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*Article*

# Population Chemorecognition in Two *Liolaemus* Lizard Species: Exploring Ethological Isolation

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**Abstract:** Reproductive isolating barriers are a crucial element in the speciation process. There are various types of barriers, and within those that act early in the speciation process, the behavioral one can play an important role in isolating populations or closely related species. It has been shown that the high diversity of some taxa correlates with the variation of sexual signals, which facilitate isolation. Here it is explored whether chemical signals may contribute to the high diversity of *Liolaemus* lizards, which has more than 290 species. Population chemical discrimination was investigated in two co-distributed species, *L. tenuis* and *L. lemniscatus*. The studied populations were closely located and likely belonged to the same genetic lineages. Lizards of both species discriminated between scents of their own and different populations, and in most cases, scents from their population were preferred. Results suggest a fast evolution of scents, and their recognition, and there for the ethological barriers associated with the chemical modality may evolve fast. However, because the studied species differed in the strength of the exhibited population chemical discrimination, ethological barriers evolve at different rates across species. It can be concluded that ethological barriers involving the chemical modality may disrupt species' cohesion, contributing to *Liolaemus* diversification, in synergy or not with geographical barriers.

**Keywords:** geographic barrier; homotypic; heterotypic; Liolaemus lemniscatus; Liolaemus tenuis; olfaction; scents; speciation; tongue flick

## 1. Introduction

A critical step in the speciation process is the occurrence of reproductive barriers that limit gene flow between populations or incipient species e.g., [1–3]. Such barriers are classified based on when they restrict gene exchange, with premating barriers precluding copulation [2], which tend to evolve early in the process of reproductive isolation [4]. Among different premating barriers, behavioral ones, also called ethological or sexual isolation barriers [2], have been proposed to play a pivotal role in isolating populations or closely related species [5]. Thus, in the context of sexual selection, signals involved in mate attraction and male-male competition can play a significant role in speciation [6–8], as supported by many studies involving different taxa and sensory modalities e.g., [9–12]. Remarkably, in some taxa, high speciation rates may result from high divergence in sexual signals e.g., [13]. This includes the *Laupala* crickets, the genus with the highest speciation rate among arthropods, with a high divergence in song characteristics across taxa [14]. Similarly, the increased diversification of African cichlid fishes has been linked to variation in male colorations involved in mate selection [15,16].

*Liolaemus*, a genus with more than 290 lizard species [17,18], is one of the most diverse tetrapod genera in the world, distributed in the southern part of South America [18,19]. Its high diversity has been related to the Andean uplift that fragmented species distribution promoting speciation [19,20], and different genetic analyses support the role of geographic barriers, i.e., rivers and mountains, in this diversification [21–23]. In fact, the diversity of different South American taxa has also been associated with changes in landscape, climate, and drainage from the Andean formation e.g., [24–26]. However, no other genus in the Andean mountains has a similar diversity to *Liolaemus*, even the genera belonging to the *Liolaemidae* family [18]. Therefore, geographic isolation per se does not explain the *Liolaemus* diversity, and other factors, such as reproductive barriers involving organismal traits, i.e., sexual signals, may play a prominent role in *Liolaemus* speciation [27]. In this context, the working hypothesis of this study is that populations of *Liolaemus* species may show differences in sexual signals allowing population discrimination, which may break species cohesion [28]. If so, ethological isolation may contribute to *Liolaemus* diversity.

Chemical signals, or scents, are relevant in the speciation process of many different taxa [29]. In *Liolaemus*, further than allowing individual recognition [30–32], scents may facilitate isolation among sympatric congeneric species [30,33,34]. The composition of precloacal secretions, a pheromone source in *Liolaemus* lizards [35–37], differ between sexes [38], among males of a population, between populations, and among congeneric species [39,40], suggesting that scents may evolve fast [33,34]. Thus, considering that scents of nearby populations can differ [40], possibly populations may discriminate between homo- and heterotypic conspecific scents, i.e., from the same and different populations, respectively, and most likely, homotypic scents would be selected e.g., [41,42], disrupting the species cohesion in the absence of geographic barriers.

This study tested the working hypothesis by determining population scent recognition of closely located populations of two *Liolaemus* species, *L. tenuis* and *L. lemniscatus*. These species are co-distributed in most of their distributional ranges, which may have shared vicariant events evolving along similar demographic trajectories [43]. Thus, by studying these species together, the effects of factors like environmental conditions that modulate scent composition e.g., [44–46], are controlled.

## 2. Materials and Methods

Lizards from *L. tenuis* and *L. lemniscatus* were collected from the south bank of the Maipo River in central Chile (Figure 1), a well-recognized geographic barrier for many lizard species [22,47–49]. The aim was to study populations that might experience gene flow. The studied populations have no genetic analyses, although data suggest they are likely from a single genetic lineage [22,43].

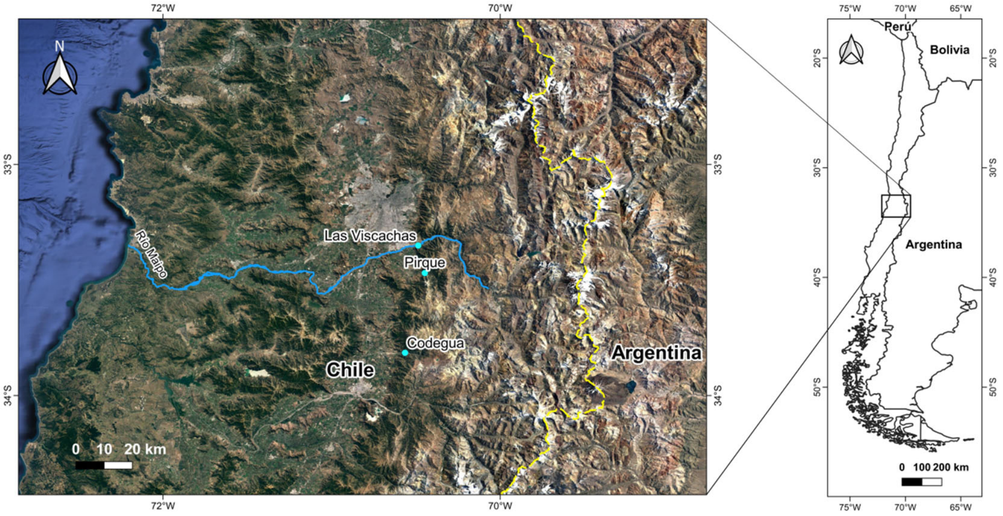

**Figure 1.** Geographic localization of the populations of *Liolaemus tenuis* and *L. lemniscatus* in central Chile.

### *2.1. Experiment I. Recognition between homo- vs. heterotypic competitors*

*Animals and their maintenance*. In January 2001, males of both species were collected from different populations (Figure 1); 19 *L. tenuis [*nine from Codegua (34.03335ºS; 70.56668ºW; Datum WGS84, 765 m), three from Las Vizcachas (33.61560°S; 70.51593ºW; 890m), and seven from Pirque (33.72283°S; 70.49030°W; 850m)*]* and 16 *L. lemniscatus* [eight from Las Vizcachas, and eight from Codegua]. In the laboratory, lizards were placed in an indoor vivarium with a glass roof, exposing them to the sun and natural photoperiod (13:11 L:D). Additionally, the vivarium was equipped with halogen lamps to maintain similar temperatures to those recorded on a typical summer day (between 12 ºC to 36 ºC). Lizards were housed individually in plastic enclosures (37 × 30 × 15 cm) with a 3 cm sand-layer substrate. Enclosures had a lid of plastic mesh and were equipped with a bowl to keep water permanently and a rock to provide shelter and a basking place. Food (mealworms) was supplied three times a week, dusted with vitamins. *Experimental design.* Animals remained for a week in their enclosures allowing habituation to experimental conditions and scent releasing, as enclosures were used as a source of substrate-borne scents [33]. For the experiments, a focal lizard was taken from its enclosure and kept in a cloth bag for 10 min to reduce handling associated stress [33]; after that, the bag was placed in the experimental enclosure, allowing the lizard to move freely. The experimental enclosure corresponded to one used by homo- or heterotypic males. Each lizard was tested in a counterbalanced design with scents from different populations. The enclosure resident was removed with the rock and the bowl for water just before the trial. *The* focal population of *L. tenuis was* Codegua, which was tested with homo- and heterotypic scents from Pirque and Las Vizcachas. In the case of L. lemniscatus, both populations, Codegua and Las Vizcachas, were tested with scents from different populations. Once the focal lizard was in the experimental enclosure, the latency to the first tongue flick was recorded as the time elapsed since the lizard was placed in the enclosure until it made the first tongue flick [50]. After this display, the lizard's behavior was filmed for 10 min with an 8-mm digital video camera placed 50 cm above the enclosure. At the end of each trial, the lizard's cloacal temperature was recorded to ensure that individuals were at the species selected body temperatures [~35- 36°C; 51] during the experiment. Finally, focal and scent-donor lizards were placed back in their enclosures. Lizards were subjected to one trial per day and had an inter-trial of at least two days.

From the videos, the following variables were recorded [32,50]: 1- Number of tongue flicks. The number of times the lizard protruded and retracted the tongue, which touched the air or a surface. Tongue flicks allow lizards to explore the chemical environment (vomerolfaction) by collecting scents (volatile and non-volatile compounds) and delivering them to the vomeronasal organ [52]. 2- Motion time. Total time that the lizard moved, which included adjustments of body posture, head movements (scanning), displacements of the body's center of gravity, and displays that occurred at too low frequency to be analyzed independently (marking behaviors, tail waving, slow motion, defecation, and digging). 3- Head displays. Time exhibiting up and down head movements combined or not with forelimb flexions.

### *2.2. Experiment II. Recognition between homo- vs. heterotypic potential sexual partners*

In December 2003, individuals of both sexes from two populations of each species were collected; *L. tenuis [*22 from Codegua *(11♂*, 11♀) and 19 from Las Vizcachas (10♂, 9♀)] and *L. lemniscatus [*35 from Codegua (21♂, 14♀) and 18 from Las Vizcachas (11♂, 7♀)]. In the laboratory, lizards were maintained as before, except that the rocks of the maintenance enclosures were replaced by inverted clay pots, which were later used as scent sources.

Experiments were performed in a round enclosure of 50 cm in diameter with a layer of 2 cm clean sand at the bottom. The arena was divided virtually into two halves, each containing a clay pot, one from a homo- and the other from a heterotypic potential sexual partner to the focal lizard. The lizard was filmed for 20 min after the first tongue flick. From the videos, five variables were quantified: time spent at each side of the experimental enclosure, time spent on the two clay pots, the number of tongue flicks performed on each side of the enclosure, number of tongue flicks directed to each clay pot, and finally, time moving on each side of the experimental enclosure. After each trial, enclosures were washed with soap, and the sand was discarded and replaced.

### *2.4. Experiment III. Scent preference for homo- vs. heterotypic potential sexual partners*

In February 2004, new individuals of both sexes from two populations of each species were collected; *L. tenuis [*11 from Codegua *(5♂*, 6♀) and 18 from Las Vizcachas (8♂, 10♀)] and *L. lemniscatus [16* from Codegua (8 ♂, 8♀) and 13 from Las Vizcachas (8♂, 5♀)]. In the laboratory, lizards were maintained as in Experiment II. To assess scent preferences, a focal lizard remained for 4.5 hrs in the round enclosure described above. Every 30 min, an observer behind a blind determined at which side of the enclosure the focal lizard was located. Scent preference was determined by calculating the percentage of time that lizards were at each side of the enclosure.

Individuals were kept in good conditions in the laboratory, and at the end of each of the three experiments, they were released at their capture sites.

### *2.5. Statistics*

Data for each experiment by species were analyzed using Generalized Linear Models for repeated measures (scent origin; homo- vs. heterotypic), followed by Fisher LSD tests. For Experiment I, analyses assessed the effect of scent origin on four responses. However, the head display was not analyzed for *L. tenuis* since it was displayed in low frequency but was included in motion time. The normality of the head display in *L. lemniscatus* was improved by log-transforming the data. The analyses of *L. lemniscatus* also included the effect of the population (Codegua vs. Las Viscachas) and its interaction with scent origin.

For Experiments II and III, tests assessed the effects of scent origin, the sex and population of the focal lizard, and their interactions on the recorded variables. The percentages of time associated with homo- and heterotypic scents (Experiment III) were transformed using the square root of the arcsine.

Statistical analyses were performed with StatSoft, Inc (2001)

## 3. Results

### *3.1. Experiment I. Recognition between homo- vs. heterotypic competitors*

*Liolaemus tenuis*. Scent origin did not modulate the latency to the first tongue flick nor motion time (Table 1A; Figure 2A and 2C, respectively), but it did affect the number of tongue flicks; lizards directed more tongue flicks toward hetero- than homotypic scents, and the scents of both heterotypic populations were similarly examined (Figure 2B).

**Table 1.** Results of the General Linear Models for repeated measures to test male scent discrimination of homo- and heterotypic competitors (Scent origin) in two species. A. *Liolaemus tenuis*. Three responses of the focal population (Codegua) were exhibited with scents from the own population and two others. Sample size: nine. B. *Liolaemus lemniscatus*. The analyses included the effect of Population (Codegua and Las Viscachas, the two focal populations) and its interaction with Scent origin upon four responses. Total sample size: 16, eight from each population. Shown values are the F-statistics [degree of freedom] (p-value); statistically significant tests ($p < 0.05$) are shown in bold.

A- Liolaemus tenuis F[2,16] (p)

| Variable | Scent origin |
|---|---|
| Latency 1st tongue flick (s) | 1.909 (0.181) |
| N° tongue flicks | **4.757 (0.024)** |
| Motion time (s) | 1.048 (0.374) |

B- Liolaemus lemniscatus F[1,14](p)

| Variable | Scent origin | Population | Scent origin*Population |
|---|---|---|---|
| Latency 1st tongue flick (s) | **5.147 (0.039)** | 0.098 (0.756) | 0.476 (0.502) |
| N° tongue flicks | 0.001 (0.978) | 0.321 (0.580) | 1.778 (0.204) |
| Motion time (s) | 0.311 (0.586) | 0.186 (0.673) | 1.872 (0.193) |
| Head displays (s) | **6.494 (0.023)** | 1.284 (0.276) | 1.984 (0.181) |

A- Liolaemus tenuis F[1,38](p)

| | Scent origin | Population | Sex | Scent origin*Pop. | Scent origin*Sex |
|---|---|---|---|---|---|
| Time on each side (min) | **5.968 (0.019)** | 0.432 (0.515) | 3.304 (0.077) | 0.001 (0.982) | 0.386 (0.538) |
| Time on each pot (min) | **4.261 (0.046)** | 0.501(0.483) | 3.578(0.066) | 0.029 (0866) | 0.371 (0.546) |

| | | | | | |
|---|---|---|---|---|---|
| Tongue flicks each side | **4.110 (0.050)** | 0.522(0.474) | 0.247(0.622) | 0.456 (0.504) | 0.754 (0.391) |
| Tongue flicks each pot | **4.902 (0.033)** | 0.306 (0.583) | 0.000 (0.980) | 0.523 (0.474) | 0.247 (0.622) |
| Motion each side (min) | **12.854 (<0.001)** | 4.472 (0.041) | 5.485 (0.025) | 0.649 (0.425) | 0.242 (0.626) |

*Liolaemus lemniscatus.* Scent origin was the only factor with an effect (Table 1B). First, males showed shorter latency to the first tongue-flick to hetero- than to homotypic male scents (Figure 3A). Second, males exhibited head displays for an extended period with homotypic scents (Figure 3D). None of the other variables, i.e., number of tongue flicks and motion time, were affected by scent origin (Figure 3B and 3C, respectively).

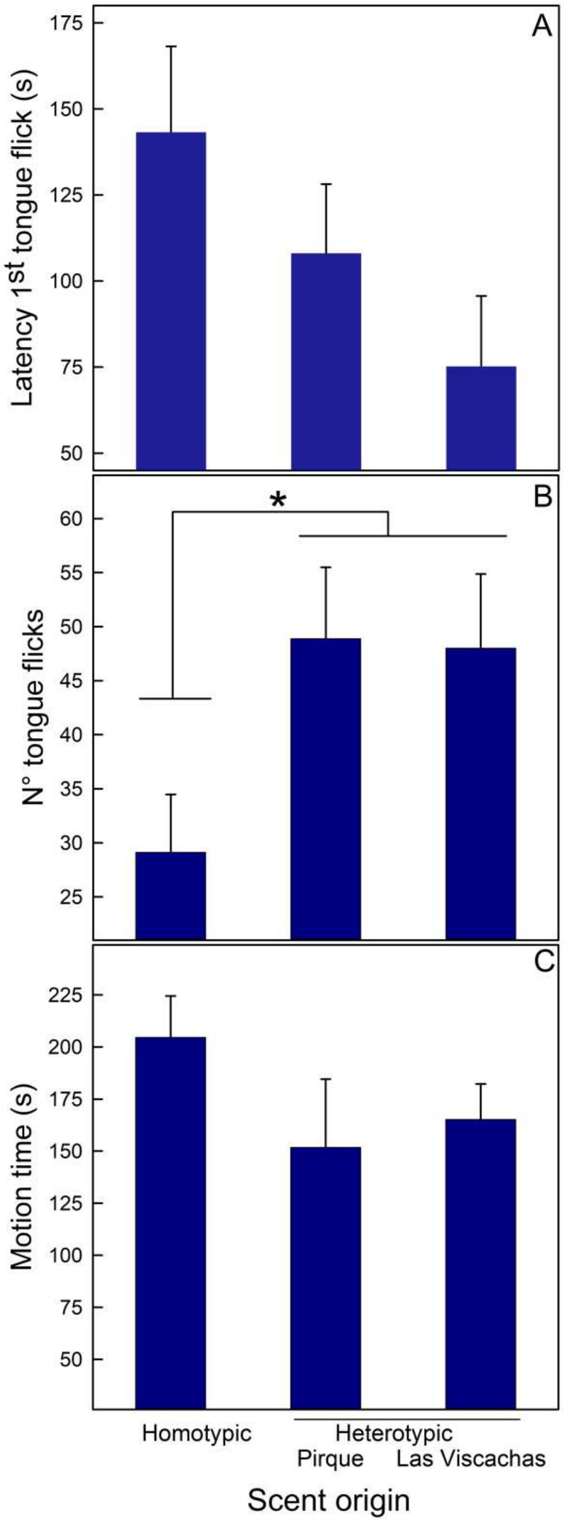

**Figure 2.** Mean (+SE) values of three behaviors recorded in males of *Liolaemus tenuis* from Codegua when exposed to male conspecific scents from the same population (homotypic), and two heterotypic populations, Pirque and Las Viscachas. **A-** Latency to the first tongue flick, **B-** Number of tongue flicks, and **C-** Motion time (s)**.** * = $p < 0.05$.

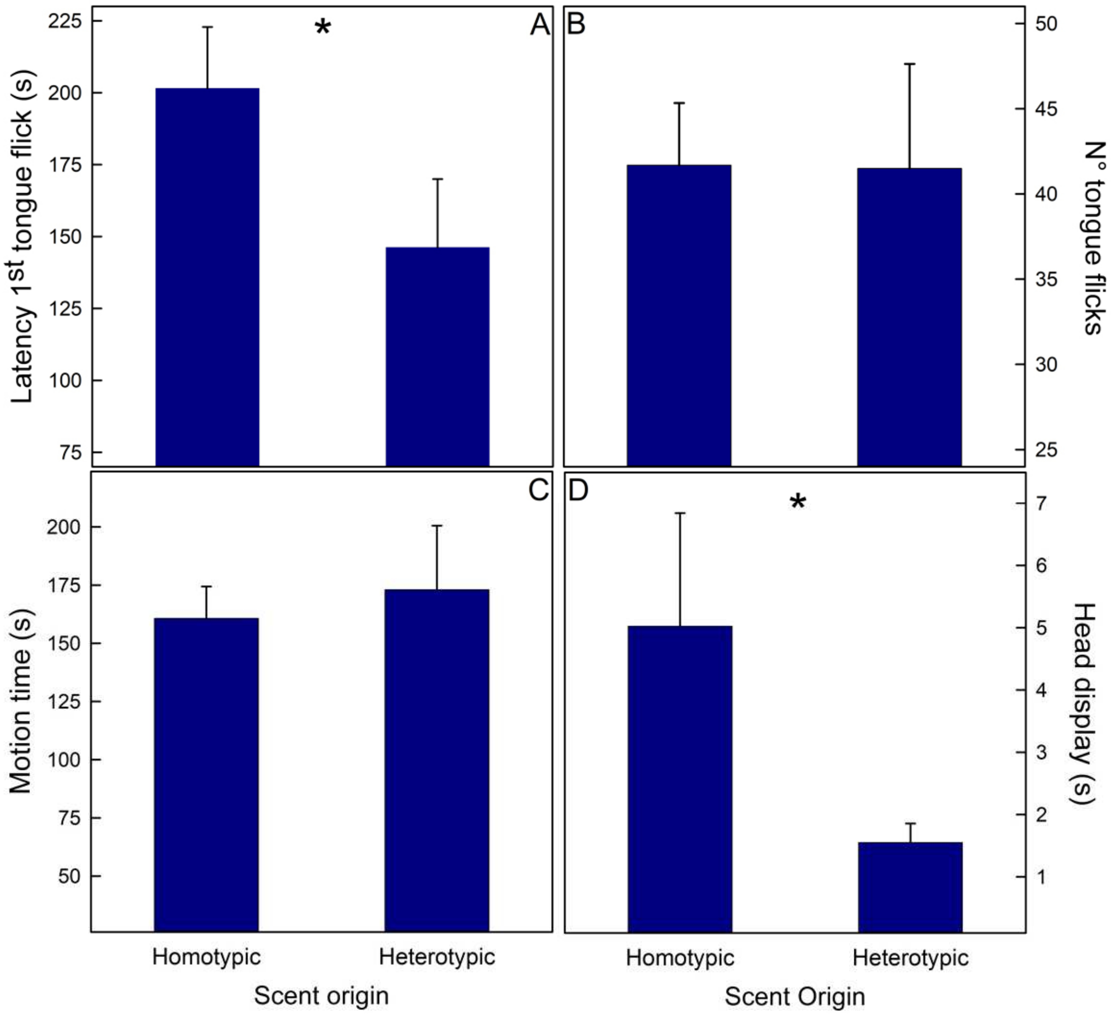

**Figure 3.** Mean (+SE) values of four behaviors recorded in males of *Liolaemus lemniscatus* from two populations (Codegua and Las Viscachas) when they were exposed to scents of homotypic or heterotypic conspecific males. Data from both populations were pooled. **A-** Latency to the first tongue flick, **B-** Number of tongue flicks, **C-** Motion time (s), and **D-** Head display (s). * = $p < 0.05$.

### *3.2. Experiment II Recognition between homo- vs. heterotypic potential sexual partners*

*Liolaemus tenuis*. Scent origin affected all the recorded variables (Table 2); lizards, independently of their sex or population, spent more time, tongue flicked more, and moved more in the area with heterospecific scents (Figure 4A,B). In addition, sex and population modulated the time spent moving (Table 2); males moved more than females (2.09 ± 0.17 vs. 1.54 ± 0.12 min, respectively), and lizards from Codegua moved more than those from Las Viscachas (2.01 ± 0.17 vs. 1.58 ± 0.12 min, respectively).

**Table 2.** Results of the General Linear Models for repeated measures to test responses to homo- and heterotypic potential sexual partner (Scent origin). Analyses included the Sex (male vs. female) and Population (Codegua vs. Las Viscachas) of the focal populations and their interactions modulating five responses. A. *Liolaemus tenuis*. B. *Liolaemus lemniscatus*. Shown values are the F-statistics $_{\text{[degree of freedom]}}$ (p-value); statistically significant tests (p < 0.05) are shown in bold.

| | B- Liolaemus lemniscatus F[1,54](p) | | | | |
|---|---|---|---|---|---|
| | Scent origin | Population | Sex | Scent origin*Pop. | Scent origin*Sex |
| Time on each side (min) | 0.373 (0.544) | 1.049 (0.310) | 0.119 (0.731) | 0.086 (0.771) | 1.583 (0.214) |
| Time on each pot (min) | 0.103 (0.750) | 0.570(0.453) | 4.454 (0.039) | 0.428 (0.516) | 4.566 (0.037) |

| | | | | | |
|---|---|---|---|---|---|
| Tongue flicks each side | 0.738 (0.394) | 1.223 (0.274) | 0.601 (0.442) | 0.186 (0.668) | 1.127 (0.293) |
| Tongue flicks each pot | 0.005 (0.945) | 0.633 (0.430) | 3.871 (0.054) | 0.001 (0.971) | 5.233 (0.026) |
| Motion each side (min) | 0.130 (0.719) | 0.785 (0.380) | 1.535 (0.221) | 0.485 (0.489) | 0.551 (0.461) |

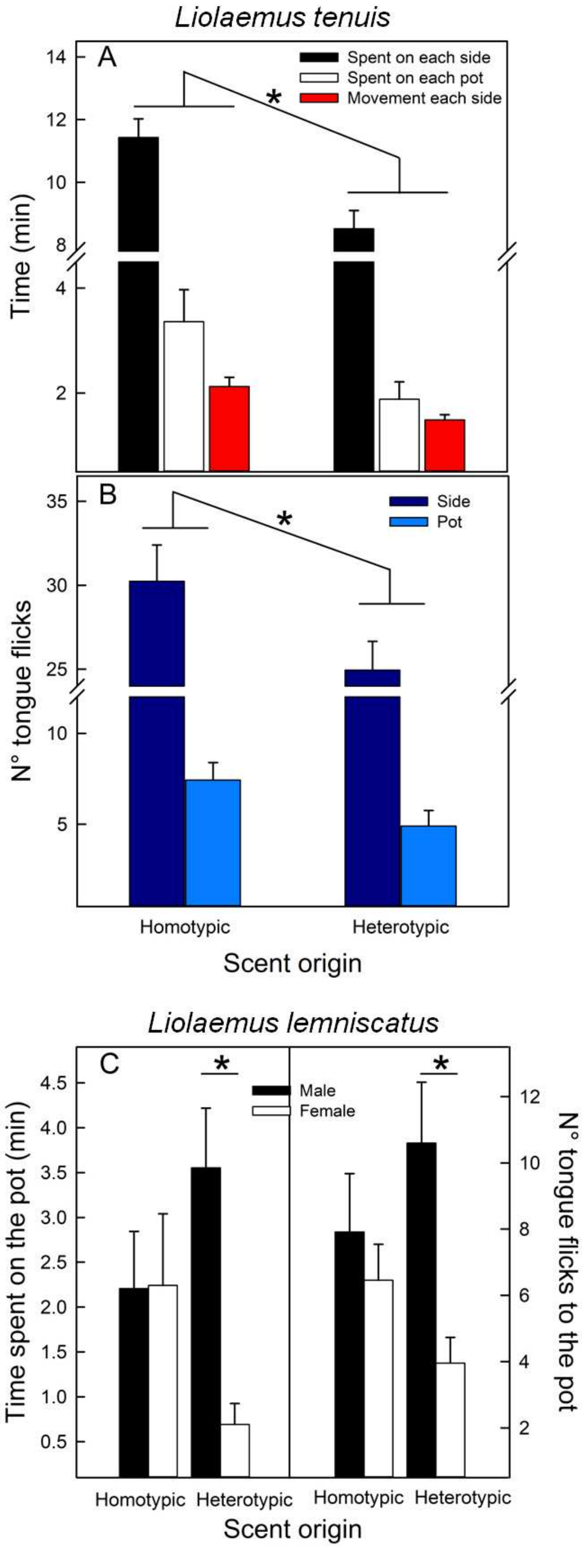

**Figure 4.** Mean (+SE) values of behaviors exhibited by lizards in enclosures with scents from homo- and heterotypic potential sexual partners. *Liolaemus tenuis*. A- Time (min) spent on each side of the

enclosure, on the pot with scents, and moving in each side of the enclosure. B- Number of tongue flicks displayed at each side of the enclosure and directed to the pot. Data from both populations and sexes were pooled. C- *Liolaemus lemniscatus*. Effect of the interaction between Scent origin and Sex upon the Time (min) spent in the pot with scents from different origins (left) and the number of tongue flicks directed to those pots (right). Data from both populations were pooled. * = $p < 0.05$.

*Liolaemus lemniscatus*. Scent origin modulated the time spent on the pot and the number of tongue flicks directed to the pot but only in interaction with the sex of the focal lizards (Table 3); females used less and directed fewer tongue flicks to pots with heterotypic scents than males (Figure 4C). However, the a posteriori tests could not exclude that both sexes behave similarly to pots with different scents (Figure 4C).

**Table 3.** Results of the General Linear Models for repeated measures to test preference for homo- and heterotypic potential sexual partner (Scent origin). Analyses included the Sex (male vs. female) and Population (Codegua vs. Las Viscachas) of the focal individuals of the two populations and the factor interactions upon scent preference. Shown values are the F-statistics [degree of freedom] (p-value); statistically significant tests (p < 0.05) are shown in bold.

| Variables | Liolaemus tenuis F[1,26](p) | Liolaemus lemniscatus F[1,26](p) |
|---|---|---|
| Scent origin | **11.953 (0.002)** | 0.262(0.613) |
| Population | 1.934 (0.176) | 0.000 (1.000) |
| Sex | 1.934 (0.176) | 0.000 (1.000) |
| Scent origin * Population | 0.048 (0.827) | **4.837(0.037)** |
| Scent origin * Sex | 1.867 (0.184) | 1.814 (0.190) |

### *3.3. Experiment III. Scent preference for homo- vs. heterotypic potential sexual partners*

Individuals of *L. tenuis*, independent of their sex and population, spent more time with homotypic scents (Table 3; Figure 5), while scent preferences of *L. lemniscatus* were influenced by the origin of the focal lizard; individuals from Las Viscachas preferred homotypic scents and individuals from Codegua preferred heterotypic scents (Table 3; Figure 5).

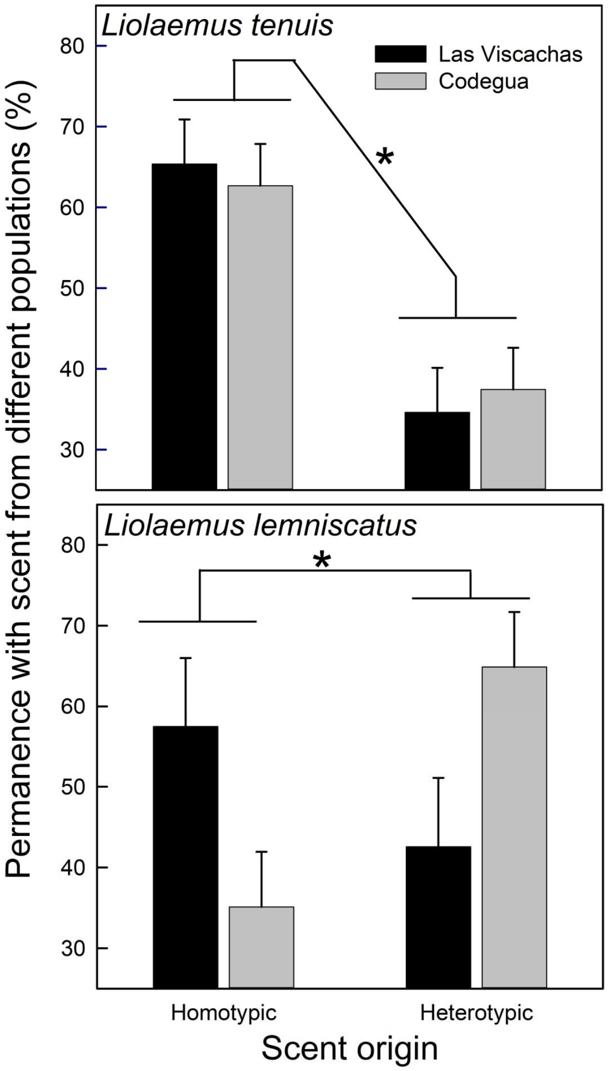

**Figure 5.** Mean untransformed (+SE) values of the Permanence (%) at each side of the enclosure contained clay pots with scents from homotypic and heterotypic conspecifics, of *Liolaemus tenuis* and *L. lemniscatus*, from two populations, Codegua and Las Viscachas. * = $p < 0.05$.

## 4. Discussion

The studied *Liolaemus* species showed discrimination between homo- and heterotypic scents, preferring homotypic scents in most cases. This suggests scent differences across populations, probably mirrored by differences in chemoreception. Because the studied populations were closely located and most likely from a unique genetic lineage [22,43], these findings indicate that behavioral barriers involving chemical modality may evolve relatively fast and may facilitate reproductive isolation even without geographic barriers. The two species, however, differed in the strength of their responses toward different populations and, therefore, in the degree of reproductive isolation conveyed by the chemical modality.

1- Recognition between homo- vs. heterotypic competitors (Experiment I)

Signals involved in competitor deterrence, as well as those implicated in mate attraction, can play a role in ethological isolation [6,8,11,53]. Thus, reproductive isolation has been studied by assessing the responses triggered by competitors from different origins e.g., [12,54]. In this study, males of both species discriminated between potential competitors from different populations, suggesting that scents may contribute to population isolation e.g., [42].

The higher rate of tongue flick made by *L. tenuis* toward non-homotypic scents suggests that these scents were perceived as different from those of the own population. These scents, however, were not as different as those from congeneric males, which elicited less exploration than homotypic scents [33,55]. Potentially, populations share a "species signature" [33], allowing recognition of non-homotypic males as conspecifics. On the other hand, since heterotypic populations were treated similarly, scents may also provide a "population signature" e.g., [56], inducing a dichotomous response, where any heterotypic c scent is treated as different, independently of how much they differ from the "population signature".

The scents from different populations triggered similar tongue-flick rates in *L. lemniscatus*. This may be because volatile scent compounds were assessed and discriminated by olfaction rather than vomerolfaction. In fact, scent evaluation by olfaction has been proposed for different *Liolaemus* species [32,35], including for *L. lemniscatus* [50]. The olfactory assessment triggered a faster exploration of heterotypic scents with the tongue and a longer exhibition of head displays toward homotypic male scents, indicating that these individuals were perceived as more threatening competitors [32,33].

2- Recognition and preference for homo- vs. heterotypic potential sexual partners (Experiments II and III)

In *L. tenuis*, the simultaneous exposition to scents from potential mates of different populations triggered a higher exploration (Experiment II) and a preference for homotypic partners (Experiment III); conspecifics from the own population were more attractive. These results suggest that these populations experience ethological isolation barriers mediated by the chemical modality and that gene flow may be restricted e.g., [42]. Consistently, genetic analyses of *L. tenuis* along its geographic distribution have revealed that the species might constitute a diverse cryptic-species complex [22,47,57]. Therefore, genetic studies should integrate scent analyses and discrimination e.g., [58].

In *L. lemniscatus*, the simultaneous exposure to scents from potential partners of the two populations did not determine clear-cut results; weak or nonexistent population recognition was observed during the short scent exposure (20 min, Experiment II) while the prolonged exposure (4.5 hrs, Experiment III) revealed population preferences. During the brief exposition, the sexes explored the heterotypic scents differently, with females spending less time investigating than males. However, because the sexes behaved similarly with homo- and heterotypic scents, data suggest a lack of population discrimination. Remarkably, the prolonged scent exposure revealed population discrimination, although preferences were asymmetric; both populations were more attracted to Las Viscachas scents, suggesting that populations may have only a weak reproductive barrier. Altogether, competitor (male) scents allowed population discrimination (Experiment I) in *L. lemniscatus*, but not scents from potential sexual partners, at least after a short exposure. Possibly, lizards may be more tolerant to deviation from the "population signature" when these are from potential sexual partners (Experiment II), requiring a prolonged scent exposure to exhibit preferences (Experiment III). Therefore, *L. lemniscatus* may have relatively strong species cohesion, and gene flow across populations may only be slightly restricted.

3- *Liolaemus* diversity and ethological isolation associated with the chemical sensory modality

Scents may evolve fast in lizards e.g., [44], showing differences across populations e.g., [40,59–61]. However, these differences do not necessarily determine population recognition e.g., [41,62] and may play a minor role in the evolution of reproductive isolation [63]. There are, however, also evidence for lizards from different genera showing population scent variations mirrored by population discrimination, driving the proposition that such variation may promote reproductive isolation, i.e., *Podarcis* [41,62,64], *Psammodromus* [65] and *Aspidoscelis* [42]. Of these genera, *Aspidoscelis* has the highest diversity, with 44 recognized species [17] and some with many subspecies [66]. This diversity possibly is determined by hybridization and introgression across taxa [66]. Therefore, ethological isolation may not contribute to the diversification of this genus. So then, although population scent recognition may allow reproductive isolation in these genera, this does not determine a high diversification of these taxa.

In *Liolaemus*, considering that the studied populations by species were closely located, without geographic barriers that prevented gene flow, and most likely belonging to a unique genetic lineage [22,43], the exhibited population discrimination suggest that scents, and their recognition, may evolve fast e.g., [33]. In this context, ethological barriers associated with the chemical modality may evolve fast, although at different rates across species, contributing to *Liolaemus* diversification, even without geographic barriers. This may explain the restricted geographic distribution of various *Liolaemus* species [18].

## 5. Conclusions

Different taxa show significant variation in speciation rates [2,27], and unraveling what causes such variation is one fundamental question in speciation research [67] but difficult to tackle, as speciation is a multi-cause process [2]. The present study provides a starting point to investigate how ethological barriers involving the chemical modality may disrupt species' cohesion, and facilitate speciation in *Liolaemus*, in synergy or not with geographical barriers e.g., [19]. However, new studies including species from different clades are needed to test the proposed role of the ethological "chemical" barriers in *Liolaemus* diversity. Furthermore, clades may differ in the relevance of chemical barriers, considering that some studies in *Liolaemus* show hybridizations and introgressions e.g., [68–70], implying that chemorecognition may not be relevant for all species.

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