Where to do number two: Lizards prefer to defecate on the largest rock in the territory

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\section*{A R T I C L E   I N F O}

Keywords:
Podarcis melisellensis
Defecation behaviour
Faecal pellets
Substrate use

\section*{A B S T R A C T}

Many animals use their excrements to communicate with cons- and heterospecifics (e.g. amphibians: Gautier and Miaud, 2003; birds: Penteriani and del Mar Delgado, 2008; mammals: Brashares and Arcese, 1999; reptiles: Fenner and Bull, 2010; invertebrates: Ślusarczyk and Rygiewska, 2004). Faeces can convey information on territory residency, reproductive status (e.g. Kean et al., 2011; Marneweck et al., 2017), and they often function as composite signals (Wickler, 1978), operating both as chemical signals (i.e. scent marks) and visual signals (Duvall et al., 1987). Such a bimodal signalling system can be beneficial as it may convey multiple messages at once (Hebets and Papaj, 2005) or increase signal effectiveness (Johnstone, 1996; Partan, 2013; Partan and Marler, 1999). In lizards, for instance, faecal deposits are often considered to function as initial cues or ‘signposts’ for conspecifics to detect, locate, and approach the deposits visually (Duvall et al., 1987). Afterwards, lizards can chemically investigate the mark more closely through (vomer)olfaction (Cooper, 1994; Cooper and Burghardt, 1990; Duvall et al., 1987). In such a scenario, where excrements operate as functional visual cues, a key requirement is that faecal deposits are easily and rapidly detectable from a large distance (Endler, 2000). To do so, lizards may adjust their defecation behaviour (Ferguson, 1978). One possible behavioural tactic of lizards to enhance visual detectability is by producing piles of faecal pellets (geckos: Carpenter and Duvall, 1995; Shah et al., 2006; skinks: Bull et al., 1999; Fenner et al., 2015) or alternatively, by favouring prominent positions as defecation site, such as high placed substrates (lacertids: Aragón et al., 2000; Moreira et al., 2006). For instance, field observations by López et al. (1998) indicate that Iberian rock lizards (Iberolacerta cyreni) tend to deposit their faecal pellets on the tip of rocks. Remarkably, empirical evidence on defecation site preferences of lizards is scarce, with a number of gaps in our understanding of lizard defecation behaviour remaining. First, it is still uncertain whether lizards prefer certain defecation sites, such as rock-tips (López et al., 1998), to enhance visual detectability, or whether these site preferences are a mere consequence of lizards selecting for rock-tips for different reasons than signal efficiency alone. In a field setting, rock-tips can also provide better opportunities for thermoregulation, foraging, escaping predators, and direct visual communication with conspecifics (Carrascal et al., 1992; 

\section*{1. Introduction}

Many animals use their excrements to communicate with conspecifics (e.g. amphibians: Gautier and Miaud, 2003; birds: Penteriani and del Mar Delgado, 2008; mammals: Brashares and Arcese, 1999; reptiles: Fenner and Bull, 2010; invertebrates: Ślusarczyk and Rygiewska, 2004). Faeces can convey information on territory residency, reproductive status (e.g. Kean et al., 2011; Marneweck et al., 2017), and they often function as composite signals (Wickler, 1978), operating both as chemical signals (i.e. scent marks) and visual signals (Duvall et al., 1987). Such a bimodal signalling system can be beneficial as it may convey multiple messages at once (Hebets and Papaj, 2005) or increase signal effectiveness (Johnstone, 1996; Partan, 2013; Partan and Marler, 1999). In lizards, for instance, faecal deposits are often considered to function as initial cues or ‘signposts’ for conspecifics to detect, locate, and approach the deposits visually (Duvall et al., 1987). Afterwards, lizards can chemically investigate the mark more closely through (vomer)olfaction (Cooper, 1994; Cooper and Burghardt, 1990; Duvall et al., 1987). In such a scenario, where excrements operate as functional visual cues, a key requirement is that faecal deposits are easily and rapidly detectable from a large distance (Endler, 2000). To do so, lizards may adjust their defecation behaviour (Ferguson, 1978). One possible behavioural tactic of lizards to enhance visual detectability is by producing piles of faecal pellets (geckos: Carpenter and Duvall, 1995; Shah et al., 2006; skinks: Bull et al., 1999; Fenner et al., 2015) or alternatively, by favouring prominent positions as defecation site, such as high placed substrates (lacertids: Aragón et al., 2000; Moreira et al., 2006). For instance, field observations by López et al. (1998) indicate that Iberian rock lizards (Iberolacerta cyreni) tend to deposit their faecal pellets on the tip of rocks. Remarkably, empirical evidence on defecation site preferences of lizards is scarce, with a number of gaps in our understanding of lizard defecation behaviour remaining. First, it is still uncertain whether lizards prefer certain defecation sites, such as rock-tips (López et al., 1998), to enhance visual detectability, or whether these site preferences are a mere consequence of lizards selecting for rock-tips for different reasons than signal efficiency alone. In a field setting, rock-tips can also provide better opportunities for thermoregulation, foraging, escaping predators, and direct visual communication with conspecifics (Carrascal et al., 1992;
Martin and Salvador, 1993, 1992). Second, while we know that there can be considerable interspecific variability in lizard defecation behaviour (Bull et al., 1999; Fenner and Bull, 2010), we only have limited information on whether defecation preferences vary among populations within a species. One might expect that lizards from populations that differ in their need to defend and signal their territory, due to differences in, for instance, ecological resources or population structure, such as sex ratio and population density (Martins, 1994), also differ in their overall defecation behaviour. Third, since it is challenging to distinguish faecal pellets of female lizards from those of males (especially in the field), there is little knowledge on whether defecation site preference differs between sexes. Most lizard species are polygynous where dominant males hold territories that contain the home ranges of several females (Bull, 2000). In such a scenario, males rely stronger on territory marking than females (Martins, 1994), and therefore, might benefit more from strategically depositing their faeces on prominent places. In polyandrous species, one might expect the opposite. Clearly, standardized indoor experiments are essential in order (1) to assess defecation preferences of lizards independently from any field-specific confounding factors that may obscure patterns of defecation behaviour (e.g. variation in temperature, prey availability, predator pressure), and (2) to determine the degree of intraspecific variability in lizard defecation behaviour.

We performed a laboratory study to experimentally assess the preferred defecation sites of lizards in a standardized setting. Using the focal lizard species Podarcis melisellensis, we examined whether lizards show a preference for a specific-sized rocky substrate to deposit their faeces on. In addition, we tested whether defecation preferences differ between sexes, and among populations. Based on the idea that faecal deposits may operate as long-distance visual cues, we hypothesized that lizards favour to defecate on the largest rock in their territory as it likely maximizes visual detectability. Previous research has shown that P. melisellensis males defend their territories and readily engage in fights with intruders (Böhme, 1986; Huyghe et al., 2012), and that male territories can contain multiple females (Huyghe et al., 2014). Therefore, we expect males to show a stronger defecation preference than females. Lastly, based on the premise that lizards from populations that carry high densities invest strongly in the protection of resources and territories (Donihue et al., 2016), we expect lizards from high-density populations to show a stronger defecation preference than those from low-density populations.

2. Materials and methods

2.1. Study species

The Dalmatian wall lizard, Podarcis melisellensis (Fig. 1a) is a diurnal ground-dwelling lacertid, occupying a variety of habitats along the Adriatic coast and on islands in the Adriatic Sea (Arnold and Ovenden, 2004; Bauwens et al., 1986), and known to rely both on visual and chemical cues for intraspecific communication (Huyghe et al., 2012, 2007). In May 2018, we captured a total of 90 lizards (45 females, 45 males) on three different Croatian islands: Vis (latitude-longitude: 43.047-16.147), Mali Barjak (43.053-16.040), and Brusnik (43.000-15.801). Population density estimations show that, of the three sampled islands, Brusnik holds the absolute largest lizard population (approximately 820 lizards per ha), Mali Barjak the second largest (400 lizards per ha), and Vis the smallest population (70 lizards per ha) (Table S1). Lizards were kept individually in cloth bags and transported to the animal facility at the University of Antwerp (Belgium), where they were housed individually in large plastic terraria (57 × 39 × 28 cm). Each terrarium contained a layer of sandy substrate, some plastic vegetation, and a slate stone. A 45-watt bulb suspended above one of the terrarium provided light (12L:12D) and heat so that lizards could maintain a body temperature within their preferred range. Lizards had access to freshwater at all times, and were fed crickets (Acheta domestica) dusted with multivitamin powder thrice a week.

2.2. Experimental procedure

In order to enable lizards to acclimatize to the novel environment, experimental trials started three months after capture. To test for defecation preferences of individual lizards, slate stones were removed from the terraria, and replaced by three (gravel) rocks of dissimilar size: a large, medium, and small rock. The three rocks differed significantly in height and width (Table S2). In each terrarium, rocks were placed next to each other, in the mid of the cage (Fig. 2). We made sure that the order of the stones was randomized per cage. The temperature of the dissimilar-sized rocks did not significantly differ ($F_{2,31} = 1.31, P = 0.282$), and averaged around $28.3 \pm 0.2^{\circ}$C (Table S2). Once the stones were placed, stones were kept untouched and lizards kept undisturbed for two weeks. After two weeks, we counted the number of faecal pellets that were deposited on each stone, and determined for each lizard its defecation stone preference; this was the stone (large, medium, or small) containing the most faecal droppings. Faecal pellets that were deposited on any other substrate in the terrarium were not counted.

2.3. Statistical analyses

To test for significant differences in defecation stone preference, a binomial generalized linear mixed-model (GLMM) was used, with stone size, population, and sex as fixed factors, and stone preference as dependent variable. With the same fixed factors, we used a Poisson model to test for differences in the number of faecal pellets. Non-significant terms were stepwise eliminated from the models. Population of origin was included as random effect in all models, with individual lizards nested within population. All analyses were conducted in R 3.5.2 (Rako and Gentleman, 1996), using the packages ‘lme4’ (Bates et al., 2015), ‘lmerTest’ (Kuznetsova et al., 2017), and ‘nlme’ (Pinheiro et al., 2019).

The package ‘emmeans’ (Lenth, 2019) was used to compute (pairwise) estimated marginal means for the factors in the linear models.

3. Results

Out of the 90 lizards, 85 defecated on (one or more) stones; the five lizards that did not defecate were excluded from further analyses (i.e. two Brusnik males, two Mali Barjak females, and one Vis male). In three cases, we counted an equal number of faecal pellets on the stones (i.e. one Brusnik female and two Vis females); these cases were documented as ‘no preference’ and were not included in the statistical analysis of stone preference.

The GLMM indicated significant and strong differences in stone defecation preference ($F_{2,243} = 45.54, P < 0.001$; Fig. 1b), with an absolute preference for the largest stone in comparison to the medium-sized stone ($\text{coeff.} = 3.55, z = 7.95, P < 0.001$) and the smallest stone ($\text{coeff.} = 4.85, z = 7.38, P < 0.001$). Defecation preference did not differ between males and females ($F_{1,243} = 0.372, P = 0.715$), or among lizards from different populations ($F_{2,243} = 0.171, P = 0.954$).

In addition, our statistics revealed significant differences in the number of faecal pellets that were found on the different-sized stones ($F_{2,243} = 90.63, P < 0.001$; Fig. 1c). On average, lizards deposited significantly more pellets on the largest rock (mean number of pellets ± SE: $3.10 \pm 0.22$), than on the medium rock ($0.89 \pm 0.14$), and the small rock ($0.23 \pm 0.08$); we also counted significantly more pellets on the medium rock than on the small rock (Fig. 1c). In addition, there were significant differences in faecal pellet counts among populations ($F_{1,213} = 5.29, P = 0.006$; Fig. 1e). Lizards from the Brusnik population ($0.88 \pm 0.17$) defecated less than lizards from Mali Barjak ($1.74 \pm 0.23$) and Vis ($1.54 \pm 0.21$), and females ($1.61 \pm 0.20$) defecated more than males ($1.24 \pm 0.14$). None of the interactions were statistically significant (all $F < 2.39, P > 0.1$).
4. Discussion

By taking an experimental approach, we, here, show that Dalmatian wall lizards (*Podarcis melisellensis*) prefer to deposit their faecal pellets on the largest stone in their territory. Moreover, we demonstrate that lizards’ defecation preference is a strong conservative behaviour within a species, showing no significant differences between sexes or among populations. Defecation rate, however, does vary intraspecifically.

Together, these findings corroborate prior field observations, suggesting that lizards select for specific defecation sites, which likely increases (visual) detectability of their faecal deposits by others, and thus, favouring the role of faeces as signals.

Our findings show that *P. melisellensis* lizards prefer to defecate on the largest rock in the environment. Earlier field observations indicated that lizards tend to select for specific locations to deposit their faecal pellets (Carpenter and Duvall, 1995; Fenner and Bull, 2010; López et al., 1998; Shah et al., 2006), yet, due to the field conditions in which these observations were made, it has remained difficult to untangle the exact origin of this behaviour. For instance, it is uncertain whether some species select rock-tips as defecation sites because it may increase the visual detectability of their faecal deposits (López et al., 1998), or because it facilitates the search for prey (Díaz and Carrascal, 1991), the escape from predators (Marshall et al., 2016), direct visual communication with conspecifics (Marshall and Stevens, 2014) or overall thermoregulation (Bauwens et al., 1996; Castilla and Bauwens, 1991; Martín et al., 1995). In our laboratory study, we created a highly standardized environment to observe lizard defecation behaviour. By doing so, we eliminated any potential ‘noisy’ environmental variation that may confound clear patterns of defecation behaviour. Our study was, therefore, able to demonstrate that in an environment where all stony substrates provide equal thermoregulatory advantage, foraging success, and predator pressure, lizards still select for the largest stone in their surroundings. While these findings may imply that lizards defecate on prominent and conspicuous substrates in order to increase visual detectability, additional behavioural assays are required to determine whether faecal deposits on larger stones are truly more easily detected by conspecifics than those on smaller stones. Moreover, since our experimental design did not allow testing for the individual effect of stone height and stone width on lizard defecation preference (as height and width were highly correlated; $R^2 = 0.82; P < 0.001$), future
experiments could focus on the specific stone dimensions that may influence defecation behaviour (do lizards prefer high stones over wide stones?). In addition, assessing the role of substrate structure might reveal valuable insight too (do lizards prefer stone over bark?).

Our experimental study not only shows that lizards prefer to defecate on the largest rock, but also that defecation preference is independent of sex or population of origin. One possible explanation on why we fail to find an effect of sex is because *P. melisellensis* might not be as polygynous as traditionally thought. Indeed, DNA microsatellite parentage analyses by Huyghe et al. (2014) revealed that not only *P. melisellensis* males, but also females have a high number of sexual partners. As lizards of both sexes behave promiscuously, there might be no strong sex differences in intraspecific signalling behaviour, and by extension, defecation behaviour. In the same line, a potential explanation for why defecation preference does not differ among the populations of study may be because the degree of territorial behaviour might not differ among populations (Carazo et al., 2008; Font et al., 2012). Although estimates of lizard densities indicate large inter-population variation (Table S1), we lack information on sex ratios and general population dynamics to make any valuable predictions or conclusion of interpopulation difference in territorial behaviour, hence, defecation behaviour. Another argument on why we did not detect an effect of sex and population on lizard defecation preference might be related to the timing of our experiment and overall lizard phenology. While Mediterranean insular lacertids can be active year-round (even during periods of warm and sunny weather in winter) with lizard activity peaking between March and October, the reproductive season typically ends in August (Bauwens, 1999; Böhme, 1986; Carretero, 2007; Castilla and Bauwens, 2005; Ortega et al., 2014; Ortega and Pérez-Mellado, 2016). Since our study started in September, lizard sex hormones levels may have been low at the onset of our trials, which might have affected the overall motivation of the lizards to communicate and socially interact with others. It is not improbable that this lack of motivation might have masked any potential differences between sexes and among populations. Regardless, the conservative character of defecation behaviour in *P. melisellensis* implies that all individuals exploit their deposits in a similar fashion, and hence, demonstrates the ubiquitous nature of defecation site preference in the species. It would be interesting to examine the generality of this behaviour among different lacertid species, and subsequently, to focus on those species that may show any deviation in general defecation behaviour in order to assess the ecological correlates of any variation in defecation behaviour. While lizards deposited the most faecal pellets on the largest stone, females deposited on average slightly more pellets than males, and lizards from the Brusnik population deposited less pellets than lizards originating from Vis and Mali Barjak. The origin of the observed intraspecific variation in faecal pellet production is most likely non-adaptive as it is known to be linked with intraspecific variation in metabolic rates (Niewiarowski and Waldschmidt, 1992), energy allocation (Warner et al., 2008), and endocrine levels (Yaron, 1972). Future research should resolve whether a higher number of pellets might influence overall visual detectability of faecal deposits.

Declaration of Competing Interest

None.

Acknowledgments

We thank Jan Scholliers, Wannes Leirs, and Jorrit Mertens for their assistance in the field. Legal and ethical authorization for the study was provided by the Croatian Ministry of Environmental Protection and Energy permit nr. S17-07-1-1-1-18-5, Department Environment and Office of Animal Welfare Flanders (TR02100325), and the Ethical Committee for Animal Testing at the University of Antwerp (ECB 2018-23). Simon Baeckens is a postdoctoral fellow of the FWO-Flanders (12F8819N).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.beproc.2019.103937.

References


References
Evolutionary and ecological consequences of tail loss


