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Article type : Research Article

For Consideration In:

Ethology

Article type: Research Article

Running title: No sex-specific personality in a lizard

**No behavioral syndromes or sex-specific personality differences in the southern
rainforest sunskink (*Lampropholis similis*)**

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/ETH.13103](https://doi.org/10.1111/ETH.13103)

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Abstract. Behavioral syndromes, when individuals within a population express consistent behavioral differences across time and context, are widespread in animal taxa. For many species, males and females experience different selective pressures after maturation, resulting in the divergence of life history and behavioral traits. However, the potential for sex-specific differences in individual behaviours and behavioural syndromes has rarely been assessed. Here we tested for sex-specific differences in behavior (activity, exploration, sociability) and personality in the southern rainforest sunskink, *Lampropholis similis*. We found that most behaviors in *L. similis* did not differ between sexes, the exception being sociability which was higher in females than males. In terms of consistency among behaviours, activity and exploration, but not sociability, were repeatable, and there were no sex-specific differences in repeatability of behavioral traits. Although a behavioral syndrome among these three traits is present in a congener (*L. delicata*), we found no evidence for such a syndrome in either sex of *L. similis*. Our study is consistent with the results of studies on other *Lampropholis* skinks that have found only limited sexual differences in behavior. More broadly, our study demonstrates there can be considerable differences in presence or absence of behavioral syndromes in closely related species.

Keywords: activity, behavioral syndrome, exploratory behavior, sexual differences, skink, social behavior

Introduction

After maturation, males and females often diverge in a wide range of morphological and life history traits including body size, longevity, and reproductive investment (Blanckenhorn 2005; Wolf et al. 2007; Schuett et al. 2010). These differences occur as a result of different selection pressures acting on each sex, as well as diverging life-history trajectories after maturity. There is also clear evidence that a range of behaviors (e.g. sociability, dispersal, and risk taking) differ between sexes in many taxa (Pardini et al. 2001; Nakagawa et al. 2007; Piyapong et al. 2010; Marentette et al. 2011; Strickland et al. 2014). For instance, in eastern water dragons (*Intellagama lesueurii*) females are more social and more readily form social bonds with other females when compared to males (Strickland et al. 2014). As well as behavioural differences, the repeatability of behaviours can also differ between sexes (Dall et al. 2004; Bell et al. 2009; Schuett et al. 2010). For example, in house sparrows (*Passer domesticus*), males show more highly repeatable parental care behaviours, both between and within years (Nakagawa et al. 2007).

A behavioral syndrome is defined as a correlation of two or more behaviors that are consistent (i.e. repeatable) across time and contexts (Sih et al. 2004a,b; Bell 2007). Studies are still exploring the degree to which males and females differ in their expression of behavioral syndromes (Schuett and Dall 2009; Chapman et al. 2013; Fresneau et al. 2014; Michelangeli et al. 2016a). Examination of such sexual differences is important, because an individual's behavioral type or personality can have a direct bearing on fitness (Bell 2007; Bell and Sih 2007). Typically, however, animal personality studies tend to focus on a single sex, ignoring the possibility that aspects of behavioral syndromes (e.g. trait means, repeatability, and behavioral correlations) may vary between sexes (Michelangeli et al. 2016a).

Skinks in the genus *Lampropholis* have become a model system for behavioural syndrome research. Behavioral syndromes have been investigated in *Lampropholis* lizards in the context of collection bias (Michelangeli et al. 2016b); habitat selection (Michelangeli et al. 2018); urbanisation (Moule et al. 2016); dispersal and aggression (Michelangeli et al. 2017); their link to thermal types and the pace of life syndrome (Goulet et al. 2017); geographic variation (Michelangeli et al. 2019); the impact of tail autotomy (Michelangeli et al. 2020); and learning capacity (Chung et al. 2017; Goulet et al. 2018). A consistent activity-exploration-sociability behavioral syndrome has been found in the widespread delicate skink (*Lampropholis delicata*); a syndrome present in both sexes (Michelangeli et al. 2016a). Generally in this species, few differences in behavior, or syndrome structure, were documented between the sexes (Michelangeli et al. 2016a). Here, we test for a behavioral syndrome in the closely related southern rainforest sunskink (*L. similis*), a recently described, range-restricted rainforest endemic (Singhal et al. 2018). Specifically, we investigate whether there are sexual differences in behavior, and whether the structure of syndromes (i.e. repeatability, trait correlations) differs between the sexes. Based on the results from *L. delicata*, we predict that a behavioral syndrome will be present in *L. similis*, and it will be expressed in both sexes, with limited behavioral differences between the sexes.

Methods

Field collection and animal husbandry

Adult *L. similis* were collected from the southern limit of the species' distribution, at Hervey Range (19° 21' 57" S, 146° 28' 40" E), between June 2013 and January 2014. This ecologically-isolated population occurs at a low elevation (182 m above sea level) where it experiences a drier and more seasonally variable (i.e., more extreme heat conditions) climate relative to montane populations (Martins et al. 2019). Field-caught skinks were transported to James Cook University in Townsville and housed individually in plastic tubs (340 x 120 x 160 mm) in a temperature-controlled room (21

°C). The tubs were placed on racks with heating mats, providing a thermal gradient from 45 °C to 21 °C inside each tub during the day (from 8:00 to 18:00). To produce offspring, after six months in captivity each skink was placed in a shared housing container with a randomly selected mating partner (Llewelyn et al. 2016). Eggs were collected as they were produced by checking containers daily and were placed individually in airtight containers (84 ml) that were two-thirds filled with moist vermiculite (50:50 ratio of vermiculite to water by weight). Egg containers were put in incubators set at one of two temperatures (23 °C or 26 °C). It should be noted that these incubation temperatures had no detectable effect on the thermal physiology of the adult lizards (Llewelyn et al. 2018). Offspring were housed individually as previously described for wild caught skinks.

In 2015, both wild-caught (referred to as the wild population herein) and captive-bred (referred to as the captive population herein) populations were transported to the temperature-controlled animal housing facility at Monash University, Clayton. Skinks were housed by population in same sex groups of six in plastic containers (300 x 230 x 370 mm) and maintained at 22 °C with a 14 h light: 10 h dark cycle (0600–2000 h). The housing containers were lined with newspaper and contained moist *Sphagnum* moss and a basking site creating a thermal gradient of 20 to 35 °C to promote natural thermoregulatory behavior. Skinks were fed crickets (*Acheta domesticus*) three times weekly and provided water *ad libitum*. Only lab-reared skinks were used in the present study to control for the potential effects arising from varied developmental conditions. Females were non-gravid during the experimental trials, and males and females were housed separately throughout the duration of their time in captivity at Monash University. All individuals were individually marked by clipping toes in a unique sequence.

Data collection

In 2016, a series of assays were conducted to measure activity, exploration, and sociability behaviors (Figure 1) of the captive population, as per the previously published methodology described in brief below (Chapple et al. 2011; Moule et al. 2016; Michelangeli et al. 2016a,b, 2019, 2020). Skinks (females: n = 34; males: n = 38) were tested individually and exposed to each behavioral assay twice with four days between repeats. Assays were conducted in opaque plastic containers (~55 x 32 x 24 cm) in a temperature-controlled room (~22 °C). Video cameras (Panasonic HC-V130) were suspended above each test arena to record behavioral responses during the experiments. Videos were imported into the program JWatcher (Blumstein et al. 2006) and behaviours scored blindly. Each experiment included a 10-min acclimation period where the skink was placed under a clear plastic container in the centre of the arena, with the trial beginning after this container was removed. Assays were conducted over 30 min. Following each test, the

equipment was washed with scentless detergent and thoroughly dried to prevent scent contamination between trials.

Activity was measured by placing skinks individually into a test arena marked with 20 equal grid squares on the base (Figure 1a). The level of activity was scored based on the number of transitions between squares. Exploration was measured by presenting skinks with a 10-cm high, opaque trapezium barrier dividing the arena into two equally sized compartments: the starting compartment and goal compartment (Figure 1b). Skinks had to squeeze between the barrier and arena wall to move from one compartment to the other. Time to reach the goal compartment was used as a measure of exploratory behavior. Lizards not reaching the goal compartment were assigned 1800 s as their time. Finally, sociability was measured by placing skinks in a test arena divided into three equally sized zones: social zone, asocial zone, and an intermediate neutral zone (Figure 1c). The social zone was comprised of a basking site (under a heat lamp) that was divided in half by a clear PerspexTM partition that ran the length of the test arena. Three stimulus skinks (the same sex as the focal lizard) that were not part of the study were placed behind the partition. The asocial zone located at the opposite end of the arena was identical however it contained no stimulus skinks. The amount of time spent basking with conspecifics was used as measures of sociability.

Statistical analysis

Data were analysed using the program R 3.50 (R Development Core Team 2016), with statistical significance set to $\alpha = 0.05$. Prior to analyses, assumptions were tested by examining diagnostic plots. SVL was previously shown not to influence behavior in this species (de Jong 2017), and thus was excluded from the analyses.

Average differences in behavioral traits among males and females was assessed using separate linear mixed-effects models ('lme4' package). For each model, sex was included as a fixed factor and skink ID as a random effect to account for repeated measures. Overall between-trial repeatability was calculated using the variance components from the mixed-effects models (ratio among-individual variation to total phenotypic variation). Parametric bootstrapping provided 95% confidence intervals and statistical significance was evaluated with likelihood ratio tests. Models were then repeated for each sex separately with skink ID as a random effect to obtain sex-specific repeatabilities. Non-overlapping confidence intervals would be indicative of differences in the level of behavioural consistency.

The presence of a behavioral syndrome among each sex was tested by estimating patterns of covariance among individuals using Markov chain Monte Carlo multivariate models following a Gaussian distribution ('MCMCglmm': Hadfield 2010). Models were run for 1,500,000 iterations and, after a burn in of 500,000 iterations, were thinned by 100 iterations. No evidence of autocorrelation between posterior samples was found. These models used the same structure as those used in the previously described univariate models and were fitted with all repeatable traits as the response variable. The multivariate models included two unstructured covariance matrices in order to partition phenotypic correlations into two levels: among-individual correlations (R_{ind}) and within-individual (residual) correlations (R_e). The R_{ind} represents consistent association between individual mean values for a series of traits over the time period within which measures were taken whereas R_e indicates whether an individual's change in one trait between time period t and $t + 1$ is correlated with its change in another trait over the same time period (Dingemanse and Dochtermann 2013). Correlation estimates whose 95% CI excluded zero were considered significant.

Results

Male and female *L. similis* differed in the amount of time they spent basking with conspecifics; females were more social (Table 1). All other behaviors were similar between the sexes (Table 1). Overall, activity (the number of transitions between grid squares) was repeatable, with no evidence of specific differences in repeatability (Table 2). Exploratory behaviour also exhibited some repeatability in males; however, no female skinks crossed the barrier during the exploration trials, and thus repeatability could not be calculated for this group (Table 2). No evidence of a behavioral syndrome involving activity and exploration was detected among the skinks as a whole. Repeatable traits were not correlated at either the within-individual ($R_e = -0.05$, CIs = -0.30, 0.19) or between-individual level ($R_{ind} = -0.80$, CIs = -1.00, 1.00).

Discussion

We found only minor sexual differences in behaviour in *Lampropholis similis*. While females were more social than males, there were otherwise no detectable differences in behavioral trait means between the sexes. Two of the three behavioral traits examined (activity and exploration) were repeatable, with activity being consistently expressed in both sexes while only males were consistent in their level of exploratory behaviour. However, there were no sex-specific differences in the repeatability of these traits. Although a behavioral syndrome among the three traits (activity, exploration, sociability) has repeatedly been documented in a congener (*L. delicata*), we found no evidence for such a syndrome in either sex of *L. similis*. Here we discuss the broader implications of these key findings.

206

207 Male and female *L. similis* were generally found to exhibit similar behaviors, as predicted. The only
208 sex-specific difference in behaviour that was identified was increased sociability in females; a
209 finding identical to that reported for *L. delicata* (Michelangeli et al. 2016a). In lizards, there is
210 evidence that gravid females are potentially more vulnerable to predators because they are
211 physically burdened by the developing brood and may also need to spend more time engaging in
212 risky behaviours, such as basking (Shine 1980). In this regard, Michelangeli et al. (2016a)
213 suggested that selection could favour higher sociability in females as a result of the antipredator
214 benefits (e.g. Downes and Hoefer 2004) and improved foraging efficiency (e.g. Martin and Lopez
215 1999) that sociability can confer which, in turn, can also lead to increased reproductive success (e.g.
216 Cote et al. 2008). Evidence also suggests that female lizards are able to use conspecific cueing to
217 identify suitable nesting sites (Refsnider et al. 2016), which might increase their propensity to group
218 with other females. More broadly, in reptiles, females are the more social sex (Baird et al. 1996;
219 Strickland et al. 2014). For instance, females form stronger social bonds than males in the eastern
220 water dragon (*Intellagama lesueurii*; Strickland et al. 2014). In contrast, male lizards, including in
221 *Lampropholis* species, may generally be more asocial due to the increased levels of male-male
222 competition and aggression (e.g. York et al. 2014; Michelangeli et al. 2017). *Lampropholis* skinks
223 are not known to be territorial, but aggression is commonly observed in the species, particularly in
224 the presence of food (Michelangeli et al. 2017; our personal observations). Regardless, there is now
225 growing evidence that sex-specific differences in behavior in *Lampropholis* skinks is restricted to
226 this sociability axis.

227

228 Contrary to our predictions, we found no evidence for an activity-exploration-sociability behavioral
229 syndrome in *L. similis*. Although activity and exploration were repeatable in *L. similis*, there was no
230 evidence for correlation between these traits. This is in direct contrast to *L. delicata*, where this
231 syndrome was documented in both sexes (Michelangeli et al. 2016a). Similarly, an activity-
232 exploration-sociability syndrome has been found in other species, including mosquitofish
233 (*Gambusia affinis*; Cote et al. 2010, 2011), and common lizards (*Zootoca vivipara*; Cote and
234 Clobert 2007). Recent studies have documented sexual differences in a range of other behavioral
235 syndromes (Rangel-Patiño et al. 2018). For instance, female Asian elephants (*Elephas maximus*) are
236 typically less aggressive, but more sociable, compared to males (Seltsmann et al. 2019). Similarly, in
237 African penguins (*Spheniscus demersus*), a relationship between boldness and foraging behavior
238 was observed in females, but not males (Traisnel and Pichegru 2019). In the black-bellied
239 bunchgrass lizard (*Sceloporus aeneus*) males had higher levels of activity, boldness and aggression
240 (Rangel-Patiño et al. 2018). Given the widespread occurrence of behavioral syndromes in the

animal kingdom, it is unclear why a syndrome was not present in *L. similis*, and more specifically why no sex-specific variation in syndromes was found. However, it could possibly be due to the fact that our lizards were raised in captivity and had not been exposed to a range of environmental pressures (e.g. predation; Bell and Sih 2007; Urszán et al. 2015). A key step in determining the significance of our result is determining how widespread behavioral syndromes are within *Lampropholis* skinks (a genus of 14 species of small, mesic-adapted skink; Wilson and Swan 2017; Singhal et al. 2018); a productive area for future research.

Acknowledgements. We thank M. Michelangeli, M. Bertram, J. Martin, N. Deal, M. de Jong and J. Fursdon for assistance during the project.

Funding information. This work was supported by an ARC Discovery Grant (DP170100684; to BMW and DGC).

Availability of data and material. All data will be deposited in Figshare upon acceptance of the manuscript.

Conflict of Interest. The authors declare that they have no conflict of interest.

Ethical approval. All animal care and experimental procedures were approved by the Monash University Animal Ethics Committee (BSCI/2016/02). All applicable international, national, and/or institutional guidelines for the use of animals were followed.

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382 **Table 1.** Mean differences ($\pm$ SE) in behavioral traits between sexes of *Lampropholis similis*. Significant differences are indicated in bold.
 383

Trait	Females (n=34)			Males (n=38)			P
	Mean ($\pm$ SE)	Minimum	Maximum	Mean ($\pm$ SE)	Minimum	Maximum	
Activity: No. Transitions	73 $\pm$ 6.49	6	361	79 $\pm$ 4.92	0	202	0.49
Exploratory behavior: Time to Goal (s)	1800 $\pm$ 34.15	1800	1800	1721.66 $\pm$ 40.01	4.24	1800	0.10
Social behavior: Time Social (s)	795.50 $\pm$ 42.50	53.68	1532.92	652.12 $\pm$ 41.07	0	1800	0.04
Social behavior: Time Asocial (s)	783.86 $\pm$ 44.03	0	1631.79	835.19 $\pm$ 42.73	0	1703.02	0.46

Table 2. Results of mixed effects models and repeatability of behavioral traits in *Lampropholis similis*. Confidence intervals not including zero indicate significant values for repeatabilities. Significant effects of fixed effects and repeatability are in bold. Repeatability of exploratory behavior was excluded among females as none crossed the barrier. V_A : between-individual variation, V_R : residual variation.

Trait	Coefficient	<i>P</i>	V_A	V_R	Repeat (95% CI)
Activity - All	6.84	0.49	1208.00	1111.00	0.53 (0.35 - 0.68)
Female			1525.00	1399.00	0.52 (0.21 - 0.72)
Male			1017.00	853.00	0.54 (0.30 - 0.50)
Exploratory behaviour - All	-78.12	0.10	14446.00	49685.00	0.23 (0.01 - 0.43)
Female			NA	NA	NA
Male			29353.00	94221.00	0.24 (0 - 0.50)
Time social - All	-141.03	0.03	26478.00	99362.00	0.22 (0 - 0.40)
Female			29356.00	95635.00	0.24 (0 - 0.52)
Male			27934.00	102715.00	0.21 (0 - 0.50)
Time asocial - All	49.70	0.45	21000.00	114524.00	0.17 (0 - 0.39)
Female			36710.00	97417.00	0.27 (0 - 0.59)
Male			9669.00	131068.00	0.07 (0 - 0.38)

390 **Figure Legends**

391 **Figure 1.** Schematic of experimental set up for behavioral assays. Frame A depicts the test arena
392 used to assess activity where the number of transitions between grid squares served as a measure of
393 activity. Frame B depicts the test arena assessing exploratory behavior where the time to cross the
394 trapezium barrier served as a measure of exploration. Frame C depicts the test arena assessing social
395 behaviour where the time spent in the social (basking site with stimulus lizards) and asocial zone
396 (empty basking site) with stimulus lizards served as a measure of sociability.

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Title:

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Date:

2021-02

Citation:

Goulet, C. T., Hart, W., Phillips, B. L., Llewelyn, J., Wong, B. B. M. & Chapple, D. G. (2021). No behavioral syndromes or sex-specific personality differences in the southern rainforest sunskink (*Lampropholis similis*). *ETHOLOGY*, 127 (2), pp.102-108. <https://doi.org/10.1111/eth.13103>.

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