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Choosing a healthy mate: sexually attractive traits as reliable indicators of current disease status in house mice

Patricia C. Lopes*, Barbara König

Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland

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Social interactions are critical for reproduction in many animals. Since several pathogens are transmitted by social contact, females searching for mating partners should select males that can signal being healthy. Not all signals, however, may be reliable, since males from a number of species can overcome behavioural symptoms of infection when mating opportunities are available. Here, we manipulated sickness status of male house mice, *Mus musculus domesticus*, by administering an immune challenge (lipopolysaccharide, LPS) and studied the consequences of this manipulation for two signals that function in mate attraction in this species: ultrasonic vocalizations and darcin (a urinary protein). Additionally, we quantified female visits to immune-challenged and control males, and the males' plasma testosterone levels. LPS-injected males had lower darcin and lower regular ultrasonic syllable production than control-injected males, while producing a larger number of high-frequency ultrasonic syllables. We conclude that immune-challenged male mice presented with a receptive female cannot maintain the production of sexually attractive signals. Females might use some of these cues when making mating decisions, since they spent significantly less time near LPS-injected males. Testosterone was reduced in LPS-injected males and could be a unifying mechanism downregulating both of the traits quantified. Darcin and ultrasonic vocalizations produced in the context of courtship may therefore function as reliable indicators of current health status.

Choosing a sick social or mating partner is risky. An individual risks disease or parasite infection or reduced benefits provided by the partner due to its inferior health. Mechanisms for the recognition and avoidance of parasitized or sick conspecifics should therefore reduce the probability of exposure and be beneficial. Indeed, animals of several species are able to distinguish between parasitized and healthy conspecifics (reviewed in Beltran-Bech & Richard, 2014). While this recognition may be beneficial for healthy individuals, it could be disadvantageous for sick animals looking to pass on their genes when the probability of survival is perceived to be low (terminal investment hypothesis; see Clutton-Brock, 1984).

Animals use different types of communication to gain information about potential partners and this is especially critical for mate choice. Females should pay close attention to signals that allow them to find potential mates. If the signals also indicate male quality, then females can use these signals to make qualitative choices among available suitors. In sexually reproducing species, honest signalling of condition and health is considered to be of prominent importance for female choice of a mating partner (Andersson, 1994). Not all signals, however, are necessarily honest, since males from a number of species are able to overcome behavioural symptoms of infection when mating opportunities are available (summarized in Lopes, 2014).

Here, we studied the consequences of experimental manipulation of sickness for two signals that function in mate attraction and mate choice in house mice, *Mus musculus domesticus*: ultrasonic vocalization and urinary proteins.

Females spend more time near male mice that are able to produce ultrasonic vocalizations (Pomerantz, Nunez, & Bean, 1983) and are attracted to the playbacks of song-like vocalizations of adult males (Hammerschmidt, Radyushkin, Ehrenreich, & Fischer, 2009; Musolf, Hoffmann, & Penn, 2010). Another important route of communication for rodents consists of olfactory cues deposited in the urine (Hurst & Beynon, 2008). Proteins with signalling functions found in mouse urine include major histocompatibility complex (MHC), major urinary proteins (MUPs) and their volatile ligands (Hurst & Beynon, 2008). MUPs consist of most of the

* Correspondence: P. C. Lopes, Institute for Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, Zürich, CH-8049, Switzerland.
E-mail address: patricia.lopes@ieu.uzh.ch (P. C. Lopes).

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protein content found in male mouse urine (Finlayson, Potter, & Runner, 1963; Humphries, Robertson, Beynon, & Hurst, 1999), and their level is sexually dimorphic with males having a three to four times higher concentration of urinary MUPs than females (Beynon & Hurst, 2004). MUP expression patterns provide different types of information, such as genetic and individual identity (Cheetham et al., 2007; Sherborne et al., 2007), but one particular MUP stands out in terms of mate attraction potential: darcin. Through a series of experiments, Roberts et al. (2010) demonstrated that darcin alone is responsible for the preference that female mice have for male versus female urine, and that females are not attracted to male urine containing low levels of darcin.

While both vocalizations and darcin serve the function of attracting females, females might extract other information from them when selecting a potential mate. Besides functioning as a way to assess genetic quality and compatibility of a male (Hoffmann, Musolf, & Penn, 2012; Hurst, 2009; Hurst et al., 2001), these types of signals could work to reflect a male's current condition, for example his disease status. From the female's perspective, detecting and avoiding mating with sick males should be important for several reasons, including: decreased likelihood of future male services (e.g. parental care, territorial defence; Andersson, 1994); poor male genetic quality (e.g. high susceptibility to disease; Hamilton & Zuk, 1982); and higher risk of disease infection (Able, 1996) and consequential loss of pregnancy (Aisemberg et al., 2010). Both acoustic signalling and urinary protein production are thought to be costly (Nelson, Colson, Harmon, & Potts, 2013; Ryan, 1988). Thus, from the perspective of the sick male, signalling can become dangerous if the energy necessary to produce the signal competes with the energy necessary to recover from the infection. However, if males can temporarily overcome symptoms of infection to attract and mate with females, this risk might be compensated for by successful fertilization (Lopes, 2014).

Immune challenges can disrupt reproductive physiology (for a review, see Tomaszewska-Zaremba & Herman, 2009), with consequences for testosterone production (Boonekamp, Ros, & Verhulst, 2008). Thus, when responding to an infection, animals may experience reduced levels of testosterone. If we consider that many secondary sexual traits, including ultrasonic vocalizations and darcin production, are known to be under androgenic influence (Knopf, Gallagher, & Held, 1983; Nunez, Nyby, & Whitney, 1978), it becomes plausible that, when animals are infected, trait production may be affected as a consequence of the associated decrease in testosterone. Therefore, testosterone is a likely candidate to link immune activation to changes in production of darcin and ultrasonic vocalizations.

In the current study, we tested whether sickness status impacted the amount of darcin and the number of ultrasonic syllables produced by wild-derived male house mice when exposed to a female in oestrus. Manipulation of sickness status was done by administering an inflammatory challenge using lipopolysaccharide (LPS) injections. When faced with deteriorating health conditions, males may attempt to invest in reproduction over recovery, and produce sexually attractive signals. We predicted that, if this system were susceptible to dishonesty, the easier signal for mice to manipulate would be vocalizations, as this signal is potentially under voluntary control (Seyfarth & Cheney, 2010). It should be harder for mice to manipulate the levels of darcin expression and we thus predicted a decrease in darcin levels during an inflammatory challenge. Given its important role as a modulator of the sexually attractive signals quantified here (Knopf et al., 1983; Nunez et al., 1978), we also measured testosterone in experimental males. We used the time females spent near sick versus control males as a proxy for attractiveness.

METHODS

Ethical Note

Animal use and experimental design were approved by the Veterinary Office Zürich, Switzerland (Kantonales Veterinäramt Zürich, no. 88/2014).

Animals

The experiments were carried out with house mice in an animal facility at the University of Zürich. Experimental animals were born in the laboratory and represented F1 to F3 descendants of wild house mice captured in the vicinity of Illnau, near Zürich. Animals were kept under standardized laboratory conditions at a temperature of 22 ± 3 °C with a relative humidity of 50–60% and on a 14:10 h light:dark cycle with a 1 h sunrise (0600–0700 hours) and dusk (1930–2030 hours) red light phase at the beginning and end of the light phase (white light started at 0630 and turned off at 2030). We tested 21 sexually mature, but nonbreeding brother pairs (mean age $\pm$ SE $= 60 \pm 1$ days) and 21 sexually mature nonbreeding females (mean age $\pm$ SE $= 105 \pm 6$ days).

In our facility, male and female siblings are separated after weaning at 23 days of age and housed in single-sex groups in standard Makrolon Type III cages. Five or six days before the start of an experimental trial, a pair of male siblings was randomly chosen from one of these cages and each was placed in a separate cage with an unrelated, unfamiliar, sexually mature, but sterile female (hybrid obtained by mating mice from different chromosomal races as part of a separate experiment). The vocalizations produced by the pair were observed for 1 h, using an Avisoft ultrasound-microphone (Ultrasound Gate CM16/CMPA) connected to a single-channel recording device (Ultrasound Gate 116Hb, Avisoft Bioacoustics, Berlin, Germany). As it has been found that not all mice vocalize when tested (C. Pfeifle, personal communication), male pairs were only used further if we observed both vocalizing during this first hour in the presence of a female. These males were housed with the sterile female for at least 5 days prior to the experiment. Given that these males were separated from their siblings before any signs of aggression, we have no reasons to believe that dominance relationships had been formed at this time. In addition, male siblings did not differ significantly in mass at the start of the experiment (paired t test: $P = 0.38$).

All animals were provided with food (laboratory animal diet for mice, Provimi Kliba SA, Kaiseraugst, Switzerland) and water ad libitum. The contents of the cages consisted of standard bedding material (Lignocel Hygienic Animal Bedding, JRS), as well as shredded paper towel and an empty toilet paper roll.

Experimental Set-up

Recording box

All experiments were conducted in the same room where the animals were housed after weaning, and we used a recording box as described in von Merten, Hoier, Pfeifle, and Tautz (2014), with a few adaptations for our experimental purposes. Briefly, our box consisted of three separate compartments, located side by side. The middle compartment was connected to the outer two via a round window (6.5 cm of diameter) on each side, covered with wire-mesh (spacing of the wire was 1 mm). Mice in the outer compartments could thus interact with the mouse in the central compartment through these windows. A round piece of PVC (same material as rest of the box) could be placed over the communication windows and tightly closed to prevent visual, physical and at least reduce olfactory and acoustic interactions.