Ethology **104,** 781—788 (1998)

ISSN 0179-1613

Department of Zoology, Oregon State University, Corvallis

Mate Choice by Chemical Cues in Western Redback (*Plethodon vehiculum*) and Dunn's (*P. dunni*) Salamanders

Adolfo Marco, Douglas P. Chivers, Joseph M. Kiesecker & Andrew R. Blaustein

Marco, A., Chivers, D. P., Kiesecker, J. M. & Blaustein, A. R. 1998: Mate choice by chemical cues in western redback (*P. vehiculum*) and Dunn's (*P. dunni*) salamanders. Ethology **104,** 781—788.

Abstract

Western redback and Dunn's salamanders (*Plethodon vehiculum* and *Plethodon dunni*, respectively) can distinguish between potential mates by using chemical cues. In laboratory choice tests, adult males of both species showed significant discrimination between chemical cues of gravid females over non-gravid females of equal body size. Furthermore, males of both species differentiated the odour of paired gravid females that differed by ≈ 5 mm snout–vent length (SVL). Given that clutch size is related to female body size in these species, adult males may be able to distinguish between females via cues that signal potentially high female reproductive success. In choice tests, *P. vehiculum* females did not discriminate between two relatively large males that differed by ≈ 5 mm SVL. However, females of *P. vehiculum* did discriminate between two relatively small males that differed by the same amount. Apparently, *P. vehiculum* females ranked males by both absolute and relative body size using chemical cues. This pattern could reflect a female preference for large males or that females avoid mating with the smallest males.

Corresponding author: Adolfo Marco, Departamento de Biología Animal, Universidad de Salamanca, E-37071, Salamanca, Spain. Email: marcoa@gugu.usal.es

Introduction

Chemical signals are important in a variety of contexts in animal communication, but they have not been well studied with regard to sexual selection (Andersson 1994). Although it has been proposed that chemical signals are important for mate choice among vertebrates (Blaustein 1981; Macdonald et al. 1990), there are few experimental tests of chemical signals in mate choice.

Chemical cues appear to be especially important in plethodontid salamanders (Dawley 1992; reviewed in Jaeger & Forester 1993; Houck & Woodley 1995). For example, chemical cues function in species recognition, reproductive isolation (Ovaska 1989; Arnold et al. 1993; Uzendoski & Verrell 1993), and territorial advertisement (Mathis 1990; Ovaska & Davis 1992). Furthermore, male courtship pheromones can have a short-term stimulating effect in female salamanders (Houck & Reagan 1990). Despite the considerable number of studies on the chemical

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ecology of salamanders, few studies have examined the importance of chemical cues in mate choice (Verrell 1985, 1995; Dawley 1992; reviewed in Mathis et al. 1995). In one study, Walls et al. (1989) found that red-backed salamander *Plethodon cinereus* females preferentially investigated faecal pellets of males with a high-quality diet over those of males with a low-quality diet. Mathis (1990) suggested that faecal pellets of males are of primary importance in the attraction of mates, but she did not test choice of males by females.

Plethodontid salamanders show significant variation in mating success in experiments where partners are presented sequentially (Houck et al. 1985). In some studies, males chose large females when given a simultaneous choice between two females (Verrell 1989, 1995). This strategy could be adaptive because there is a strong relationship between female body size and clutch size (Kaplan & Salthe 1979; Verrell 1995). However, females may mate with several males and may not reject small and inexperienced males when they are the only partners available (Houck 1988; Verrell 1991). These studies provide limited evidence of female mate choice in plethodontid salamanders (reviewed in Sullivan et al. 1995). However, large males could have either a higher survival capacity or a greater growth rate, which could be advantageous to offspring if heritable. Large *P. cinereus* males may also have better quality territories (Mathis 1991).

In laboratory experiments, we tested whether adults of *Plethodon vehiculum* and *Plethodon dunni* can determine the quality of potential mates using chemical signals, during their natural mating period. Plethodontids from the coastal mountains of Oregon, USA, have a prolonged mating period (autumn to spring), with oviposition occurring in spring and early summer (Nussbaum et al. 1983; Blaustein et al. 1995). These species show patchy distribution, usually occupying steep rocky areas in close proximity to streams (Dumas 1956). These areas can have relatively high population densities and multiple captures (adults within 10 cm of each other) are frequent (Dumas 1956; Ovaska 1988, 1993; pers. obs.), as occurs in other plethodontids (Jaeger 1980; Mathis 1990). In choice tests, adult males of both species were exposed to (1) chemical cues of gravid females vs. non-gravid females, both of similar size, and (2) chemical cues of large gravid females vs. small gravid females. Gravid *P. vehiculum* females were also tested for their ability to distinguish between chemical cues of large vs. small adult males. If salamanders can distinguish potential mates using chemical cues, we hypothesize that males will choose the cues of gravid and large females, and females will choose the cues of large males.

Materials and Methods

Collection and Maintenance of Salamanders

In the autumn of 1995, we collected *P. vehiculum* and *P. dunni* adults from the Oregon Coastal Mountains at Fall Creek, 10 km west of Alsea, Lincoln County, Oregon (250 m above sea level). We differentiated classes of salamanders as follows. Adult males were those salamanders that possessed a mental gland and protruding teeth. Gravid females were those that possessed ova visible through the abdominal wall. Non-gravid females were those individuals having a body size typical for gravid females, but lacking visible ova, a mental gland, or protruding teeth. Salamanders were maintained in individual Petri dishes (150 mm diameter, 25 mm deep) with a damp paper towel substrate. They were kept on a 14 h light/10 h dark photoperiod at 13–16°C and were fed periodically with crickets. Animals were in captivity for ≈3 months. They were returned to the wild at the end of the study.

General Methods

We conducted five experiments in the laboratory at 20°C during the natural mating period of each species (25 Jan.–29 Feb. 1996). The average snout–vent length (SVL) of test and donor salamanders are provided in Table 1. Each test animal was used in only one trial.

As odour sources, we used the paper towels that had been used as substrates for salamanders for 6 d prior to the experiment. Salamanders were not fed during this 6-d period, thus towels did not bear cricket debris. However, paper towels did bear salamander faecal pellets. For each trial, we used a rectangular plastic container (32 × 18 × 8 cm) as a testing chamber. Each half of the floor of a container was lined with paper towels from the appropriate stimulus salamanders. The placement of the stimulus paper towels on the left or right side of the container was determined randomly. Paper towels were placed 2 cm apart to minimize diffusion of cues between sides. Before each test, paper towels were sprayed with dechlorinated water to control for differences in moisture level between the sides.

For each trial, we introduced a single salamander into the centre of the container and recorded the side occupied by the salamander every 2 min for 1 h. If the salamander was located in the middle of the enclosure we used the position of the salamander's snout to assign location. All containers were carefully rotated 180° every 15 min to avoid any bias from salamander orientation in the room.

Table 1: Mean (±SD) body size (mm) of test and donor salamanders used in the three experiments

	N	Test	Donor 1	Donor 2
Plethodon dunni				
1	16	m 64.3 (3.65)	Gf 68.8 (2.93)	NGf 67.2 (2.53)
2	14	m 61.1 (3.91)	Lf 71.3 (1.55)	Sf 66.3 (0.64)
3	8	f 68.8 (2.93)	Lm 67.4 (1.39)	Sm 62.1 (1.17)
Plethodon vehiculum				
1	16	m 47.4 (2.73)	Gf 52.1 (1.74)	NGf 51.0 (2.12)
2	21	m 48.2 (3.07)	Lf 56.4 (1.87)	Sf 51.6 (1.33)
3	23	f 53.8 (2.42)	Lm 50.5 (1.49)	Sf 45.5 (1.33)

f = Female, m = male, G = gravid, NG = non-gravid, S = small, and L = large.

Analyses of Data

For each trial, we calculated the number of observations out of 30 (one observation every 2 min for 60 min), that the test animal spent on each side of the testing container. We then used a Wilcoxon-signed ranks test (two-tailed) to determine whether the salamanders spent significantly more time on one stimulus side compared with the expected 50% (15 observations). In experiments of female mate choice we used the Spearman correlation index to test the possible effect of the size of the donor animals on the number of observations of the test animals on the predicted side of the containers.

Results

Experiment 1. Male Discrimination Between Cues of Gravid vs. Non-gravid Females

In 14 out of 16 trials, males were more often found on the side of the testing container with the cues of gravid females than non-gravid ones. The median number of observations spent on the gravid female's side was significantly greater than 50% (Wilcoxon test, $T = 3.10$, $p = 0.004$, $n = 16$, Fig. 1). There was no difference in body size between gravid and non-gravid donor females of *P. dunni* (matched pairs t-test: $t = 1.656$, $p = 0.108$, $n = 16$, Table 1).

P. vehiculum males also had significantly greater than 50% of their observations on the side of the testing chamber with odours from gravid conspecific females (Wilcoxon test, $T = 3.54$, $p < 0.001$, $n = 16$, Fig. 1). All 16 males tested were more frequently found on the side with cues from the gravid female. There was no difference in body size between gravid and non-gravid donor females (matched pairs t-test: $t = 1.676$, $p = 0.104$, $n = 16$, Table 1).

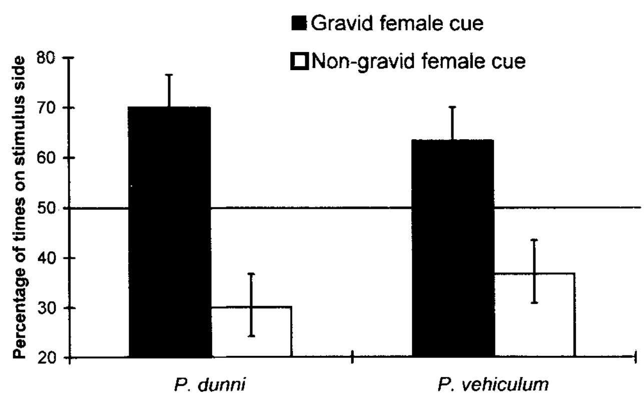

Fig. 1: Male choice by chemical cues of gravid vs. non-gravid females for *Plethodon dunni* and *Plethodon vehiculum*. Median percentage (± quartile) of times that males were on each side of the chamber

Experiment 2. Male Discrimination Between Cues of Large vs. Small Gravid Females

The SVL difference between the paired gravid *P. dunni* donor females was, on average, 5.05 mm (SD = 0.785, range = 4.9–6.2 mm, Table 1). Eleven out of 14 *P. dunni* males were more often located on the side of the chamber with cues from the larger female. The median number of observations on the side with cues from the larger female was significantly greater than 50% (Wilcoxon test, $T = 2.95$, $p = 0.006$, $n = 14$, Fig. 2).

For *P. vehiculum*, SVL differences between large and small donor females were, on average, 4.78 mm (SD = 0.688, range = 4–6 mm). Sixteen out of 21 males of this species were more often located on the side with the cues from the large female. The median number of observations on the side with cues from the larger female was significantly greater than 50% (Wilcoxon test, $T = 2.45$, $p = 0.028$, $n = 21$, Fig. 2).

Experiment 3. Female Discrimination Between Cues of Large vs. Small Males

The SVL differences between large and small donor males of *P. vehiculum* were, on average, 5.00 mm (SD = 0.337, range = 4.5–5.5 mm). Sixteen out of 23 females were more often located on the side of the testing chamber with cues from the larger male vs. the smaller male. This difference was not significantly different from random; however, a trend was apparent (Wilcoxon test, $T = 2.126$, $p = 0.067$, $n = 23$). Subsequent analysis showed that there was a negative correlation between the number of observations of females on the side with the large male cues and the body sizes of the smaller donor males (Spearman correlation, $r_S = -0.57$, $p < 0.01$, $n = 23$). This result indicates that females can determine both relative and absolute male body sizes based on chemical cues. Average body sizes are shown in Table 1.

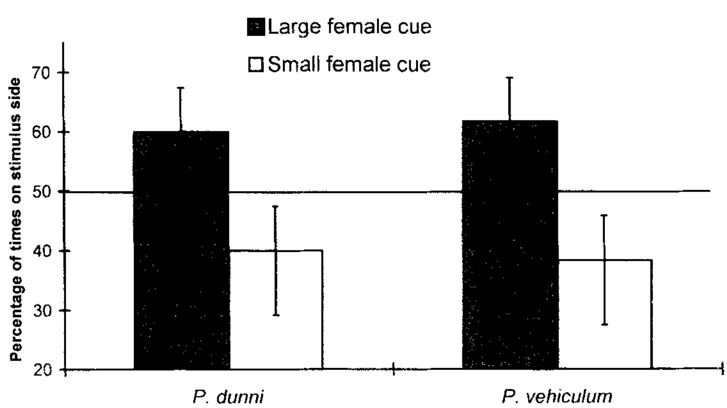

Fig. 2: Male mate choice by chemical cues of large vs. small females for *Plethodon dunni* and *Plethodon vehiculum*. Median percentage (±quartile) of times that males were on each side of the chamber

Discussion

Our results suggest that chemical cues play an important role in mate choice in plethodontid salamanders. Our experiments showed that males of these species can distinguish whether females are gravid or non-gravid based on chemical cues. Further, males of both species distinguished differences in female body size based on chemical cues and associated more often with the cues of larger females.

Female plethodontids show great variation in their reproductive success in a given breeding season. *P. vehiculum* females usually have a biennial reproductive cycle and, consequently, only a portion of adult females lay eggs in a given year (Peacock & Nussbaum 1973). The reproductive cycle of *P. dunni* is less well known than that of *P. vehiculum*. During the mating period, we found females of *P. dunni* with developed eggs and others without them. Consequently, *P. dunni* likely does not have an annual reproductive cycle and only a portion of females lay eggs during any given year. Clutch size is also related to female body size in both *P. vehiculum* (Peacock & Nussbaum 1973) and *P. dunni* (Nussbaum et al. 1983). Given that in our experiments males discriminated among the chemical cues of different females, we assume that there are qualitative or quantitative differences among the chemical cues of female salamanders in different reproductive condition or with different clutch sizes. Males discriminating these differences may mate more often with more fecund females and may consequently increase their own reproductive success.

Mathis (1990) showed that male and female red-backed salamanders gain information about the size of conspecifics through chemical signals associated with faecal pellets. She suggested that females use this information mainly in territorial advertisement. During the mating period, female pheromones in faecal pellets could also be used by male plethodontids, perhaps to choose larger females as mates or to distinguish between females in different reproductive conditions.

Substances related to gravidity could be detected by males. During late stages of yolk formation, female salamanders have high levels of vitellogenin, gonadotropins and oestrogens (Zerani et al. 1991; Jorgensen 1992). These substances show seasonal fluctuations, with a pattern that follows that of the gonadal steroids (Herman 1992). Perhaps non-gravid or small female plethodontids have low levels of these substances and males could assess, using chemical cues, different levels of these substances.

Plethodontid females mate with multiple males, mate with small and inexperienced individuals, store sperm and exhibit sperm competition (Houck et al. 1985; Verrell 1991). In *Desmognathus ocoee*, there is no female preference related to male body size, male age, or male sexual experience (Houck & Francillon-Vieillot 1988; Verrell 1991). In *P. cinereus* there is some evidence of female preference for males feeding on superior prey items (Walls et al. 1989; Jaeger & Wise 1991) and larger males that hold superior territories (Mathis 1991). In our experiments, *P. vehiculum* females did not choose between the odour of different sized males when the males were relatively large. However, females did preferentially associate with the cues of the larger male when the two donor males were relatively small. The quality or the ability to produce attractive chemical signals to females could be limited in very small or young adult males.

Acknowledgements

We thank Lynne Houck, Paul Verrell, Alicia Mathis and Lee Kats for their helpful suggestions on the manuscript and Erica Wildy, Jill Devito, Lisa Belden and Mike Anderson for their help in collecting salamanders and commenting on the manuscript. This study was supported by Oregon State University (Department of Zoology Research Funds), the Ministry of Education and Science of Spain (Grant E95–16796691), the Natural Sciences and Engineering Research Council of Canada and the National Science Foundation of USA (Grant DEB 9423333).

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Received: October 20, 1997

Accepted: March 2, 1998 (J. Brockmann)