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Research Article

Microgeographical Variations in Coloration of Male Iberian Wall Lizards May Be Related to Habitat and Climatic Conditions

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Intraspecific variations in coloration may represent a compromise between selection for intraspecific communication and selection for thermoregulation and predator avoidance. Iberian wall lizards, *Podarcis hispanica*, exhibit substantial levels of intraspecific variation that cannot be necessarily attributed to genetic differences. We compared variations in coloration and habitat use of three phenotypically distinct populations of *P. hispanica* in Central Spain. Results suggested that differences in coloration may be related to habitat characteristics and climatic conditions. Thus, lizards from populations with colder temperatures were darker and larger, which may favor thermoregulation. Lizards that lived in habitats with more vegetation and darker granite rocks showed a dark brown to black dorsal coloration. In contrast, lizards from habitats with gypsum and light sandy soil without vegetation or large rocks had a brighter yellow to green dorsal coloration. These differences may increase crypsis to predators in each habitat. There were also differences in the characteristics and relative importance of sexual visual signals (i.e., ventrolateral coloration and number of lateral blue spots) and chemical signals (i.e., number of femoral pores) that might increase efficiency of communication in each environment. Natural selection for traits that allow a better thermoregulation, predator avoidance, and communication might lead to population divergence.

1. Introduction

Intraspecific variation in coloration is an intriguing phenomenon found in a widespread number of animal taxa, particularly vertebrates [1–3]. Animal coloration represents a compromise between selection for signaling functions in intraspecific communication (i.e., sexual signals) and selection for thermoregulation and defense against visually oriented predators [2, 4–10]. According to this, differences in coloration between species and populations or between sexes and age classes are the result of subtle differences in the balance between natural and sexual selection [4, 5, 9, 11]. Variation in coloration on a large geographical scale is essentially found in response to variations in climate, habitat, and predators [3, 12–14]. Correlational selection will promote that coloration will become associated genetically and developmentally with other traits [15].

In reptiles, color variations have long been studied as examples of adaptive evolution [14, 16, 17]. On the one hand, coloration impacts thermoregulation in ectotherms because darker reptiles are able to warm faster and maintain higher body temperatures for longer [18–20]. Coloration also affects detectability by avian predators, so natural selection for substrate color matching is predicted to be strong for diurnal reptiles [21–24]. On the other hand, many reptiles use visual sexual signals based on colourful conspicuous traits in intraspecific communication and sexual selection [1]. However, the characteristics and relative importance of visual sexual signals against alternatives such as chemical signals may also depend on the relative efficacy of these signals in each environment [25–29].

In this study, we examined the variations in coloration of three phenotypically distinct populations of Iberian wall lizards, *Podarcis hispanica* from Central Spain. This is a small diurnal lizard, widespread in the Iberian Peninsula, where it inhabits different contrasting environments. Molecular and morphological studies suggest that this lizard is paraphyletic and actually forms part of a "species complex," which includes at least five monophyletic lineages and some well-recognized species, such as *P. bocagei*, *P. carbonelli,* and *P. vaucheri* [30–34]. Iberian wall lizards exhibit substantial levels of morphological variation, being highly variable in size and coloration (e.g., for the human eye, lizards have a brown, greenish, or greyish back with variable black patterns, ventrolateral small blue spots, and white, red, orange, pink, or yellow bellies) [35]. This variation occurs not only between currently recognized species but also among populations within genetically related lineages [33, 36]. For example, several apparently distinct populations of *P. hispanica* with a not well-studied variety of morphologies and colorations can be found in different habitats of the relatively small area (8000 km^2) of the Madrid Region (Central Spain) [37–41]. These differences cannot be necessarily attributed only to neutral genetic drift [33], and we hypothesized that variations in coloration may represent adaptations to different microhabitats and climatic characteristics.

Here, we examined and compared the characteristics of dorsal and ventrolateral coloration, by using spectrometry, of male lizards from these populations. We also characterized the habitat (vegetation and substrate) used by lizards at each population and described climatological conditions in each area. We tested whether differences in habitat and climatic characteristics could explain the variations in coloration observed among these lizard populations. For example, we predicted that thermoregulatory requirements may favor that lizards from populations with colder conditions would be larger and had darker dorsal colorations to facilitate thermoregulation. Also, adaptations to avoid predation would favor that dorsal colorations would match habitat coloration and depend on visibility within the habitat.

In addition, to test for the relative importance of different types of communication (visual versus chemical) among populations, we compared the numbers of lateral blue spots, which are used in visual intraspecific communication [42, 43], and femoral pores, which produce secretions used as chemical signals [43–46]. We predicted that visual communication would be favored in open habitats. Additionally, the persistence and efficacy of scent marks are expected to be lower in hot and dry climatic conditions [25, 29], which may select against the use of chemical signals in these habitats.

2. Material and Methods

2.1. Study Populations. During March 2007, we captured *P. hispanica* lizards by noosing at three localities within the Madrid Province (Central Spain) (Table 1). One of the lizard populations inhabits dark granite rock-cliffs at the edge of a mountain pine forest in the upper part of "Fuenfría" Valley (40°47′N, 04°03′W). The second lizard population is found on dark granite rocky outcrops in a montane oak forest ("Golondrina") near Cercedilla village (40°44′N, 04°02′W). The third lizard population occupies whitish chalk and gypsum rocks and soils in deforested bushy hills and plains near "Aranjuez" village (40°02′N, 03°37′W). From north to south, Fuenfría was located at 7 km from Golondrina, which is 75 km from Aranjuez.

Table 1: Habitat characteristics of Fuenfría, Golondrina and Aranjuez populations and morphological characteristics (mean ± 1 SE; range between brackets) of *Podarcis hispanica* lizards from these populations.

Population	Fuenfría ($n = 20$)	Golondrina ($n = 19$)	Aranjuez ($n = 8$)
Habitat:			
Elevation (m)	1760	1200	500
Vegetation type	Pine forest	Oak forest	Grassland/shrub
Substrate	Dark rock	Dark rock	White chalk
Morphology of lizards:			
Weight (g)	5.3 ± 0.2 (3.6–7.5)	4.1 ± 0.1 (2.5–5.2)	3.6 ± 0.2 (2.2–4.6)
SVL (mm)	61 ± 1 (53–72)	58 ± 1 (53–65)	52 ± 1 (47–55)
Femoral pores	37 ± 1 (31–45)	38 ± 1 (32–44)	34 ± 1 (31–37)
Blue spots	3.5 ± 1.1 (0–13)	6.9 ± 1.3 (0–17)	9.1 ± 1.6 (2–16)

All lizards were individually housed at "El Ventorrillo" Field Station (Cercedilla, Madrid), in indoor 60 × 40 cm PVC terraria containing sand substratum and rocks for cover. Cages were heated with 40 W spotlights during 6 h/day and lit overhead (36 W full-spectrum daylight tubes) using a 10 : 14 light/dark cycle. Terraria were separated visually from each other using cardboard. Every day, lizards were fed mealworm larvae (*Tenebrio molitor*) dusted with multivitamin powder for reptiles, and water was provided *ad libitum*. Lizards were held in captivity while coloration measurements were taken and to be used in other experiments. Lizards maintained a good health condition through the study and were returned to their exact capture sites (determined with a GPS) at the end of studies, one month after being captured.

2.2. Coloration of Lizards. In the laboratory, we measured reflectance of coloration of male lizards from 300 to 700 nm using an Ocean Optics USB2000 spectroradiometer (Ocean Optics, Inc., Dunedin, FL) with a DT-1000-Mini Deuterium-Halogen light source (215–2500 nm spectral range), one or two days after capture [47]. To exclude ambient light and standardize measuring distance, a cylindrical metallic tube was mounted on the bifurcated fiber optic probe. The probe was held at a 90° degree angle to the skin, and reflectance was measured always by the same person (MG). We measured dorsal coloration of two standardized spots on the dorsum: one in the middle point between the two forelimbs and one in the middle point between the hind limbs. Because measurements at these two locations were highly repeatable (intraclass correlation coefficient, $r = 0.76$, $P < 0.0001$), we later calculated an average value for the two dorsal spots of each individual. We also measured ventral coloration on four standardized spots in the ventrolateral part: one on the middle of the throat (between the last chin shields and the collar; "throat"), one between the two forelimbs (at the middle of the second row of ventral scales from the collar; "breast"), one close to the end of the vent (at the middle of the fourth row of ventral scales from the cloacae; "belly"), and one on the proximal portion of the tail ("tail"). We measured coloration at three closely haphazardly selected places at each body location, and, thereafter, we calculated means and used these values for further analyses. Reflectance was calculated relative to a white standard (WS-1-SS) with the OOIBase32 software (Ocean Optics, Inc.). Recalibration of the spectroradiometer was made for each individual lizard. Mean reflectance was summarized over 6 nm steps ("binned" [48] Grill and Rush, 2000) before statistical analysis.

We mathematically summarized the spectra using principal component analysis (PCA) [6, 48, 49]. This method makes no assumptions about which aspects of the spectrum might be important [49]. In PCA of spectral data, PC1 represents variation in brightness and subsequent PCs represent combinations of hue and chroma [6, 48, 49]. Principal component analysis was performed separately on all spectra from the dorsum and the venter. Then, we used mixed effects GLMs to examine the variation in PC scores among populations (random factor) and among body positions within the same individual (within effect). Pairwise comparisons were based on Tukey's honestly significant difference (HSD) tests [50].

In addition, we measured body "weight" (with a digital balance to the nearest 0.01 g) and the snout-to-vent length ("SVL"; with a ruler to the nearest 1 mm) of each lizard. We also noted the number of small but distinctive and conspicuous blue spots that run along each of the body sides on the outer margin of the belly and calculated the total number of spots in the two sides for each lizard. These spots seem to have a role in sex recognition and intrasexual social relationships between males in this lizard [43] and in other lacertid lizard species [51]. Finally, we counted the number of femoral pores on the right and left hind limbs under a magnifying glass and calculated the total number of pores in the two limbs for each lizard.

We used general lineal models (GLMs) to test for differences in $\log_{10}$ transformed body weight and SVL among populations (random factor). Pairwise comparisons were based on Tukey's HSD tests [50]. Because number of blue spots and femoral pores were count data, we used generalized linear models (GLZ) with a Poisson distribution and a log-link function to test for population differences without prior transformation of the data [52]. Pairwise comparisons were made with nonparametric post hoc multiple comparisons by simultaneous test procedures [50].

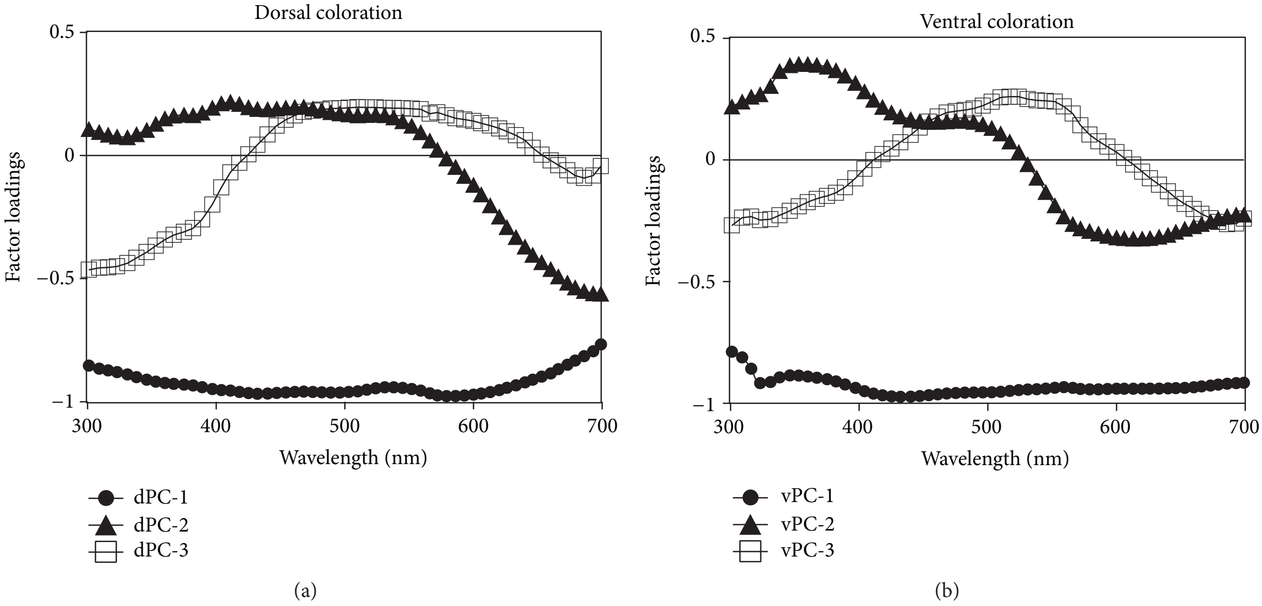
2.3. Habitat and Climatic Characteristics. To describe characteristics of habitat used by lizards in each of the three populations, we walked haphazardly in each habitat during May-June 2007. These walks were performed on days with favorable climate conditions (warm sunny days) and between 09:00 and 13:00 GMT, when lizards were fully active. When we detected an adult male lizard, we marked the point where it was first observed. For each point we took four 4 m transects, one at each cardinal orientation radiating from the point where each individual was sighted. We noted the substrate type in the central point and at 25, 50, and 100 cm in each of the four cardinal directions from the original lizard point. Substrates were classified as bare soil, leaf litter, herbaceous vegetation or grass, medium semiperennial plants or bushes, trees, or rocks and classified according to their size as small (1–5 cm), medium (5–20 cm), large (20–200 cm), or very large (>2 m). We also noted the presence of canopy tree cover above each sample point and the height from the ground to the first contact with the stick of leaves of subarboreal vegetation at each point. For each lizard observation, we calculated percent cover values for each habitat variable (i.e., percentage of contacts with each substratum and vegetation type).

Given the large size of the areas surveyed (>5 km^2 each) and the high lizard density and because we avoided sampling the same location twice, the probability of repeated sampling of the same individual was very low. We therefore treated all measurements as independent. We used principal component analysis (PCA) to reduce all the habitat variables to a smaller number of independent components (PCs). Original data were normalized by means of square root transformation. The initial factorial solutions were rotated by the Varimax procedure to find a rotation that maximizes the variance on the new axes [53]. Thus, we aimed to obtain a clear pattern of loadings on each factor, that is, factors that were somehow clearly marked by high loadings for some variables and low loadings for others (i.e., that approximated simple structure), lending itself to easier interpretation. We used GLMs to analyze whether the habitat characteristics defined by the PC scores differed among populations (random factor). Pairwise comparisons were based on Tukey's HSD tests [50].

To describe climatic characteristics of the three populations, we used statistical data obtained from long-term records of meteorological stations located close to each lizard population, at similar altitude and in the same type of habitat. These stations have recorded, among others, temperatures and precipitation every day during at least a 25-year period (data available in the web of the Spanish Meteorological Agency, "Agencia Española de Metereología (AEMET)"; http://www.aemet.es). We used data from the meteorological stations of "Puerto de Navacerrada" (40°46′N, 04°00′W, 1894 m altitude), "Colmenar Viejo" (40°41′N, 03°45′W, 1004 m altitude), and "Getafe" (40°18′N, 03°43′W; 617 m altitude), to characterize climate of the Fuenfría, Golondrina, and Aranjuez lizard populations, respectively. We used only data from March to September, coinciding with the main activity season of lizards, and from 1985 to 2010. We used GLMs to compare average monthly temperatures or total monthly precipitation among populations (random factor).

3. Results

3.1. Dorsal Coloration of Lizards. The PCA on reflectance data of all spectra of dorsal coloration produced three dorsal



(a) (b)

Figure 1: Factor loadings of the first three principal components from two PCAs on all the reflectance spectra that characterize (a) dorsal or (b) ventrolateral coloration of male lizards *P. hispanica*.

principal components (dPCs) that together accounted for 98.48% of the variation in the original spectra. The first PC (dPC1) accounted for 86.60% of variation (eigenvalue = 50.23). The coefficients relating dPC1 to the original reflectance data were all negative and of similar magnitude (Figure 1(a)), and thus dPC1 represented achromatic brightness variation in the original spectra. The second PC (dPC2) accounted for a further 6.47% of the variation (eigenvalue = 3.75) in the original spectra. The values of dPC2 below 580 nm were all positive, while above 580 nm they were negative (Figure 1(a)). This variation suggested that it represented variation in the relative amounts of short-medium (300–580 nm) wavelengths in the positive side to long (580–700 nm) wavelengths in the negative side. The third PC (dPC3) accounted for 5.41% of the variation (eigenvalue = 3.14), and the coefficients relating dPC3 to the original reflectance values between 420 nm and 660 nm were all positive, while below 420 nm and above 660 nm they were all negative (Figure 1(a)). Thus, dPC3 could represent variation in the relative amount of medium (420–660 nm) to both short (<420 nm; that is, UV coloration) and very long (>660 nm) wavelength reflectance of dorsal coloration.

There were significant differences in the characteristics of dorsal coloration defined by the dPC1 (i.e., brightness) among populations (GLM; random factor, $F_{2,43} = 17.58$, $P < 0.0001$) (Figures 2 and 3(a)). Lizards from Fuenfría had significantly darker (dPC1) dorsal colorations than lizards from Golondrina (Tukey's test: $P = 0.0027$) and from Aranjuez ($P = 0.00012$), and lizards from Golondrina had darker dorsal colorations than lizards from Aranjuez ($P = 0.015$) (Figures 2 and 3(a)).

With respect to the characteristics of dorsal coloration defined by the dPC2 (i.e., relative amounts of short-medium to long wavelengths), there were significant differences among populations (GLM; random factor, $F_{2,43} = 3.31$, $P = 0.046$) (Figures 2 and 3(b)). Lizards from Golondrina had significantly higher dPC2 values (i.e., lower amounts of long wavelengths) than lizards from Fuenfría (Tukey's test: $P < 0.05$) and from Aranjuez ($P = 0.006$), but lizards from Fuenfría and Aranjuez did not differ ($P = 0.95$) (Figures 2 and 3(b)).

Finally, there were no significant differences in the characteristics of dorsal coloration defined by the dPC3 (i.e., relative amounts of medium to both short and long wavelengths) among populations (GLM; random factor, $F_{2,43} = 1.68$, $P = 0.20$) (Figures 2 and 3(c)).

3.2. Ventrolateral Coloration of Lizards.

The PCA on reflectance data of all spectra of ventrolateral coloration produced three principal components (vPCs) that together accounted for 97.16% of the variation in the original spectra. The first PC (vPC1) accounted for 87.06% of variation (eigenvalue = 50.49). The coefficients relating vPC1 to the original reflectance data were all negative and of similar magnitude (Figure 1(b)), so vPC1 represented achromatic brightness variation in the original spectra. The second PC (vPC2) accounted for a further 6.44% of the variation (eigenvalue = 3.73) in the original spectra. The coefficients relating vPC2 to the original reflectance values below 535 nm were all positive, while above 535 nm they were negative (Figure 1(b)). Thus, vPC2 represented variation in the relative amount of short- to long-wavelength reflectance. The third PC (vPC3) accounted for 3.67% of the variation (eigenvalue = 2.13), and the pattern of coefficients suggested that it represented variation in the relative amounts of medium (415–615 nm) wavelengths in the negative side to both short (300–415 nm; i.e., UV wavelengths) and long (615–700 nm) wavelengths in the positive side (Figure 1(b)).

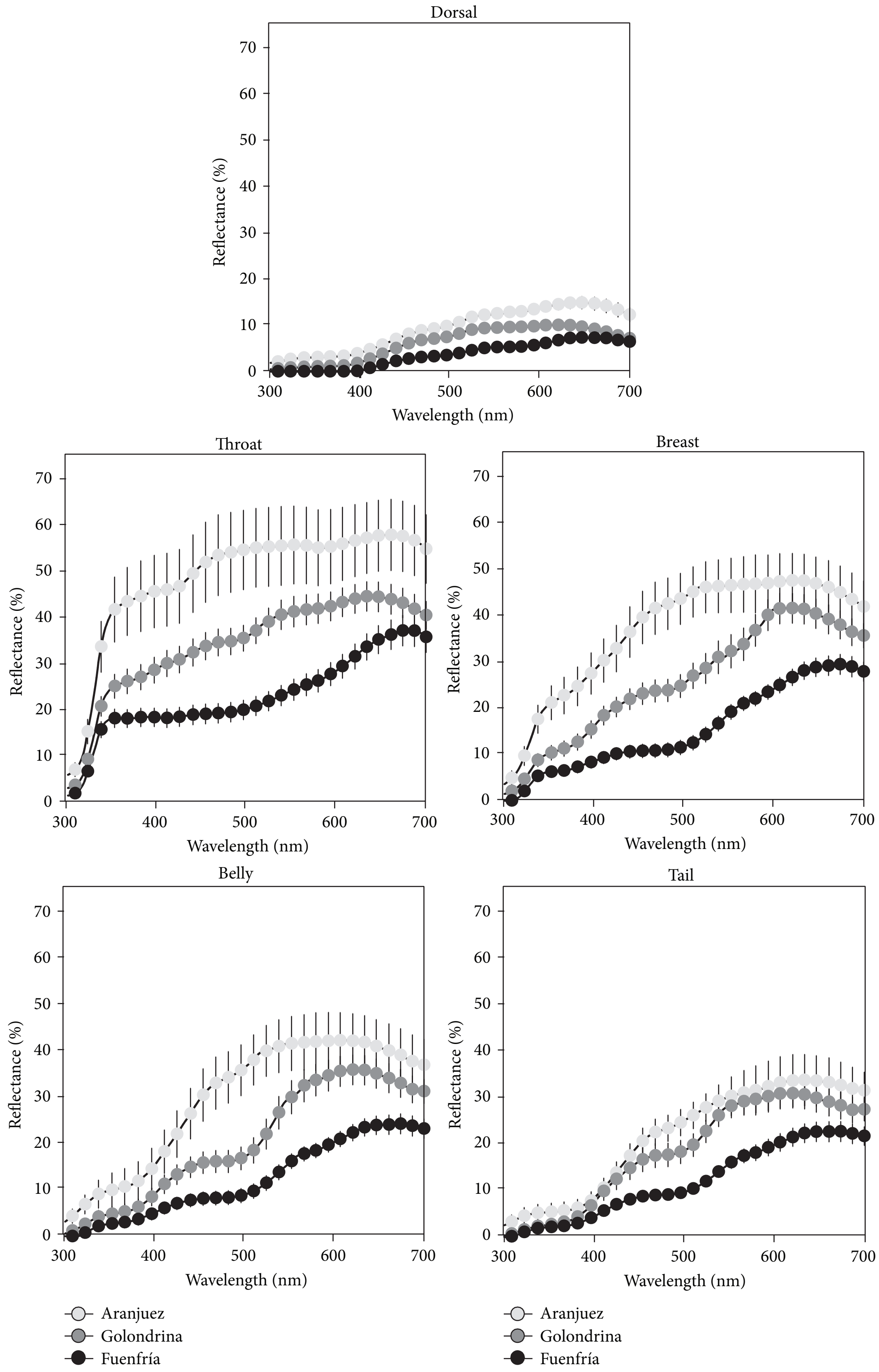


Figure 2: Mean (± SE shown at 50 nm intervals) reflectance spectra of dorsal coloration and ventrolateral coloration at four positions (throat, breast, belly, and tail) of male lizards *P. hispanica* from three populations (Fuenfría: (black); Golondrina: (dark grey); Aranjuez: (light grey)).

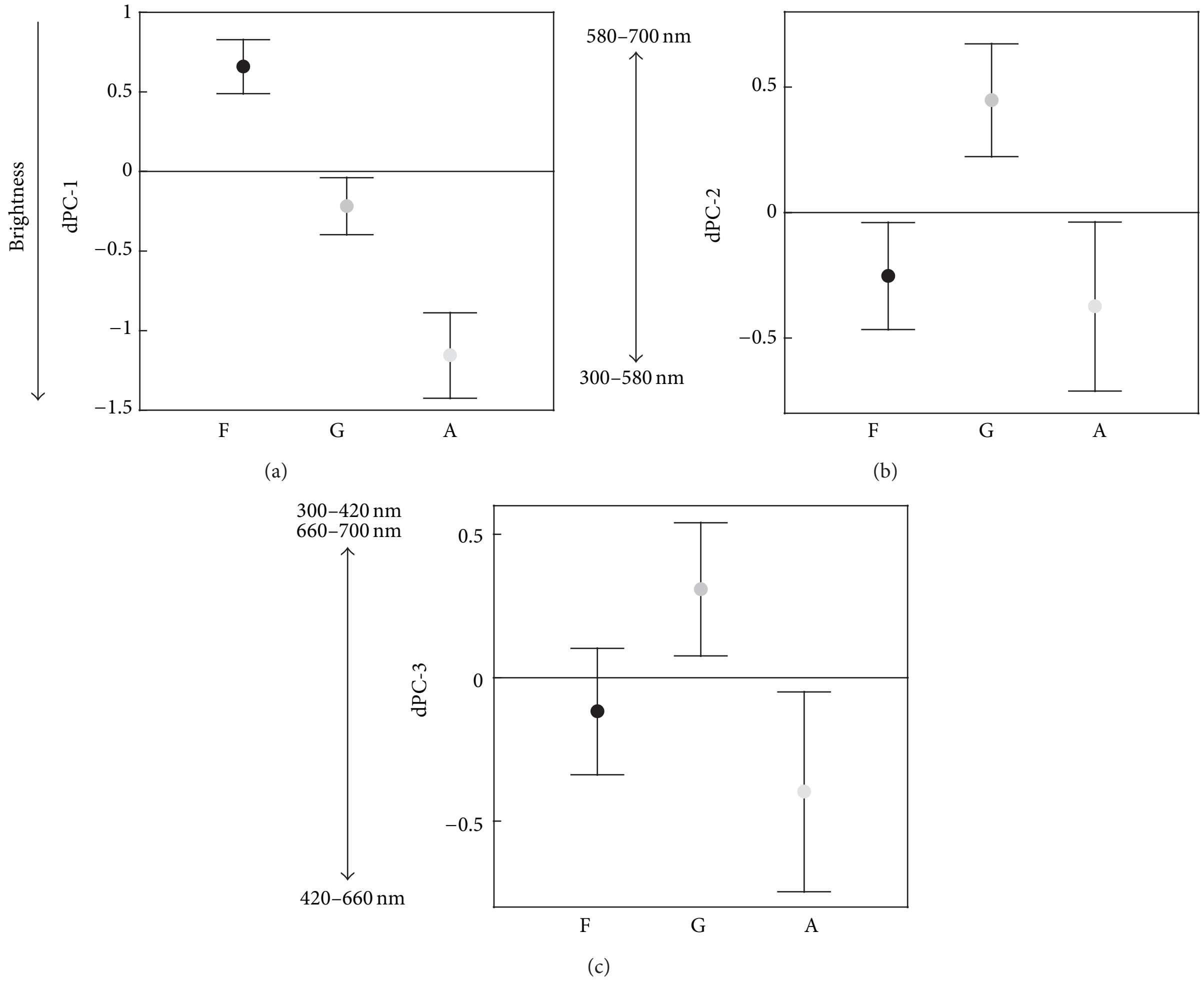
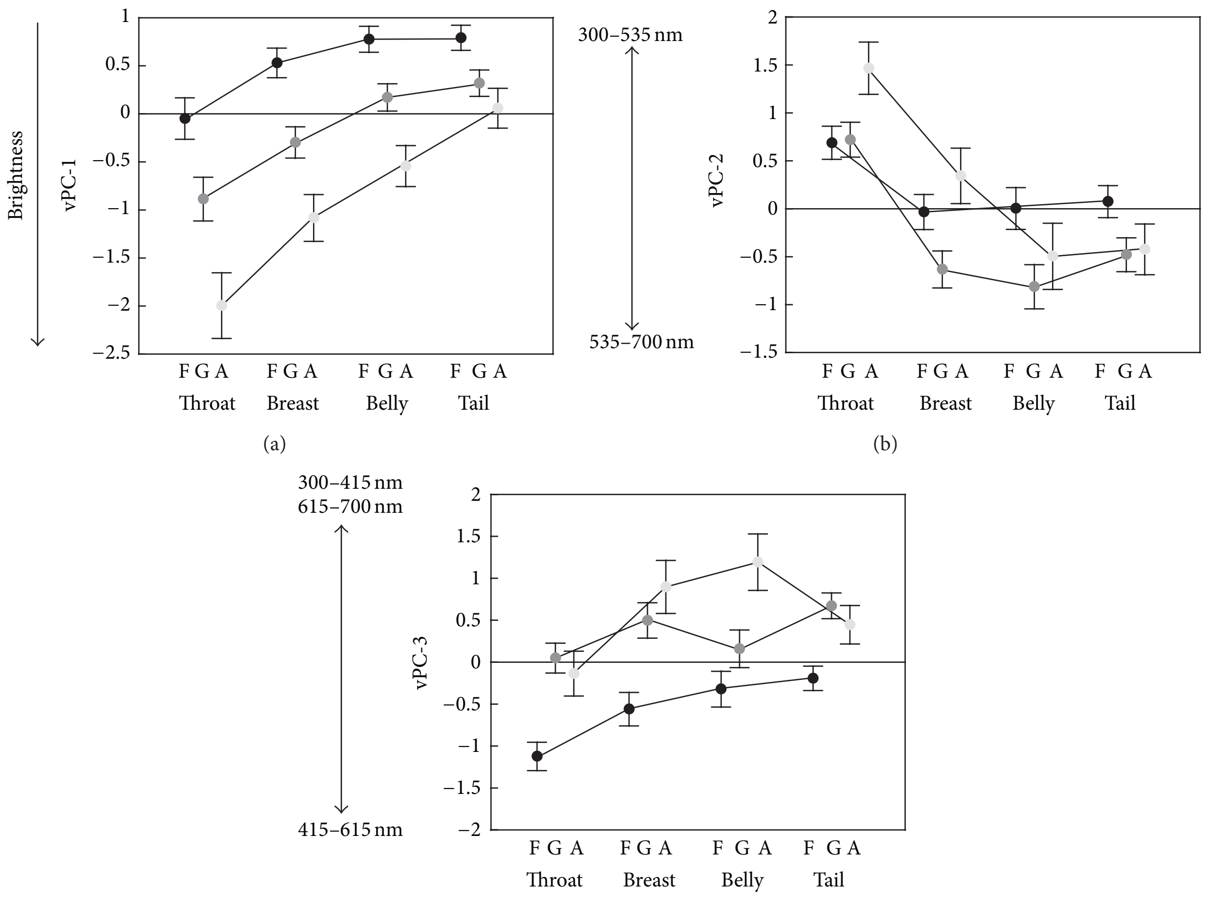


FIGURE 3: Mean (±SE) PC scores from a PCA on all the reflectance spectra that characterize dorsal coloration of male lizards *P. hispanica* from three populations (Fuenfría, F: (black); Golondrina, G: (dark grey); Aranjuez, A: (light grey)).

Characteristics of ventrolateral coloration defined by vPC1 (i.e., brightness) differed significantly among populations (mixed effects GLM; random factor, $F_{2,172} = 31.26$, $P = 0.0007$) and among body positions ($F_{3,172} = 18.50$, $P = 0.001$) and the interaction was not significant ($F_{6,172} = 1.47$, $P = 0.19$) (Figures 2 and 4(a)). Lizards from Fuenfría had significantly darker (vPC1) ventrolateral colorations than lizards from Aranjuez (Tukey's test: $P < 0.0001$) and from Golondrina ($P < 0.0001$), and lizards from Golondrina had darker ventrolateral colorations than lizards from Aranjuez ($P < 0.0001$) (Figures 2 and 4(a)). Coloration was significantly brighter at the throat than at the other positions ($P < 0.0001$ in all cases) and brighter at the breast than at the belly ($P < 0.05$) and at the tail ($P = 0.0015$) and did not significantly differ between the belly and the tail ($P = 0.68$) (Figures 2 and 4(a)).

Characteristics of ventrolateral coloration defined by vPC2 (i.e., relative amount of short- to long-wavelengths) did not differ significantly among populations (mixed effects GLM; random factor, $F_{2,172} = 3.59$, $P = 0.09$), but they differ significantly among body positions ($F_{3,172} = 10.89$, $P = 0.006$) and the interaction was significant ($F_{2,172} = 2.16$, $P < 0.05$) (Figures 2 and 4(b)). Values of vPC2 scores were positive and significantly higher at the throat than at other positions (Tukey's tests: $P < 0.0001$ in all cases), while vPC2 values were negative and similar at the breast, belly, and tail ($P > 0.50$ in all cases). Thus, ventrolateral coloration of the anterior body part (throat and breast) was characterized by greater relative amounts of short wavelengths (<535 nm), whereas the posterior body part (belly and tail) had higher relative amounts of long wavelengths (>535 nm).

Finally, the characteristics of ventrolateral coloration defined by vPC3 (i.e., relative amounts of medium to both short and long wavelengths) differed significantly among populations (mixed effects GLM; random factor, $F_{2,172} = 18.31$, $P = 0.003$) but not among body positions ($F_{3,172} = 1.97$, $P = 0.12$) and the interaction was not significant ($F_{6,172} = 1.79$, $P = 0.10$) (Figures 2 and 4(c)). Lizards from Fuenfría had vPC3 scores significantly lower than lizards from Golondrina and Aranjuez (Tukey's test: $P < 0.0001$ for both), which did not significantly differ ($P = 0.31$) (Figures 2 and 4(c)). Thus, the relative amount of medium



(a)

(b)

(c)

Figure 4: Mean (±SE) PC scores from a PCA on all the reflectance spectra that characterize ventrolateral coloration at four positions (throat, breast, belly, and tail) of male lizards *P. hispanica* from three populations (Fuenfría, F: (black); Golondrina, G: (dark grey); Aranjuez, A: (light grey)).

wavelengths (415–615 nm) was higher in ventrolateral colorations of lizards from Fuenfría.

3.3. Body Size and Numbers of Blue Spots and Femoral Pores. There were significant differences among populations in body weight (GLM, random factor, $F_{2,44} = 16.48$, $P < 0.0001$) and SVL ($F_{2,44} = 19.29$, $P < 0.0001$) (Table 1). Lizards from Fuenfría were significantly heavier and larger than lizards from Golondrina and Aranjuez (Tukey's tests; $P < 0.001$ in both cases), and lizards from Golondrina were significantly heavier and larger than lizards from Aranjuez ($P < 0.03$ in both cases).

The total number of blue spots differed significantly among populations (GLZ; Wald statistic = 34.64, $\chi^2_2 = 37.26$, $P < 0.0001$) (Table 1). Males from the Fuenfría population had significantly fewer number of blue spots than males from Golondrina (nonparametric post hoc tests; $P = 0.047$) and Aranjuez ($P = 0.024$). However, males from Aranjuez and Golondrina did not significantly differ ($P = 0.31$). The number of blue spots was not significantly related to body size within any population (Spearman rank order correlation; $r_s < -0.15$, $P > 0.40$ in all cases).

The number of femoral pores of males was significantly different among populations (GLZ; Wald statistic = 8.52, $\chi^2_2 = 8.23$, $P = 0.016$) (Table 1). Lizards from Aranjuez had significantly fewer femoral pores than lizards from Golondrina (nonparametric post hoc tests; $P = 0.049$) or Fuenfría ($P = 0.0041$), but there were no significant differences between lizards from Golondrina and Fuenfría ($P = 0.26$). The number of femoral pores was not significantly related to body size within any population (Spearman rank order correlation; $r_s < 0.14$, $P > 0.60$ in all cases).

3.4. Habitat and Climatic Characteristics. The PCA for microhabitats used by lizards produced five principal components (PCs) with eigenvalues greater than 1 that together accounted for 71.67% of the variance. The first PC (PC-1) (eigenvalue = 2.68) had positive values representing increasing tree cover. The second PC (PC-2) (eigenvalue = 2.37) had positive values representing cover of medium semiperennial plants and bushes. The third PC (PC-3) (eigenvalue = 2.26) had positive values that characterized grass or herbaceous vegetation cover. The fourth PC (PC-4) (eigenvalue = 1.50) characterized the rock types, with negative values corresponding to very large rocks and positive values representing large rocks. Finally, the fifth PC (PC-5) (eigenvalue = 1.13) had positive values corresponding to small and medium rocks and negative values representing sand substrates.

There were significant differences among populations in the PC-1 (GLM; random factor, $F_{2,73} = 3.45$, $P = 0.037$), PC-3 ($F_{2,73} = 4.14$, $P = 0.02$), and PC-5 ($F_{2,73} = 8.37$, $P = 0.0005$). Thus, populations differed in the cover of trees, grasses, small and medium rocks, and sand. With respect to the PC-1, post hoc test showed that Fuenfría had significantly more cover of trees than Golondrina and Aranjuez populations (Tukey's test: $P < 0.05$ for both), which did not significantly differ ($P = 0.85$). Differences in the PC-3 showed that Fuenfría had significantly more grass and herbaceous plants than Golondrina ($P = 0.016$), but the rest of comparisons among populations were not significant ($P > 0.22$ in all cases). Finally, with respect to cover of small rocks (PC-5), Fuenfría had a significantly larger cover of small and medium rocks and a smaller cover of sand than Aranjuez ($P = 0.0006$) and Golondrina ($P = 0.03$), which did not significantly differ ($P = 0.32$). For the rest of PCs, there were no significant differences among populations in the cover of semiperennial plants and bushes (PC2: $F_{2,73} = 1.81$, $P = 0.17$) or of large rocks (PC4: $F_{2,73} = 0.24$, $P = 0.78$).

With respect to climatic characteristics from March to September (i.e., during the main activity season of lizards), there was a gradient of increasing average monthly temperatures from Fuenfría (mean ± SE = 10.5 ± 0.4°C, range = 1.2–19.5°C), through Golondrina (17.3 ± 0.4°C, range = 6.2–26.0°C), and to Aranjuez (19.4 ± 0.4°C, range = 8.5–27.9°C) (GLM; random factor, $F_{2,543} = 116.90$, $P < 0.0001$) (Tukey's tests: $P = 0.001$ in all cases). Conversely, there was a gradient of decreasing average monthly total precipitation from Fuenfría (64.8 ± 4.7 mm, range = 0–374.6 mm), through Golondrina (32.3 ± 2.4 mm, range = 0–162.7 mm), and to Aranjuez (mean ± SE = 23.3 ± 1.6 mm, range = 0–101.0 mm) (GLM; random factor, $F_{2,542} = 46.52$, $P < 0.0001$) (Tukey's tests: $P < 0.05$ in all cases).

4. Discussion

Our results show that the three populations of *P. hispanica* lizards studied here differ in several aspects of coloration and also occupy different habitats with different climatic conditions. The habitat in Fuenfría is characterized by a higher cover of trees, grassy vegetation, and rocks compared to Golondrina or Aranjuez. Climatic conditions are also different, with a clear gradient of temperature and precipitation from the southern plains of Aranjuez (hot temperatures and dry conditions) to the northern mountains of Fuenfría (low temperature and high humidity). This raises the possibility that observed differences in coloration have been driven by differences in climate and habitat characteristics.

There are important differences among populations with respect to dorsal coloration; southern lizards (from Aranjuez) have brighter dorsal colorations than northern lizards (Golondrina and Fuenfría). Geographic variation in reptile coloration is frequently a consequence of changes in melanin, the pigment responsible for darkness of the body [1]. Thermoregulation requirements may affect coloration because darker lizards are able to warm faster and maintain higher body temperatures for longer [18–20, 54]. Also, lizards from the northern populations (Fuenfría and Golondrina) are larger and heavier than lizards from the southern population (Aranjuez), which may be an adaptation to increase effectiveness of thermoregulation (i.e., low cooling rates [54, 55]). Similarly, many vertebrate species show variations in body size where individuals from colder environments are larger than individuals from warmer environments as an adaptation to different climatic conditions (e.g., [56–58]; but see [59, 60]). Therefore, differences in thermoregulatory requirements under different climatic conditions may contribute to explain differences among populations in dorsal coloration and body size of *P. hispanica* lizards.

A lizard's coloration will also affect detectability by visually guided predators, and there could be selection for substrate color matching [21–23]. This might explain that lizards of Fuenfría and Golondrina, which live in areas with darker granite rocks, show a dorsal coloration of dark brown to black. In contrast, lizards from Aranjuez populations from areas with gypsum and light sandy soil with scarce vegetation or rocks have a dorsal coloration of brighter yellow to green. These differences in coloration may allow lizards to be more cryptic to predators in their respective habitats. Similarly, in three New Mexican lizard species: *Holbrookia maculata*, *Sceloporus undulatus*, and *Aspidoscelis inornata,* each species shows dark morphs, cryptic on the brown adobe soils of the Chihuahuan Desert, and white morphs, cryptic on the gypsum substrate of White Sands [23, 24, 61].

Interpopulational differences in ventrolateral coloration were also observed. In this lizard species, this coloration could be considered as a secondary sexual signal because it is sexually dichromatic (females and juveniles have white bellies compared to what males show) and because it is dependent on hormones, being more developed in males during the mating season [43, 62]. This also occurs in many other lizards [1]. Ventrolateral coloration extends through the belly but can be visually perceived by other individuals in vertical rock surfaces where these lizards live. Intraspecific sex identification at a long distance is based on the presence/absence of this coloration [42, 43]. Lizards from Aranjuez have a brighter yellow ventrolateral coloration than individuals from Golondrina (i.e., orange) and Fuenfría (i.e., darker red). As in other lizards, yellow, orange, and red colorations are probably produced by carotenoid and pteridine pigments [1, 63] together with integumentary components that provide pigments with a reflective background (i.e., iridophores or melanophores [64, 65]). Differences in coloration might be explained by differences in availability of different pigments in the diet [65, 66], adaptation to different light conditions tending to maximize the efficiency of the visual signal [11, 26, 67], or differences in relative importance of visual signals in sexual selection [27, 28, 62].

In addition, the studied populations of Iberian wall lizard differ in the number of blue spots and femoral pores, with males from the northern populations having fewer blue spots and more femoral pores than males from the southern population. Because blue spots and secretions from femoral glands connected to the pores are used in visual and chemical intraspecific communication, respectively [43–46], these results could suggest that these populations may differ in the relative importance of chemical and visual sensory modes. A larger number of blue spots may represent a greater use of visual signals [51], whereas a higher number of femoral pores may be related to a higher production of chemical secretions [68]. The differential relative importance of visual and chemical signals might be explained by the effectiveness of these two types of communication in habitats with different microclimatic conditions [25, 27–29]. This suggests that habitat and climatic differences among populations of *P. hispanica* lizards might have affected the evolution of their sexual signals. For example, in Fuenfría population, the higher cover of rocks and trees, which provides shadow, and the low temperatures may favor a higher use of scent marks on rock substrates where chemical compounds may persist for longer. In contrast, in the Aranjuez population, a higher cover of open sandy microhabitats and exposure to sun radiation and hot temperatures may increase the evaporative rate of chemicals left on the substrate rendering scent marks less effective [25, 29]. In fact, these environmental conditions might rather favor the use of long distance visual signals in this population because visibility of individuals may be higher in open and more illuminated habitats [26, 67]. Differences among populations of *P. hispanica* in chemical compounds in femoral secretions and the magnitude of chemosensory responses to these compounds also suggest that the importance of chemical communication is lower in populations from hot-dry areas [69]. Lizards from Golondrina would be in an intermediate position, where relatively low temperatures may favor chemical signals (and hence the similar number of femoral pores compared to lizards from Fuenfía), but a more sunny open habitat may also favor visual signals (and hence the similar number of blue spots compared to lizards from Aranjuez).

In summary, our study shows that in the region of Madrid there is a significant variation in coloration and other traits among three populations of *P. hispanica* lizards. These variations among populations could be explained by adaptation to the different climate and habitat conditions in each population. Nevertheless, future studies that analyze more populations and take into account potential phylogenetic differences between them are needed to test this hypothesis. Natural selection for coloration that allows a better thermoregulation and avoidance of predators, coupled with selection for a differential use of sensory modes in intraspecific communication, is expected to lead to the observed interpopulational differences. Moreover, differences in sexual signals and morphological traits might lead to population divergence and further reproductive isolation, which may help to explain the previously published genetic differences among these populations.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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