancing effort when each male was sequentially paired with strongly gravid females of different sizes.

METHODS

Animal Collection and Maintenance

We collected 60 adult male and 100 adult female *P. shermani* in Macon County, North Carolina, U.S.A. (035°10'48"N 083°33'38"W) during early August 2011 with the appropriate permits from the North Carolina Wildlife Resources Commission (permit no. 13-SC00345). Animals were housed in individual boxes when not being used in behavioural trials. In most cases, animals were fed two waxworms (*Galleria mellonella*) weekly, with one exception: females used in experiment 1 were not fed for the duration of the experiment to prevent their condition from changing between trials with different males. Females were fed at the end of the experiment (approximately 2 weeks after collection). Because these animals are only fed weekly in the laboratory (Eddy et al., 2012; Houck et al., 2008; Wilburn et al., 2015), withholding food for one extra week was likely to be within the normal variation in feeding for this ectothermic species and should not have negatively impacted their body condition. In both laboratory locations (described below), each animal was housed individually in a clear plastic box (17 × 9 × 13 cm) with a substrate of moist paper towels and crumpled damp towels for refugia when not being used in courtship trials. All animal care and experimental protocols used in this study were approved by the Oregon State University Animal Care and Use Committee (LAR 3549 to L.D.H.). After the behavioural trials were concluded, all animals from experiment 1 were released at their original collection site. Those that were used in experiment 2 were subsequently

ethanized for use in unrelated terminal experiments of olfactory neurostimulatory pathways following IACUC-approved protocols.

Gland Removal

Mental gland removal was necessary to control for the delivery of male courtship pheromones in our experiments. Male pheromones can influence female receptivity (by shortening the duration of courtship) (Rollmann et al., 1999). We were interested in how males adjust their courtship behaviours in response to female gravidity, and wanted to control for any changes in female receptivity due to the males pheromones. Thus, we chose to control for pheromone delivery by surgically removing the males' mental glands. It is well documented that mental gland removal has no effect on male courtship behaviour (Houck et al., 2007; Rollmann et al., 1999; Wilburn et al., 2015). To remove each gland, we first anaesthetized each male in a 7% ether solution. Each gland was surgically excised using iridectomy scissors (after Houck et al., 1998). All males were allowed at least 2 weeks to recover before use in trials, during which time all males completely healed.

Experiment 1: Male Display Effort for Females of Varying Fecundity

Courtship trials were initially set up at Highlands Biological Station (HBS; Highlands, NC, U.S.A.) to identify deglanded males willing to mate in the laboratory (prescreening as described in Vaccaro et al., 2009). Of the males that initiated courtship at least once in the laboratory, 20 were randomly chosen to participate in the behavioural trials for experiment 1. In

contrast, the 60 females collected for this experiment were not used in any courtship trials before the start of the experiment. For each female, we recorded snout–vent length (SVL; mm) and degree of gravidity. To quantify gravidity, we categorized females into one of three groups: nongravid (NG; no ova present in either ovary), weakly gravid (WG; small, poorly developed ova present), or strongly gravid (G; both ovaries containing at least some well-developed ova). Females measuring less than 46 mm in SVL were not considered reproductively mature and were not used in our study (cf. Hairston, 1983). The median difference in female size was minimal (<1.8 mm) between the three gravidity groups. For the trials, we randomly assigned one female from each category to each male on subsequent nights.

Three nights of courtship trials were staged in mid-August 2011 (10, 12 and 16 August) from 2100–2230 hours Eastern Standard Time (EST). To ensure that males would have access to an individual female’s scent (and potentially sense fecundity via olfaction), each time a male was paired with a nongravid, weakly gravid or strongly gravid female, the courtship trial occurred in the female’s home box with the refugia removed (leaving only the bottom moist paper towel liner). The order in which each male was exposed to the three different types of females was randomized. One of four observers watched and scored the same five males each night to eliminate interobserver variation within male observations. Commencement of behavioural observations began as soon as the animals were introduced into the boxes and continued for 90 min because preliminary observations suggested that males will start foot dancing near a female after spending as little as 10 min in a box with her. Every minute an observer recorded whether or not each male was foot dancing by scan sampling (Altmann, 1974). Behavioural observations were performed under low-light conditions using dim white light that mimicked nocturnal light levels in the field. White light was used because male red-legged salamanders have red patches

on their legs and use of red light might have impacted female perception of male foot-dancing signals. Observers watched the courtship observations from a darkened section of the room with their arms below the height of the courtship arenas so animals could not see movements of their hands. This set-up is a standard procedure that has been used previously in this species (Houck et al., 2008; Rollmann et al., 1999; Wilburn et al., 2015).

We used generalized linear mixed modelling (GLMM) with the `lmer` function in the R package `lme4` (Bates & Maechler, 2008) to determine whether female reproductive condition influenced male foot-dancing behaviour. GLMMs were employed because our analyses incorporated repeated measures and our response variable was a proportion (binomial distribution), and they are more robust against deviation from normality than repeated measures ANOVAs (Bolker et al., 2009). GLMMs can also account for nonindependence of observations by incorporating a clustering variable (in this case, male ID) into the model as a random effect (Bolker et al., 2009). Because the response variable (percentage of observations with observed foot dancing) was a proportion, we used a binomial distribution with the probit link function to model our data. The full model included the proportion of observations that a male spent foot dancing as the response variable, female reproductive condition as a fixed effect and male ID as a random effect: `glmer(cbind(fd.observations,total.observations-fd.observations)~female.cond+(1|male.id),family=binomial)`. We used a likelihood ratio test to compare the fit of this full model to a reduced model that did not include female reproductive condition. A full model that yielded a significantly better fit to our data would indicate that female reproductive condition was a significant variable influencing male foot-dancing effort. Within-factor effects were compared by post hoc Z tests with corrected standard errors.

Experiment 2: Male Display Effort for Gravid Females of Varying Size

The experiments assessing male foot-dancing effort related to female size were performed at Oregon State University (OSU; Corvallis, OR, U.S.A.). The *P. shermani* were maintained at 15–18 °C on a late-August North Carolina photoperiod and each was fed two waxworms (*G. mellonella*) weekly. Forty deglanded males and 40 strongly gravid females were chosen for behavioural trials from a pool of animals that mated under laboratory conditions. Scoring of gravity and courtship prescreening followed the procedures described in experiment 1. The chosen animals were randomly placed into four blocks (10 males and 10 females in each). The design of the experiment was to pair each male with every female in his block; consequently, each female would be paired with every male in her block. Trials were conducted from mid-September to early October 2011. Animals had 1–2 nights off between each trial night.

On each trial night, beginning at 2030 hours EST, 40 male–female pairs were placed into individual plastic containers (17 × 9 × 13 cm) that were lined with a newly dampened paper towel substrate. Each trial lasted 3 h, and each block of 10 animals was watched by the same observer across all 10 trial nights. Light conditions and observer positioning were set up in the same manner as experiment 1. Observers scanned each pair every 3 min and recorded whether the male was foot dancing or whether the pair had progressed successfully to the next stage of courtship (tail-straddling walk). If a pair was observed in tail-straddling walk for two consecutive scans (6 min), scoring was terminated. The observer then disrupted the courtship to prevent the female from becoming inseminated. This precaution was necessary because inseminated females will not remate for at least 3 days (Eddy 2011) and thus would not be receptive in subsequent trials. Although our objective was to pair every female with 10 males, not every female met this

condition for two reasons: (1) two females were inseminated in our trials and were subsequently removed from the experiment; these females were replaced with two new females, and (2) one male became sick during the trials and was removed, but not replaced.

As in experiment 1, we used GLMM in R to statistically test whether males varied their foot-dancing efforts across the strongly gravid females that varied in size. We determined whether female SVL influenced male foot-dancing effort (response variable) by comparing a full model with female SVL as a fixed factor and male ID as a random factor:

```
glmer(cbind(fd.observations,total.observations-  
fd.observations)~svl+(1|male.id),family=binomial). We used a likelihood ratio test to compare  
the full model to the model that did not include female SVL, and we compared within-factor  
effects by post hoc Z test.
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RESULTS

Experiment 1: Males Display More for Females with Higher Gravidity

A likelihood ratio test revealed that female reproductive condition accounted for a significant amount of the variance in the proportion of time a male spent foot dancing ($\chi^2 = 217.9$, $P < 0.0001$; Fig. 1). Tail-straddling walk was not initiated in any of the 90 min trials, so every male had 90 observations per trial. When paired with strongly gravid females, males foot-danced for an average of 27.5% of the trial period (Fig. 1). In contrast, males were observed foot dancing on average 2.3 times less often when paired with nongravid females ($z = -9.352$, $P < 0.0001$) and 1.4 times less often when paired with weakly gravid females ($z = -4.344$, $P < 0.0001$). We used a post hoc Z test to compare whether males foot-danced significantly more or

less for weakly gravid versus nongravid females and found no significant difference between these groups ($P = 0.2877$).

Experiment 2: Males Display More for Larger Females When Their Reproductive Value Is Similar

Comparison of mixed-effect models with and without female SVL (including male identity as a random effect) showed that male foot-dancing effort significantly increased when female SVL was included and females were all of similar reproductive condition ($\chi^2 = 106.46$, $P < 0.0001$; Fig. 2). On average, males foot-danced ~2.3 times more for the largest female (71 mm; 16.3%) than for the smallest female (54 mm; 7.9%). The average proportion of persuasion and orientation stages that males spent foot dancing in these trials was 18.63% (range 0–83%).

DISCUSSION

Plethodon shermani males clearly exhibit male mate choice, as demonstrated by a differential allocation of courtship display effort (amount of foot dancing) to females in different reproductive conditions. Foot dancing is a visual display performed during the early persuasion stage of courtship and is likely energetically expensive, by placing a cost on males that must be factored into the trade-offs associated with courtship. When paired with females with large differences in fecundity (nongravid, weakly gravid, strongly gravid), males allocated the most foot-dancing effort towards fecund females. In contrast, when males were paired with females of similar reproductive value (all strongly gravid), they used female size to determine how much effort to allocate to courtship displays.

The more time a male spends foot dancing, the more likely a female is to progress to the next stage of courtship (Eddy et al., 2012). In a previous study, males foot-danced for strongly gravid females about 20% of a given courtship trial (Eddy et al., 2012). In addition, if a female did not enter into tail-straddling walk within the 3 h period allotted, the male would display (on average) for 36 min across those 3 h. This may not seem like much effort, but these salamanders are relatively sedentary: typically, *P. shermani* move only short distances and have relatively small home ranges (e.g. 5.04 m²; Nishikawa, 1990). Given these conditions, 36 min of foot dancing may be a substantial investment of energy. Furthermore, *P. shermani* are only active on the surface for about 2–4 h on rainy or humid nights (Petranka, 1998). Courtship duration in this species is lengthy ($\bar{x}$ = 120 min in the laboratory), so investment in a single courtship for a male is also costly because the time burden may limit his opportunity for additional courtships. Another cost of foot dancing may be the attention it draws from rival males. Both Organ (1960) and Arnold (1972) noted that rival males will attack those that are courting. All of these observations are concordant with our findings that males exhibit mate choice, and that differential investment is likely to be made in the early stages of courtship to further reduce the cost of courting females of lesser reproductive value.

Although we found that male *P. shermani* foot-danced more intensely for strongly gravid females, males did not seem to discriminate between nongravid or weakly gravid females. Males may not differentiate between nongravid and weakly gravid females because their reproductive value is equally low or because the cues from females in these categories are indistinguishable. Female *P. shermani* store sperm obtained during the late summer and autumn, with oviposition and fertilization occurring the following late winter or early spring (Hairston, 1983). Eggs must be sufficiently large to produce viable offspring, and a female developing her ova may require up

to two seasons (Highton, 1962). Thus, neither nongravid nor weakly gravid females (with their small eggs) are as likely to oviposit in a given year as are strongly gravid females. Nongravid and weakly gravid females therefore may have similarly low reproductive values for a male. In this context, males receive the greatest reproductive payoff in courtships with strongly gravid females and modify their signalling investments accordingly.

Unlike most other salamander taxa in which males engage in mate choice, male *P. shermani* preference for female size appears to be contextual, a trait males only assess among gravid females. In experiment 1, when males were presented with females of similar size but differing levels of fecundity, males were able to distinguish female reproductive quality. However, once a female was assessed to be gravid, males would increase their foot dancing by ~13% per millimetre of SVL (experiment 2). Although male mate preference for large females has been documented in several other plethodontids and salamandrids (*Notophthalmus viridescens*: Verrell, 1982; *Triturus vulgaris*: Verrell, 1986; *Desmognathus ochrophaeus*: Verrell, 1989; *Desmognathus santeelah*: Verrell, 1995; *N. viridescens* subspecies: Takahasi et al., 2010), gravidity of the females was not directly determined in these studies. In *T. vulgaris* and *D. santeelah*, Verrell (1986, 1995) did find a strong correlation between female size and the number of yolked oocytes in the ovaries of a subset of females. The strong correlation between female reproductive quality and size suggests that male salamanders commonly use size as an indicator of female quality (Verrell, 1986, 1995). Our study extends these previous findings to show that male *P. shermani* can assess gravidity directly and probably use size as a secondary cue after assessing gravidity.

Differences in study design, especially in how potential mates are presented, also need to be considered when comparing results among studies. Differences in how mates are presented

can influence whether or not mate choice occurs (Werner & Lotem, 2006). While our study employed sequential choice trials, previous male mate choice studies in salamanders have primarily used simultaneous presentation of potential mates. These studies have demonstrated male choice based on female size in a number of salamanders (Verrell, 1982, 1986, 1989, 1995; Takahasi et al., 2010). But, in the few experiments that used sequential choice, a preference for larger females was not demonstrated (*D. ochrophaeus*: Verrell, 1989; *D. santeelah*: Verrell, 1995). Thus, *P. shermani* is the first salamander species for which males have been observed to invest more time in courtship displays for larger females in a sequential mate choice study.

Compared to many conserved aspects of plethodontid salamander courtship, foot dancing is a relatively recent acquisition: it has been documented in nine large eastern *Plethodon* species, and presumably extends to the entire *Plethodon glutinosus* complex of 28 species that radiated ~10 million years ago (Highton et al., 2012). While the major stages of courtship are similar across all plethodontid salamanders, more ancestral lineages vary in many respects; in particular, male mental glands are much smaller, their pheromones are delivered directly to the female bloodstream via ‘scratching’ behaviour (instead of slapping) during the persuasion phase, prior to tail-straddling walk (Houck et al., 2008). It has been postulated that the transition from transdermal to olfactory delivery permitted greater bursts of gene duplication and positive Darwinian selection by exploiting the sensitivity of olfactory receptors. These many gene duplications are thought to provide greater diversity in male pheromone composition and permit stimulation of any female in the breeding population (Wilburn et al., 2012; Wilburn & Swanson, 2015). The simultaneous change in timing of pheromone delivery may have allowed for a second ornament (foot dancing) to evolve as a replacement during the persuasion stage. As an honest signal of male quality, positive sexual selection on female preference would have quickly

selected for males that performed longer displays until male displays became sufficiently balanced by negative natural selection (from energetic and/or time costs), leading to increased levels of male mate choice in this system.

Two alternative indicators may be involved in male mate assessment of different levels of female gravidity: (1) female behavioural feedback (i.e. visual and/or tactile signals), or (2) female chemical odorants. In many systems, the male is attuned to the female's behavioural responses and modifies his courtship displays accordingly. For example, a male whitethroat, *Sylvia communis*, will reduce courtship display when the female does not respond to his displays with an appropriate vocalization (Balsby & Dabelsteen, 2002). Thus, by assessing female behavioural signals, males lessen their investment in courtship with less responsive (and likely less receptive) females. Unfortunately, female *P. shermani* exhibit minimal observable (to our eyes) receptivity signals during the orientation and persuasion stages of courtship. Receptive females simply either do not move away or do not move as far away when a male approaches her (Arnold, 1976).

In addition to behavioural signals, chemical cues may be indicators of female reproductive condition in salamanders. In many salamander species, including other plethodontids, males can distinguish between gravid and nongravid females using only olfactory cues (*Plethodon vehiculum* and *Plethodon dunni*: Marco et al., 1998; *Plethodon cinereus*: Dantzer & Jaeger, 2007; *N. viridescens*: Verrell, 1985; *T. vulgaris*: Verrell, 1986). *Plethodon shermani* males are therefore likely to have this same ability; other members of the *P. glutinosus* complex can use volatile cues to identify the sex, species and identity of conspecifics (Dawley, 1984; Palmer & Houck, 2005). As our fecundity-based experiments were performed in the females' home boxes, there were likely many semiochemicals with which the male could

identify and assess individual females. The sophisticated chemical signalling system in this family of salamanders (including the olfactory detection of gravidity) strongly supports the conclusion that chemical cues probably serve as an indicator of female reproductive condition and thus can facilitate male mate choice. In future experiments, we plan to test this hypothesis through cross-validation studies, exchanging gravid/nongravid females with chemical cues of the other reproductive class.

Overall, the results of the present study revealed that male *P. shermani* demonstrate mate choice through differential investment in their visual display in the earliest stages of courtship. Males invested the most in strongly gravid females and did not show differential investment between nongravid and weakly gravid females. Male mate choice and courtship effort is impacted by male condition in *P. shermani* (Eddy et al., 2012). As the amount of foot dancing was positively correlated with courtship success, males with higher condition are likely to be more successful in courtship. Unlike many other instances of male mate choice, males probably do not use female size as the primary cue of female reproductive value but may be directly assessing female gravidity through olfactory mechanisms or behavioural signals. Plethodontid salamanders have long been studied in regard to female mate choice (e.g. Chouinard, 2012; Mathis, 1991; Walls et al., 1989), but seemingly less attention has been paid to potential importance of male mate assessment (see Dantzer & Jaeger, 2007; Marco et al., 1998; Verell, 1995). Because of the apparent energetic and temporal constraints placed upon *P. shermani*, it is likely that males are also under selective pressure to be discriminating of their potential mating partners. Further research will help us better understand the complexities of mutual mate assessment and selection by both sexes in vertebrate mating systems.

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Figure 1. Foot-dancing effort by male red-legged salamanders ($N = 18$) for three female reproductive conditions (G: strongly gravid; WG: weakly gravid; NG: nongravid). Boxes represent interquartile range and middle line marks the median for each group. $*P < 0.001$.

Figure 2. Foot-dancing effort by male red-legged salamanders in relation to female size. Individual data points are fitted mixed-effect estimates of the proportion of time that individual males ($N = 40$) spent foot dancing with females of varying snout–vent length (SVL); the line of best fit is shown.

figure 1

figure 2