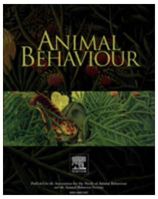
Animal Behaviour 80 (2010) 983–989

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav



A pheromone mechanism for swaying female mate choice: enhanced affinity for a sexual stimulus in a woodland salamander

Elyse A. Vaccaro[a,*], Pamela W. Feldhoff[b,1], Richard C. Feldhoff[b,1], Lynne D. Houck[a]

[a] Department of Zoology, Oregon State University, Corvallis
[b] Department of Biochemistry and Molecular Biology, University of Louisville School of Medicine

ARTICLE INFO

Article history:
Received 2 March 2010
Initial acceptance 26 May 2010
Final acceptance 20 August 2010
Available online 26 September 2010
MS. number: A10-00142R

Keywords:
amphibian
animal signal
chemical communication
female preference
locomotion
mate choice
pheromone
Plethodon shermani
red-legged salamander
sexual motivation

Traditionally, male signals have been thought to function in satisfying female choice by conveying meaningful information about a potential mate. However, the male signal, rather than merely providing raw material for female evaluation, may actively modulate a female's intrinsic preferences or decision-making capabilities in favour of a given male. We propose two broad mechanisms by which male signals could modulate female behaviour: (1) specific augmentation of sexual motivation or (2) heightened general arousal. Specifically, we investigated the ability of a generic male pheromone mix to elicit changes in general activity or affinity for different classes of stimuli in female terrestrial salamanders (*Plethodon shermani*). Attraction to male olfactory stimuli was significantly increased by pheromones, but attraction to visual stimuli and nonsexual olfactory stimuli remained unaffected, as did locomotor activity. These results are consistent with the hypothesis that sex pheromones activate specific behavioural subsystems associated with augmented sexual motivation. This pheromone action may still function within the context of information-transfer signalling, for example, if pheromones influence female choice by affecting (1) sensory processing of relevant stimuli, (2) the value assigned to a set of sexual stimuli, or (3) the criteria used to decide whether to mate.

Within the paradigm of the signalling male and the discriminating female, sexual selection is dictated by the primacy of satisfying female choice. Sexual signals have been researched in terms of the personal information (such as sex, strength, status, size, age) that a signaller can convey to a potential mate. This information is gathered from multiple senses and integrated over an array of male characteristics, then used to assess male attractiveness (Burley 1981; Zuk et al. 1992). This assessment, or female preference, dictates how mate quality is determined and therefore drives mate choice. However, can sexual signals influence mate choice by means other than conveying information for female assessment?

Ongoing debate over the definition of information has caused some researchers to call for an eschewal of the idea of a message or meaning in animal communication and instead focus on the effect of the signal on its receiver (Dawkins & Krebs 1978; Rendall et al. 2009). Regardless of whether semantic information (Krebs & Dawkins 1984) is conveyed by the male signal, many factors are known to influence female preference and mate choice without directly providing input for mate assessment. Female preference can vary both among individuals and within an individual, and is susceptible to the vicissitudes of (1) reproductive state (Lea et al. 2000; Lynch et al. 2005) (2) previous experience (Kodric-Brown & Nicoletto 2001; Aisenberg & Costa 2005) and (3) social context (e.g. cues from conspecifics: Alonzo & Sinervo 2001; Royle et al. 2008). Additionally, factors unrelated to mate preference can influence mate choice, including female physiological condition (e.g. food energy reserves: Dickerman et al. 1993; Jones & Lubbers 2001; Fisher & Rosenthal 2006) and ecological context (e.g. threat of predation: Moore & Miller 1984; Moore & Zoeller 1985). Finally, male signals can act as sensory modulators that activate female-specific behaviours. Recent studies on pheromone effects on female mice (Kimchi et al. 2007) and mating calls in túngara frogs (Hoke et al. 2008) indicate that male signals can trigger a sex-specific 'sensory switch' or neural 'gatekeeper nucleus' that regulates

---

[*] Correspondence: E. A. Vaccaro, Department of Zoology, Oregon State University, 3029 Cordley Hall, Corvallis, OR 97331, U.S.A.
E-mail address: vaccaroe@science.oregonstate.edu (E.A. Vaccaro).

[1] P. W. Feldhoff and R. C. Feldhoff are at the Department of Biochemistry and Molecular Biology, University of Louisville School of Medicine, Louisville, KY 40292, U.S.A.

0003-3472/$38.00 © 2010 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.anbehav.2010.08.020

female behavioural response to male stimuli. Thus, the male signal, rather than being merely the raw material for female evaluation, may actively modulate a female's intrinsic preferences or decision-making criteria in favour of a given male.

By definition, pheromones have held unique status among animal communication signals. Karlson & Luscher (1959, page 55) characterized pheromones as 'substances which… release a specific reaction, for example, a definite behavior or a developmental process'. Thus, these signals were defined as immediate modifiers of recipient behaviour or eventual modulators of recipient physiology rather than information to be evaluated by the receiver. In this context, sex pheromones and their potential role influencing mating decisions are of particular interest. Plethodontid salamander pheromones, like many other sex pheromones, are chemical signals that can coordinate or otherwise influence male–female mating interactions. However, plethodontid pheromones belong to a distinct subclass of 'courtship pheromones' that are delivered by the male only after initial contact with a female, and only if the female is not immediately responsive to the male's overtures (Arnold 1976; Houck & Sever 1994). These pheromones activate distinct neural pathways to areas of the brain known to regulate mating behaviour (Schmidt et al. 1988; Wirsig-Wiechmann et al. 2002, 2006; Laberge et al. 2008). Behavioural studies of plethodontid salamanders have demonstrated pheromone-induced enhancement of sexual receptivity, as measured by decreased courtship duration (Houck & Reagan 1990). The effect of courtship pheromones in reducing courtship duration has been well documented (Houck et al. 1998, 2008a, b; Rollmann et al. 1999). However, we still lack a mechanistic understanding of the forces underlying a female's tendency to respond to, and cooperate with, a pheromone-producing partner.

Here, we explore two broad mechanisms by which a female may be swayed in favour of a courting male: a specific mechanism of direct augmentation of sexual motivation and a general mechanism of central nervous system arousal. In the case of the specific mechanism, pheromones would activate behavioural subsystems affecting the tendency of a female to approach or affiliate with functionally related stimuli (in this case sexual stimuli) (Giraldi et al. 2004). In particular, we would expect a sexually motivated female to show (1) unaffected locomotor activity, (2) a greater interest in sexual stimuli (i.e. scent or sight of a reproductive male) and (3) unaffected or diminished interest in nonsexual stimuli. Alternately, in the case of the general mechanism, sexual receptivity would be heightened as a secondary consequence of broad central nervous system arousal, previously known as 'general excitement' (Tinbergen 1952). This internal state of general arousal has been operationally defined by Pfaff (2006) as being more alert to sensory stimuli in all sensory modalities, engaging in more voluntary motor activity, and being more reactive emotionally (as measured by patterns of change in autonomic activity). Thus, we would expect a generally aroused female to demonstrate (1) increased locomotor activity and (2) a broad tendency to approach attractive stimuli, regardless of the class or sensory modality of the stimulus.

We investigated these specific and general mechanisms of swaying female mate choice in plethodontid salamanders. Specifically, we examined whether pheromones altered female general activity or attraction to stimuli in two classes (sexual, ingestive) and two sensory modalities (olfactory, visual). We used a generic or 'every male' signal (pheromones pooled from ca. 200 males) that prevented the transmission of individual-specific information. Our results revealed that male pheromones increased female affinity for olfactory sexual stimuli alone, consistent with the mechanism of augmented sexual motivation. This pheromone action may still function within the context of information-transfer signalling if a male signal affects the recipient's sensory processing, stimulus evaluation standards or decision-making criteria.

STUDY SPECIES

We studied the mechanism of pheromone-enhanced receptivity in the red-legged salamander, *Plethodon shermani*. Courtship in *P. shermani* is highly stereotyped and has been well described (Arnold 1977), and is summarized here. First, the male approaches and solicits the female with an array of behaviours such as physical contact, foot dancing and tail arching. If the female is amenable, she steps over the male's tail and positions herself with her head resting on the base of the male's tail. A 'tail-straddling walk' ensues in which the female roughly matches the male step for step. During the tail-straddling walk, the male pauses periodically to deliver pheromones by tapping his mental gland (pheromone-producing chin gland) to the female's nares. The male lacks an intromittent organ, and insemination occurs via the deposition of a spermatophore. Immediately following deposition, sperm transfer occurs when the male guides the female over the spermatophore and the female draws the apical sperm mass into her cloaca.

Courtship for *P. shermani* can be considered a period of reciprocal assessment. The female may appraise the male's desirability through multiple modes of input (e.g. visual, pheromonal, somatosensory), while the male simultaneously evaluates the moment-to-moment level of female receptivity by her willingness to match his pace during the tail-straddling walk (Arnold 1976). Thus, a reluctant female can prolong courtship duration and will likely receive a greater quantity of pheromone.

METHODS

Study Species Collection, Maintenance, Gland Removal and Prescreening

Methods followed those of Vaccaro et al. (2009) and are summarized here. Male and female *P. shermani* in reproductive condition were collected during the August 2008 mating season from Macon County, NC, U.S.A. Animals were housed individually in plastic boxes (31 × 17 × 9 cm) lined with damp paper towels as substrate and crumpled moist paper towels as refuges. Animals fed ad libitum and were offered 10 fly larvae weekly (*Calliphora vomitoria*, GrubCo, Hamilton, OH, U.S.A.). Shortly following salamander collection, we removed the pheromone-producing mental glands from anaesthetized males. Each male recovered from anaesthesia with his chin resting on a pillow made from moistened surgical gauze treated with antibiotics. Because of the superficial location of the mental gland, the excision site usually healed within 1 week. Males were allowed to recover fully in the laboratory before being released at the collection site. Pheromones were extracted from the gland tissue following established protocols (Houck et al. 1998). Each female was prescreened for a willingness to mate under laboratory conditions. Reproductively active females were shipped to Oregon State University (OSU), Corvallis, U.S.A. where behavioural experiments were conducted. Animals were kept in conditions similar to the field: 15–18 °C on a late-August North Carolina photoperiod. North Carolina scientific collecting permits were obtained, and animals were cared for using a protocol approved by the Institutional Animal Care and Use Committee at OSU (LAR 3549 to L.D.H.).

Observational Arena

All behavioural trials were conducted in an arena consisting of an array of eight observation boxes (245 × 245 × 20 mm, Square