



Original Article

The size of a smell: assessment of rival's relative size from femoral secretions in the common wall lizards, *Podarcis muralis* (Laurenti, 1768)

Stefano Scali^{a,○}, Roberto Sacchi^{b,○}, Elisabetta Gozzo^a, Stefano Chiesa^a, Alan J. Coladonato^b, Marco A. L. Zuffi^c and Marco Mangiacotti^{b,○}

^aMuseo di Storia Naturale di Milano, Corso Venezia 55, I-20121 Milano, Italy, ^bDipartimento di Scienze della Terra e dell'Ambiente, Università di Pavia, Viale Torquato Taramelli 24, I-27100, Pavia, Italy, and

^cMuseo di Storia Naturale dell'Università di Pisa, Via Roma 79, I-56011 Calci, PI, Italy

Received 28 June 2022; revised 14 December 2022; editorial decision 18 December 2022; accepted 23 December 2022

Animal communication depends on signals conveying information to a receiver who must perceive and decode them. Signals involved in territoriality are usually complex stimuli that should be correctly interpreted to avoid unnecessary conflicts. Lacertids use both visual and chemical stimuli in modulating their aggressive response against conspecifics and the rival's size is one of the most important information, affecting the success probability in combat. To assess the actual ability of decoding information about a rival's size based on its chemical stimulus alone, 60 males of *Podarcis muralis* were tested for three consecutive days in an arena bearing a mirror (to simulate an equal-sized intruder), and the chemical cues (femoral secretions) from an unknown individual of different size. Significant differences were observed in tongue-flicks number, which grew as the size difference between the focal lizard and the secretion donor decreased. This can be interpreted as the need for the lizard to better evaluate the potential competitor's characteristics. The size difference also affected the number of bites against the mirror. They increased when the size of the focal lizard was larger than the donor triggering the aggressive response with a higher probability of winning the contest. This confirms that the focal lizard had correctly decoded the information about the opponent's size by chemical stimulus. Although previous studies have shown that some components of the chemical signals are potentially informative about the signaler's size, this is the first demonstration that male *P. muralis* is actually able to decode and use such information.

Key words: *Podarcis muralis*, chemical stimuli, visual stimuli, intraspecific communication, opponent's size assessment.

INTRODUCTION

Animal communication is the result of the evolution of complex systems that allow individuals to make decisions based on the morphology, physiology, and behavior of other individuals (Endler 1993). Communication occurs by means of signals, that is, changes in the environment caused by the emitter to convey information to the receiver, and these signals must be perceived and recognized on a background of other stimuli coming from the surrounding environment (Fuller and Endler 2018). The need for correctly decoding the signal and reducing interpretation errors is critical when signals

are involved in aggressive behaviors, because their misinterpretation could lead to unnecessary conflicts and to high costs for the contenders (Immelmann 1983).

Intraspecific aggression is a well-known case of interaction, particularly common in territorial species, and is often used to define a hierarchy, territorial boundaries or to guarantee access to some resources, such as food, shelters, reproductive sites, or mates (Brown 1964; Myrberg and Thresher 1974; Van den Berghe 1974; Stamps 1977; Kaufmann 1983). Once established, individuals occupying adjacent territories usually respect them, avoiding unnecessary conflicts with neighbors, according to the paradigm of the dear enemy effect (Fisher 1954). This choice allows them to save costs for aggression towards known individuals which already own a defined territory (Wilson 1978). Under such social contexts, intraspecific communication plays a central role, allowing individuals to gain

Address correspondence to Stefano Scali, Museo di Storia Naturale di Milano, Corso Venezia 55, I-20121 Milano, Italy. E-mail: stefano.scali@comune.milano.it

information about their conspecifics' characteristics (e.g., size, age, sex, kinship, identity, etc.), and modulate their behavior according to a decision-making process (Bradbury and Vehrencamp 2011).

Many cases of territorialism are documented for reptiles, such as chelonians, tuataras, crocodiles, and in particular, lizards (Pough et al. 2004); the latter have been widely used as model species to study the role of intraspecific communication on aggressive behavior (Pianka 1973; Fox et al. 2003; Whiting and While 2017). Although many factors can influence the individual predisposition to escalate into aggressive interactions (e.g., level of circulating sexual hormones: Adkins and Schlesinger 1979; Coladonato et al. 2020; DeNardo and Licht 1993; DeNardo and Sinervo 1994; body temperature: Hertz et al. 1982; Mautz et al. 1992; resource value: Leuck 1995; Lailvaux et al. 2012; Sacchi et al. 2021; population density: Stamps 1995), the evaluation of the opponent's characteristics eventually determines the actual outcome of the decision process (Whiting 1999; López and Martín 2001, 2002, 2011; Aragón et al. 2006, 2007; Sacchi et al. 2009; Titone et al. 2018). This underscores the importance of intraspecific communication and information exchange in aggressive interactions (López and Martín 2011).

Intraspecific communication in lizards can be achieved thanks to different sensory channels, in particular using acoustic, visual, and chemical stimuli. The former are used only by a small number of species of reptiles, whereas the other two are commonly used, even if their relative importance may vary in different clades (Robinson et al. 2015; Baeckens et al. 2016; Pruett et al. 2016; Martins et al. 2018; Scali et al. 2019; Romero-Díaz et al. 2021). Semiochemicals are used by many animals in intraspecific communication to convey information about sex, physiological and reproductive state, territorial marking, kinship, and identity. Terrestrial species typically use pheromones for these purposes by means of feces, urine, and gland secretions left on the substrate or on trunks and rocks, whereas aquatic species usually disperse chemical cues in the water. The advantage of these kinds of marking is that chemical cues are long lasting and less costly than other mechanisms of advertizing their presence (Brennan and Kendrick 2006). Chemical communication has, anyway, a metabolic cost that makes them honest signals that cannot be bluffed, under the paradigm of handicap theory (Zahavi 1975).

Chemical cues are particularly important in Lacertoidea lizards, thanks to the evolution of a complex vomeronasal organ that freed the tongue from its ancestral role (Mason 1992; Schwenk 1993; Cooper 1994), and the occurrence of specialized epidermal glands in the cloacal and femoral region (Cole 1966; Mayerl et al. 2015; García-Roa et al. 2017), which produce waxy secretions used in intraspecific communication (Martín and López 2011, 2015). Such cues can convey many different pieces of information about quality (Martins et al. 2006; Martín and López 2011, 2015; Mangiacotti et al. 2019b, 2019c, 2020), allowing other individuals to quickly assess the fighting ability of a rival (Martín and López 2007). In particular, chemical signals alone are known to potentially inform conspecifics about size (Martín and López 2000; López et al. 2006; Aragón et al. 2007; Khannoon et al. 2011a), a parameter which may be used as a proxy for fighting ability (Labra 2006; Aragón et al. 2007; Martín and López 2007; Khannoon et al. 2011a). Previous work with staged contests in lizards has indeed demonstrated that size, together with residency, plays a major role in determining the winner of a territorial fight (Olsson 1992; Aragón et al. 2006; Sacchi et al. 2009, 2021; Titone et al. 2018). Nonetheless, the actual ability of lizard to specifically decode and use information about size from chemical signals has been assumed on the basis

of indirect considerations, supported by the correlation between the size and chemical composition of the secretions (López et al. 2006; Martins et al. 2006), or discrimination behavioral tests (Labra 2006; Martín and López 2007; Khannoon et al. 2011b).

Here we aim at explicitly addressing this question, by simulating contests where the opponent's visual and chemical information mismatches. Notably, we use mirror tests to keep constant the visual information (i.e., rivals of the same size, status, and motivation; Scali et al. 2019, 2021), but providing chemical cues from a real donor of different sizes. In this way, only if the focal lizard could actually decode and use the chemically conveyed information about the opponent's size, it should show an aggressive behavioral pattern coherent with the difference in size with the donor.

As model species, we chose the Common wall lizard, *P. muralis*, which shows many characteristics that make it suitable for a study involving chemical and visual stimuli. First, it is a territorial species and males vigorously fight during the reproductive period (Edsman 1990). Second, as all the other lacertids, it uses chemical secretions to communicate with other individuals (Carazo et al. 2008; Mangiacotti et al. 2019a). Third, mirror tests have already been used with this species and they proved to be a good tool to investigate intraspecific aggression (Scali et al. 2019, 2021; Sacchi et al. 2021). Furthermore, many studies have been conducted over the last decades on its behavior, physiology, and ecology (Sacchi et al. 2007, 2021; Martín et al. 2008; Pérez i de Lanuza et al. 2012; Galeotti et al. 2013; Scali et al. 2013; Sannolo et al. 2014), including those focusing on the effect of the size difference between opponents on the aggressive behavior in staged encounters (Sacchi et al. 2009). Therefore, we assessed if common wall lizard males are able to decode contestant's size information coming from femoral secretions and if they use them in the modulation of aggressive interactions. We expect that creating a mismatch between the visual and the chemical stimuli causes the focal lizards to behave differently when the rival is larger, smaller, or size matched. In particular, the aggressive response will be higher when the winning probability is higher and the exploratory behavior will be modified by contrasting information.

METHODS

Field and laboratory methods

Podarcis muralis is a small lacertid lizard (snout to vent, SVL up to 7.5 cm); it is sexually dimorphic, with males larger than females. Males are territorial (Edsman 1990; Sacchi et al. 2009), as supported by data about circulating testosterone and homing behavior (Scali et al. 2013; Coladonato et al. 2020). Chemical communication of this species has been deeply studied during the last years analyzing femoral pores secretions, and it was demonstrated that lipids give information about individual quality, whereas proteins could give information about individual identity (Martín and López 2015; Mangiacotti et al. 2019b). The common wall lizard is a polymorphic species, with three discrete morphs (white, yellow, and red) showing alternative strategies as far as behavior and physiology are concerned (Sacchi et al. 2007; Galeotti et al. 2013; Scali et al. 2013). A previous study demonstrated that the males of all morphs are aggressive toward other morphs (Sacchi et al. 2009; Abalos et al. 2016), but that the maximum aggression occurs during homomorphic contests (Scali et al. 2021). Thus, we decided to use males without manipulating colors to obtain the maximum aggressive response during the experiments. We captured 60 adult males (SVL

> 55 mm) by noosing, 20 for each morph, in an urban garden near Milan (Borromeo Park in Cesano Maderno, UTM 32T 511782E, 5052935N) from April to June 2019, when territorial contests occur. Lizards were transported in cotton bags to the Natural History Museum of Milan, measured for snout-to-vent length with a caliper (accuracy 0.5 mm), and individually housed in plexiglass boxes (40 × 40 × 30 cm), with water at libitum and fed with three mealworms/day (*Tenebrio molitor*). A second sample of 60 adult males were captured in other localities (more than 3 km from the focal site) to avoid familiarity, and used as donors of femoral pores secretions. All the available secretions were collected from each individual gently squeezing the pores and they were collected in vials and kept at 4 °C in a refrigerator. The quantity of secretion could change in relation to the donor's size, but we are confident that this aspect should not affect the response to the stimulus, because wild individuals usually leave only a small portion of it scratching the thighs on the substrate, while we used the whole sample from one donor in each trial. Donor males were then measured for snout-to-vent length with a caliper and released healthy at their capture sites.

The individuals used for the experiments were left in the box for 3 days before tests for acclimation, so that the terraria could keep the odor of the animals. The boxes were kept at the environment temperature with natural sunlight.

Before starting the experiment, individuals were heated under a lamp (ZOO-MED 150W) for 10 min to reach a normal activity temperature between 28 and 37 °C (Sannolo et al. 2014). Body temperature was measured using a contactless infrared thermometer (GBC KTD810), with a measure range of −50.0 to 330 °C and a precision of ±2 °C. The box was then moved under a photographic set with LED lighting and the individual was hidden by a small opaque box. After this operation, a mirror (30 × 15 cm) was inserted on the opposite side of the box and a thin plastic strip smeared with the secretion of an unknown individual of the same morph was positioned in front of the mirror (donor secretions were used only once). We chose to use donors of the same morph of the focal individuals to maximize the aggressive response (Scali et al. 2021). After 1 min, the box hiding the lizard was removed and all the behaviors after the first movement were recorded using a webcam (Microsoft Life Cam HD-3000) for 15 min. We conducted all the experiments from 09.00 to 14.00 h in accordance with the more intense activity period of lizards. All the individuals were tested once and then released healthy at the capture site.

Video analyses

The videos were analyzed using BORIS (Behavioral Observation Research Interactive Software, Friard and Gamba 2016, available at www.boris.unibo.it), and the observer was blind as far as the size difference between the focal and the donor is concerned. We a priori defined the half of the terrarium bearing the mirror as the interaction zone with the stimuli (Scali et al. 2021), and we extracted three response variables. We used the first two variables to assess aggressive behavior as the time (seconds) spent in the half of the arena containing the mirror (Time), and the total number of bites against the mirror image (Bites). The third variable was the number of tongue flicks (TF) measured in the half of the arena containing the mirror. TF are used by lizards to collect chemicals from the environment and can be regarded as a proxy for the interest of an individual for an external stimulus (Cooper 1994). The TF in the half cage without the mirror were not counted because they were not considered as interactions with the mirrored image.

Statistical analyses

In order to quantify the size difference between the focal lizard and the donor of the femoral secretions, a new variable (dSVL) was computed as the standardized difference of size between the focal male (SVL_{focal}) and the donor (SVL_{donor}). Positive values of dSVL indicate that the focal is larger than the donor and the opposite for negative values. Then, generalized linear models (GLMs) were fitted to the data in order to assess any effect of dSVL on each behavioral variable. In the models, the following variables were used as predictors: dSVL, is the size difference between focal and donor individuals (standardized); SVL_f is the size of the focal individual (standardized); T is the body temperature at the beginning of the trial (standardized); Time is the (standardized) time spent in the half cage with the mirror (to control for the individual-specific duration of the interaction with the mirror; not included in the Time model). Previous work on the common wall lizard demonstrated that aggressive behaviors can be modulated in relation to the size difference between resident and intruder (the mirrored image, in our case), both according to a linear pattern (dSVL) and to a quadratic pattern (dSVL²) (Sacchi et al. 2009). In the former case, a proportional effect of the donor's size compared with the focal's size is hypothesized, in the latter case a proportional effect as the difference gets away from zero is expected. We translated the above relations into two alternative hypotheses by including only the linear term or the quadratic term in the models, and applying the Akaike information criterion (AIC) to choose the best model (Burnham and Anderson 2001). Time is modeled using a normal error distribution; TF and Bites (count variables) were modeled assuming Poisson error distribution. A constant, zero-inflation term was added for the Bites model, since in many trials Bites was equal to zero (Zuur et al. 2012). Analyses were performed under the R 3.5.2 statistical environment (R Development Core Team 2018), using the package “glmmTMB” (Brooks et al. 2017).

RESULTS

Five out of the 60 planned experiments (one for each focal lizard) were excluded because the individuals never moved (one), or never entered the half cage with the mirror (four). Thus, the final sample for the analyses included 55 trials from 55 individuals.

The mean time spent in the half of the cage bearing the mirror was 635 s ± 215 SD (range: 62–900). In all the experiments, lizards showed TF behavior (mean = 73.71; range = 2–296), while Bites occurred at least once in 33 cases (60%; mean = 11; range = 0–63).

The model incorporating linear dSVL performed sharply better than the one using the quadratic form only for Bites (Table 1). On the opposite, the quadratic models outperformed the linear one for TF (Table 1). For Time the linear and quadratic models scored similarly: so, the simplest one, where only linear predictors are included, was eventually chosen.

None of the chosen predictors revealed significant effects on the time spent in the half-cage bearing the mirror (Time; Table 2). TF was negatively affected by dSVL² (Table 2; Figure 1), increasing as the size difference between the focal and donor lizard symmetrically approaches zero. Considering the control variables, body temperature, T , decreased TF, which is also proportional to the time spent in front of the mirror (Time; Table 2). The same predictors showed significant effects on the number of bites (Bites), but with different relations: the number of bites increased with dSVL, i.e., with the focal lizard becoming larger than the donor (Table 2; Figure 1);

Table 1

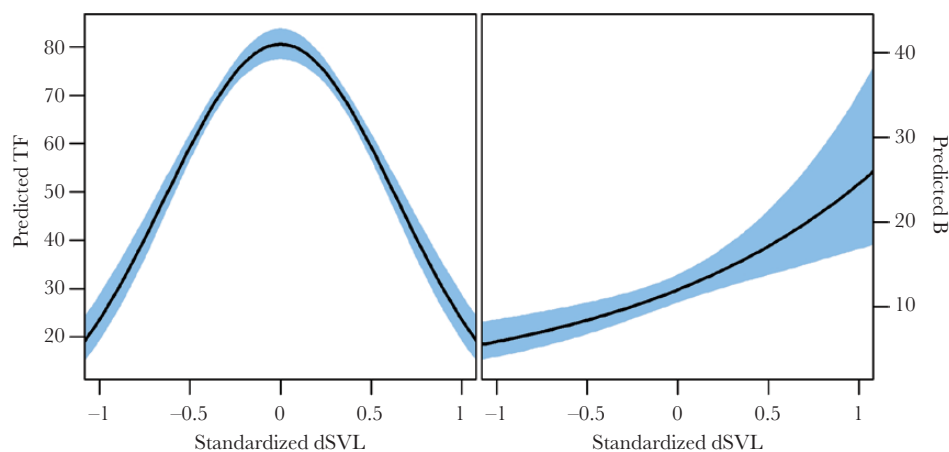
Model selection between linear and quadratic term of dSVL (difference in SVL between focal and donor lizard) for the four response variables: mirror = standardized time spent in the half cage with the mirror; TF = number of tongue-flicking; B = number of bites against the mirrored image; AIC = value of the Akaike information criterion; Δ AIC = AIC difference with the best model of the pair; w = Akaike weights. Models with strong support are bolded. For mirror the linear and quadratic models scored similarly and worse than the null model (in italic)

Response	dSVL term	AIC	Δ AIC	<i>W</i>
Mirror	<i>Null</i>	<i>160.158</i>	<i>0.000</i>	<i>0.519</i>
	Linear	161.468	1.310	0.269
	Quadratic	161.946	1.788	0.212
TF	Null	2357.143	136.590	0.000
	Linear	2303.485	82.931	0.000
	Quadratic	2220.553	0.000	1.000
B	Null	636.522	14.114	0.001
	Linear	622.408	0.000	0.998
	Quadratic	637.194	14.786	0.001

Table 2

Parameters significance and estimation for the best models associated to each response variable. Mirror = time spent in the half-cage with the mirror; TF = number of tongue-flicking in the half-cage with the mirror; B = number of bites against the mirror; dSVL = difference between focal and donor's snout-to-vent length; SVL_f = snout-to-vent length of the focal lizard; T = body temperature (°C) at the beginning of the trial. χ^2 , degrees of freedom (d.f.) and *P*-values are the outcome of a likelihood ratio test for each parameter; coefficient estimations and their standard error ($\beta \pm SE$) are reported only for significant parameters. Significant values are bolded

Response	Parameter	χ^2	d.f.	<i>P</i>	$\beta \pm SE$
Mirror	SVL_f	1.435	1	0.231	
	T	1.487	1	0.223	
TF	dSVL²	113.638	1	<0.001	-1.224 \pm 0.115
	SVL_f	1.246	1	0.264	
	T	249.616	1	<0.001	-0.255 \pm 0.016
B	Mirror	45.968	1	<0.001	0.122 \pm 0.018
	dSVL	16.767	1	<0.001	0.711 \pm 0.174
	SVL_f	2.869	1	0.090	
	T	14.618	1	<0.001	0.164 \pm 0.043
	Mirror	113.457	1	<0.001	0.932 \pm 0.088

**Figure 1**

Prediction by significant models according to standardized dSVL (difference in SVL between focal and donor lizard; positive values of dSVL indicate that focal is larger than the donor and the opposite for negative values) variation: *left*, predicted number of tongue-flicking (TF); *right*, predicted number of bites (B).

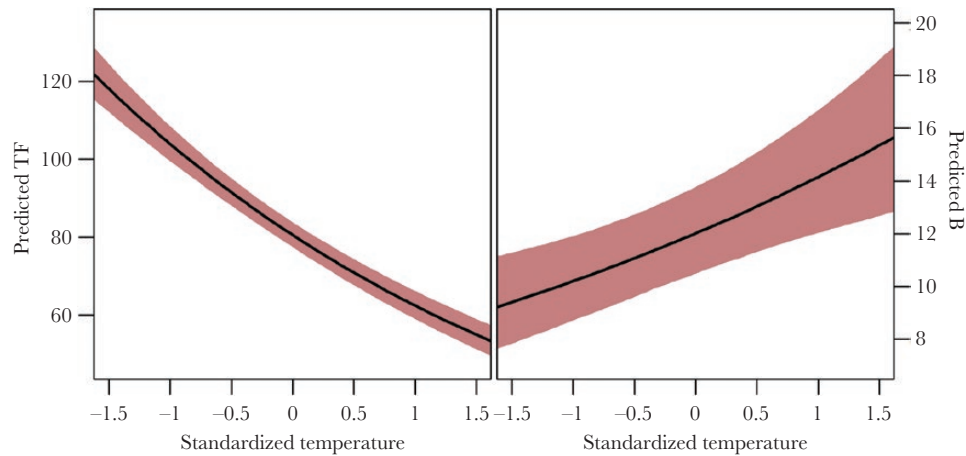


Figure 2

Prediction by significant models according to variation in standardized body temperature: *left*, predicted number of tongue-flicking (TF); *right*, predicted number of bites (B).

Bites was also positively affected by body temperature controlling for time spent in the same half as the mirror (Table 2; Figure 2).

DISCUSSION

Our data demonstrate that common wall lizard males are able to perceive, decode, and use size-related information coming from femoral secretions of conspecific individuals. Indeed, only by completing all these steps, they can perform an overall behavior during aggressive interactions which is coherent with the chemical-informed size difference between opponents. Indeed, chemical information about the rival's size seem to affect interest (TF) and aggressive behaviors towards the contender (Bites), and this kind of information can be obtained only through femoral secretions in our experimental setting, since the mirrored image is size-matched with the focal individual (Scali et al. 2019).

Previous work demonstrated that femoral secretions contain compounds whose concentration is related to size/age (Martín and López 2007; Khannoon et al. 2011b). In particular, the amount of some alcohols (e.g., 1-octadecanol) and lipids (e.g., cholesterol) is positively correlated to male size and could convey information to contenders or potential partners about fighting abilities, namely, size (López et al. 2006; Martín and López 2007; Khannoon et al. 2011a). This hypothesis is consistent with the experimental outcomes of our study, in that chemical cues are informative about individual size. In particular, not only the overall behavior was conditioned by chemically perceived size difference between the focal and donor lizards but the direction of the aggressive response modulation agreed with the direction of the size asymmetry. Bites are indeed considered the maximum expression of overt aggression against a contender, even against a mirrored image (Scali et al. 2019, 2021). Here we found their number to increase with the increasing size of the focal lizard compared to the secretion's donor. This is perfectly in line with what is expected from previous studies also including *P. muralis*, which underscored that, independently to other factors, a positive difference in size compared to the opponent triggers aggressive behavior and escalation into contests (Olsson 1992; Sacchi et al. 2009; Titone et al. 2018).

A different result came by the TF, whose intensity was low when the secretion's donors were both larger and smaller than the focal individual, and grew up only when individuals perceived a thin

difference between them and the contenders. TF is considered an exploration behavior used to collect and discriminate chemical cues (Cooper 1994) and significant results are interpreted as discrimination ability, even if their absence does not necessarily mean a lack of this skill (Cooper 1998). Many data about vomeronasal organ functionality suggest that the discrimination of some individual features could require only few TF (Mason 1993; Shine et al. 2003; Barbosa et al. 2006). Scent marks can convey information about age (i.e., size) of other individuals, as demonstrated for *Psammotromus algirus* (Nisa Ramiro et al. 2019) and *Liolaemus* sp. (Labra 2008), thus variation in TF rates can be interpreted not only as a chemical discrimination action, but also as an expression of interest towards other individuals. Our data support this hypothesis, since TF rate grows when the focal's and the donor's sizes are similar. Size is a prominent cue in territorial competition among lizards (Tokarz, 1985; Sacchi et al. 2009), but when the difference between contestant is minimal, individuals need more precise information before escalating into a fight with a rival (Labra 2008). In this case, any error in signal decoding may lead to the exposition to unnecessary risks, such as wounds or predation. Risk evaluation is indeed a primary goal during territorial defence, and understanding if a contest will be symmetric or asymmetric is a crucial task for any individual, thus avoiding costly aggressive interactions (Maynard-Smith and Price 1973; Stamps and Krishnan 1994; Aragon et al. 2006; Khannoon et al. 2011b). Similar results were obtained for *Anolis aeneus*, where the probability of fighting increases when two like-sized individuals encountered for the first time (Stamps and Krishnan 1994).

Two control predictors (Bites and TF) were statistically significant in the models. More interestingly, the body temperature affected Bites and TF in opposite ways, positive for the former, negative for latter. The tendency of individuals with higher body temperature to be more aggressive may be just the effect of increased general activity with temperature proper to ectotherms, as it was already observed for other species, such as *Liolaemus sarmientoi* (Fernández et al. 2018) and *Sceloporus occidentalis* (Engbretson and Livezey 1972). However, in some species colder individuals may show more aggressive behaviors and stronger bite force, probably as a defensive response compensating the diminished capacity for struggle at low body temperature (Hertz et al. 1982; Mautz et al. 1992; Woolrich-Piña et al. 2021). On the opposite, TF decreased

with increasing body temperature. This counter-intuitive result, compared to other squamates (e.g., Stevenson et al. 1985; van Damme et al. 1990), may be explained by considering that in our experiments body temperatures were intentionally kept within the optimal range for the species (range: 26.0–37.4 °C, Brana et al. 1991) in order to favor behavioral responses. This temperature range corresponds to the one where Cooper and Vitt (1986) found a decrease in TF rate, according to a quadratic relation with temperature (with the maximum at about 30 °C). Despite being tested on a skink (*Plestiodon laticeps*), the same may have occurred in our study species.

Finally, the time spent in the half-cage with the mirror (Time) was not affected by the donor's relative size: this suggests Time to be mainly driven by other factors, namely residency (Sacchi et al. 2021): the interest towards the cage portion where the visual stimulus (mirrored image) is presented raises when the focal lizard is the owner of the territory, that is, when the subjective resource value is high (Sacchi et al. 2021). The observed values for the “mirror” response (635 ± 216 s) are indeed within the range recorded in the resident trial of Sacchi et al. (2021). Additional, not exclusive, explanations may be that it is not at this stage of the interaction that the information about the opponent's size comes into play in driving the behavior, or that the size-related components of the chemical signal could have not been spread enough in the arena to be detected.

Evidence of semiochemicals used to assess rival's social status, health, and nutrition were found in many mammals, including mice, carnivores, primates and elephants (Eisenberg and Kleiman, 1972; Schulte et al. 2007; Scordato and Drea 2007; Wyatt 2014), plethodontid salamanders (Mathis 1990; Anthony and Wicknick 1993) and insects (Prokopy et al. 1984). In some cases, pheromones are sprayed against rivals with urine to assess the fighting abilities and social rank, as in the case of some crustaceans (Breithaupt and Eger 2002), but also in some primates like ringtailed lemurs (Scordato and Drea 2007). In these cases, chemical cues are used in conjunction with aggressive behaviors and are useful to establish or reinforce social status and dominance, avoiding unnecessary fighting before escalation (Breithaupt and Eger 2002). No information is available about the possibility of making mistakes when the signals are discordant. Our results suggest that lizards could start a conflict with a visible male in presence of chemical cues in the environment coming from a smaller male, but this hypothesis should be tested with real contests and not only with mirror tests, because other signals could intervene to correct false information.

In conclusion, the present study demonstrated the ability of common wall lizards to decode size information from femoral gland secretions of conspecifics, and to modulate the intensity of the aggressive response according to the inferred information. Femoral secretions are known to be used by lizards to convey information to other individuals for territorial and reproductive purposes. Chemical cues are made of a plethora of components, thus it would be crucial to understand which portion of secretions is specifically involved in coding individual size information.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.beheco.oxfordjournals.org/>

This work was conducted under permits for Italy by “Ministero dell'Università e della Ricerca”—MIUR, prot. 0000685/PNM/15.01.2019. We want to thank two anonymous reviewers for their helpful comments on

manuscript. Analyses reported in this article can be reproduced using the data provided by Author (<https://doi.org/10.5061/dryad.gmsbcc2s7>)

FUNDING

No funding was received for this work.

ETHICAL APPROVAL

The experiment was conducted in accordance with the ARRIVE guidelines (<https://arriveguidelines.org>).

Conflict of Interest: The authors declare no conflicts of interest.

Data Availability: Analyses reported in this article can be reproduced using the data provided by Scali et al. (2022).

Handling Editor: Mark Briffa

REFERENCES

- Abalos J, Pérez i de Lanuza G, Carazo P, Font E. 2016. The role of male coloration in the outcome of staged contests in the European common wall lizard (*Podarcis muralis*). *Behaviour*. 153(5):607–631.
- Adkins E, Schlesinger L. 1979. Androgens and the social behavior of male and female lizards (*Anolis carolinensis*). *Horm Behav*. 13(2):139–152.
- Anthony CD, Wicknick JA. 1993. Aggressive interactions and chemical communication between adult and juvenile salamanders. *J Herpetol*. 27(3):261–264.
- Aragón P, López P, Martín J. 2006. Roles of male residence and relative size in the social behavior of Iberian rock lizards, *Lacerta monticola*. *Behav Ecol Sociobiol*. 59:762–769.
- Aragón P, López P, Martín J. 2007. Familiarity modulates social tolerance between male lizards, *Lacerta monticola*, with size asymmetry. *Ethol Ecol Evol*. 19(1):69–76.
- Baeckens S, Driessens T, Van Damme R. 2016. Intersexual chemosensation in a “visually-oriented” lizard, *Anolis sagrei*. *PeerJ* 2016(3):e1874.
- Barbosa D, Font E, Desfilis E, Carretero MA. 2006. Chemically mediated species recognition in closely related *Podarcis* wall lizards. *J Chem Ecol*. 32:1587–1598.
- Bradbury JW, Vehrencamp SL. 2011. Principles of animal communication. 2nd ed. Sunderland, MA: Sinauer Associates.
- Braña F. 1991. Summer activity patterns and thermoregulation in the wall lizard, *Podarcis muralis*. *Herpetol J*. 1:544–549.
- Breithaupt T, Eger P. 2002. Urine makes the difference: chemical communication in fighting crayfish made visible. *J Exp Biol*. 205(9):1221–1231.
- Brennan PA, Kendrick KM. 2006. Mammalian social odours: attraction and individual recognition. *Philos Trans R Soc B Biol Sci*. 361(1476):2061–2078.
- Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J*. 9(2):378–400.
- Brown JL. 1964. The evolution of diversity in avian territorial systems. *Wilson Bull*. 76(2):160–169.
- Burnham KP, Anderson DR. 2001. Kullback-Leibler information as a basis for strong inference in ecological studies. *Wildl Res*. 28(2):111–119.
- Carazo P, Font E, Desfilis E. 2008. Beyond “nasty neighbours” and “dear enemies”? Individual recognition by scent marks in a lizard (*Podarcis hispanica*). *Anim Behav*. 76(6):1953–1963.
- Coladonato AJ, Mangiacotti M, Scali S, Zuffi MAL, Pasquariello C, Matellini C, Buratti S, Battaia M, Sacchi R. 2020. Morph-specific seasonal variation of aggressive behaviour in a polymorphic lizard species. *PeerJ*. 8:e10268.
- Cole CJ. 1966. Femoral glands in lizards: a review. *Herpetologica*. 22(3):199–206.
- Cooper WE. 1994. Chemical discrimination by tongue-flicking in lizards: a review with hypotheses on its origin and its ecological and phylogenetic relationships. *J Chem Ecol*. 20(2):439–487.
- Cooper WE. 1998. Prey chemical discrimination indicated by tongue-flicking in the eublepharid gecko *Coleonyx variegatus*. *J Exp Zool*. 281(1):21–25.

- Cooper WE, Vitt LJ. 1986. Thermal dependence of tongue-flicking and comments on use of tongue-flicking as an index of squamate behavior. *Ethology*. 71(3):177–186.
- van Damme R, Bauwens D, Vanderstighelen D, Verheyen RF. 1990. Responses of the lizard *Lacerta vivipara* to predator chemical cues: the effects of temperature. *Anim Behav*. 40(2):298–305.
- DeNardo DF, Licht P. 1993. Effects of corticosterone on social behavior of male lizards. *Horm Behav*. 27(2):184–199.
- DeNardo DF, Sinervo B. 1994. Effects of corticosterone on activity and home-range size of free-ranging male lizards. *Horm Behav*. 28(1):53–65.
- Edsman L. 1990. Territoriality and competition in wall lizards [PhD Thesis]. Stockholm, Sweden: Department of Zoology, Division of Ethology, University of Stockholm.
- Eisenberg JF, Kleiman DG. 1972. Olfactory communication in mammals. *Annu Rev Ecol Syst*. 3:1–32.
- Endler JA. 1993. Some general comments on the evolution and design of animal communication systems. *Philos Trans R Soc London B*. 340(1292):215–225.
- Engbretson GA, Livezey RL. 1972. The effects of aggressive displays on body temperature in the fence lizard *Sceloporus occidentalis occidentalis* Baird and Girard. *Physiol Zool*. 45(3):247–254.
- Fernández JB, Bastiaans E, Medina M, Méndez De la Cruz FR, Sinervo BR, Ibarreguiyotía NR. 2018. Behavioral and physiological polymorphism in males of the austral lizard *Liolaemus sarmientoi*. *J Comp Physiol A*. 204(2):219–230.
- Fisher J. 1954. Evolution and bird sociality. In: Huxley J, Hardy AC, Ford EB, editors. *Evolution as a process*. London, UK: Allen & Unwin. p. 71–83.
- Fox SF, McCoy K, Baird TA. 2003. *Lizard social behavior*. Baltimore, MD: Johns Hopkins University Press.
- Friard O, Gamba M. 2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol Evol*. 7(11):1325–1330.
- Fuller RC, Endler JA. 2018. A perspective on sensory drive. *Curr Zool*. 64(4):465–470.
- Galeotti P, Sacchi R, Pellitteri-Rosa D, Bellati A, Cocca W, Gentili A, Scali S, Fasola M. 2013. Colour polymorphism and alternative breeding strategies: effects of parent's colour morph on fitness traits in the common wall lizard. *Evol Biol*. 40:385–394.
- García-Roa R, Jara M, Baeckens S, López P, Van Damme R, Martín J, Pincheira-Donoso D. 2017. Macroevolutionary diversification of glands for chemical communication in squamate reptiles. *Sci Rep*. 7(1):9288.
- Hertz PE, Huey RB, Nevo E. 1982. Fight versus flight: body temperature influences defensive responses of lizards. *Anim Behav*. 30(3):676335–676679.
- Immelmann K. 1983. *Introduction to ethology*. New York, USA: Plenum Press.
- Kaufmann JH. 1983. On the definitions and functions of dominance and territoriality. *Biol Rev*. 58(1):1–20.
- Khannoon ER, El-Gendy A, Hardege JD. 2011a. Scent marking pheromones in lizards: Cholesterol and long chain alcohols elicit avoidance and aggression in male *Acanthodactylus boskianus* (Squamata: Lacertidae). *Chemoecology*. 21(3):143–149.
- Khannoon ER, Flachsbarth B, El-Gendy A, Mazik K, Hardege JD, Schulz S. 2011b. New compounds, sexual differences, and age-related variations in the femoral gland secretions of the lacertid lizard *Acanthodactylus boskianus*. *Biochem Syst Ecol*. 39(2):95–101.
- Labra A. 2006. Chemoreception and the assessment of fighting abilities in the lizard *Liolaemus monticola*. *Ethology*. 112(10):993–999.
- Labra A. 2008. Multi-contextual use of chemosignals by *Liolaemus* lizards. In: Hurst JL, Beynon RJ, Roberts SC, Wyatt TD, editors. *Chemical signals in vertebrates*, 11. New York, NY: Springer. p. 357–365.
- Lailvaux SP, Huyghe K, Van Damme R. 2012. Why can't we all just get along? Interspecific aggression in resident and non-resident *Podarcis melisellensis* lizards. *J Zool*. 288(3):207–213.
- Leuck BE. 1995. Territorial defence by male green anoles: an experimental test of the role of residency and resource quality. *Herpetol Monogr*. 9:63–74.
- López P, Amo L, Martín J. 2006. Reliable signaling by chemical cues of male traits and health state in male lizards, *Lacerta monticola*. *J Chem Ecol*. 32(2):473–488.
- López P, Martín J. 2001. Fighting roles and rival recognition reduce costs of aggression in male lizards, *Podarcis hispanica*. *Behav Ecol Sociobiol*. 49(2–3):111–116.
- López P, Martín J. 2002. Chemical rival recognition decreases aggression levels in male Iberian wall lizards, *Podarcis hispanica*. *Behav Ecol Sociobiol*. 51(5):461–465.
- López P, Martín J. 2011. Male Iberian rock lizards may reduce the costs of fighting by scent matching of the resource holders. *Behav Ecol Sociobiol*. 65(10):1891–1898.
- Mangiacotti M, Fumagalli M, Cagnone M, Viglio S, Bardoni AM, Scali S, Sacchi R. 2019a. Morph-specific protein patterns in the femoral gland secretions of a colour polymorphic lizard. *Sci Rep*. 9(1):8412.
- Mangiacotti M, Gaggiani S, Coladonato AJ, Scali S, Zuffi MAL, Sacchi R. 2019b. First experimental evidence that proteins from femoral glands convey identity-related information in a lizard. *Acta Ethol*. 22(1):57–65.
- Mangiacotti M, Martín J, López P, Reyes-Olivares CV, Rodríguez-Ruiz G, Coladonato AJ, Scali S, Zuffi MAL, Sacchi R. 2020. Proteins from femoral gland secretions of male rock lizards *Iberolacerta cyreni* allow self—but not individual—recognition of unfamiliar males. *Behav Ecol Sociobiol*. 74(6):68.
- Mangiacotti M, Pezzi S, Fumagalli M, Coladonato AJ, D'Etorre P, Leroy C, Bonnet X, Zuffi MAL, Scali S, Sacchi R. 2019c. Seasonal variations in femoral gland secretions reveals some unexpected correlations between protein and lipid and components in a lacertid lizard. *J Chem Ecol*. 45:673–683.
- Martín J, Amo L, López P. 2008. Parasites and health affect multiple sexual signals in male common wall lizards, *Podarcis muralis*. *Naturwissenschaften*. 95(4):293–300.
- Martín J, López P. 2000. Chemoreception, symmetry and mate choice in lizards. *Proc R Soc Lond B Biol*. 267(1450):1265–1269.
- Martín J, López P. 2007. Scent may signal fighting ability in male Iberian rock lizards. *Biol Lett*. 3(2):125–127.
- Martín J, López P. 2011. Pheromones and reproduction in reptiles. In: Norris DO, Lopez KH, editors. *Hormones and reproduction of vertebrates*, Vol. 3. San Diego, California, USA: Academic Press. p. 141–167.
- Martín J, López P. 2015. Condition-dependent chemosignals in reproductive behavior of lizards. *Horm Behav*. 68:14–24.
- Martins EP, Ord TJ, Slaven J, Wright JL, Housworth EA. 2006. Individual, sexual, seasonal, and temporal variation in the amount of sagebrush lizard scent marks. *J Chem Ecol*. 32(4):881–893.
- Martins EP, Ossip-Drahos AG, Vital García C, Zúñiga-Vega JJ, Campos SM, Hews DK. 2018. Trade-offs between visual and chemical behavioral responses. *Behav Ecol Sociobiol*. 72(12):189.
- Mason RT. 1992. Reptilian pheromones. In: Gans C, Crews D, editors. *Biology of the Reptilia: Vol. 18. Physiology E*. Chicago, USA: University of Chicago Press. p. 114–228.
- Mason RT. 1993. Chemical ecology of the red-sided garter snake, *Thamnophis sirtalis parietalis*. *Brain Behav Evol*. 41(3–5):261–268.
- Mathis A. 1990. Territorial salamanders assess sexual and competitive information using chemical signals. *Anim Behav*. 40(5):953–962.
- Mautz WJ, Daniels CB, Bennett AF. 1992. Thermal dependence of locomotion and aggression in a xantusiid lizard. *Herpetologica*. 48(3):271–279.
- Mayerl C, Baeckens S, Van Damme R. 2015. Evolution and role of the follicular epidermal gland system in non-ophidian squamates. *Amphib-Reptilia*. 36(3):185–206.
- Maynard-Smith J, Price GR. 1973. The logic of animal conflict. *Nature*. 246(2):15–18.
- Myrberg AA, Thresher RE. 1974. Interspecific aggression and its relevance to the concept of territoriality in Reef fishes. *Am Zool*. 14(1):81–96.
- Nisa Ramiro C, Rodríguez-Ruiz G, López P, da Silva Junior PI, Trefaut Rodrigues M, Martín J. 2019. Chemosensory discrimination of male age by female *Psammmodromus algirus* lizards based on femoral secretions and feces. *Ethology*. 125(11):802–809.
- Olsson M. 1992. Contest success in relation to size and residency in male sand lizards, *Lacerta agilis*. *Anim Behav*. 44(2):386–388.
- Pérez i de Lanuza G, Font E, Carazo P. 2012. Color-assortative mating in a color-polymorphic lacertid lizard. *Behav Ecol*. 24(1):273–279.
- Pianka ER. 1973. The structure of lizard communities. *Annu Rev Ecol Syst*. 4:53–74.
- Pough FH, Andrews RM, Cadle JE, Crump ML, Savitzky AH, Wells KD. 2004. *Herpetology*. 3rd ed. Upper Saddle River, NJ, USA: Pearson Prentice Hall.
- Prokopy RJ, Roitberg BD, Averill AL. 1984. Resource partitioning. In: Bell WJ, Cardé RT, editors. *Chemical ecology of insects*. New York: Springer-Science + Business Media, B.V. p. 301–330.
- Pruett JA, Zúñiga-Vega JJ, Campos SM, Soini HA, Novotny MV, Vital-García C, Martins EP, Hews DK. 2016. Evolutionary interactions between visual and chemical signals: chemosignals compensate for

- the loss of a visual signal in male *Sceloporus* lizards. *J Chem Ecol.* 42(11):1164–1174.
- R Development Core Team. 2018. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. <http://www.rproject.org>.
- Robinson CD, Patton MS, Andre BM, Johnson MA. 2015. Convergent evolution of brain morphology and communication modalities in lizards. *Curr Zool.* 61(2):281–291.
- Romero-Diaz C, Pruett JA, Campos SM, Ossip-Drahos AG, Zúñiga-Vega JJ, Vital-García C, Hews DK, Martins EP, Romero-Diaz C. 2021. Evolutionary loss of a signalling colour is linked to increased response to conspecific chemicals. *Proc R Soc B Biol.* 288(1947):20210256.
- Sacchi R, Coladonato AJ, Battaiola M, Pasquariello C, Buratti S, Matellini C, Mangiacotti M, Scali S, Zuffi MAL. 2021. Subjective resource value affects aggressive behavior independently of resource-holding-potential and color morphs in male common wall lizard. *J Ethol.* 39:179–189.
- Sacchi R, Pupin F, Gentilli A, Rubolini D, Scali S, Fasola M, Galeotti P. 2009. Male-male combats in a polymorphic lizard: residency and size, but not color, affect fighting rules and contest outcome. *Aggress Behav.* 35(3):274–283.
- Sacchi R, Rubolini D, Gentilli A, Pupin F, Razzetti E, Scali S, Galeotti P, Fasola M. 2007. Morph-specific immunity in male *Podarcis muralis*. *Amphib-Reptilia.* 28(3):408–412.
- Sannolo M, Mangiacotti M, Sacchi R, Scali S. 2014. Keeping a cool mind: head-body temperature differences in the common wall lizard. *J Zool.* 293(2):71–79.
- Scali S, Mangiacotti M, Sacchi R, Coladonato AJ, Falaschi M, Saviano L, Rampoldi MG, Crozi M, Perotti C, Zucca F, et al. 2021. Close encounters of the three morphs: does color affect aggression in a polymorphic lizard? *Aggress Behav.* 47(4):430–438.
- Scali S, Sacchi R, Azzusi M, Daverio S, Oppedisano T, Mangiacotti M. 2013. Homeward bound: factors affecting homing ability in a polymorphic lizard. *J Zool.* 289(3):196–203.
- Scali S, Sacchi R, Falaschi M, Coladonato AJ, Pozzi S, Zuffi MAL, Mangiacotti M. 2019. Mirrored images but not silicone models trigger aggressive responses in male Common wall lizards. *Acta Herpetol.* 14(1):35–41.
- Scali S, Sacchi R, Gozzo E, Chiesa S, Coladonato AJ, Zuffi MAL, Mangiacotti M. 2022. Data from: the size of a smell: assessment of rival's relative size from femoral secretions in the common wall lizards, *Podarcis muralis* (Laurenti, 1768). *Behav Ecol.* doi:10.5061/dryad.gmsbcc2s7.
- Schulte BA, Freeman EW, Goodwin TE, Hollister-Smith J, Rasmussen LEL. 2007. Honest signalling through chemicals by elephants with applications for care and conservation. *Appl Anim Behav Sci.* 102(3–4):344–363.
- Schwenk K. 1993. The evolution of chemoreception in Squamate Reptiles: a phylogenetic approach. *Brain Behav Evol.* 41(3–5):124–137.
- Scordato ES, Drea CM. 2007. Scents and sensibility: information content of olfactory signals in the ringtailed lemur, *Lemur catta*. *Anim Behav.* 73(2):301–314.
- Shine R, Phillips B, Wayne H, LeMaster M, Mason RT. 2003. Chemosensory cues allow courting male garter snakes to assess body length and body condition of potential mates. *Behav Ecol Sociobiol.* 54(2):162–166.
- Stamps JA. 1977. The relationship between resource competition, risk, and aggression in a tropical territorial lizard. *Ecology.* 58(2):349–358.
- Stamps JA. 1995. Territory acquisition in lizards: III. Competing for space. *Anim Behav.* 49(3):679–693.
- Stamps JA, Krishnan VV. 1994. Territory acquisition in lizards: I. First encounters. *Anim Behav.* 47(6):1375–1385.
- Stevenson RD, Peterson CR, Tsuji JS. 1985. The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. *Physiol Zool.* 58(1):46–57.
- Titone V, Marsiglia F, Mangiacotti M, Sacchi R, Scali S, Zuffi MAL. 2018. Better to be resident, larger or coloured? Experimental analysis on intra-specific aggression in the ruin lizard. *J Zool.* 304(4):260–267.
- Tokarz RR. 1985. Body size as a factor determining dominance in staged agonistic encounters between male brown anoles (*Anolis sagrei*). *Anim Behav.* 33(3):746–753.
- Van den Berghe PL. 1974. Bringing beasts back in: toward a biosocial theory of aggression. *Am Sociol Rev.* 39(6):777–788.
- Whiting MJ. 1999. When to be neighbourly: differential agonistic responses in the lizard *Platysaurus broadleyi*. *Behav Ecol Sociobiol.* 46(3):210–214.
- Whiting MJ, While GM. 2017. Sociality in lizards. In: Rubenstein DR, Abbot P, editors. *Comparative social evolution*. New York: Cambridge University Press. p. 354–389.
- Wilson EO. 1978. *Sociobiology: the new synthesis*. 6th ed. Cambridge (MA): The Belknap Press of Harvard University.
- Woolrich-Piña GA, Smith GR, Martínez-Méndez N, Lemos-Espinal JA, Gadsden-Esparza H. 2021. Effects of body temperature on initial bite force in three species of rock- and crevice-dwelling lizards from Mexico. *Herpetozoa.* 34:163–168.
- Wyatt TD. 2014. *Pheromones and animal behaviour*. Cambridge, UK: Cambridge University Press.
- Zahavi A. 1975. Mate selection-a selection for a handicap. *J Theor Biol.* 53(1):205–214. doi:10.1016/0022-5193(75)90111-3.
- Zuur AF, Saveliev AA, Ieno EN. 2012. *Zero inflated models and generalized linear mixed models with R*. Newburgh, UK: Highland Statistics Ltd.