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Climate and Socio-Sexual Environment Predict Interpopulation Variation in Chemical Signaling Glands in a Widespread Lizard

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ABSTRACT

Many animal species show considerable intraspecific phenotypic variation. For species with broad distributions, this variation may result from heterogeneity in the strength and agents of selection across environments and could contribute to reproductive isolation among populations. Here, we examined interpopulation variation in a morphological trait related to chemical communication, femoral pore number (FP), using 3437 individuals from 55 Pyrenean populations of the common wall lizard (*Podarcis muralis*). Specifically, we tested the relative roles of genetic relatedness and gene flow, and adaptation to local conditions in generating this variation, with particular interest in the influence of climate and the socio-sexual environment (i.e., the intensity of sexual selection, estimated using sexual size dimorphism [SSD] and adult sex ratio as proxy measures). We found significant interpopulation variation and sexual dimorphism in FP, as well as high genomic differentiation among populations driven by both geographic and environmental distances. Specifically, FP differences across populations were best predicted by a combination of positive allometry and the local intensity of sexual selection, as determined by SSD, or local climatic conditions. Higher FP in more male-competitive environments, or with higher temperature and vegetation complexity, is consistent with adaptation to maintaining signaling efficacy of territorial scent marks. These results suggest that adaptation to local conditions contributes to interpopulation divergence in FP and thus environmental changes can potentially impact the fine-tuning of chemical communication mediating social and sexual behavior.

1 | Introduction

Variation in phenotypic traits across populations of the same species generally results from a combination of shared common ancestry, population-specific effects, including local selection and genetic drift, and gene flow (Stone, Nee, and Felsenstein 2011). When interpopulation variation concerns signaling traits or systems mediating social and sexual behavior, it can have significant evolutionary consequences (Schaefer and Ruxton 2015; Servedio and Boughman 2017). In particular, population divergence in recognition signals, or sexually selected signals involved in male competition or female choice, can contribute to reproductive isolation and speciation (Ryan and Rand 1993; Smadja and Butlin 2009; Tibbetts, Mullen, and Dale 2017). Such is the case of variation in the composition of the female sex pheromone between denning populations of red-sided garter snakes (LeMaster and Mason 2003) and in the acoustic properties of male advertisement calls between neighboring populations of an Amazonian frog (Boul et al. 2007).

Disentangling among sources of phenotypic variation is especially challenging among populations of the same species due to their shared evolutionary history, life history, and ongoing gene flow (Zamudio, Bell, and Mason 2016). Populations that are more closely related or exchange more migrants are expected to have more similar trait values, and trait variation is often associated with geographical or environmental gradients (Mayr 1956). A common pattern in nature is isolation by distance (IBD; Wright 1943), whereby gene flow between populations decreases as the geographic distance between them increases. Here, phenotypic divergence is expected from gradual genetic drift under geographically restricted gene dispersal (e.g., due to physical barriers). Alternatively, but non-mutually exclusive, isolation by environment (IBE; Wang and Summers 2010) describes a pattern in which gene flow is reduced among habitats with dissimilar ecological characteristics. Although IBE makes no assumptions about the underlying processes that generate divergence, it is often interpreted as selection against migrants, or adaptation to divergent local conditions, which encompass both abiotic (e.g., climate) and biotic (e.g., vegetation) factors (Wang and Bradburd 2014; Campbell-Staton, Edwards, and Losos 2016; Wishingrad and Thomson 2023).

Climate can exert strong selection on signaling traits, either directly or through its influence on habitat characteristics (e.g., vegetation structure, trophic resources; Candolin and Wong 2012). The abiotic conditions of the habitat impose constraints on signal design that ultimately favor environmentally tuned signals, which have increased transmission efficacy through the local environment (Guilford and Dawkins 1991; Endler 1992; Endler 1993), and thus spatially heterogeneous environments can contribute to genetic and phenotypic differentiation among populations (Boughman 2002; Wang and Bradburd 2014; Zamudio, Bell, and Mason 2016). The same habitat conditions that drive signal divergence may also lead to divergence in morphological, physiological, or behavioral traits that indirectly impact signal development and expression, for example, through changes in body size or condition, such as the more complex calls of frog populations in which males are larger (Boul and Ryan 2004), or the more disrupted facial patterns (a quality signal) of wasps from colonies fed *ad libitum* and thus in better condition (Tibbetts 2010).

Sexual selection is an important driver of diversification in animal signaling systems involved in male competition and mate attraction (West-Eberhard 1983; Endler and Basolo 1998; Schaefer and Ruxton 2015). The strength of sexual selection often varies across populations (Siepielski et al. 2013) and thus can also contribute to the divergence of sexually selected traits among populations. Sexual selection can be difficult to quantify, and it is often estimated using proxy measures based on (i) traits assumed to have evolved as a result of sexual selection, such as indices of sexual size dimorphism (SSD, e.g., body size dimorphism; Abouheif and Fairbairn 1997; Cox, Butler, and John-Alder 2007; Janicke and Fromonteil 2021), or (ii) mating or reproductive success variation, which depend on the number of competing individuals and the availability of potential mates, reflected by demographic properties such as the proportion of males in the adult population (adult sex ratio [ASR]; Emlen and Oring 1977; Schacht et al. 2017; Kappeler et al. 2023). SSD and ASR can both vary substantially within species and populations over space and time, even reverting from female- to male-biased and vice versa (Stamps 1983; Székely, Weissing, and Komdeur 2014; Tarr et al. 2019). SSD is expected to increase with body size when strong sexual selection on male size (via competition for mating opportunities) causes this allometric pattern (Rensch 1950; Stamps 1983; "Rensch's rule"; Abouheif and Fairbairn 1997; Meiri and Liang 2021). Changes in ASR can affect breeding behavior, including mate choice, mating system, and parental care, and thus the intensity of competition and sexual selection (Trivers 1972; Clutton-Brock and Parker 1992; Kokko and Jennions 2008).

In most lacertids, chemical signals are primarily produced by epidermal holocrine glands located on the ventral surface of the inner thighs (García-Roa et al. 2017), which release their waxy secretion through femoral pores that open in the center of modified scales. These gland secretions are the main source of semiochemicals in male lizards (Fleishman and Font 2019) and are involved in territory marking, rival assessment, and/or individual recognition (Carazo, Font, and Desfilis 2007, 2008; Font et al. 2012). Secretions are smeared onto substrates or passively deposited as lizards move through their home ranges, and they are perceived by receivers via olfaction and/or vomerolfaction and chemosensory behavior such as tongue-flicking (Font et al. 2012). In general, the number of femoral pores affects the amount of secretion that can be released and has thus been associated with the degree of investment in chemical signaling (Alberts 1992; Escobar, Labra, and Niemeyer 2001; García-Roa et al. 2017). Both secretion abundance and chemical composition, which determines volatility and vulnerability to degradation, affect chemical signal efficacy (Alberts 1992; Martín and López 2013; Apps, Weldon, and Kramer 2015; Romero-Diaz et al. 2021), and thus, differences in femoral pore number among populations could result from adaptation to local habitat conditions and/or the intensity of sexual selection.

Here, we use an integrative and comparative approach to study the processes that generate variation in femoral pore number (FP) in the European wall lizard (*Podarcis muralis*). Specifically, we test the relative contribution of local habitat conditions (climate) and the social environment reflecting the intensity of sexual selection in shaping interpopulation variation in FP, accounting for the effects of shared common ancestry and gene flow. Previous macroevolutionary phylogenetic studies indicate that shared ancestry is a strong predictor of the number of epidermal glands in most lizard groups (García-Roa et al. 2017; Jara et al. 2018), but it is unclear to what extent this applies to inter-population variation. Moreover, the evidence supporting the role of ecological and/or climatic factors has been so far inconsistent (Pincheira-Donoso, Hodgson, and Tregenza 2008; Baeckens et al. 2015; Jara et al. 2018; Fleishman and Font 2019), perhaps because these factors likely operate at local scales and significant patterns may be obscured in macroecological studies based on a single or few populations per species, which do not capture intraspecific variation at microecological scales. Similarly, despite the generally accepted role of epidermal glands in lizard sexual communication, the intensity of sexual selection has rarely been found to explain interspecific variation in FP (but see Font and Pérez i de Lanuza 2017). If FP is influenced by local habitat conditions and/or the socio-sexual environment, we predict an effect of climate and/or any of our proxies for the intensity of sexual selection on inter-population variation in FP, particularly on males.

2 | Materials and Methods

2.1 | Ethical Statement

All protocols and procedures were performed in accordance with Spanish (Real Decreto 53/2013) and European (Directive 2010/63/EU) legislation and complied with the ASAB/ABS Guidelines for the Use of Animals in Research.

2.2 | Study Species

P. muralis is a small lacertid (adult snout-to-vent length [SVL] up to 79 mm), widespread across most of Western and Central Europe. In South-Western Europe, it occupies habitats from 0–2400 m.a.s.l. in the Eurosiberian Region of the Pyrenees, Pre-Pyrenees, the Cantabrian Range, and in the Iberian and Central Systems (Diego-Rasilla 2015; Pérez i de Lanuza, Carretero, and Font 2017). It is largely a rock-dwelling species of broad climatic niche, occurring across a variety of habitats, including anthropogenic constructions in urbanized areas (e.g., stone walls), sunny open habitats with scattered rocky outcrops, and edges or clearings of deciduous forests (Diego-Rasilla 2015). It is iteroparous, multivoltine, producing 1–3 clutches per breeding season during spring and summer (Diego-Rasilla 2015). Individuals become sexually mature (i.e., adults) at 2–3 years of age, when body size is at least 54 mm (Barbault and Mou 1988). Sexual dimorphism is often present, with males having a relatively larger head and limb-to-body size ratio (Diego-Rasilla 2015). Males are territorial, setting out exclusive areas that overlap the home ranges of several females. It has been argued that intrasexual selection is an important force acting on signaling traits used between male competitors, such as the ultraviolet-reflecting patches located on some of the outer ventral scales (Pérez i de Lanuza, Font, and Monterde 2013; Pérez i de Lanuza, Carazo, and Font 2014; Pérez i de Lanuza, Carretero, and Font 2017) and scent marks (Edsman 1990; Carazo, Font, and Desfilis 2007; Font et al. 2012; MacGregor et al. 2017; but see MacGregor et al. 2017). Individuals possess between 12 and 24 ontogenetically invariant femoral pores in each hindlimb (Diego-Rasilla 2015). Epidermal glands exhibit peak secretory activity during the reproductive season and are the main source of male chemosignals (Font et al. 2012; Baeckens et al. 2017). Males typically have more and larger femoral pores and produce more secretion than females, suggesting that this trait is influenced by sexual selection and may have a role in male intrasexual agonistic interactions (Edsman 1990). There is substantial variation in ASR and SSD among populations (Pérez i de Lanuza, Carretero, and Font 2017; Eroğlu et al. 2018), which could affect the level of intra-sexual competition (territorial behavior) and thus the strength of local sexual selection.

There is strong support for six major genomic lineages within *P. muralis* (Yang et al. 2021), and despite relatively low nuclear genetic diversity, the species shows high mitochondrial variability, with at least 23 well-supported mitochondrial clades representing evolutionary independent lineages with clear and coherent geographic structures (Salvi et al. 2013). All the populations sampled in this study belong to the so-called western France mitochondrial clade, with the exception of Penyagolosa, located in the Iberian System (population #2, Figure 1).

2.3 | Population Sampling and Morphological Measurements

Between 2018 and 2020, we sampled 55 sites within the natural range of *P. muralis* in the Central and Eastern Pyrenees (Figure 1; mean and range distance between sites: 75.71 [1.78–330.26] km). We collected an average of 34 adult (i.e., SVL $\geq$ 55 mm) males (range: 17, 98) and 29 adult females (range: 9, 77) per population, between April and September, spanning the reproductive season and period of highest activity of the species. We visited each site for 1–3 days for at least 5 h at a time between 9 a.m. and 5 p.m. to avoid capture biases related to differences in activity patterns between males and females. Upon capture, we measured lizard SVL ($\pm$1 mm) with a ruler and took high-resolution (600 DPI) scans (Canon LiDE 700F) from the ventral side of each lizard, placing a piece of graph paper in the scanned area for scaling. From scans, two observers counted the number of femoral pores (FP) in the hindlimbs of each lizard, discarding cases where counts were unreliable (e.g., due to incorrect positioning of the hindlimb) or differed between observers. This resulted in a higher sample size for FP of the right hindlimb, compared to the left, and thus, we chose to analyze the right FP count only. Right and left hindlimb FP counts did not significantly differ ($\chi^2_1 = 0.93$, $p = 0.333$; Figure S1). When possible, we also measured the right side "femur" length (FL, $\pm$0.01 mm) in scans of a random subset of 15 males and 15 females per population using the straight line tool in ImageJ (Rasband 1997). We only took FL of individuals whose scans showed a clear and full view of the right hind leg, with the upper hindlimb flat against the scanner's surface, reminiscent of the hind leg's position when the lizard is in a natural resting position (Figure 2). This method was validated in a previous pilot study with 24 *P. muralis*, where hindlimb measurements from scans showed a significant, strong correlation ($r > 0.6$; $p < 0.001$) with digital caliper measurements made by the same person (Figure S2), thus confirming that measurements from scans provide a reasonable estimate of upper hindlimb length. We calculated averages for male and female traits for each population and estimated sexual dimorphism in body size (SSD) as the size of the largest sex/size of the smaller sex: −1 if females were larger, or +1 if males were larger (Lovich and Gibbons 1992), arbitrarily expressed as positive when females were larger and negative in the opposite case; and ASR as the proportion of males in the adult population (m/(m + f)) (Wilson and Hardy 2002).

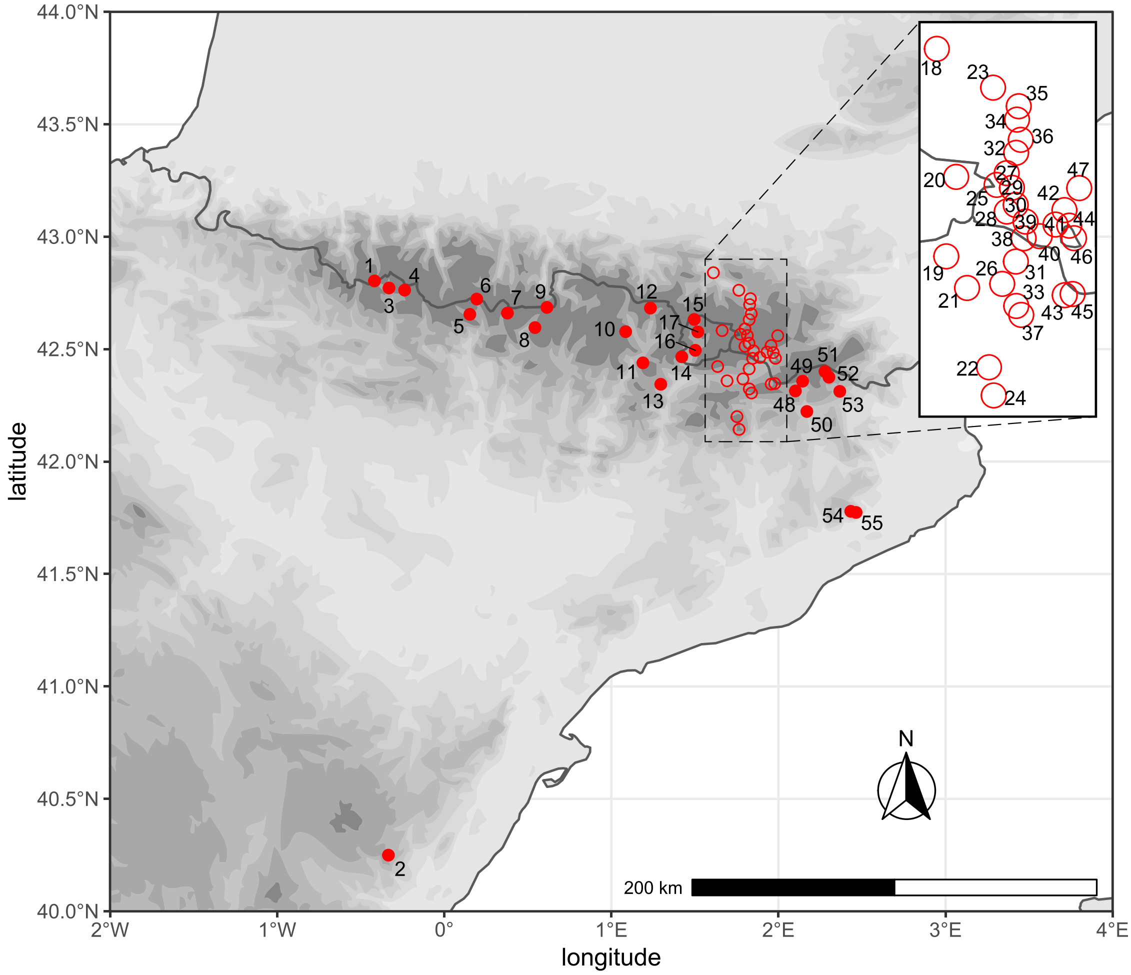

FIGURE 1 | Map of 55 sampled populations of *Podarcis muralis*. See Table S2 for population names, GPS coordinates, and sample sizes. Shading represents orography, with darker areas denoting higher elevation.

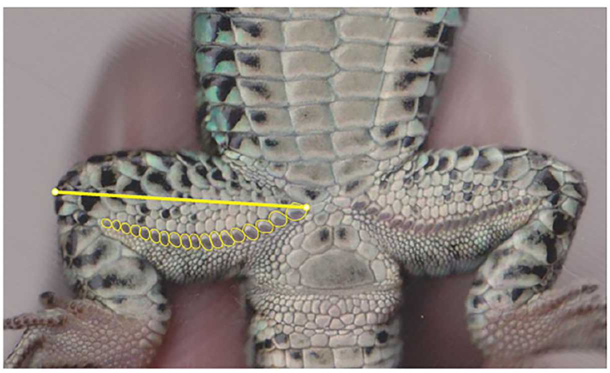

FIGURE 2 | Femur length and femoral pore number were measured on the right-side hindlimb of lizards. Shown is a ventral scan of the pelvic region of an adult male *Podarcis muralis* with femoral pores in the right hindlimb encircled in yellow.

2.4 | Climate and Vegetation Data

We downloaded climate data from WorldClim (v2.1) (Fick and Hijmans 2017) for each of our 55 sites with a 30 arc second (~1 km^2) spatial resolution for the 1970–2000 period with the package geodata (v. 0.4-11; https://github.com/rspatial/geodata) in R (v. 4.1.0; R Core Team 2021). Downloaded variables included the standard 19 bioclimatic WorldClim variables (BIO1-19) describing different annual temperature and precipitation trends, seasonality, and limiting environmental factors (see Table S1 for a complete list), as well as monthly averages of solar radiation (SRad), wind speed (Wind), and water vapor pressure (Vp), from which we calculated annual averages. We also downloaded elevation data from the Shuttle Radar Topography Mission (SRTM) for the same period and at the same resolution as climate data, and the normalized difference vegetation index (NVDI) between April and September for the period 2000–2020 from NASA's MODerate-resolution Imaging Spectroradiometer (MODIS; v6) product MOD13Q1 using MODISTools (v. 1.1.4) (Tuck et al. 2014). Specifically, we downloaded the 250 m 16 days NDVI band, buffered with 1 km left-right and top-bottom for each location, and calculated averages to obtain an equivalent spatial resolution to that of climate and elevation.

We estimated the Spearman correlation among all climatic variables and retained only those with $\rho < 0.7$, informing the decision of which variables to keep with our knowledge on the ecology of this species and the published literature (e.g., Carneiro et al. 2015; Pérez i de Lanuza, Sillero, and Carretero 2018). The final subset consisted of six climatic variables: BIO1, BIO2, BIO9, BIO15, SRad, and NDVI, from which we ran a principal component analysis (PCA) with the base R function prcomp to reduce the dimensionality of the dataset. The resulting PCs were then used in subsequent analyses (see 2.5 and 2.6).

2.5 | Genetic Sampling and Estimation of Genetic Distances

To determine the degree of genetic differentiation among populations, we gathered double digest restriction-site associated (ddRAD) genomic data from two previous studies (Aguilar et al. 2022; Aguilar et al. 2024). We recovered 14 225 single-nucleotide polymorphisms (SNPs) for 495 individuals across 55 populations (each population represented by 6–10 random individuals after filtering; see Methods in Supporting Information for more details). These data were obtained from a ddRAD sequencing library (ddRAD-seq; Peterson et al. 2012) paired-end sequenced in four lanes of an Illumina NovaSeq 6000 platform to a length of 150 bp through protocols described in Aguilar et al. (2024). We estimated pairwise genetic divergence based on the fixation index (F_{ST}) using the StAMPP (v. 1.6.3) R package (Pembleton, Cogan, and Forster 2013). We inspected population structure in ADMIXTURE (Alexander, Novembre, and Lange 2009) using 20 replicates for $K = 1–20$ and calculated a 20-fold cross-validation error to estimate the most suitable value of K.

We estimated the effects of IBE and IBD by performing a multiple matrix regression with randomization analysis using 9999 permutations with the function MMRR in R (Wang 2013). We used the pairwise F_{ST} genetic distance matrix, and we calculated a pairwise geographic distance matrix as the shortest surface distance between two points on an ellipsoid, using our populations' GPS coordinates and the R package geosphere (Hijmans et al. 2022). We also obtained a matrix of pairwise environmental distances between populations by calculating the Euclidean distance in the retained principal components of the PCA performed on our standardized subset of climatic variables. All distance matrices were scaled and centered internally, and thus, MMRR provides comparable standardized linear regression coefficients. To determine whether geographic and environmental distances covary, we also conducted a Mantel test implemented in the mantel function of R package vegan (v. 2.5-7) (Oksanen et al. 2020) between geographic and environmental distance matrices using 9999 permutations.

2.6 | Statistics

The study of biological data sampled across multiple populations within a species requires accounting for the non-independence of populations (Stone, Nee, and Felsenstein 2011). To determine the existence of sex and overall population differences in phenotypic traits of *P. muralis*, namely FP, SVL, and FL, we used linear mixed models (LMM) and the R package nlme (Pinheiro et al. 2023). LMMs included sex as a fixed factor, SVL as a covariate, and population as a random effect. We used quantile-quantile plots and Bartlett's tests to confirm the normality and homoscedasticity of model residuals, respectively, and used a *varIdent* structure to correct for heteroscedasticity in models of FL.

To determine which factors best predict interpopulation variation in FP, we tested, separately for each sex, the correlation between population-average FP with site-specific climate data (summarized via PCA) and two different proxies of the local strength of sexual selection: ASR and SSD using phylogenetic mixed models and the MCMCglmm package in R (Hadfield 2010). We also included FL or SVL as a covariate, to control for the effects of allometric scaling, and used the residuals of SSD regressed on FL or SVL to avoid collinearity between these two variables. When using FL as a covariate, we had to exclude seven (for males) and eight (for females) populations from the total 55 because these had FL averages based on less than 15 individuals, either due to their small sample size (e.g., "Gréixer") or insufficient measurable scans. Since head size in lizards consistently exhibits sexual dimorphism, and similarly to body size, this dimorphism is assumed to have arisen through sexual selection (Braña 1996; Kaliontzopoulou, Carretero, and Llorente 2007; Toyama, Mahler, and Goodman 2023), we additionally estimated and used either head length or head width sexual dimorphism in lieu of SSD as the size dimorphism index in MCMCglmm models of FP. Neither were associated with FP, and we provide the results in Figure S3 and Tables S4 and S5. MCMCglmm allows accounting for shared ancestry and ongoing gene flow among populations by including the pairwise genetic distances between populations in the variance–covariance model matrix. The genetic divergence matrix (F_{ST}) obtained from ddRAD-seq (see 2.5) was decomposed with the svd function and used as a random effect (Stone, Nee, and Felsenstein 2011). We ran models for 125 000 iterations, with a burn-in phase of 5000 and a thinning interval of 60 for the estimation of parameters. We used default, weakly informative/flat priors for fixed effects, and a stronger prior with degree-of-belief parameter (nu) equal to 1, attributing the largest proportion (95%) of the variation to G, for the random effect to ensure proper mixing. Changing random effect priors did not qualitatively alter model results. We assessed model convergence by visual examination of three independent MCMC chain traces for each model and posterior density distributions and calculating the potential scale reduction factors, which were always very close to 1 ($\leq$1.01). We performed model simplification by backward elimination of nonsignificant terms, and here, we show results from the minimum adequate models only. Posterior modes did not differ qualitatively from posterior means.

3 | Results

3.1 | Interpopulation Variation and Sexual Dimorphism in Morphology

Using biometric data from 3437 adult lizards (1859 males, 1578 females) captured in 55 populations (Table S2), we found high intraspecific variation in morphological traits across populations and between sexes, resulting in overall statistically significant differences in the number of femoral pores, body size, and femur length (Table 1; Figure 3). Males were larger than females in both SVL and FL, and FL but not FP scaled tightly with body size (Table 1). Average pore number (and range) across populations was 18 (14–24) for males and 17 (13–22) for females. The average adult SVL was 63.89 mm for males and 62.87 mm for females, with values ranging from 55 to 79 and 55 to 77 mm, respectively, while

TABLE 1 | Results from LMMs showing significant differences between sexes and variance attributable to population on morphological traits of *Podarcis muralis*.

	FP			SVL			FL		
	χ^2	df	*p*	χ^2	df	*p*	χ^2	df	*p*
Sex (fixed)	227.66	1	<0.0001	48.16	1	<0.0001	1477.0	1	<0.0001
SVL	1.358	1	0.244	–	–	–	1509.2	1	<0.0001
Population (random)	416.91	1	<0.0001	470.02	1	<0.0001	248.01	1	<0.0001

Note: The significance of random effects was tested via likelihood ratio test (LRT, which approximately follows a chi-square distribution) between the full and a reduced model, with df equal to the number of additional parameters in the more complex model. Sample sizes are as in Figure 3.
Abbreviations: FP: femoral pore number; SVL: body size; FL: femur length.

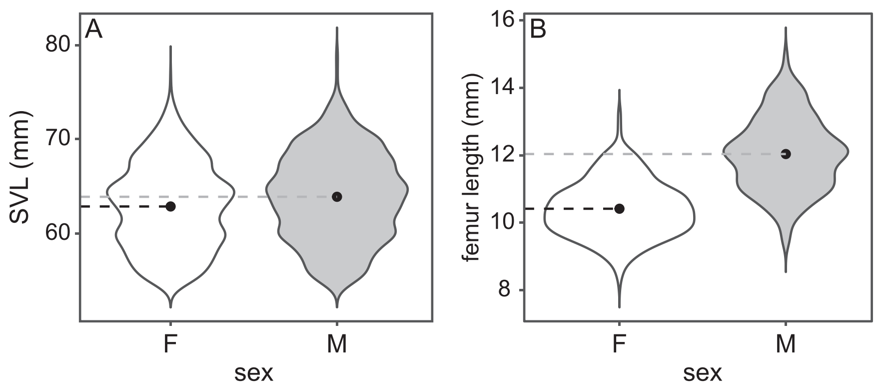

FIGURE 3 | Intraspecific and sexual variation in SVL (A) and femur length (B) across populations of *Podarcis muralis* in the Central and Eastern Pyrenees. Dashed lines indicate species averages for females (black) and males (gray). Sample size for SVL: $M = 1859$, $F = 1578$; FL: $M = 784$, $F = 778$.

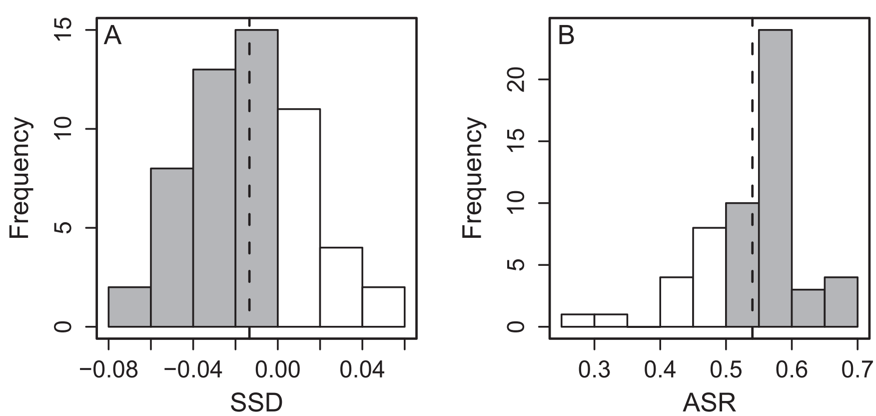

FIGURE 4 | Frequency distribution of sexual size dimorphism, SSD (A) and adult sex ratio, ASR (B) for 55 populations of *Podarcis muralis*. Frequency refers to the number of populations. Shaded bars indicate male-biased values and empty bars female-biased values. Vertical dashed lines depict the average across populations.

FL was 12.03 (9.3–15.0) mm for males and 10.4 (8.1–13.3) mm for females.

SSD was more often biased toward males (38 out of 55 populations, Figure 4a) but varied greatly across populations, ranging from a male (−0.07) to female-biased (0.046). Similarly, ASR was more often male-biased (Figure 4b), although it was nearly unbiased on average (0.54) and showed great variation, with populations strongly skewed toward either sex, from 0.3 (roughly, one male to every two females) to 0.7 (about five males to every two females). However, ASR and SSD were uncorrelated (Pearson's $r = -0.26$, $p = 0.051$).

3.2 | Environmental and Social Predictors of Femoral Pore Number Variation

PCA on the retained subset of climatic variables revealed that 63% of the variation in these data was captured by two primary axes. PC1 loaded most heavily on annual mean temperature

TABLE 2 | Principal component (PC) loadings of retained climatic variables, with their eigenvalues (SD^2). See results and Table S1 for a complete description of each variable.

Variable	PC1	PC2
BIO1	**−0.571**	0.233
BIO2	0.048	**0.545**
BIO9	**0.494**	−0.271
BIO15	0.379	**0.485**
SRad	0.148	**0.580**
NDVI	**−0.512**	0.057
Eigenvalue	2.16	1.63

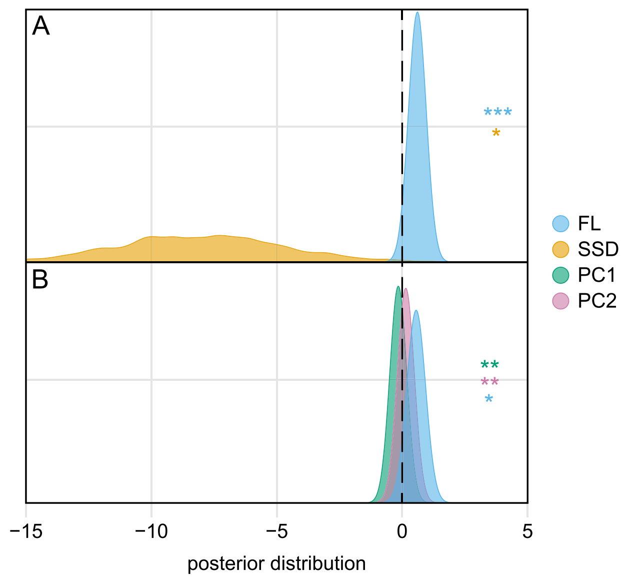

FIGURE 5 | Posterior density distributions from the minimum adequate MCMC mixed model for factors influencing interpopulation variation of femoral pore number of male (a) and female (b) *Podarcis muralis*. FL: femur length; SSD: sexual size dimorphism; PC1: first environmental PC; PC2: second environmental PC. Asterisks indicate that the 95% CI do not overlap 0.

(BIO1), the vegetation index (NDVI) and mean temperature of the driest quarter (BIO9), while PC2 did on solar radiation (SRad), mean diurnal range (BIO2), and precipitation seasonality (BIO15) (Table 2).

In males, FP consistently and positively correlated with FL (or SVL) and negatively with residual SSD (Table 3a; Figure 5). Thus, we found higher average FP count in populations with larger average femur or body size and more male-biased (i.e., more negative) SSD. Comparatively, FP showed a stronger scaling with FL than SVL, and PC1 significantly predicted FP only when SVL was used instead of FL as the covariate (Tables S3–S5). In females, average FP positively correlated with FL but not SVL. It negatively correlated with PC1 and, to a lesser degree, positively correlated with PC2 (Table 3b). As in males, populations with higher FP had on average larger femurs. These populations were also characterized by higher (less negative) annual mean temperatures, more positive (less negative) NDVI values, indicating sparse to dense green vegetation, and lower mean temperatures during the driest quarter (typically the summer). In addition, populations where females had more FP experienced higher solar radiation, wider mean diurnal temperature ranges, and more precipitation seasonality. Last, no proxy of the strength of sexual selection predicted FP of females, with the exception of SSD when SVL was used instead of FL (Table S3).

3.3 | Genetic Diversity and Population Structure

Genetic differentiation among populations (F_{ST}) ranged from 0.004 to 0.51, with a mean of 0.16. Variability in the ancestry coefficients from the ADMIXTURE analysis seemed to be partially explained by both latitude and longitude (Figure S5). The assignment of individuals into $K = 9$ ancestral population clusters was the best supported (i.e., showed the smallest CV error).

We found evidence of genetic IBD as well as IBE even though pairwise environmental and geographic distances moderately covaried (Mantel test: r = 0.45, p = 0.003). The MMRR linear model including geographic and environmental distance between populations significantly explained the observed patterns of genetic distance within *P. muralis* ($R^2 = 0.4$, $p = 0.001$). The effect of geographic distance was highly significant (β_G = 0.544, p = 0.001) and larger than that of Euclidean environmental distances ($\beta_E = 0.161$, $p = 0.032$).

4 | Discussion

Using data from 55 populations representing diverse environmental niches over a relatively small geographic range, we found high intraspecific and interpopulation variation in the number of femoral pores of *P. muralis* (Table 1). On average, there was a high degree of genomic differentiation among populations, and this divergence was not simply the result of isolation by distance (reduced migration and genetic drift), but it was also environmentally driven (IBE). In agreement with this, femoral pore number (FP) differences across populations were significantly explained by a combination of positive allometry and the local intensity of sexual selection, as determined by SSD, or local climatic conditions (Table 3; Figure 5). Since these effects were observed when accounting for shared ancestry and ongoing gene flow among populations, our results suggest that interpopulation variation in FP of *P. muralis* is partly driven by local adaptation.

The specific combination of factors responsible for FP variation differed for males and females, suggesting that different selective regimes underlie the sexual dimorphism of this trait or, more likely, that female FP is the result of incomplete sexual dimorphism. Males seem to respond more strongly to allometry and proxy measures of the intensity of male competition, and thus male FP presumably evolves under the influence of sexual selection (Font and Pérez i de Lanuza 2017). This result is consistent with *P. muralis*' polygynous resource defense-based mating system (Edsman 1990; Font et al. 2012), where intrasexual competition among males for territories and/or mates is an important determinant of male mating success and favors large males (male-biased SSD) that defend larger territories (Stamps

TABLE 3 | Effects of allometry (FL: femur length), local environmental conditions, and sexual size dimorphism (SSD) on femoral pore number variation across populations for male (a) and female (b) *Podarcis muralis*. Posterior means with 95% credible intervals (CI) and *p*-values from the MCMCglmm minimum adequate model are shown.

	Posterior mean	Lower 95% CI	Upper 95% CI	Effective sample	*p*
a. Males					
Intercept	10.87	7.71	13.87	2000	<0.0001
FL	0.60	0.35	0.85	2000	<0.0001
SSD (resid)	−8.02	−14.49	−1.35	2000	0.020
b. Females					
Intercept	11.68	7.65	15.14	2000	<0.0001
FL	0.55	0.22	0.93	2000	0.003
PC1	−0.15	−0.25	−0.06	2000	0.002
PC2	0.14	0.007	0.27	2119	0.038

1983; Olsson and Madsen 1998; Cox, Butler, and John-Alder 2007). In contrast, female pores are typically reduced and nonfunctional, producing almost no secretion, and may simply be a by-product of nonadaptive genetic correlations between the sexes (Lande 1980). In other words, selection occurring on FP of males can cause a correlated response of FP in females, which would explain why female pores also showed great variation in number.

Genetic divergence is a good predictor of morphological differentiation among populations of *P. muralis* (While et al. 2015; Uller et al. 2019). Natural selection via local adaptation to climate has been identified as a driver of phenotypic (e.g., morphology, physiology, behavior) and/or genetic differentiation, even in the presence of gene flow, at small spatial scales, or over short evolutionary time (Uller et al. 2019; Ruiz Miñano et al. 2022). Here, we also found that local climatic and vegetation conditions predict morphological variation among populations of *P. muralis*. Annual mean temperature, which positively correlates with the vegetation index, seemed to be the main contributor to increased FP in both sexes, with higher temperatures associated with a higher FP. The number of pores is typically used as a proxy for the amount of secretion produced by the epidermal glands, and thus a higher FP may be one way to counter the increased volatility of chemicals at higher temperatures (Fleishman and Font 2019). For example, *Psammodromus algirus* from a population occupying a warmer, low-elevation habitat had more femoral pores than those in a high-elevation habitat (Iraeta et al. 2006). In females, additional climatic factors such as solar radiation and mean diurnal temperature range also predicted higher FP. Temperature is a strong selective factor of lizard morphology and physiology and the source of intraspecific geographic variation in other widespread taxa like *Anolis carolinensis* (Campbell-Staton, Edwards, and Losos 2016; Jaffe, Campbell-Staton, and Losos 2016). In *Liolaemus* lizards, male-limited precloacal gland variation within subclades is also partially driven by some climatic factors, including solar radiation, topographic heterogeneity, net primary production, and precipitation range (Jara et al. 2018). In lacertids, Baeckens et al. (2015) found covariation between male FP and substrate usage, which may closely reflect habitat availability and thus structure. Together, these studies support a role for ecological and/or climatic factors in epidermal gland evolution in lizards, which may be more apparent at microecological scales and differ between males and females.

Allometric scaling was stronger in relation to limb length (FL) than overall body size (SVL), especially in females, and thus, limb morphology could impose an upper limit to FP, given that only populations with large enough upper hindlimbs, on average, express the highest number of femoral pores. Similarly, in a study comparing three populations of *P. liolepis*, Ortega et al. (2019) found that highland males were larger and had longer femurs and more femoral pores than those from the lowlands. In female lizards, SVL is likely under strong selection for fecundity (Cox, Butler, and John-Alder 2007) whereas large male size confers an advantage in male aggression and territoriality (Edsman 1990; Cox, Butler, and John-Alder 2007), which may explain why SVL is a better predictor of FP in males than it is in females. If higher FP confers better scent marking capacity, for example, via faster turnover, this may contribute to the higher resource-holding potential of larger males, which typically defend larger territories. Limb morphology is strongly shaped by ecological selection via microhabitat use (Vanhooydonck and Van Damme 1999; Herrell, Meyers, and Vanhooydonck 2002), and thus, some of the interpopulation variations in FP could be the indirect result of hindlimb adaptation to local habitat characteristics for improved locomotion performance and even hindlimb length plasticity (Losos et al. 2000). In *P. muralis*, however, sexual dimorphism in limb morphology does not seem to translate into ecological or performance differences between the sexes (Braña 2003), suggesting that sexual selection is also contributing to the evolution of FL dimorphism (Figure 3b).

Among proxy measures of the local strength of sexual selection, only SSD predicted FP, primarily in males (Table 3 and Figure 5; Table S3; Font and Pérez i de Lanuza 2017). Males from populations with more male-biased SSD exhibited more femoral pores, which is what we would expect if local SSD reflected increased male competition. Assuming that gland secretions function in a scent-marking context, males may need to replenish territory markings more frequently or for longer periods of time in more competitive environments, where encounters with rivals or intruders are bound to be more frequent (Edsman 1990).

Interestingly, we found additional effects of SSD on FP that are unrelated to allometry (e.g., male body size *per se*) since we used the residuals from the regression of SSD against FL (or SVL) as a predictor in our models. Furthermore, we found no clear pattern of hyperallometry across populations (Figure S4), and thus, populations with more male-biased SSD were not necessarily those in which males were larger on average. In lizards, SSD may also vary along environmental gradients such as temperature, becoming more male-biased in populations that experience higher temperatures, presumably due to stronger male–male competition in these warmer environments (Tarr et al. 2019; Toyama, Mahler, and Goodman 2023). ASR variation was similar to that found in *Anolis* lizards (Schoener and Schoener 1980; Muralidhar and Johnson 2017) and was not associated with SSD or FP. ASR may be difficult to accurately measure (e.g., due to differences in detectability between sexes), requiring larger sample sizes, or be a better predictor of the intensity of sexual selection over a different time or taxonomic scale (Kokko and Jennions 2008). Additional studies testing the reliability of these and other measures of the intensity of sexual selection would benefit future research (Janicke and Fromonteil 2021).

Our results suggest that, in addition to historical and neutral processes, local selection drives interpopulation divergence in the FP of *P. muralis*. Local selection may act both directly and indirectly on signaling trait variation; for example, natural selection via climate (e.g., increased temperature) can directly and negatively impact chemical signal efficacy by causing faster signal degradation (Martín and López 2013) and selecting for increased secretion abundance (Alberts 1992). Warmer environments can also affect hindlimb morphology via changes in habitat structure, and thus, indirectly, FP. Stronger intra-sexual selection may directly favor increased FP of males to improve chemically mediated territoriality (Font et al. 2012), or indirectly, by acting on male body size for a physical advantage during combat (Stamps 1983). Changes in the number (or size) of signaling glands may have a significant impact on the chemical communication of lizards, especially if pore number is related to gland secretion activity or chemical signaling reliance. Nevertheless, changes in the chemical composition of femoral gland secretions, the rate of production, or aspects of signaling behavior (e.g., substrate selection), rather than morphology itself (pore number or size) could all be potential, and non-exclusive, targets of selection for improved communication (Alberts 1992; Martins et al. 2006). Chemical composition of the gland secretion, for example, is known to show high intraspecific variation in lizards (Pellitteri-Rosa et al. 2014; Campos 2018; Ortega et al. 2019) as well as other terrestrial vertebrates (Apps, Weldon, and Kramer 2015). Whether and how gland number, secretion abundance, and scent composition relate to one another, along with other aspects of chemical signal production, will determine the functional impact of high interpopulation variation on chemical signaling glands mediating competitive and reproductive behavior.

Phenotypic variation can scale up from individuals, through populations to species; however, whether variation at different levels of hierarchical organization responds to the same evolutionary influences is unclear. Here, we found that both genetic and environmental factors are important in shaping FP variation across populations of *P. muralis*. Genetic divergence (e.g., F_{ST}), allometry, and environmental factors such as temperature have also been found to yield phenotypic variation among *Podarcis* species (Kaliontzopoulou, Pinho, and Martínez-Freiría 2018; Taverne et al. 2021), suggesting there is some similarity in the mechanisms that may drive diversification at the intra- and interspecific levels. Changes in the number of secretory glands in different environments could contribute to the fine-tuning of chemical communication to local conditions and may reduce gene flow among populations, and this process may be affected by current and future changes in the signaling environments (e.g., due to climate change; Candolin and Wong 2012). More generally, our findings show that accounting for intraspecific variation would be desirable when using macroevolutionary comparative approaches since individuals from different classes (e.g., age, sex) or populations may reflect a unique selective regime.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are provided as supplementary material.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.