

Nonvisual mate choice in the Pyrenean mountain newt (*Euproctus asper*): females prefer small males

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Abstract In many species, females prefer large males as mates. In this paper, we examined whether female Pyrenean mountain newts (*Euproctus asper*) would show a preference for large males in a simultaneous choice situation. When we tested for a visual preference relative to male body size using visual cues, females did not prefer large males. When chemical cues were presented, females did show a preference, but contrary to our prediction, they preferred the smaller of two males. *E. asper* males exhibit coercive mating behavior (amplexus), keeping females from feeding for several hours or even days, which may explain why females avoid large, physically stronger, and thus, more coercive males. The predominantly nocturnal lifestyle may explain why visual cues are less important for mate discrimination in this species.

Keywords Cave animals · Chemical communication · Mate choice · Mating preferences · Sexual conflict

Introduction

Mate choice for large body size has been described for numerous species throughout the animal kingdom (Andersson 1994; Ryan and Keddy-Hector 1992). In many species, individuals use visual cues to assess the size of their mates (e.g., Schlupp et al. 1994; Rosenthal and Evans 1998; Plath et al. 2004, 2006), but nonvisual cues (such as pheromones) can also be employed for this purpose (e.g., Marco et al. 1998; LeMaster and Mason 2002; Poschadel et al. 2006). For example, females of the surface-dwelling Mexican fishes *Poecilia mexicana* (Poeciliidae) and *Astyanax fasciatus* (Characidae) have an intrinsic preference for large males, which is mediated exclusively by visual cues (Plath et al. 2004, 2006). On the contrary, in the cave-dwelling forms of those species, which live in completely lightless subterranean habitats, females use nonvisual cues for selecting large males (Plath et al. 2004, 2006). In such studies, a female can typically choose between two males differing in body size in a simultaneous choice situation.

In the present study, we examined female mate choice regarding male body size in the Pyrenean mountain newt, *Euproctus asper* (Salamandridae, Urodela). The Pyrenean mountain newt is an aquatic species inhabiting high altitude mountain creeks in the French and Spanish Pyrenees with a predominantly nocturnal lifestyle (Montori 1988; Clergue-Gazeau 1999). They inhabit the uppermost springheads of creeks and effluents of cold mountain lakes (Salthe and Meham 1997; Clergue-Gazeau 1999). Mountain newts mostly hide under stones and in crevices of rocks to avoid the very strong water current and predation. Furthermore, *E. asper* is a facultative cave dweller, which means that several cave-dwelling populations have been described, but individuals inhabiting caves show no pronounced morphological divergence from typical surface-dwelling newts

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(i.e., eyes and pigmentation are not reduced like in other cave animals; Clergue-Gazeau 1969, 1971, 1999; Guillaume 2001). This suggests that the cave populations are not isolated and genetically differentiated, but that exchange with adjacent surface populations may still occur. Indeed, some creeks in the French Pyrenees that are inhabited by *E. asper* may desiccate in the summertime, and the newts appear to seek shelter near the groundwater in the furrowed, cragged underground (O. Guillaume, personal communication), thereby possibly entering caves.

The predominantly nocturnal lifestyle of *E. asper* and its ability to survive in subterranean habitats suggests that nonvisual communication could play an important role for the behavior of this species, but visual cues may also play a role for the assessment of male traits—at least in surface habitats. Therefore, we asked which role visual and nonvisual cues play for female mate choice in *E. asper*. Specifically, we gave surface-dwelling females a choice between a large and a small male when (a) only visual cues, or (b) only nonvisual (chemical) cues from the males could be assessed. Because female choice for large male size is a common phenomenon in many taxa (Andersson 1994; Ryan and Keddy-Hector 1992), we predicted female *E. asper* to prefer to associate with the larger of the two males.

Materials and methods

Animals and housing conditions

Seven females and 12 males of *E. asper* were maintained at the Biozentrum Grindel in Hamburg for at least 1 year before the tests. Individuals originated from Río Flumen (Sierra Bones near Huesca, Spain), or they were descendants of animals collected near Aulus-les-Bains (Ariège, France). Three additional females of French origin were tested in the Laboratoire Souterrain de Centre national de la Recherche Scientifique (CNRS) in Moulis under conditions identical to those in Hamburg (two in experiment 1 and one in experiment 2). Due to the limited number of specimens available, we could not use different males (large vs small) for each trial, but we maximized the number of different combinations of individual stimulus male pairs. Hence, a male of intermediate size may have been used as a “smaller male” when paired with a very large male, but may have acted as “larger stimulus male” when paired with a very small male. Large males were probably older than smaller males, but the exact age was unknown for most animals. All males used in the experiments were sexually mature; that is, they were observed showing sexual behaviors.

All newts were maintained in several large aerated and filtered stock tanks at 11°C under an artificial 12:12 h light–dark cycle. The water level in the stock tanks was

18–20 cm. Stones, bricks, and artificial, as well as live, plants provided shelter. The newts exhibited intense mating behavior, and females frequently laid eggs. Newts were individually identified by color differences, namely, the pattern of ventral black spots, size differences, or, in some cases, missing toes. All specimens were isolated in small aquaria (25×16.5×18 cm), filled with water to 10 cm in height, for at least 1 week before the tests. A tile served as a hiding place. The sides of the tanks were covered with black plastic foil, preventing any visual contact between individuals. All newts were fed twice a week with live *Daphnia*, live *Tubifex* sp., and frozen chironomid larvae, and the water was exchanged on the following day to avoid the presence of chemical cues other than those originating from the newts. Individual females were tested in two experiments (see below). The order of the experiments was random. After each trial, females were placed into their individual tanks again, and were not tested earlier than at least 1 week after the last trial.

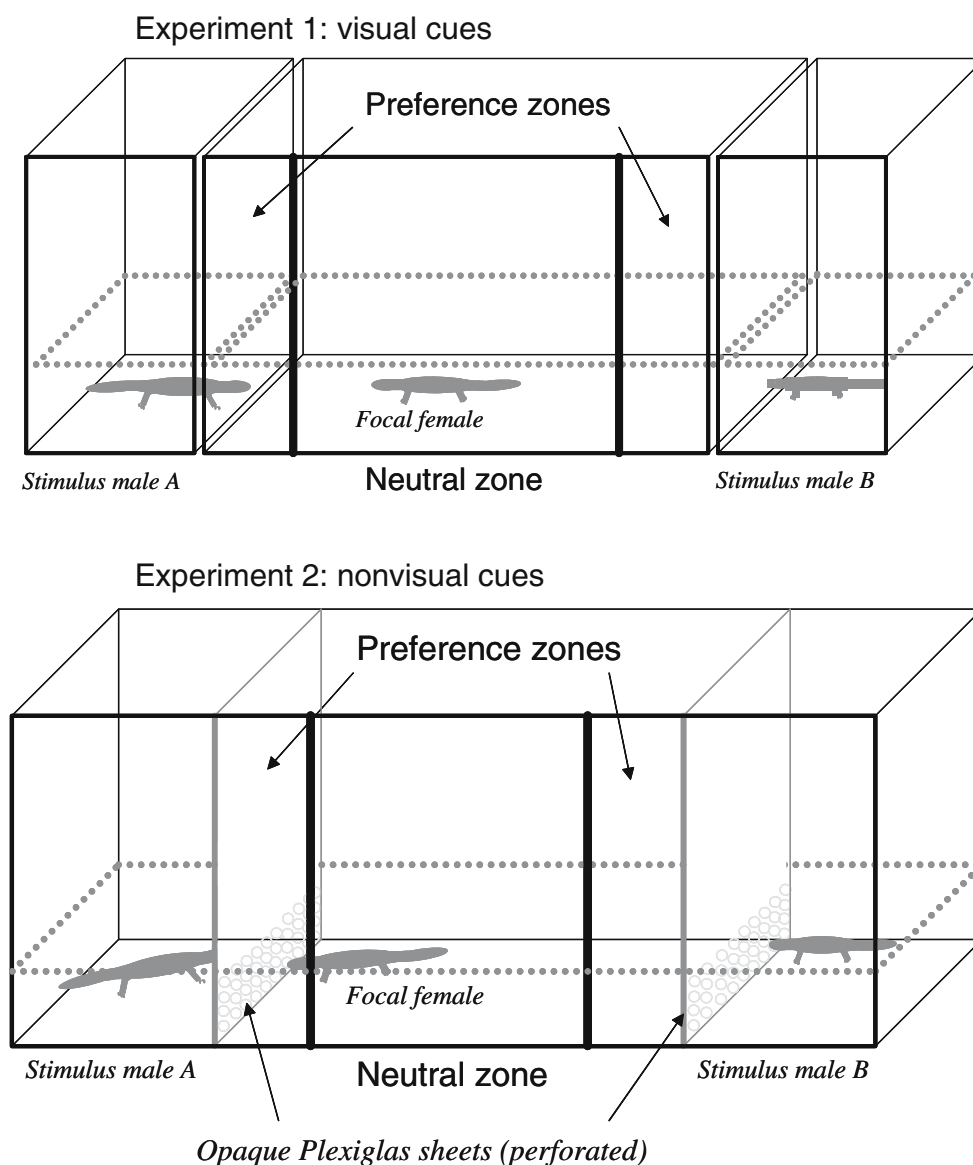
Choice tests

Experiment 1: visual cues

For the visual mate choice experiment, we placed two small stimulus tanks (30×19×19.5 cm) directly adjacent to the two outer sides of a test aquarium (50×30×29 cm; Fig. 1). All three tanks were filled with water to 5 cm in height. The bottom and the back of the test tank, as well as the bottom and two outer sides of the stimulus tanks, were covered with brown plastic foil. This setup guaranteed that focal animals had only visual contact with the males inside the stimulus tanks. A 60-W lamp centrally above the test tank provided light. We marked two lateral preference zones (8 cm width) adjacent to each of the stimulus tanks by vertical lines, drawn on the front of the test tank. White opaque plastic sheets were placed between the three tanks to prevent visual contact before the test was started.

To initiate a trial, two males (a large and a smaller one) were introduced into each of the stimulus tanks. A focal female was then placed into a Plexiglas cylinder in the center of the test tank. After 5 min of acclimatization, the cylinder and the opaque plastic sheets were gently removed. A camera, installed approximately 1 m in front of the test tank, transferred the signal to a monitor in a neighboring room, where the trials were scored directly. As mountain newts move slowly and may rest for considerable time periods, time measurements were started when a focal animal had moved into one of the preference zones and both front legs were inside the preference zone. For a period of 15 min, we measured the time the female spent in each preference zone. Then, the opaque sheets were positioned between the three aquaria again, the stimulus

Fig. 1 Experimental setup. For details see main text



animal was placed into the opposite stimulus tank, and the focal animal was placed into the cylinder again. After 5 min, the measurement was repeated. This procedure was conducted to avoid a possible influence of side bias. Time measurements from both parts of the trial were summed before statistical evaluation.

Experiment 2: chemical cues

For the nonvisual mate choice experiment, we used a larger (81.5×31.5×30.5 cm) test tank filled with tap water to 8 cm in height. The bottom and three sides were covered with brown foil. Two opaque plastic sheets divided two stimulus compartments (17 cm) from the center (Fig. 1).

The sheets were perforated by four rows of each 18 small holes (2-mm diameter) at their lower end so that water-soluble pheromones could pass through. Two vertical

lines, drawn on the front of the central compartment, visually divided two preference zones (each 10 cm), adjacent to the stimulus compartments, from a central neutral zone. Before each test, two additional nonperforated opaque plastic sheets were fixed in parallel to the perforated sheets (see above) to prevent exchange of water before a trial was started. To initiate a trial, a large and a smaller male were placed into the outer compartments (one male per side; Fig. 1). Additionally, 1 l of water from the residence tank of the stimulus animal was carefully poured into the respective compartment. The stimulus males had spent at least 24 h without feeding in their holding tanks before the test. A focal female was then placed into a Plexiglas cylinder in the center of the tank. After 5 min of acclimatization, the cylinder and the two nonperforated plastic sheets were gently removed. We measured the time the females spent in each of the preference zones during a

20-min observation period. After every trial, the tank and plates were thoroughly cleaned with hot water, KMnO_4 solution, and ethanol and were then thoroughly rinsed with tap water. All individuals involved were measured for body length from the head to the tip of the tail. Males classified as large were 12.2 ± 0.2 cm long (mean \pm SD), those classified as small were 10.5 ± 0.3 cm long, and the focal females were 13.3 ± 1.4 cm long.

Results

We compared the amount of time the *E. asper* females spent near both types of males. In the visual cues experiment, females spent roughly the same time near large and small males (Fig. 2a). There was no statistically significant difference in association times (paired *t*-test: $t_9 = 0.018$, $P = 0.99$). Five females spent more time near the larger, and five spent more time near the smaller male.

When chemical cues were presented, the focal females showed a significant preference (paired *t*-test: $t_7 = -2.67$, $P = 0.032$); however, contrary to our prediction, they preferred the odor of the smaller male (Fig. 2b). Seven of the eight females (88%) preferred the smaller male.

Discussion

Chemical communication has been reported as a pattern of social behavior for a variety of amphibians, such as terrestrial salamanders (Dawley 1986; Houck and Reagan 1990; Jaeger and Gabor 1993; Simons et al. 1994), newts (Kikuyama et al. 1997; Kikuyama and Toyoda 1999; Yamamoto et al. 2000; Park and Propper 2001), and frogs (Waldman and Bishop 2004). Chemical communication may be especially relevant for a nocturnal species like *E. asper*, in which the possibility for visual communication is limited.

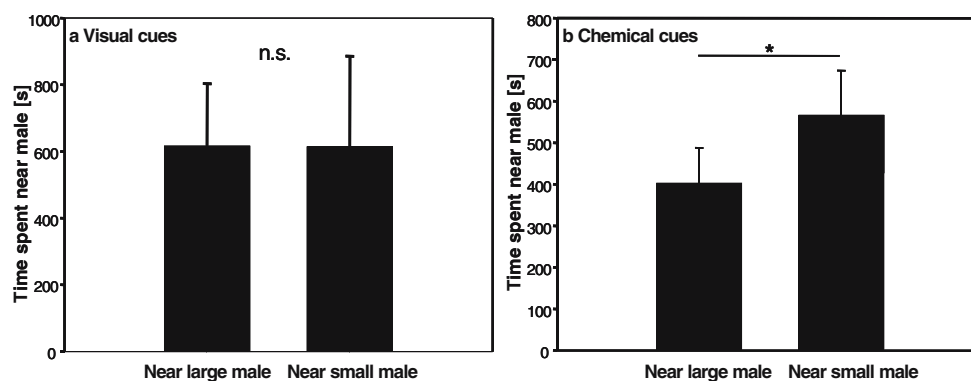
Sample sizes are low in this study, because only a small number of animals could be maintained in the laboratory.

Therefore, the nonsignificant result in experiment 1 (visual cues) needs to be considered with caution due to low statistical power. We did not collect more animals in the field, because *E. asper* is a protected species, endemic to a relatively small area in Europe. However, our data suggest that nonvisual communication using chemical cues is more important than visual communication in *E. asper*.

There is clear evidence that *E. asper* use chemical cues to detect hiding places that have previously been marked by conspecifics (Parzefall et al. 2000). Parzefall et al. (2000) also found evidence for a response of both males and females to water-soluble pheromones in a binary Y-maze choice experiment in which “stimulus water” (two males and two females) vs “control” water was filled into two choice tubes (Parzefall et al. 2000). In a previous study using the same setup as in the present study, we gave single *E. asper* an opportunity to choose between a conspecific and no stimulus (Poschadel et al. 2005). When focal females were presented with cues from male stimulus animals, they showed no significant discrimination when visual cues were available, but significantly preferred the compartment with a male over the empty compartment when chemical cues were presented (Poschadel et al. 2005). The results from the present study confirm our previous results that water-soluble pheromones play an essential role for communication in this species. Based on the observation that females sometimes sniff the male cloacal region (Behrens 1995; Thiesmeier and Hornberg 1986), the cloacal glands of *E. asper* likely are a source of pheromone production (Guillaume 2000). We conclude that *E. asper* females are generally attracted by the odors of males (Poschadel et al. 2005), but, when they have a choice between two males (this study), they prefer the smaller of two males as mating partner.

Females clearly preferred the odor of a smaller male over the odor of a larger male (experiment 2). Potentially, large and small *E. asper* males differ in the composition of (water-soluble) pheromones. For example, it has been shown that, in garter snakes (*Thamnophis sirtalis*), small and large females differ in the composition of skin lipids

Fig. 2 Female choice in *E. asper*. A focal female was given the opportunity to choose between two males (a large and a small one), while (a) only visual cues were available or (b) chemical cues were presented. The mean (\pm SD) time spent near both types of males. * $P < 0.05$; n.s. not significant (paired *t*-tests)



(LeMaster and Mason 2002). An alternative explanation would be that females alter their response in relation to the concentration of male pheromones in the water, assuming that the amount of pheromones produced increases with large male body size. It remains unsolved so far which mechanism *E. asper* females use to estimate the size of conspecific males. Experiments by Kamio et al. (2005) revealed a stronger response of female helmet crabs (*Telmessus cheiragonus*) to higher concentrations of male sex pheromones. Similar experiments with *E. asper* would offer an elegant approach to the question whether differences in the chemical structure of the pheromone(s) or different concentrations are responsible for the described size discrimination in this species.

Another potential explanation would be that females also detect vibrational cues from males. To rule out this possibility, or to determine the relative roles of chemical and vibrational cues on female mate choice behavior in this species, we propose a future experiment in which only “stimulus water” is presented to the focal female, but no males are present in the test tank.

The observed female preference for small males in *E. asper* seems to be an exception from the general pattern of females typically preferring large males (Andersson 1994; Ryan and Keddy-Hector 1992). Female preferences for small males in this species may be explained in the context of coercive male mating behavior (see also Watters 2005 for salmon, *Oncorhynchus kisutch*): Male *E. asper* use amplexus, during which the male grasps and holds the female for an extended time (Despax 1923; Ahrenfeldt 1955; Salthe 1967). The costs of this behavior for females may include reduced feeding opportunities and increased predation risk. Amplexi can last for several hours or even days (Thiesmeier and Hornberg 1986, 1990), although spermatophores are usually transferred by males within the first minutes of an amplexus (O. Guillaume, personal communication). Females typically try to escape from males during amplexus (M. P., personal observation). It is tempting to speculate that small, physically weaker males are less successful in monopolizing females and that females are more successful escaping from smaller males. A preference for less harassing males has also been demonstrated for female Japanese quail (*Coturnix coturnix japonica*), which avoid males that they had seen winning fights with other males (Ophir and Galef 2003; Persaud and Galef 2003). As most spermatophores are transferred within a few minutes in *E. asper* (see above), even short copulations with small males are probably sufficient to guarantee complete fertilization of a female’s oocytes.

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