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Original Article

Lateralization in refuge selection in *Podarcis hispanica* at different hierarchical levels

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The traditional explanation of brain lateralization is that it avoids costly duplication of neural circuitry with the same function as well as decreasing the interference between different functions. This advantage could have important ecological implications in terms of predator–prey interactions. If species are lateralized in escape behavior at an individual level, any predator is facing individually asymmetric prey. On the other hand, if species are lateralized at a higher hierarchical level, such as at the population or species level, the predator can learn from experience arising from earlier encounters. In the present study, we investigate patterns of lateralization in 5 lizard populations of the *Podarcis hispanica* species complex, with the aim of analyzing the occurrence of lateralization at different hierarchical levels. Our results show that refuge selection during escape behavior is not universal in the *P. hispanica* species complex at different hierarchical levels, some individuals and populations are lateralized, with a consistent bias toward the refuge on the right. However, 2 populations showed no refuge preference, with lack of lateralization mainly arising due to the dominance of individuals with no preference rather than from a mix of right- and left-biased individuals. When considered at the *P. hispanica* species complex level, a pattern for right-refuge preference was found. *Key words:* antipredatory behavior, behavioral asymmetry, ectotherms, hierarchical levels, wall lizards. *[Behav Ecol]*

INTRODUCTION

The traditional interpretation of brain lateralization is that it avoids costly duplication of neural circuitry with the same function (Levy 1977) as well as decreasing the interference between different brain functions (e.g., Rogers 2000). Another advantage of lateralization is that dominance of one brain side prevents the simultaneous initiation of incompatible responses in organisms with laterally placed eyes (Andrew 1991; Cantalupo et al. 1995; Vallortigara 2000), which is crucial for refuge selection when fleeing from a predator's attack and may result in strong ecological implications in terms of predator–prey interactions.

Although these aspects have been investigated in other vertebrate groups (Rogers 2010), little is known concerning lateralization in antipredatory behavior of reptiles. Bonati et al. (2010) recently reported a form of lateralization in the escape behavior of the lacertid lizard, *Podarcis muralis*, which tended to escape to the right after a simulated predator attack from behind. However, no comparative studies are available to infer to what extent antipredator behavior is laterally biased at different hierarchical levels in these lacertids lizards.

In lacertid lizards, the eyes are positioned laterally, minimizing binocular vision and making them interesting subjects to study lateralization. In addition, they do not have a large corpus callosum (Bonati et al. 2008; Rogers 2010; Wade 2010), or great commissure of nerve fibers system connecting the 2 hemispheres, and are restricted to mesencephalic levels connecting them via a well-developed tectal commissure (Aboitiz and Montiel 2003). Furthermore, the genus *Podarcis* represents a key element of Mediterranean ecosystems because they play a major ecological role in food webs being at the same time both prey of multiple terrestrial (Valverde 1967) and aerial vertebrate (Martín and López 1996) predators that use visual cues to search for their prey (Carretero 2004), and predator, using visual cues to forage invertebrate faunas. Moreover, the group is highly diversified in the Mediterranean Basin (Arnold 1973, 1989; Harris and Arnold 1999; Poulakakis et al. 2005; Carretero 2008).

If escape behavior is lateralized at an individual level, any predator is confronted by individual prey displaying heterogeneous asymmetric behaviors that are unpredictable for the predator which cannot develop a laterally systematic predation strategy (Vallortigara 2006). However, if escape behavior is lateralized at higher hierarchical levels, such as at the population or species level, experience arising from previous encounters can be used by the predator to modify its behavior following a laterally biased strategy to maximize predation success (Vallortigara 2006).

In the present study, we investigate patterns of lateralization in 5 Iberian populations of the *P. hispanica* species complex (these populations according to Kaliontzopoulou et al. 2011 represent 5 distinct phylogenetic lineages whose taxonomic status still deserves further analysis), with the aim of analyzing the occurrence of lateralization at different hierarchical levels, from individuals to the species complex level in escape behavior. Specifically, the objectives are 1) to test the preference in the selection of left/right refuge in the escape

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behavior from individual to the species complex level, 2) to test whether the lack of lateralization at population levels could arise either from individuals with no preference or from a mix of right- and left-biased individuals; 3) to test whether left-right–biased individuals are equally common at the population and species complex level.

MATERIALS AND METHODS

In May 2011, we collected by noosing (following methods described by García-Muñoz and Sillero (2010)) 20 adults, 10 males and 10 females, from 5 different populations of the *P. hispanica complex* (sensu Kaliontzopoulou et al. 2011: *P. vaucheri* south-central Spain, PvJ; *P. hispanica* Albacete/Murcia, PhAl; *P. hispanica* Galera, only 8 females, PhCa; *P. hispanica* Type 1B, PhT1B; *P. hispanica* Type 2A; PhT2A, only 9 males) from across the Iberian Peninsula. Lizards were taken to the laboratory, were identified with a small painted number on the venter, and kept in terraria (30 × 40 × 100 cm; width × height × length; 10 individuals each). After experiments (48 h), all individuals were released at the original capture sites. Lizards were fed with mealworm larvae (*Tenebrio molitor*) daily, and water was provided ad libitum. A photothermal gradient (~25 to 45 °C, 30 × 40 × 100 cm length experimental terrarium) was produced by a 100 W reflector bulb fixed 15 cm above the substrate. Lizards were maintained on a natural photoperiod with natural light from outside (Veríssimo and Carretero 2009).

Before the beginning of the experiments, we allowed lizards at least 2 h to thermoregulate (Veríssimo and Carretero 2009) and attain a temperature allowing maximal locomotor performance, necessary to express normal escape behavior. In each test, an individual lizard was placed in a 100 × 5 × 400 cm plexiglass experimental maze with a natural cork floor that provided excellent traction for running. No cover was added. Two refuges were attached to extremes of the experimental maze, one on the right side and another on the left (Figure 1). The experimental maze walls were covered with white panels to prevent external stimuli influencing the lizard's choice of escape direction. The experimental maze was cleaned after each individual experiment with 70% ethanol.

During tests, the observer stayed against the back of the experimental maze, gently put the lizard in the extreme opposite end from the refuges and then immediately stimulated it with a stick in order to induce the escape. The stimulation was made by beating the stick at the back of the experimental terrarium, simulating a predator attack, by the same experimenter using the right and left arm, alternately, to exclude influences on direction. When lizards escaped and arrived to the end of the experimental terrarium, they could only choose the left or right refuge. If the lizard stopped running before reaching either refuge of the experimental terrarium, the experimenter beat the stick again. Ten tests per each individual were conducted on 2 consecutive days, with the minimal period between 2 consecutive trials of the same individual being 2 h.

Statistical analysis

Log-linear analysis was performed in order to detect interactions between 1) Populations (5: PvJ, PhAl, PhCa, PhT1B, PhT2A), 2) Sex (2: Male and Female), 3) Trial (10), and 4) Refuge Side (2: left or right).

Furthermore, for repeated tests of the same individual, we used the laterality index (LI) developed by Stancher et al. (2006), calculated as: frequency of right runs/(total frequency of right runs + left runs) × 100. Values of LI lower or greater than 50% indicate a left or right preference, respectively, whereas a 50% value indicated no preference. In addition a binomial test (Fisher exact *P*, one-tailed) was used to analyze the preference used of left or right refuge compared with theoretical no preference, at individual, population, and the *P. hispanica* complex level. Furthermore, a binomial test (Fisher exact *P*, one-tailed) was used, with lateralized individual, to test if left- and right-type individuals are equally common at the population and the *P. hispanica* complex level. The software Statistica 10 (Statsoft Inc. 2011) was used in all statistical analysis.

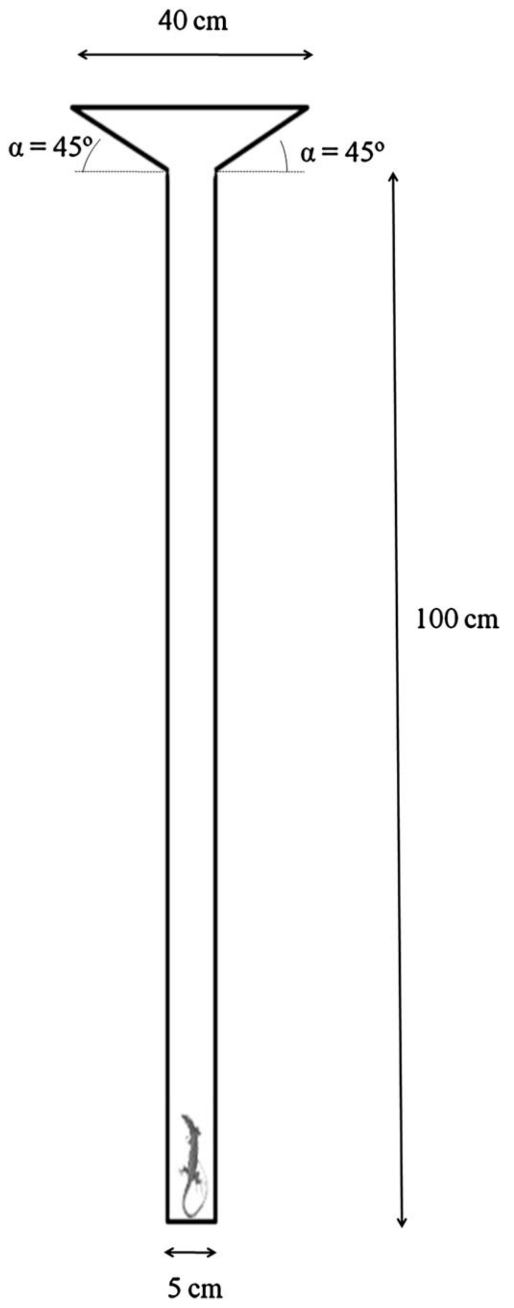

Figure 1
Schematic design of experimental maze used in the escape behavior test.

RESULTS

Log-linear analysis showed significant effects in the selection of left/right refuge in escape behavior, Refuge Side, and between the interaction Population × Refuge Side. No significant effects were detected in the interaction Sex × Refuge Side and between the interactions Trial × Refuge Side (Table 1).

At the *P. hispanica* complex level, the 97 lizards used in this experiment pooled showed a LI = 0.67, indicating an overall preference for the right refuge (Table 2, Figure 2A). Table 2 also shows the preference for the left or right refuge at a lower hierarchical level, namely at the population level (Figure 2B). Whereas populations PvJ, PhT2A, and PhAl showed preference for the right refuge, populations PhCa and PhT1B failed to show an overall preference for one side over the other. In both populations, lack of lateralization mostly derives from a high frequency of individuals with no preference instead of being a mix of right- and left-biased individuals (Table 3). At an individual level, 62 lizards (64% for the total, $N = 97$) showed a preference for one refuge, either left (9 lizards) or right (53 lizards), whereas 35 individuals showed no preference (Table 3).

DISCUSSION

Our results show that the escape behavior of *Podarcis* lizards is lateralized at different hierarchical levels, from an individual to the *P. hispanica* species complex level, preferring a specific side to escape from a simulated predator attacks. Nevertheless, in 2 of the 5 populations studied, the majority of individuals show no refuge preference. In these 2 populations, the lack of lateralization mainly arises from a majority of individuals with no preference, although the possibility that in PhCa, this also arose due to a mix of right- and left-biased individuals need to be tested with larger sample sizes. It is here suggested, following Brown et al. (2007), that this heterogeneity would be derived from different predator–prey interactions between populations. These authors found that a high predatory pressure could be important in increasing the number of lateralized individuals in a population. Nevertheless, because the different populations used in this study belong to different mitochondrial DNA lineages (Harris and Arnold 1999; Poulakakis et al. 2005; Carretero 2008; Kaliontzopoulou et al. 2011), we cannot rule out an effect of phylogeny on antipredatory behavior.

At lower levels, the lateralized individuals displayed a strong preference for the right refuge. Recent studies conducted on common wall lizards, *P. muralis*, showed that lizards predominantly monitor a predator with the left eye while escaping in both laboratory (Bonati et al. 2010) and field (Martín et al. 2010) experiments. In contrast, experiments conducted with *Anolis* lizards by Deckel (1995) showed a left-side preference when attacked by attacking other *Anolis*. Apparently, *Anolis* tend to attack with the left side because they monitor potential threats with the left eye. If lizards monitor the predator (attacker) with the left eye, the right eye could be controlling the presence of a possible refuge to escape from the attack; this could explain why lizards escaped preferentially to the right refuge.

Table 1

Results of log-linear analysis to detect interactions between 1) Populations (P) (5: PvJ, PhAl, PhCa, PhT1B, PhT2A), 2) Sex (2: Male and Female), 3) Trial (10), and 4) Refuge Side (Side) (2: left or right).

		Partial association		Marginal association	
	df	Chi square	*P*	Chi square	*P*
P	4	1.513	>0.05	1.513	>0.05
Sex	1	0.092	>0.05	0.092	>0.05
Trial	9	<0.001	>0.05	<0.001	>0.05
Side	**1**	**106.021**	**<0.001**	**106.021**	**<0.001**
P × Sex	4	2.533	>0.05	2.387	>0.05
P × Trial	36	0.522	>0.05	0.000	>0.05
P × Side	**4**	**75.370**	**<0.001**	**74.701**	**<0.001**
Sex × Trial	9	0.001	>0.05	<0.001	>0.05
Sex × Side	1	0.155	>0.05	0.009	>0.05
Trial × Side	9	7.868	>0.05	7.345	>0.05
P × Sex × Trial	36	0.628	>0.05	<0.001	>0.05
P × Sex × Side	4	2.673	>0.05	2.695	>0.05
P × Trial × Side	36	30.390	>0.05	30.150	>0.05
Sex × Trial × Side	9	4.105	>0.05	3.674	>0.05

Model: P × Side; chi square = 70.879, degrees of freedom [df] = 190, $P = 1.000$. (P values lower than 0.05 are marked in bold).

Table 2

Numbers of individuals used in each population (*N*)

	N Males	*N* Females	Left%	Right%	*P*
PvJ	10	10	22	78	**0.000**
PhAl	10	10	36	64	**0.035**
PhCa	10	8	47	53	0.388
PhT1B	10	10	46.5	53.5	0.360
PhT2A	9	10	13	87	**0.000**
Podarcis hispanica species complex	49	48	33	67	**0.010**

Left% and Right% represent the percentage of times that all individuals chose the left or right refuge. Fisher exact test P one-tailed was used in order to test the null hypothesis: no refuge preference (see also Figure 2) LI = frequency of right runs/(total frequency of right runs + left runs) * 100. (P values lower than 0.05 are marked in bold).

Being lateralized at an individual level could provide the advantage to individual lizards to more effectively escape from predator's attacks by enabling separate and parallel processing to take place in the 2 hemispheres (left eye—right hemisphere controlling the predator; right eye—left hemisphere searching for a refuge). However, in a population context, as in the cases of PvJ, PhAl, and PhT2A, if most individual lizards are biased in the same direction, their behavior would become more predictable to predators (Hori 1993), resulting in a disadvantage for the majority-type individuals. Ghirlanda and Vallortigara (2004) explored the possibility that population-level lateralization may arise when the fitness of an individual asymmetrical organism depends on what other individual asymmetrical organisms do. In this situation, Ghirlanda and Vallortigara (2004) showed that the majority of prey gain protection by keeping together but pay a cost because predators are better at handling them. On the other hand, a minority of prey enjoy the same escape success by trading off protection from the group against an advantage in the face of predators.

Table 3

Numbers of lateralized individuals found in each population

	Left	Right	No preference	Left%	Right%	*P*
PvJ	1	14	5	7	93	0.000
PhAl	2	10	8	17	83	0.000
PhCa	2	5	11	29	71	0.001
PhT1B	4	7	9	36	64	0.031
PhT2A	0	17	2	0	100	0.000
Podarcis hispanica species complex	9	53	35	15	85	0.000

Left% and Right% represent the percentage of times that all the lateralized individuals (left and right) chose the left or right refuge. Fisher exact test P one-tailed was used in order to test the null hypothesis: no refuge preference (see also Figure 2).

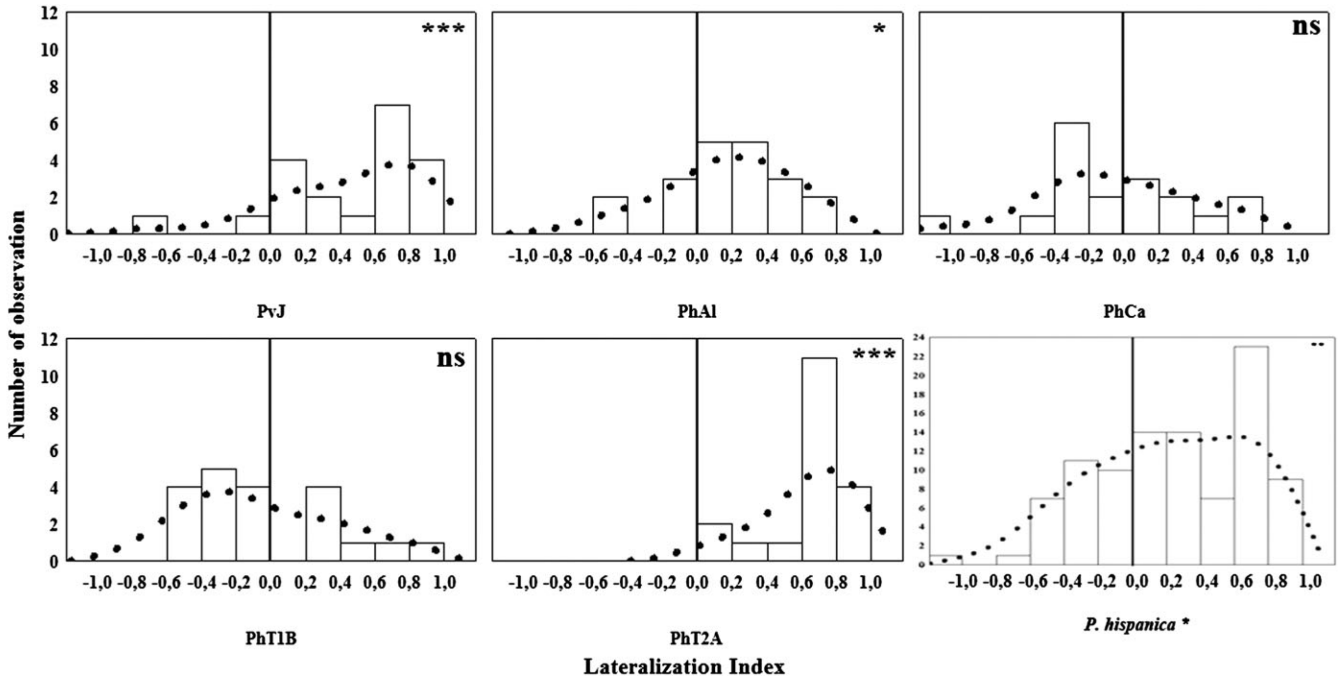

Figure 2
Frequency plots showing the number of observations of each value plotted on the *x* axis. The *x* axis is the LI for each individual, LI = right/(right − left). (ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; Fisher exact test P one-tailed).

In conclusion, our study has shown how refuge selection during escape behavior in the *P. hispanica* species complex at different hierarchical level is lateralized, using preferentially the right refuge. However, we found 2 populations that showed no refuge preference. Future studies will be developed in order to understand the possible effect of the predator in the selection of left- or right-biased prey and the possible relationship between phylogeny and lateralization.

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