Behavioural Processes 94 (2013) 109–114

Contents lists available at SciVerse ScienceDirect

Behavioural Processes

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

# Responses of female rock lizards to multiple scent marks of males: Effects of male age, male density and scent over-marking

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ARTICLE INFO

*Article history:*
Received 15 November 2012
Received in revised form 5 January 2013
Accepted 7 January 2013

*Keywords:*
Chemical communication
Pheromones
Mate choice
Scent marking
Alternative strategies

ABSTRACT

Scent-marked substrates may inform conspecifics on the characteristics of territorial males. Scent-marks of male Carpetan rock lizards (*Iberolacerta cyreni*) affect space use of females, which by selecting an area may increase the probability of mating with the male that has scent-marked that area. However, males do not hold exclusive territories, and scent-marks of different individual males are often together. This may provide complex information from multiple sources on the social structure. Here, we examined female preference in response to scent marks of various males and combinations in a laboratory experiment. Females preferred areas scent-marked by territorial old males against those scent-marked by young satellite-sneaker males. This reflected the known preference of females for mating with old males. In a second experiment, females preferred areas scent-marked by two males to areas of similar size marked by a single male. This may increase the probability of obtaining multiple copulations with different males, which may favour sperm competition and cryptic female choice, or may be a way to avoid infertile males. Finally, when we experimentally over-marked the scent-marks of an old male with scent-marks of a young male, females did not avoid, nor prefer, the over-marked area, suggesting that the quality of the old male may override the presence of a satellite male. We suggest that, irrespective of the causes underlying why a female selects a scent-marked area, this strategy may affect her reproductive success, which may have the same evolutionary consequences that "direct" mate choice decisions of other animals.



## 1. Introduction

Chemoreception is one of the main sensory systems used by many animals, and chemical signals (pheromones) play an important role in intraspecific communication, social organization and sexual selection of many types of animals, including vertebrates (Wyatt, 2003; Müller-Schwarze, 2006). Pheromones are very often incorporated into faeces, urine or other substrate scent marks with the purpose of marking territory boundaries or attracting mates (reviewed in Gosling and Roberts, 2001; Brennan and Kendrick, 2006; Müller-Schwarze, 2006; Mason and Parker, 2010). For example, in many lizards, scent marks may give information on sex, body size, or age recognition (Aragón et al., 2001a; López and Martín, 2001; reviewed in Mason, 1992; Mason and Parker, 2010; Martín and López, 2011), or even provide more detailed information on morphological traits and health condition of the signaller (López et al., 2006; Martín et al., 2007a). This information seems important in intrasexual relationships between males (López and Martín, 2002, 2011; Carazo et al., 2007, 2008; Martín and López, 2007; Martín et al., 2007b) and in female mate choice (Martín and López, 2000, 2006a,b; López and Martín, 2005a; Olsson et al., 2003; Martín et al., 2007a). Therefore, scent marking a territory might initially be a simple and effective method to inform on the identity and characteristics of the male that defends that territory. If the information in the scent mark is reliable (Gosling and Roberts, 2001; Martín and López, 2006b; Kopena et al., 2011), the signaller will benefit from this advertisement, for example by attracting mates (Martín and López, 2012), and the receivers of the signal will benefit in that they may incorporate this information from scent marks into their fighting (e.g. Carazo et al., 2007; López and Martín, 2011) or mate choice decisions (e.g. Martín and López, 2006a).

However, male lizards do not hold exclusive territories, and conspecifics may often find many scent marks of different individual males together in the same area. In some cases, males of different ages or social status, or even with different reproductive strategies, overlap their home ranges extensively (Aragón et al., 2004). Thus, scent marks could not only provide information on the characteristics of a particular male, but also on the density of males or "social structure" in that area (delBarco-Trillo and Ferkin, 2004, 2006; Vaughn and Ferkin, 2011). For example, conspecifics may use multiple scent marks to assess the number of different individual adult males occupying an area (Ferkin et al., 2008), or to

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http://dx.doi.org/10.1016/j.beproc.2013.01.002

detect the presence of young, but reproductively mature, males that can adopt a satellite strategy within the territory of a dominant male. In addition, males of some animals, such as rodents, may actively over-mark or counter-mark the scent marks of other males that they find (Johnston et al., 1994, 1995; Hurst and Rich, 1999; reviewed in Johnston, 2005). This information may be used by females to assess the competitive ability of the top-scent males, which, based on this trait alone, may be the preferred partners (Johnston et al., 1997a,b). Other animals, such as lizards, might not actively over-mark, but, in some species, the high spatial overlapping of home ranges would similarly lead to over-marks, especially if males tend to mark on specific preferred places such as on the top of high rocks or other preferred basking places (López et al., 1998; Martins et al., 2006). Also, territorial lizards preferentially visit areas in their territories recently marked by other lizards, which may result in over-marking (Carazo et al., 2008). Therefore, in many cases the receivers of the signal may obtain simultaneous and complex information from scent marks of several individual males. However, it is not entirely understood how this information from multiple sources is incorporated into decision making by the receivers of the signal.

The Carpetan rock lizard, *Iberolacerta cyreni* (formerly *Lacerta monticola cyreni*), is a lacertid lizard found in rocky mountain habitats of Central Spain (Martín and Salvador, 1997a). This is a polygynandrous species, and, there are at least two distinct ontogenetic classes of sexually mature males with alternative social and reproductive tactics (Martín and Salvador, 1997b; Aragón et al., 2004; Salvador et al., 2008). Old males (4–5 years old; with dorsal green colouration) are larger, dominant, and defend more exclusive and larger territories than young males (3 years old; with dorsal brown colouration), which overlap extensively their smaller home ranges with territories of green males (Aragón et al., 2001b, 2004). Green old males guard females more often and sire most of the offspring, while brown young males adopt a satellite sneaking strategy with forced matings, which allow them to sire some offspring (Aragón et al., 2004; Salvador et al., 2008). Females prefer mating with old males, and reject copulation attempts by young males (López et al., 2003). Similar ontogenetic-dependent social and mating alternative strategies occur in other lizard species (e.g. Baird et al., 1996; Baird and Timanus, 1998; Martín and Forsman, 1999; reviewed in Baird et al., 2003).

Male rock lizards scent mark substrates with faeces and femoral gland secretions (Aragón et al., 2000, 2001a; López and Martín, 2005b). Experimental evidence suggests that females may use proportions of some lipophilic compounds in scent marks of males to choose potential mates of high quality (Martín and López, 2006a,b, 2010, 2012). These chemosensory preferences of females affect their space use, which may increase the opportunities for mating with the male that has scent marked a particular area (Martín and López, 2012).

We designed a laboratory experiment where we offered to female rock lizards scent marked substrates experimentally manipulated to simulate different scenarios of social organization of males and examined the use of space of females in relationship to these scent marked areas. We specifically studied the female choice of areas scent marked by (1) males of different ages that also show different mating strategies (young sneakers vs. old territorial), (2) males of the same age but with different densities (scent marks of 1 vs. 2 old males in the same area without overmarking), and (3) scent marks of old males alone or over marked by young males. We predicted that if females used scent marks to select males of higher quality, females should select areas in each experiment scent-marked by (1) old males, (2) 1 old male alone, or (3) an old male alone without overmarking. This is because we could expect that old males were of higher quality than younger males, and also that better males could hold more exclusive territories.

## 2. Materials and methods

### 2.1. Study animals

We captured by noosing adult *I. cyreni* lizards during May 2010 at "Alto del Telégrafo" (Guadarrama Mountains, Madrid Prov., central Spain; 40°47′N, 04°01′W) at an elevation of 1900 m. The study area is characterized by a high cover of granite rock boulders and screes interspersed with shrubs (*Cytisus oromediterraneus* and *Juniperus communis*) and meadows of *Festuca* and other grasses (Martín and Salvador, 1997a). Activity of lizards lasts from late April to early October, and the mating season occurs in May–June, with females producing a single clutch in July (Aragón et al., 2004; Salvador et al., 2008). Captured lizards were kept in individual numbered cloth bags and their capture locations were recorded using a hand-held GPS. Lizards were transferred by car immediately after capture to "El Ventorrillo" Field Station, 5 km from the capture site, where they were individually housed in outdoor 80 cm × 50 cm × 40 cm PVC terraria containing rocks for cover, and food (mealworms) and water ad libitum. Cages of males and females were in different places to avoid contact between them.

Lizards were weighed and their snout-vent length (SVL) measured (males: SVL: $\overline{X} \pm \text{SE} = 72 \pm 1$ mm, range = 69–77 mm; body mass: $7.8 \pm 0.2$ g, range = 6.5–9.5 g; $N = 24$; females: SVL: $\overline{X} \pm \text{SE} = 75 \pm 1$ mm, range = 68–86 mm; body mass: $6.3 \pm 0.3$ g, range = 4.5–9 g; $N = 18$). Based on colouration, size and data from recapture observations of other lizards in the field (Aragón et al., 2004), we classified the males in two age classes (young males: $70 \pm 1$ mm, range = 69–71 mm, $N = 12$; old males: $76 \pm 1$ mm, range = 75–77 mm, $N = 12$). Lizards were captured before the start of the mating season. We confirmed that females had not mated yet because they lacked characteristic mating scars on the belly (unpublished data). The experiments were carried out during May and June, which coincided with the mating season of lizards in their original natural population (Aragón et al., 2001b; Salvador et al., 2008).

All individuals were healthy and in good condition during the tests, did not show any sign of stress, and, at the end of the experiments (middle June), all of them had maintained or increased their body mass. Lizard were then returned and released at their exact initial sighting location before capture.

### 2.2. Scent preference tests

At the beginning of the experiments, we placed in the males' cages several absorbent paper strips (35 cm × 10 cm) fixed to the floor, and left them there for 2 weeks to allow males to scent mark them with faeces and femoral gland secretions. The ventral location of the femoral pores allowed the secretions to be passively deposited on the paper substrate as male lizards moved through their terraria, but active rubbing of the legs on the substrate has also been observed in these and other lizards (Martín and López, 2006a; Martins et al., 2006).

Female choice of scent experiments were performed at the end of May. Female cages (80 cm × 50 cm × 40 cm) had two identical flat tiles (30 cm × 10 cm), used as basking platforms, placed symmetrically at each end of the cage, and rocks for basking and cover in the centre. At the beginning of each test (07:00 h GMT), when females were still inactive, we fixed with fresh gloves on one tile one paper strip scent marked by a male, and another paper scent marked by a different male on the other tile. We conducted three different experiments. In the first experiment, we tested whether females discriminated and preferred areas scent marked by green old males alone against areas scent marked by brown young males alone. Thus, we fixed on one tile one paper strip from 1 young male, and on the other tile one paper from an old male.