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Chemical Polymorphism and Chemosensory Recognition between *Iberolacerta monticola* Lizard Color Morphs

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Abstract

In the lizard *Iberolacerta monticola*, there are 2 discrete color morphs ("blue" vs. "green") that seem to have alternative reproductive strategies. Because scent marking of territories and chemoreception are important in social organization of these lizards, we explored whether there is also chemical polymorphism and chemosensory recognition between color morphs. Analyses by gas chromatography–mass spectrometry showed that adult males of different morphs had similar chemical compounds in femoral gland secretions, but the proportions of some shared chemicals were different; blue morph males had higher proportions of steroids and lower proportions of fatty acids than green males. Differential tongue-flick rates to scents from femoral secretions of males indicated that males were able to detect the scent of any male from a baseline odorless control (water) but did not discriminate between the scent of males of different morphs. In contrast, females detected and discriminated between scents of the 2 male morphs by chemical cues alone. We suggest that differences in males' chemical signals between morphs and the ability of females of discriminating chemically between male morphs might be required to maintain spatial behavior, social organization, and mate choice decisions and, thus, be important for the maintenance of alternative reproductive strategies and polymorphism in lizards.

Key words: chemosensory recognition, femoral glands secretion, lizards, morphs, polymorphism

Introduction

In many animals, 2 or more different morphs can coexist within the same population (e.g., Sinervo and Lively 1996; Roulin 2004). This polymorphism can be evolutionarily maintained if it is selectively neutral, and all morphs have the same fitness (e.g., Hoffman et al. 2006). Alternatively, polymorphism can be maintained by temporal or spatial heterogeneity in selective pressures (Sinervo and Lively 1996; Sinervo et al. 2007). Frequency-dependent selection in color polymorphism is sometimes linked to a parallel polymorphism in life history strategies (e.g., Sinervo et al. 2001) or mating tactics (Sinervo and Lively 1996), the success of which is influenced by the frequency of players with alternative strategies.

Examples of color polymorphism have been described in many species of lizards, both iguanians (Thompson and Moore 1991; Sinervo and Lively 1996; Sinervo et al. 2000) and lacertids (Huyghe, Vanhooydonck, et al. 2007; Sacchi et al. 2007; Sinervo et al. 2007). Iberian rock lizards are small lacertids of highland rocky habitats, which comprise several closely related species (Mayer and Arribas 2003; Carranza et al. 2004). In Portugal, *Iberolacerta monticola* (formerly *Lacerta monticola monticola*) is restricted to a single population located at Serra da Estrela mountains. Two discrete and very distinct color morphs can be found in this species (Arribas 1996; Moreira 2002) and in closely related species (Arribas et al. 2008). "Green" morph adult lizards, both males and females, have greenish dorsum and yellow-greenish or bright green venter, whereas "blue" morph adult lizards have bluish dorsum and white-bluish or bright-blue venter (Arribas 1996; Moreira 2002). Lizards do not show ontogenetic changes in coloration between these 2 morphs. Field observations indicated that blue morph lizards use smaller home ranges than green morph lizards, which suggest that the 2 morphs might perform different alternative reproductive strategies (Moreira 2002).

In reptiles, the chemical senses play important roles in intraspecific communication and social organization (Mason 1992). In many lizards, chemical cues used in intraspecific communication come from femoral gland secretions (Mason 1992; Aragón et al. 2001). The femoral pores are epidermal structures on the ventral surface of the thigh connected to glands that produce copious amounts of holocrine secretion, especially in males and during the mating season (reviewed in Mason 1992; Alberts 1993). Only a few studies have described chemical composition of gland secretions of few lizard species (e.g., Alberts 1990; Weldon et al. 1990; Alberts et al. 1992; Escobar et al. 2001; López and Martín 2005a, 2005b, 2006; Louw et al. 2007; reviewed in Weldon et al. 2008). These studies showed that secretions are composed of both proteins and lipids. Lipids, however, are thought to be the main compounds involved in communication in lizards (Mason 1992; Martín and López 2006a, 2006b). The ventral location of the femoral pores allows secretions to be passively deposited on the substrate as lizards move through their home ranges. Behavioral experiments with several lacertid lizard species showed that both male and female lizards responded by tongue flicking to substrate scent marks and that scent marks may advertise residence and/or convey information about dominance status and competitive ability (Aragón et al. 2001; Carazo et al. 2007; Martín, Moreira, and López 2007a), or transmit information about a male's characteristics, which females may use to select mates or take residence in a male's territory (López et al. 2002; Olsson et al. 2003; Martín and López 2006a, 2006b).

Chemosensory recognition is well-developed in Iberian rock lizards. Male *I. monticola* discriminate their own femoral secretions from those of other males and also discriminate the dominance status of other males based on chemical cues arising from femoral secretions (Moreira et al. 2006). Moreover, proportions of specific chemicals (i.e., hexadecanol) in femoral secretions may function as a reliable signal of dominance status (Martín, Moreira, and López 2007a). In the closely related lizard *Iberolacerta cyreni*, females seem to base their mate choice decisions on proportions of some chemicals (e.g., pro-vitamin D_3, ergosterol) in scent marks of males (Martín and López 2006a, 2006b). Therefore, intraspecific chemical communication based on femoral secretions of males seems to play an important role in social organization of Iberian rock lizards.

It has been suggested that cycling mate choice games and context-dependent mate choice might maintain the genetic variation for polymorphisms even in the presence of mate choice for good genes (Alonzo and Sinervo 2001). We hypothesized that because chemosensory cues are important in rock lizards to maintain spatial behavior, social organization, and mate choice decisions, chemosensory recognition between lizard morphs might be required for the maintenance of alternative strategies and polymorphism in *I. monticola* lizards. Males might decide whether to enter a territory of a rival male based on chemicals in scent marks. Females might also use scent marks to assess the morph of a territorial male and decide whether to increase their probabilities of mating with that particular male by remaining in his territory.

In this paper, to explore whether there is chemical polymorphism between *I. monticola* male lizard color morphs, we first report the results of an analysis by gas chromatography–mass spectrometry of the lipophilic fraction of femoral gland secretions of males of the 2 morphs. We also designed a laboratory study to examine chemical recognition between these 2 morphs of the Iberian rock lizard. We analyzed the latter to discover whether chemical polymorphism allows chemosensory recognition within or between morphs and their potential implications in social organization of this lizard.

Materials and methods

Study animals

The subspecies of Iberian rock lizard *I. monticola monticola* is restricted to a single population located at Serra da Estrela, Portugal. It is distributed between approximately 1400 m of altitude and the top of the mountain—"Torre"—at 1993 m and covers a range of approximately 57 km^2 (Moreira et al. 1999). Lizards are active from March to November, the mating season begins in May–June, and females produce a single clutch per year (Moreira 2002). Lizards of the green morph are more abundant than blue morph ones. However, abundance of blue morph lizards increases with altitude. At about 1500 m, green morph lizards comprise 95% of the animals, whereas at the mountain top they comprise 75% of the animals (Moreira 2002).

We captured adult male and female lizards of the 2 morphs at the highest elevations of Serra da Estrela (surroundings of Torre) during March and April, before the start of the mating season. At the beginning of May, lizards were transferred to indoor facilities at "El Ventorrillo" field station of the Museo Nacional de Ciencias Naturales (Madrid province, Spain). We used glass terraria ($50 \times 25 \times 25$ cm^3) provided with a cardboard substrate, cardboard rolls for refuge, and a hardware cloth lid. Terraria were lighted from 9:00 to 19:00 h with full spectrum fluorescent lamps and were heated by 60 W incandescent lamps. Mealworm larvae (*Tenebrio* sp.) dusted with multivitamin powder and water were provided ad libitum. Males and females were housed in individual terraria. Different individual animals were used as secretion donors for chemical analyses before trials, as focal animals in behavioral experiments, and as secretion donors for experiments. Lizards maintained good conditions throughout the study and were returned to their capture sites at the end of the trials.

Analyses of femoral gland secretions

We extracted femoral gland secretion of adult males of similar body size (snout-to-vent length; blue morph: mean ± standard error (SE) = 76.6 ± 0.4 mm, $n = 20$; green morph: mean ± SE = 76.3 ± 0.5 mm, $n = 19$) by gently pressing with forceps around the femoral pores, but without touching the secretion, and collected secretion directly in glass vials with Teflon-lined stoppers. Forceps were washed with hexane between collections. Vials were stored at –20 °C until analyses were carried out. We also used the same procedure but without collecting secretion, to obtain blank control vials that were treated in the same manner. Control samples were compared with the lizards' samples to exclude contaminants from the handling procedure or from the environment and for further examining impurities in the solvent. Before the analyses, we added 200 μl of *n*-hexane (capillary gas chromatograph [GC] grade) to each vial and mixed the solution with a vortex. A nonsoluble fraction of the secretion containing proteins remained in the bottom of the vial, whereas the lipid fraction was fully dissolved into hexane. Although proteins may be the major component in secretions (Alberts 1990; Alberts et al. 1993), most recent evidences points to lipids as the main compounds involved in pheromonal communication in Iberian rock lizards (Martín and López 2006a, 2006b; Martín, Moreira, and López 2007a).

We analyzed lipophilic compounds in samples by using a Finnigan-ThermoQuest Trace 2000 GC fitted with a poly(5% diphenyl/95% dimethylsiloxane) column (Equity-5, 30 m length × 0.25 mm ID, 0.25-μm film thickness) and a Finnigan-ThermoQuest Trace mass spectrometer as detector. Sample injections (2 μl of each sample dissolved in *n*-hexane) were performed in splitless mode using helium as the carrier gas, with injector and detector temperatures at 270 °C and 250 °C, respectively. The oven temperature program was as follows: 50 °C isothermal for 10 min, then increased to 280 °C at a rate of 5 °C/min, and then isothermal (280 °C) for 30 min. Mass spectral fragments were recorded in the range of mass–charge ratios (m/z) = 39–646, at a scan rate of 1.8 scans/s. Impurities identified in the solvent and/or the control vial samples are not reported. Initial tentative identification of secretion components was done by comparison of mass spectra in the National Institute of Standards and Technology/Environmental Protection Agency/National Institutes of Health (NIST/EPA/NIH) 1998 computerized mass spectral library. Identifications were confirmed when possible by comparison of spectra and retention times with those of authentic standards. For unidentified or unconfirmed compounds, we used their retention times and characteristic ions and m/z ratios to confirm whether these compounds were present in a given individual.

For the statistical analyses of chemicals in secretions, the relative amount of each component was determined as the percent of the total ion current (TIC). The relative areas of the peaks were transformed following Aitchison's formula: $[Z_{ij} = \ln(Y_{ij}/g(Y_j)]$, where Z_{ij} is the standardized peak area i for individual j, Y_{ij} is the peak area i for individual j, and $g(Y_j)$ is the geometric mean of all peaks for individual j (Aitchison 1986; Dietemann et al. 2003). This is the recommended mathematical transformation for statistical analyses of these types of compositional data (Aitchison 1986). The homogeneity of variance of these variables was tested with Levene's test (after applying Bonferroni's correction).

The transformed areas were used as variables in a repeated-measures multivariate analysis of variance (MANOVA), testing for differences between the 2 males' morphs. Because we found significant differences between morphs in the multivariate analyses (see Results), we could perform protected 1-way analyses of variance (ANOVAs) separately on each chemical, to locate which chemicals (dependent variables) changed more between morphs and explained the significant difference found in the previous MANOVA model (Scheiner 2001).

Detection of chemical cues

Lizards react to a variety of chemical stimuli with increased and differential rates of tongue extrusions (Cooper and Burghardt 1990). Tongue-flick (TF) rate can, therefore, be used as a quantitative bioassay of detection of chemical cues of conspecifics (e.g., Aragón et al. 2001; Cooper and Pérez-Mellado 2002). Thus, to test for differential responses to femoral secretions of *I. monticola* males of the 2 morphs, we made comparisons of TF rate by lizards (males and females) in response to chemical stimuli arising from cotton applicators impregnated with femoral gland secretions of males of blue and green morphs, or with deionized water (odorless control) (Cooper and Burghardt 1990). Water was used to gauge baseline TF's rates in the experimental situation. We prepared stimuli dipping the cotton tip (1 cm) of a wooden applicator attached to a long stick (50 cm) in deionized water. Femoral secretions consisted of a waxy substance, which was easily extracted by gently pressing with forceps around the femoral pores and collected directly on cotton tips of applicators. We used approximately the same amount of femoral secretion in each stimulus (about 2 × 1 mm of solid secretion from each of 3 pores) to minimize likelihood that differences in TF rates were due to differences in the amount of secretions presented to lizards. Only one secretion was collected per source animal. Swabs were used in the trials within 2 min after collection to avoid fading of the stimuli, and a new swab with water or femoral secretions from different donor individuals was used in each trial. Donor lizards were different individual lizards than the lizards tested in the trials.

Every focal lizard (16 blue males, 16 green males, 16 blue females, and 16 green females) was exposed to each scent stimulus, and order of presentation was determined using a stratified randomization procedure. One trial was conducted per day for each animal. Trials were conducted at the beginning of June, which coincided with the mating season of lizards in their original natural population (Moreira 2002), and between 1100 and 1300 h (GMT) when lizards were fully active.

To begin a trial, the experimenter slowly approached the terrarium and slowly moved the cotton swab to a position 1 cm anterior to the lizards' snout. Lizards usually did not flee from the swab but explored it repeatedly by tongue flicking or ignored it after the firsts TFs. The numbers of TFs directed at the swab were recorded for 60 s beginning with the first TF. Latency to the first TF was computed as the period elapsed between presentation of the cotton swab to the first TF directed at the swab. The swab tester was blind respect to the scent stimuli tested.

To examine differences in number of TFs directed at the swab and latency to first TF among treatments, we used repeated-measures 2-way ANOVAs examining the effects of scent stimuli (within factor: "own morph" vs. "other morph" vs. "water") and morph of the responding lizard (between factor; "blue" vs. "green"). We included the interaction in the model to analyze whether responses to the different scents differed as a function of the morph of responding lizard. Analyses were made separately for responding males and females. Data were log-transformed to ensure normality. Tests of homogeneity of variances (Levene's test) showed that in all cases variances were not significantly heterogeneous after transformation. Pairwise comparisons were planned using Tukey's honestly significant difference tests (Sokal and Rohlf 1995).

Results

Chemical composition of femoral gland secretions

We found in the lipophilic fraction of femoral gland secretions of male *I. monticola* a mixture of 49 compounds, including steroids (89.3% of TIC), carboxylic acids ranged between n-C_9 and n-C_{22} and some of their esters (8.4%), and alcohols between C_{12} and C_{20} (1.7%), but we also found 4 waxy esters (0.2%), squalene (0.28%), and 2 furanones (0.20%) (Table 1). On average, the 5 most abundant chemicals were cholesterol (69.9%), campesterol (9.7%), hexadecanoic acid (3.2%), sitosterol (2.7%), and dodecanoic acid (2.0%).

All compounds were shared by males of the 2 morphs, but some minor compounds were not detected in all individuals, although frequencies of appearance of these minor compounds were similar in both morphs, and we attributed interindividual differences to difficulties of detection in chemical analyses. However, in comparison with males of the green morph, males of the blue morph had significantly higher proportions of steroids (blue vs. green, mean ± SE = 90.1 ± 0.9% vs. 88.2 ± 0.9%; 1-way ANOVA, $F_{1,37} = 5.03$, $P = 0.03$) and significantly lower proportions of fatty acids (7.4 ± 0.9% vs. 9.6 ± 0.9%; $F_{1,37} = 5.70$, $P = 0.02$), but the 2 morphs did not differ significantly in proportions of alcohols (1.8 ± 0.2% vs. 1.6 ± 0.2%; $F_{1,37} = 0.61$, $P = 0.44$).

Moreover, multivariate analyses on the relative proportions of compounds showed that there were significant differences in the relative proportions of some compounds between male morphs (MANOVA, Wilks'$\lambda = 0.30$, $F_{20,18} = 2.38$, $P = 0.035$). Univariate protected 1-way ANOVAs showed that blue males had secretions with significantly lower relative proportions of sitosterol ($F_{1,37} = 20.91$, $P < 0.0001$), dodecanoic acid ($F_{1,37} = 12.99$, $P < 0.001$), and octadecenoic acid ($F_{1,37} = 11.01$, $P = 0.002$). These were the only significant differences noted in chemical composition of femoral gland secretions between male morphs (all other univariate tests had $P > 0.20$).

Chemosensory recognition by males

All male *I. monticola* directed TFs to the swab in all conditions. Mean latency to first TF differed significantly between scent stimuli treatments (2-way repeated-measures ANOVA: $F_{2,60} = 128.63$, $P < 0.0001$; Figure 1a), there were no significant differences between the overall responses of both male morphs ($F_{1,30} = 0.07$, $P = 0.79$), but the interaction was significant ($F_{2,60} = 3.94$, $P = 0.02$). All males responded to any male's scent significantly earlier than to water (Tukey's tests: $P = 0.00013$ in all cases), but responses to males of their own or of the other morph were not significantly different (blue: $P = 0.20$; green: $P = 0.57$).

There were significant differences between scent stimuli in numbers of TFs directed by males to the swab (2-way repeated-measures ANOVA: $F_{2,60} = 73.00$, $P < 0.0001$; Figure 1b), there were no significant differences between the response of the 2 male morphs ($F_{1,30} = 1.49$, $P = 0.23$), which responded in a similar way to the different stimuli (interaction: $F_{2,60} = 2.32$, $P = 0.11$). Males discriminated between scents of any male and water (Tukey's tests: $P = 0.0012$ in both cases), but there were no significant differences in the responses to scent of males of their own or of the other morph ($P = 0.87$).

Chemosensory recognition by females

All female *I. monticola* directed TFs to the swab in all conditions. Mean latency to first TF differed significantly between scent stimuli (2-way repeated-measures ANOVA: $F_{2,60} = 15.12$, $P < 0.0001$; Figure 2a), and there were no significant differences between the overall responses of both morphs of females ($F_{1,30} = 0.04$, $P = 0.84$), which responded in a similar way to the different stimuli (interaction: $F_{2,60} = 0.60$, $P = 0.55$). Females responded later to water than to scents of males of their own (Tukey's test: $P = 0.00012$) or the other morph ($P = 0.03$) and earlier to scent of males of their own morph than of the other morph ($P = 0.014$).

There were significant differences between treatments in numbers of TFs directed by females (2-way repeated measures ANOVA: $F_{2,60} = 135.41$, $P < 0.0001$; Figure 2b), there were no significant differences between the response of the 2 morphs of females ($F_{1,30} = 2.06$, $P = 0.16$), which responded in a similar way to the different stimuli (interaction: $F_{2,60} = 0.01$, $P = 0.99$). Females discriminated between scents of any male morph and water (Tukey's tests: $P = 0.00012$ in both cases) and had significantly higher TF rates to scent of males of their own morph than to scent of males of the other morph ($P = 0.00012$).

Table 1 Major lipophilic compounds found in femoral gland secretions of male lizards, *I. monticola* of 2 color morphs

Compound	Blue morph (N = 20)	Green morph (N = 19)
Steroids		
Cholesterol	69.83 ± 4.13	67.34 ± 5.09
Campesterol	9.63 ± 1.30	9.72 ± 2.12
γ-Sitosterol	2.47 ± 0.53	3.02 ± 0.47*
Cholestanol	2.01 ± 0.74	2.02 ± 0.65
4,4-Dimethyl-cholesta-5,7-dien-3-ol	1.97 ± 0.66	1.91 ± 0.95
Stigmasta-5,24(28)-dien-3-ol	0.99 ± 0.41	1.06 ± 0.30
Ergosta-5,8-dien-3-ol	1.04 ± 0.62	0.97 ± 0.54
Cholestan-3-one	0.68 ± 0.20	0.71 ± 0.22
Ergosterol	0.45 ± 0.27	0.40 ± 0.30
4,4-Dimethyl-cholest-7-en-3-ol	0.34 ± 0.47	0.22 ± 0.27
4-Methyl-cholest-7-en-3-ol	0.14 ± 0.21	0.17 ± 0.19
2,2-Dimethyl-cholest-8(14)-en-3-ol	0.13 ± 0.21	0.19 ± 0.10
Cholesta-4,6-dien-3-ol	0.11 ± 0.25	0.14 ± 0.22
24-Propylidene-cholest-5-en-3-ol	0.11 ± 0.12	0.07 ± 0.12
Lanost-8-en-3-ol	0.08 ± 0.12	0.12 ± 0.14
Cholesta-3,5-diene	0.06 ± 0.07	0.04 ± 0.05
Unidentified steroid 1	0.04 ± 0.07	0.03 ± 0.08
Unidentified steroid 2	0.04 ± 0.08	0.03 ± 0.06
Anthiaergosta-5,7,9-trien-3-one	0.01 ± 0.03	0.01 ± 0.05
Cholesta-2,4-diene	0.01 ± 0.04	0.01 ± 0.05
Stigmasterol	0.01 ± 0.01	0.01 ± 0.01
Carboxylic acids and their esters		
Nonanoic acid	0.01 ± 0.04	0.01 ± 0.04
Decanoic acid	0.01 ± 0.02	0.01 ± 0.03
Dodecanoic acid	1.70 ± 0.71	2.32 ± 0.98*
Tetradecanoic acid	0.03 ± 0.06	0.03 ± 0.07
Pentadecanoic acid	0.01 ± 0.03	0.01 ± 0.02
Hexadecanoic acid	2.85 ± 1.85	3.55 ± 2.48
Hexadecenoic acid	0.01 ± 0.04	0.01 ± 0.02
Heptadecanoic acid	0.01 ± 0.01	0.01 ± 0.03
Octadecanoic acid	0.93 ± 0.47	1.20 ± 0.65*
Octadecenoic acid	1.14 ± 0.16	1.57 ± 0.57
Octadecadienoic acid	0.50 ± 0.29	0.66 ± 0.48
Octadecenoic acid, methyl ester	0.01 ± 0.03	0.01 ± 0.02
Eicosanoic acid	0.13 ± 0.11	0.14 ± 0.12
Eicosatetraenoic acid, methyl ester	0.01 ± 0.02	0.01 ± 0.01
Docosanoic acid	0.01 ± 0.02	0.01 ± 0.03
Erucic acid	0.02 ± 0.03	0.02 ± 0.04
Alcohols		
Dodecanol	0.02 ± 0.01	0.02 ± 0.01
Tetradecanol	0.01 ± 0.01	0.01 ± 0.01
Hexadecanol	1.51 ± 0.60	1.42 ± 0.68
Octadecanol	0.24 ± 0.24	0.14 ± 0.19
Eicosanol	0.01 ± 0.04	0.04 ± 0.03
Waxy esters		
Hexadecyl tetradecenoate	0.01 ± 0.21	0.01 ± 0.15
Hexadecyl hexadecenoate	0.01 ± 0.11	0.01 ± 0.08
Hexadecyl octadecenoate	0.15 ± 0.18	0.16 ± 0.20
Octadecyl hexadecenoate	0.01 ± 0.05	0.01 ± 0.05
Others		
Squalene	0.30 ± 0.22	0.26 ± 0.13
4-Hydroxy-hexadecanoic acid γ-lactone	0.19 ± 0.08	0.18 ± 0.16
4-Hydroxy-octadecanoic acid γ-lactone	0.01 ± 0.02	0.01 ± 0.02

The relative amount of each component was determined as the percent of the TIC and reported as the average (±1 standard deviation). An asterisk denotes a significant difference between morphs in protected 1-way ANOVAs (see Results).

Discussion

Our results show that polymorphism of male *I. monticola* is not limited to coloration differences but that there is also polymorphism in chemical signals. Femoral gland secretions of males of the 2 different color morphs contain similar lipophilic chemicals, but some major compounds vary in relative proportions. These differences in chemical composition are apparently enough to allow females the chemosensory discrimination of the 2 male morphs. However, males do not seem to discriminate between male morphs by chemical cues alone.

The lipophilic fraction of femoral secretions of male *I. monticola* lizards is very similar to those described in other lacertid lizards in that it is mainly composed of steroids, carboxylic acids, and alcohols. In particular, in comparison with the closely related *I. cyreni*, secretions differ qualitatively in few chemical compounds (López and Martín 2005a; López et al. 2006). Thus, most compounds (ca. 70%) were shared with *I. cyreni*, but 16 compounds were exclusive of *I. monticola*, and 6 compounds previously found in *I. cyreni* were not detected in *I. monticola* (López and Martín 2005a; Martín, Moreira, and López 2007a). Differences between male morphs of *I. monticola* are much fewer; males of the 2 morphs shared all chemicals found in secretions, but

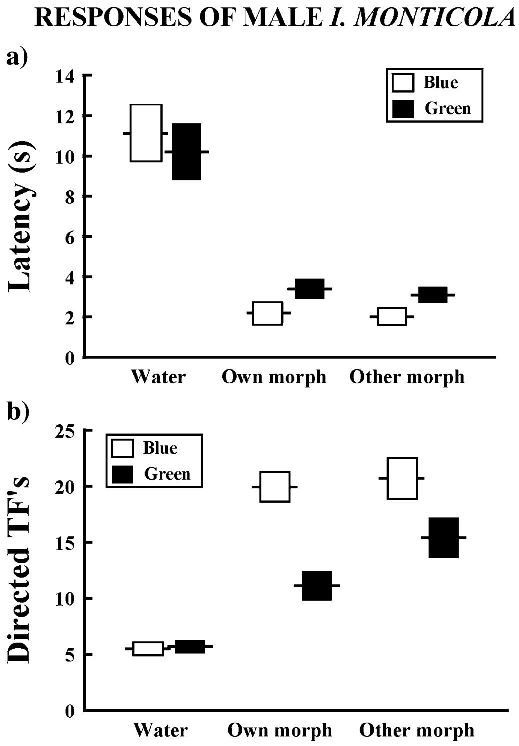

Figure 1 Mean (±SE) **(a)** latency (s) to the first TF and **(b)** number of TFs directed to the swab by male *I. monticola* of 2 color morphs (open boxes: blue; closed boxes: green) in response to control deionized water or scents from femoral gland secretions of males of their own or of different morph, presented for 60 s on cotton-tipped applicators.

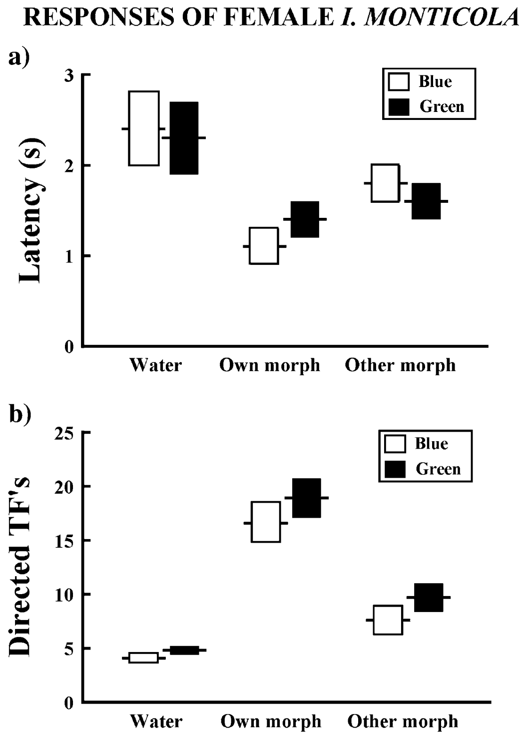

Figure 2 Mean (±SE) **(a)** latency (s) to the first TF and **(b)** number of TFs directed to the swab by female *I. monticola* of 2 color morphs (open boxes: blue; closed boxes: green) in response to control deionized water or scents from femoral gland secretions of males of their own or of different morph, presented for 60 s on cotton-tipped applicators.

relative proportions of some major compounds differ between morphs. Blue morph males have femoral secretions with more steroids and less fatty acids than green morph males, and at least 3 major compounds (sitosterol, and dodecanoic and octadecenoic acids) are more abundant in green males. The simpler explanation might be that these observed differences might be directly related to small genetic differences between morphs.

However, differences in chemicals might be linked to the apparent different reproductive strategies of each male morph (green males defending larger territories with more overlap between neighbors, whereas blue males having smaller and more exclusive territories) (Moreira 2002). The different strategies might depend on different circulating levels of steroid hormones as occurred in the 3 morphs of male lizards *Uta stansburiana* (Sinervo et al. 2000) and in at least 2 of the 3 male morphs of the Dalmatian wall lizard *Podarcis melisellensis* (Huyghe, Herrel, et al. 2007, 2009). Lipid metabolism is mediated by levels of glucocorticoids and other hormones (Sheridan 1994). Thus, it is likely that, if the 2 male *I. monticola* morphs differ in circulating hormone levels, these differences would be reflected in proportion of lipids allocated to femoral secretions. Interestingly, in males of the related *I. cyreni* lizard, experimentally increased predation risk, which increases stress, and probably corticoid levels results in increased proportions of steroids and lowered proportions of fatty acids in femoral secretions (Aragón et al. 2008). Thus, we could speculate that blue male *I. monticola* might have higher levels of glucocorticoids, which would affect lipids in femoral secretions in a similar way. Nevertheless, the relationships between hormones, reproductive strategies, and chemical signals of the 2 morphs of male *I. monticola* remain to be studied in detail.

Other possible explanations for inter-morph differences in femoral secretions may be considered. The occurrence of blue and green morphs in the study population varies linearly with altitude, with blue morph lizards being more abundant at high altitude (Moreira 2002). This suggests that the maintenance of this polymorphism could be related to environmental factors which might vary with altitude. In this sense, differences in proportions of chemicals in secretions might result from selection for the persistency and efficiency of chemical signals in different environments (Alberts 1992; Martín and López 2006c); less volatile and more stable compounds (e.g., steroids) being favored in lizards inhabiting high-altitude habitats with subalpine conditions, in comparison with the low-altitude Mediterranean environments. This would be in agreement with the observed higher proportions of blue morph lizards, which have more steroids in secretions, at high altitude (Moreira 2002). Similar environmental related differences in chemicals in femoral secretions between populations have been described in other lizards (Martín and López 2006c).

TF rates of male *I. monticola* in response to different scents showed that males were able to detect scent of males from a baseline odor (water), but males did not seem to discriminate between the 2 male morphs based on chemical cues alone. This result was initially not expected given the chemosensory ability of *I. monticola* to discriminate between conspecific males, possibly even at the individual level (Moreira et al. 2006). Chemosensory recognition mechanisms between male lizards are important because they may contribute to identify and assess rival fighting ability or dominance status, thus reducing the intensity and the costs of fighting (Aragón et al. 2001; López and Martín 2002; López et al. 2003; Carazo et al. 2007). However, chemicals that signal dominance in *I. monticola* lizards (i.e., hexadecanol and octadecanol) and that elicit aggressive responses of males (Martín, Moreira, and López 2007a) did not differ between male morphs. It remains possible that overall differences between morphs might not be so great in comparison with interindividual differences in chemicals signalling dominance as to be revealed with the TF bioassay. Alternatively, the result of a potential discrimination might not elicit an increase in TF rate for the own morph males. Nevertheless, even if males do not discriminate between male morphs per se, they could still use individual characteristics of each male's scent in deciding their social behavior, which may lead to different outcomes if the 2 male morphs vary in dominance status and social strategies.

In contrast, female *I. monticola* clearly detected and discriminated between the scents of the 2 male morphs. Thus, it is likely that chemosensory identification of the males' morph by females was based on one of some of the chemicals that vary in proportion between morphs. Although the cotton swab method has been widely used in many studies as a quantitative bioassay of detection of chemical cues of conspecifics (Cooper and Burghardt 1990; Aragón et al. 2001; Cooper and Pérez-Mellado 2002), it also has some limitations. In our study, a higher TF rate by females clearly indicates discrimination of the scent of males of their own morph, but it is not clear without additional behavioral experiments whether females may prefer or avoid males of their own morph. However, other studies have suggested that a higher exploration rate indicates a greater interest for exploring that scent (Martín, Civantos, et al. 2007b). Thus, it could be predicted that females of each morph might prefer the scent of males of their own morph. Therefore, this preference could lead to assortative matings if females look for and prefer to remain in home range areas scent marked by males of their own morph. Nevertheless, because females can discriminate between male morphs, females could also be able to identify and select the areas of males of the other morph, favoring inter-morph matings. In fact, successful copulations between males and females of the 2 morphs can occur, leading to viable offspring (P.L. Moreira, unpublished data). The social environment might decide which pairing strategy would be more adequate in each circumstance.

Irrespective of the causes that might explain the intermorph differences in chemicals in femoral secretions of males, the observation that females showed different chemosensory responses depending on the male morph stimuli, and on the own morph of the responding female, suggests that the chemical polymorphism of males may be important in social organization in this lizard. Thus, chemical polymorphism in femoral secretions might be implied in the maintenance of this polymorphism, which might be mediated by the role of chemical signals in mate choice decisions of females. Because chemical cues are important in many other lizard species, it is also likely that there are also differences in chemicals of male morphs and chemosensory responses of females in other known lizard polymorphisms (Thompson and Moore 1991; Sinervo and Lively 1996; Sinervo et al. 2000, 2007; Huyghe, Vanhooydonck, et al. 2007; Sacchi et al. 2007). Further studies are clearly needed to clarify the implications of chemical signals in the maintenance of alternative morphs in lizards.

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