Received: 15 July 2024 | Accepted: 7 October 2024

DOI: 10.1111/1365-2435.14687

Functional Ecology BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

# Temporal dynamics of scent mark composition in field-experimental lizard populations

**Simon Baeckens**[1,2] | **José Martín**[3] | **Panayiotis Pafilis**[4,5] | **Anthony Herrel**[1,2,6,7] | **Colin M. Donihue**[8]

[1]Department of Biology, University of Antwerp, Antwerp, Belgium; [2]Department of Biology, Ghent University, Ghent, Belgium; [3]Department of Evolutionary Ecology, Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain; [4]Department of Biology, National and Kapodistrian University of Athens, Athens, Greece; [5]Museum of Zoology, National and Kapodistrian University of Athens, Athens, Greece; [6]UMR 7179 CNRS/MNHN, Paris, France; [7]Naturhistorisches Museum Bern, Bern, Switzerland and [8]Institute at Brown for Environment and Society, Brown University, Providence, Rhode Island, USA

**Correspondence**
Simon Baeckens
Email: simon.baeckens@uantwerp.be

**Funding information**
Division of Biological Infrastructure, Grant/Award Number: 1609284; Muséum National d'Histoire Naturelle; Ministerio de Asuntos Económicos y Transformación Digital, Gobierno de España, Grant/Award Number: CGL2014-53523-P; Fonds Wetenschappelijk Onderzoek, Grant/Award Number: 12I8822N; Institute for Biospheric Studies, Yale University; National Geographic Society; Belgian American Educational Foundation

**Handling Editor:** Pau Carazo

## Abstract

1. Animal signals are dynamic traits that can undergo considerable spatial and temporal changes and that are influenced by factors such as age, health condition and interactions with both the abiotic and biotic environment. However, much of our understanding of signal changes throughout an individual's lifetime stems from cross-sectional, often laboratory-based, studies focused on visual and auditory signals. Longitudinal field investigations of temporal variation in chemical signals, especially in vertebrates, remain rare despite chemical communication being the most ubiquitous form of information exchange in the natural world.
2. To remedy this, we conducted a unique, replicated field experiment to study the temporal signal dynamics in free-living lizard populations on natural islands. Specifically, we collected scent marks from individually marked lizards across five populations during the spring of two consecutive years and analysed the lipophilic chemical composition of these scent marks.
3. Our findings demonstrate that the overall scent mark composition of individual lizards changed over time, shifting consistently in both direction and magnitude from year to year among individuals and across replicate populations. Similar patterns were observed for the chemical richness and diversity of scent marks. Temporal variation in the relative proportions of three potentially socially relevant signalling compounds in lizard scent marks revealed a more complex pattern: $\alpha$-tocopherol remained stable over time, oleic acid decreased and the change in octadecanoic acid proportion was body size-dependent.
4. Together, our results provide novel insights into how individual vertebrate chemical signals may fluctuate across space and time. We discuss the potential causes of the observed temporal variability and its consequences for chemical signal evolution.

KEYWORDS
animal chemical communication, chemical signal evolution, island introduction experiment, Lacertidae, lizard ecology, *Podarcis erhardii*

## 1 | INTRODUCTION

The bewildering diversity of animal signals, ranging from the intricate displays of birds of paradise to the complex calls of frogs, has long captivated scientists (Fögen, 2014). Traditionally, research has focused on the differences in signalling repertoire among species and among populations. However, recent decades have seen a shift towards investigating inter- and intra-individual variation in animal communication (Groot et al., 2024; Patricelli et al., 2016). This body of research has highlighted that animal signals are dynamic traits that can undergo considerable changes throughout an individual's lifetime. Factors such as age, health and nutritional state, and interactions with both abiotic and biotic environments can trigger physiological or behavioural responses, producing among- and within-individual signal variability in populations over time. For example, studies have shown that the expression of ornamental white plumage in collared flycatchers increases with age (Evans et al., 2011), song frequency in field crickets decreases when infested by parasites (Beckers & Wagner, 2011), beak colour brightness increases in zebra finches feeding on carotenoid-rich seeds (McGraw & Ardia, 2003), head-bob display in anole lizards accelerates in response to strong winds affecting background vegetation movement (Ord et al., 2007), and pheromone release in American bird grasshoppers escalates under crowded conditions (Stahr et al., 2013). Understanding the extent of an individual's phenotypic variation over time is crucial as this variation determines the temporal dynamics of phenotypic selection and shapes population responses to novel or changing environments (Fox et al., 2019; Siepielski et al., 2009).

Despite the recent surge in knowledge on signal variation among and within individuals, significant gaps in our understanding persist for several reasons. First, many studies on the topic have used a cross-sectional design, which do not allow tracking individual changes over time. Longitudinal studies, though more labour-intensive, can offer more robust and detailed insights into the temporal dynamics of animal signals (Diamond, 1986). Second, much of the published research has been conducted in controlled, often ecologically unrealistic laboratory settings, failing to capture the environmental variability and unpredictability of the natural world (Hendry, 2017). Third, the focus of most investigations on animal signal diversity is skewed towards visual and auditory signals, with chemical signals being relatively understudied (Symonds & Elgar, 2008). This preference may be a reflection of human's own sensory biases or the increased technical complexity associated with analysing chemical signals. Finally, research on chemical signals has primarily centred on insects, while studies on chemical signals in vertebrates, particularly reptiles, have been comparatively underrepresented in the literature in terms of absolute number of publications (Baeckens, 2019; De Pasqual et al., 2021; Symonds & Elgar, 2008).

Lizards are a promising clade for studying chemical signal variability in vertebrates (Baeckens, 2019; Martín & Lopez, 2014; Mason & Parker, 2010). Males of most species have specialized epidermal glands on the underside of their hind legs (Baeckens & Whiting, 2021; García-Roa, Jara, et al., 2017), which exude waxy secretions used as scent marks composed of both proteins and lipids (Cole, 1966; Mangiacotti et al., 2021, 2023; Mayerl et al., 2015). Over the past decade, extensive behavioural assays on various lizard species, coupled with studies in natural product chemistry, have highlighted the importance of the lipophilic chemical composition of these glandular secretions for intraspecific signalling (Baeckens, Huyghe, et al., 2017; Campos et al., 2017; Khannoon et al., 2011; MacGregor et al., 2017; Martín & López, 2000; Pruett et al., 2016; Romero-Diaz et al., 2020, 2021; Zozaya et al., 2019). Variations in the lipophilic composition or the proportions of specific lipid compounds in secretions can convey individual differences in features such as age (Martín & López, 2006a), fluctuating asymmetry (Martín & López, 2000), access to quality thermal resources (Heathcote et al., 2014), immune response levels (López et al., 2009), parasite load (Martín, Civantos, et al., 2007), nutritional condition (Kopena et al., 2011, 2014) and hormone levels (Martín, López, et al., 2007). The temporal changes in the lipophilic chemical composition of scent marks in individual lizards have not been previously investigated.

In response, we set up a large-scale field experiment to track scent mark composition of individual lizards over time across replicate island populations. Briefly, we translocated lizards (*Podarcis erhardii*) from a source population on a large Greek island to five small experimental islets nearby (Figure 1). Four years after translocation, we revisited these islets to examine scent mark composition of a subset of lizards in each population (Donihue et al., 2020). The same individuals were recaptured and re-examined the following year to assess potential changes in their scent mark composition. The aim of this study was to determine whether scent mark composition of lizards exhibit temporal variability. If such variability is affected by external conditions, we predicted that the direction and degree of change over time would be consistent across replicate populations. This unique field experiment with free-living lizards on natural islands offers a rare opportunity to examine whether and how individual vertebrate chemical signals fluctuate across space and time.

## 2 | METHODS

### 2.1 | Study species and system

The study species, the Aegean wall lizard (*Podarcis erhardii*), is a small-bodied lacertid lizard (Figure 1) and invertebrate generalist feeder (Adamopoulou et al., 1999; Donihue et al., 2023;

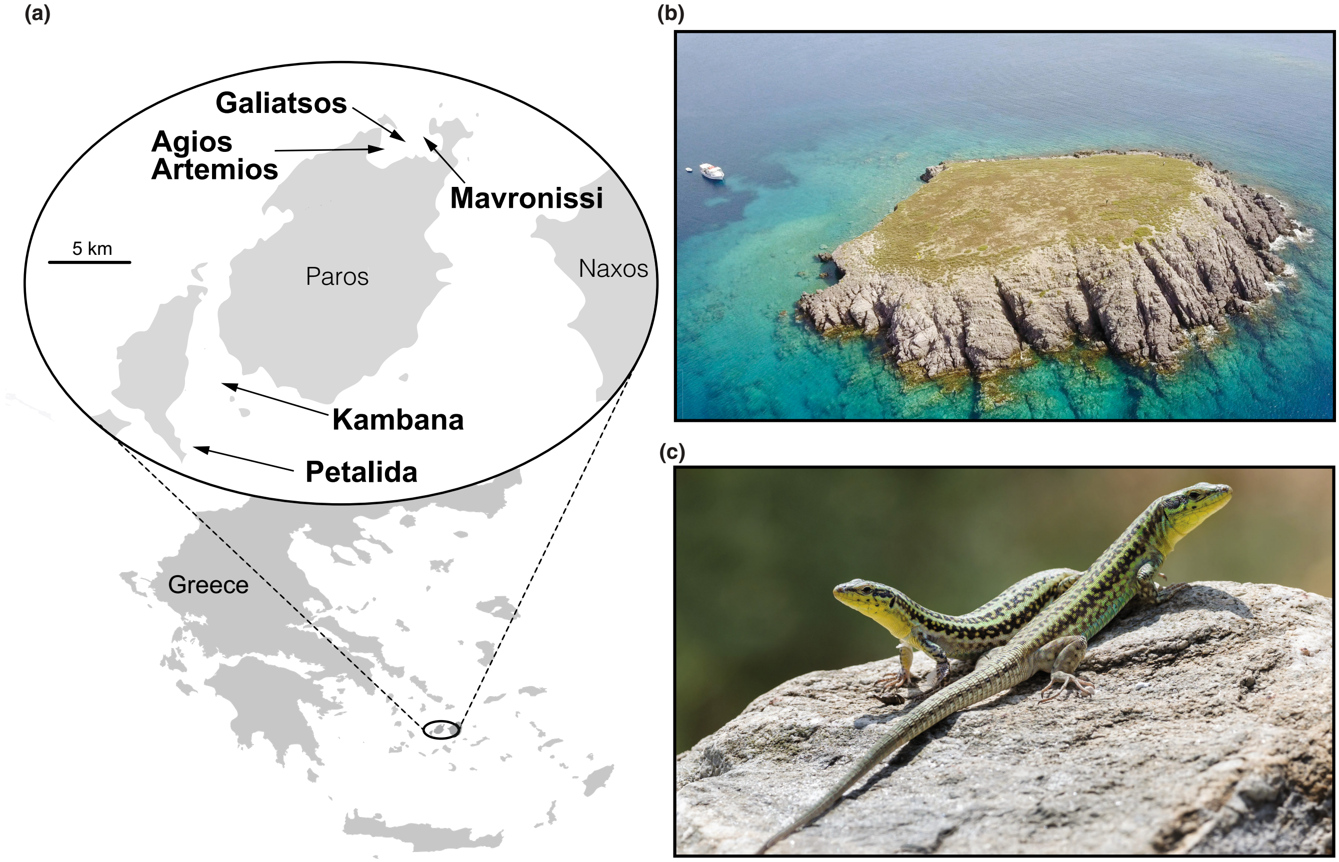

**FIGURE 1** Study system. (a) Map showing the location of the study area within the Greek Cyclades, and the distribution of the five replicate experimental islets (indicated at the arrow tips) near the large islands of Paros and Naxos. (b) Oblique drone photograph of a representative experimental island, Petalida, with a boat, approximately 8 m long, to provide a sense of scale; photo credit: Colin Donihue. (c) The Aegean wall lizard (*Podarcis erhardii*), the study species; photo credit: Peter Oefinger.

Valakos, 1986). It is widely distributed throughout the Greek Cyclades island group in the Aegean Sea (Lymberakis et al., 2018). Like all other members of the genus, *P. erhardii* possesses active follicular epidermal glands that produce secretions used for chemical signalling (Brock et al., 2020; Donihue et al., 2020). In June 2014, we opportunistically captured 100 *P. erhardii* lizards from a population on Naxos, the largest Cycladic island. We then randomly assigned 12 female and 8 male lizards to each of the five experimental islets: Agios Artemios, Galiatsos, Kambana, Mavronissi and Petalida (Figure 1). We selected these specific islets as replicate units for our experiment due their similar vegetation community (namely, *Pistacia* shrubs, low flowering forbs and grasses), weather conditions (due to their close geographical proximity) and surface areas (ranging from 0.002 to 0.004 km$^2$). Notably, these islets lacked *Podarcis* lizards and predators prior to the introduction event, and they have consistently remained predator-free throughout the experiment, as verified by annual surveys. During those surveys, we also captured all the lizards on each islet until the capture rate decreased to one lizard per two person-hours; lizards were re-released after measuring. A more detailed account of the experimental system can be found in (Donihue et al., 2020, 2022, 2023; Pafilis et al., 2019). This study was approved by the Ethics Committee of Yale University (2013-11548) and by special permits issued by the Greek Ministry of Environment (Permits ΑΔΑ: 7Θ9Ω4653Π8-0ΞΨ and Ω4534653Π8-Γ06).

## 2.2 | Secretion collection and chemical analysis

Over two consecutive years, we collected and analysed gland secretions from a consistent cohort of 23 adult male lizards from the five replicate island populations. The initial sampling took place in May 2018, followed by a subsequent sampling in May 2019, resulting in a total of 46 unique samples. Each lizard could be individually identified based on subcutaneously implanted PIT tags (Loligo systems, #AB10320; 7 × 1.35 mm). Upon (re)capture, epidermal gland secretions were collected by applying gentle pressure around the femoral pores. The secretions were then transferred to glass vials filled with glass inserts and sealed with Teflon-line lids. Subsequently, they were stored at −20°C before undergoing chemical analysis. Gas chromatography–mass spectrometry (GC–MS) was used to separate and identify the volatile lipophilic chemical compounds present in the secretions and to estimate their relative abundance, following established methodologies (García-Roa et al., 2018; Martín & López, 2006b, 2007, 2015). We used the same analytical procedure, GC–MS equipment and parameters consistently over the 2 years; for detailed information see Donihue et al. (2020). After the chemical analysis, we assessed the chemical complexity of each individual secretion sample by calculating its chemical 'richness' and 'diversity' (Baeckens, García-Roa, et al., 2017; Baeckens, Martín, García-Roa, Pafilis, et al., 2018; Baeckens, Martín, García-Roa, & Van Damme, 2018). Chemical richness was determined by the total number of different lipophilic compounds present in each lizard secretion sample, and chemical diversity was quantified using the Shannon–Wiener diversity index (Shannon, 1948). Furthermore, each chemical compound was categorized into one of several main chemical classes, and the relative proportion of each class was calculated. The 10 main chemical classes included alcohols, aldehydes, amides, carboxylic or fatty acids, furanones, ketones, steroids, terpenoids, tocopherols and waxy esters (Martín & Lopez, 2014; Mayerl et al., 2015).

## 2.3 | Statistics

To correct for the non-independence of proportions, we first performed a compositional analysis which consists of a logit transformation of the proportion data by taking the natural logarithm of proportion divided by (1 − proportion) (Aebischer et al., 1993). We then used unconstrained multivariate statistics, here a principal component analysis (PCA) with all 10 main chemical classes as input variables, to assess and visualize patterns of temporal variation in scent mark composition across replicate populations ('prcomp' function; no rotation). We focused on principal components with eigenvalues greater than 1 or those explaining at least 10% of the variation, and we visually assessed the scree plot for breakpoints (Morton & Altschul, 2019) (see Figure S1). Using the scores of the main principal components as response variables, we then ran linear mixed-effect models (LMMs) to test whether the chemical profiles of individual lizards' scent marks have changed over time ('lme4' package, 'lmer' function; Bates et al., 2015) with 'year' (two level-factor: 2018, 2019) as fixed effect, and 'individual' and 'population' as random effects. We included lizard body size (log-transformed 'snout-to-vent length', SVL) as covariate (López et al., 2006) to account for potential effects of changes in body size confounding temporal changes in scent mark composition. Next, we fitted a LMM with chemical diversity as response variable and a generalized LMM ('glmer' function; Poisson distribution) with chemical richness (i.e. count data) as response variable; fixed and random effects were equal to the previous models. In the same way, we tested for temporal changes in the proportions of three specific compounds that have previously been suggested to be important for intraspecific signalling in closely related lacertid lizards: Octadecanoic acid (Aragón et al., 2008; Martín, Civantos, et al., 2007), oleic acid (Heathcote et al., 2014; López & Martín, 2012; Martín & López, 2010a) and α-tocopherol (García-Roa, Sáiz, et al., 2017; Kopena et al., 2011; Martín & López, 2010b). *R*-squared statistics ('MuMIn' package; Bartoń, 2023) provided information on the explanatory value of the model predictors. In reporting the results of the (G)LMMs, we provide *t*-values (or *z*-values in Poisson regressions) for the predictor variables. In addition, we report *F*-values alongside their estimated degrees of freedom using Kenward-Roger df-approximation. All data were analysed in R version 4.4.0 (R Core Team, 2024). The replication statement can be found in Table 1.

**TABLE 1** Replication statement.

| Scale of inference | Scale at which the factor of interest is applied | Number of replicates at the appropriate scale |
|---|---|---|
| Individual | Island population | 23 individuals per year |

## 3 | RESULTS

Our analysis revealed a total of 104 different lipophilic compounds in the secretions of lizards sampled across years. These compounds included steroids (average percent of the total ion current ± SE: 83.04 ± 1.68%), waxy esters (7.10 ± 0.94%), tocopherol (2.74 ± 0.34%), alcohols (1.79 ± 0.16%), fatty acids (0.64 ± 0.30%), aldehydes (1.48 ± 0.14%), terpenoids (1.06 ± 0.35%), amides (0.75 ± 0.20%), ketones (0.28 ± 0.02%) and furanones (0.12 ± 0.02%) (Table S1). The three most abundant compounds in both sampling years were cholesterol (2018: 40.93 ± 2.24%; 2019: 64.18 ± 2.06%), campesterol (2018: 6.62 ± 0.74%; 2019: 5.03 ± 0.38%) and cholest-4-en-3-one (2018: 10.36 ± 1.96%; 2019: 5.19 ± 1.80%).

A PCA revealed strong temporal variability in the scent mark composition of lizards from five replicate experimental populations. The first two principal components (PC1 and PC2) accounted for 78.5% of the variation in the composition and proportions of chemical compounds in the secretions (PC1: 63.8%, eigenvalue = 2.41; PC2: 14.7%, eigenvalue = 0.55; Figure 2a; Figure S1). The seven classes that contributed most to these axes were waxy esters (scores for PC1 = −0.62; scores for PC2 = +0.18), steroids (PC1 = +0.55; PC2 = +0.19), fatty acids (PC1 = −0.37; PC2 = +0.21), amides (PC1 = +0.25; PC2 = +0.05), aldehydes (PC1 = −0.22; PC2 = +0.07), terpenoids (PC1 = −0.20; PC2 = −0.22) and tocopherols (PC1 = −0.08; PC2 = −0.90).

Time of sampling emerged as a significant factor, accounting for a substantial portion of the variation in PC1, but its impact was less pronounced for PC2. The predictor 'year' explained 88.4% of the variation in PC1, while the random effects 'individual' and 'population' combined contributed only 2.7% (Table 2). Overall, the chemical profile of lizard scent marks shifted in the same general direction from 2018 to 2019, from negative to positive values on the PC1 axis (Figure 2a). Specifically, individual chemical profiles transitioned to one with a higher proportion of steroids and a lower proportion of waxy esters, fatty acids, amides, and aldehydes compared to those observed in 2018 (Figure 2a; Table 2). This temporal shift in PC1 was consistent across replicate populations (Figure 2a; Table S2). In contrast to PC1, most of the variation in PC2 was attributed to the random effects (38.8%), with only a minor portion explained by 'year' (3.1%) (Table 2; Tables S2 and S3). Indeed, variation in PC2 expressed more nuanced differences in chemical profiles among replicate populations from year to year (Figure 2; Table S3). As illustrated

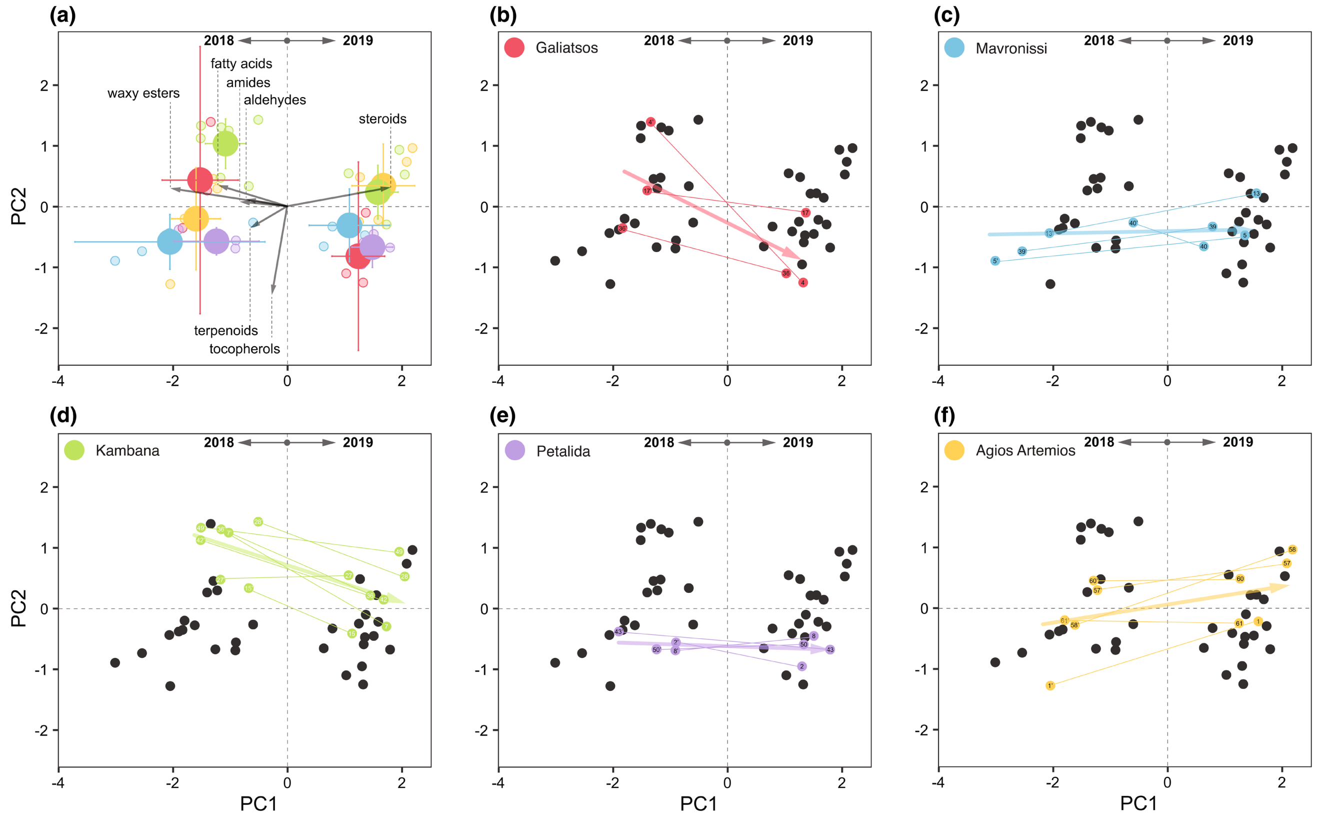

FIGURE 2 Temporal variation in the lipophilic chemical composition of scent marks in individual lizards across field-experimental populations. Biplots A to F show the output of the same principal component analysis with all ten main chemical classes as input variables. (a) Biplot displaying the scores of the two primary principal components (PC1 and PC2) and their seven most contributing loadings vectors (waxy esters, steroids, tocopherols, fatty acids, amides, terpenoids and aldehydes). Centroids of the five populations are shown for 2018 and 2019 separately; error bars denote the 95% confidence intervals of the distribution of points around the centroids. Colour denotes population. (b–f) Biplots focusing on temporal shift per population. Individual lizards are identified by numbers and samples from the same individual, collected in different years, are paired (single quotes indicate 2018 samples). Black dots in the biplot represent data points from lizards belonging to the different populations. The intercept and slope of the thick-lined arrow illustrates the average temporal change observed in that population.

TABLE 2 Outcome of the (generalized) linear mixed models testing for the effect of year on various features of the chemical signal profile of lizards.

| | Year | | | | | | $R^2$ | |
|---|---|---|---|---|---|---|---|---|
| **Chemical signal profile feature** | $\beta$ | **SE** | $t/z$ | $F$ | **df** | $p$ | **Marginal** | **Conditional** |
| PC1 (63.8%) | **2.88** | **0.14** | **20.64** | **424.52** | **23.24** | **<0.001** | **0.884** | **0.911** |
| PC2 (14.7%) | −0.27 | 0.18 | −1.53 | 2.35 | 23.26 | 0.133 | 0.031 | 0.419 |
| Chemical richness | **−0.13** | **0.04** | **−3.45** | **11.98** | **21.00** | **<0.001** | **0.223** | **0.223** |
| Chemical diversity | **−0.81** | **0.06** | **−12.66** | **159.51** | **23.18** | **<0.001** | **0.775** | **0.795** |
| Oleic acid (CH24) | **−0.14** | **0.03** | **−4.67** | **21.70** | **23.14** | **<0.001** | **0.323** | **0.346** |
| Octadecanoic acid (CH25) | **−1.15** | **0.10** | **−11.38** | **128.67** | **23.10** | **<0.001** | **0.746** | **0.746** |
| α-tocopherol (CH62) | −0.09 | 0.17 | −0.55 | 0.31 | 23.26 | 0.583 | 0.008 | 0.395 |

*Note*: This table presents the estimates ($\beta$), standard errors (SE), *t*- or *z*-value, *F*-value, degrees of freedom (df) and *p*-value for the predictor variable 'year'. Additionally, the proportion of variance explained by the fixed effects (marginal $R^2$) and by both the fixed and random effects (conditional $R^2$) are provided. All models included 'individual' and 'population' as random effects and 'body size' as covariate. Statistical significance ($p < 0.05$) is denoted in bold.

by the among-island variation in slopes (Figure 2), the proportion of terpenoids and tocopherols slightly increased over time in the scent marks of lizards from Galiatsos and Kambana (decrease in PC2 value; Figure 2b,d), but slightly decreased for Agios Artemios lizards (increase in PC2 value; Figure 2f). The values for PC2 remained stable for lizards from Petalida and Mavronissi (Figure 2c,e). Temporal variation in PC1 and PC2 was unrelated to individual changes in body size (PC1: $\beta \pm \mathrm{SE} = -2.33 \pm 3.48$, $t = -0.67$, $p = 0.506$; PC2: $\beta \pm \mathrm{SE} = 1.70 \pm 4.43$, $t = 0.35$, $p = 0.704$). A principal components analysis that included the 104 single compounds instead of the 10 major chemical classes showed similar temporal patterns in lizard scent mark composition, that is a notable increase in the proportion of steroids over time across all populations, and subtle inter-population variability in the proportions of terpenoids and tocopherols (data not shown).

Over the course of 2 years, chemical richness of individual lizards' scent marks decreased by an average (± SE) of 12% (±2) across replicate populations. Following the same trend, chemical diversity decreased with an average of 32% (±2) over time and with a slight difference among populations (Figure 3: Table 2; Tables S4 and S5). Specifically, the drop in chemical diversity was more pronounced in lizards from Agios Artemios (mean ± SE decrease = 1.10 ± 0.12) compared to those from Kambana (0.58 ± 0.10) (Table S4). Overall, the random effects contributed minimally (<5%) to the overall observed variation in chemical richness and diversity (Table 2). Similar to PC1 and PC2, body size did not affect temporal changes in chemical richness ($\beta \pm \mathrm{SE} = -0.26 \pm 0.85$, $z = -0.31$, $p = 0.756$) and diversity ($\beta \pm \mathrm{SE} = -1.15 \pm 1.55$, $t = -0.74$, $p = 0.462$).

The temporal dynamics of the three socially relevant signalling compounds exhibited distinct patterns. While the proportion of α-tocopherol remained stable across time, a significant decrease was observed in the proportion of oleic acid (Table 2). Neither compound showed a significant association with body size (α-tocopherol: $\beta \pm \mathrm{SE} = -1.73 \pm 4.31$, $t = -0.40$, $p = 0.690$; oleic acid: $\beta \pm \mathrm{SE} = 0.99 \pm 0.71$, $t = 1.41$, $p = 0.167$). The temporal pattern in the proportion of the third component, octadecanoic acid, was more complex due to its body size-dependence (interaction between body size and year: $\beta \pm \mathrm{SE} = -12.57 \pm 4.82$, $t = -2.61$, $p = 0.015$; Figure S2). Specifically, across populations, the proportion of octadecanoic acid increased with body size in 2018 ($\beta \pm \mathrm{SE} = 0.08 \pm 0.02$, $t = 3.28$, $p = 0.004$), yet this relationship disappeared and became non-significant the following year ($\beta \pm \mathrm{SE} = -0.01 \pm 0.01$, $t = -0.36$, $p = 0.725$; Figure S2). While temporal variation patterns were largely consistent across replicate populations for oleic and octadecanoic acid (with random effects explaining <3% of the variation), such consistency was less pronounced for α-tocopherol (with random effects explaining 38.7% of the variation; see Table 2).

## 4 | DISCUSSION

We have tracked scent mark composition of individuals over time using a large-scale field experiment with free-living lizards on natural islands. Our findings reveal significant year-to-year changes in various features of the chemical composition of scent marks in individual *P. erhardii* males, demonstrating the temporal variability of lizard semiochemicals. Notably, these changes in scent mark composition exhibit largely consistent patterns both among individuals and across replicate populations, suggesting a deterministic mechanism underlying this temporal phenotypic change. While the larger portion of chemical compounds shows consistent temporal trends across populations, a smaller subset exhibits variable shifts between populations; nevertheless, year-to-year changes within each population remain stable. Below, we explore potential causes and implications of these convergent changes in scent mark composition across replicate populations.

Age is one potential factor explaining this temporal pattern. Ontogenetic changes in chemical signal profiles are well-established

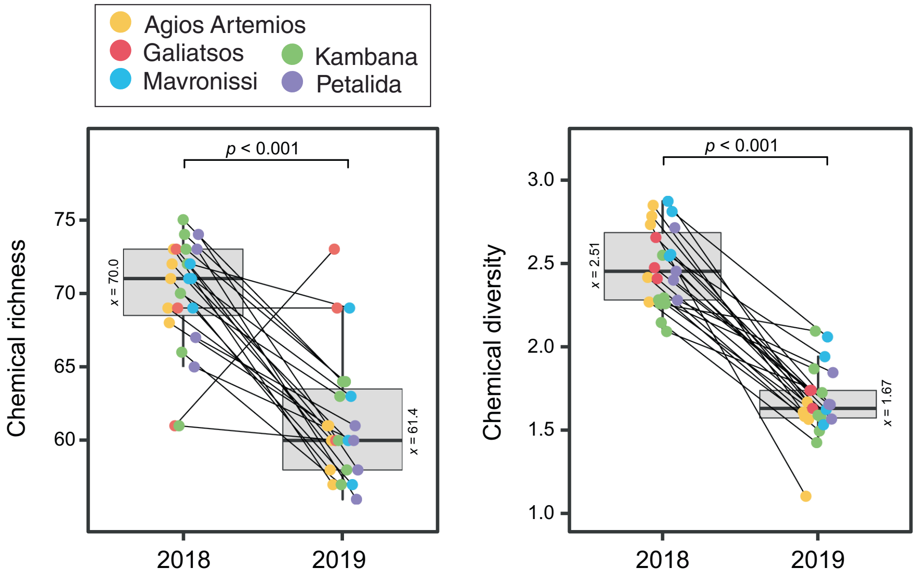

FIGURE 3 Fluctuations in the chemical richness and diversity of lizard scent marks. Boxplots overlayed with raw data points illustrate the significant decrease in the chemical richness and diversity of scent marks over time across replicate lizard populations. The plots display the distribution of the data, with average values plotted adjacent to each box. Individual data points sampled in 2018 and 2019 are connected with a thin black line, providing a visual representation of individual lizard trajectories over time. Each population is distinguished by a unique colour.

in lepidopterans (Nieberding et al., 2012; Umbers et al., 2015) and mammals (Ferkin, 2010; Müller-Schwarze, 1971; Osada et al., 2003, 2008). In lizards, age-related variation in scent mark composition has been reported in two species: the lacertids *Acanthodactylus erythrurus* (López & Martín, 2005) and *Psammodromus algirus* (Martín & López, 2006a). Although these researchers compared lizards among age classes rather than tracking individuals over time, they observed that older *P. algirus* males exhibited lower proportions of oleic acid in their scent marks compared to younger males; this finding is in line with our longitudinal study of *P. erhardii* showing that individuals secreted lower proportions of oleic acid in 2019 compared to the year before. Due to its design, our study additionally provides evidence that age-related variation in lizard scent mark composition is not due to differential survival—a conclusion that could not be drawn from these earlier studies. Such temporal changes in scent mark composition may arise simply from the physiological or hormonal changes inherently associated with aging (Patnaik, 1994), such as a reduced immune response, increased metabolic rate, and elevated testosterone levels (Arslan et al., 1980; Cox et al., 2005; Massot et al., 2011; Plasman et al., 2019). Moreover, ontogenetic dietary shifts (Ballinger et al., 1977; Best & Pfaffenberger, 1987; Durtsche, 2000) can influence the chemical signal profiles of lizards (García-Roa, Sáiz, et al., 2017; Kopena et al., 2011, 2014; Martín & López, 2006c). Irrespective of the proximate mechanisms, age-related differences in chemical composition of lizard scent marks can have significant functional implications, as they can be detected by conspecifics and influence on species closely related to *P. erhardii* have demonstrated that oleic acid can elicit strong chemosensory responses (Cooper et al., 2002) and that females can discriminate between males of different age classes based solely on the scent of their secretions (Martín & López, 2013; Nisa Ramiro et al., 2019). This ability allows female *Iberolacerta cyreni* lizards to actively choose their preferred older mates (López et al., 2003), whose longevity attests to their viability (Kokko, 1998; Trivers, 1972). Note that López et al. (2003) did not account for body size in their analyses, which may conflate the effects of size and age. Further behavioural research is needed to first test whether *P. erhardii* can use chemosensory cues to differentiate the observed temporal differences in scent mark composition, and second, to determine whether and how conspecifics use this ability for individual assessment.

An alternative explanation for the observed temporal variation in scent mark composition is a shift in diet, independent of ontogeny. In some animal species, certain components and precursors of their chemical signals are sequestered from their diet (Martín & Lopez, 2014; Wyatt, 2003). Tocopherols in lizard scent marks, for instance, are derived from plants and are acquired by consuming plant material, phytophagous arthropods, or both (García-Roa, Sáiz, et al., 2017; Kopena et al., 2011; Martín & López, 2015; Weldon et al., 2008, p. 2015). The production of these diet-derived signals depends on resource availability and is, therefore, susceptible to environmental influences. Consequently, temporal changes in arthropod abundance on our experimental islets may have spurred dietary shifts in the lizards, which, in turn, altered the chemical composition of their scent marks from 1 year to the next. Although we lack the appropriate data on prey availability and lizard diet to statistically test this assumption, we can still reflect on the likelihood of diet-dependent temporal changes within our study system. Generally, lacertid lizards are opportunistic mesopredators, so their diet typically reflects local prey availability (Arnold, 1987). As arthropod abundance is sensitive to climate extremes (Dalton et al., 2023; Soroye et al., 2020; Uhl et al., 2022), prey availability for lizards can decline during unusually warm and dry seasons. During our study period, the spring of 2018 in the south Aegean was significantly drier than that of 2019 (Figure S3), with no precipitation in April and May, possibly affecting the arthropod communities on our experimental islets. Since all islets experienced the same drought, the potential climatic effect on prey availability, and consequently on lizard diet, would be consistent across islets; if this scenario holds true, it could explain the consistent temporal change in overall glandular secretion composition across the replicate islets. The observed temporal decline in chemical richness and diversity of lizard scent marks suggests a decrease in diet diversity over time (Baeckens, García-Roa, et al., 2017). A higher diet diversity in the first year of sampling may have resulted from a drought-induced decrease in arthropod abundance, forcing lizards to expand their dietary range to ensure sufficient food intake. The Aegean wall lizard is known to adapt its feeding repertoire to environment-induced changes, such as preying on clumped preys during summer (Adamopoulou et al., 1999) or adopting oophagy, frugivory and even cannibalism during periods of food scarcity (Brock et al., 2014; Madden & Brock, 2018). Conversely, if arthropod abundance increased in 2019, lizards may have been able to be more selective in choosing their preferred prey, in line with ecological theory (Roughgarden, 1972; Schoener, 1971), leading to a decrease in diet diversity from 2018 to 2019. Of course, the relationship between lizard prey selectivity and local prey abundance is likely more complex and may involve additional nuances (Pyke, 1984). Our observation of temporally stable levels of α-tocopherol suggests that there was no significant temporal change in the lizards' vitamin E-rich diet over time (García-Roa, Sáiz, et al., 2017; Kopena et al., 2011). Unfortunately, the biosynthesis pathways of most lipophilic compounds found in the scent marks of lizards remain unknown, hindering a thorough assessment of the significance of dietary variation. Further investigation is required to determine whether individual signal compounds are synthesized de novo, or acquired through sequestration or simple chemical modification of dietary compounds. Note that not only the diversity of food consumed but also the amount of food eaten might influence scent mark composition, as food deprivation is known to affect hormone levels in wild lizards (Dunlap, 1995).

Other environmental factors can alter an animal's physiological state and induce changes in individual scent marks, such as thermal habitat (Boullis et al., 2016), pathogens (Rantala et al., 2002), and interactions with conspecifics (Guo et al., 2020) and predators (Verheggen et al., 2010). This is also true for lizards. First, changes in thermal habitat can influence the time lizards spend at optimal body temperatures, affecting their average metabolic rates and the rates at which chemicals are sequestered, metabolized, and secreted as scent marks (Heathcote et al., 2014). Although slight variations in thermal habitat quality among our experimental islets (Pafilis et al., 2019) may explain the minor differences in scent mark composition among populations, there is no clear indication that the thermal habitat (e.g. exposure to sunlight; Figure S3) has changed over time and in a similar way on all islets, causing alternations in the lizards' scent mark composition. Second, changes in the degree of parasite infestation can alter the composition of lizard scent marks (Martín et al., 2008; Martín, López, et al., 2007), likely due to the activation of the immune system affecting the allocation of certain compounds to the glandular secretions. As we lack data on lizard ecto- and endoparasite counts for our study populations, it is uncertain whether a consistent shift in parasite infestation has occurred across replicate populations. Third, increases in agonistic interactions and predatory encounters can elevate levels of corticosterone and testosterone in male lizards (Knapp & Moore, 1995, 1996; Thaker et al., 2009), inducing changes to the chemical composition of their scent marks, such as decreased proportions of octadecanoic acid (Aragón et al., 2008) and cholesta-5,7-dien-3-ol (Martín, López, et al., 2007). Although the islets have remained predator-free throughout the study, agonistic encounters may have fluctuated from year-to-year due to changes in population densities. Our annual lizard capture efforts indicate that population densities changed on average 23% (range: 11%–34%) between 2018 and 2019 (Figure S4). Overall, the number of lizards caught decreased, but slightly increased on one of the islets (i.e. Mavronissi). Density-dependent changes in antagonistic interactions over time may have driven temporal changes in lizard scent mark composition. The variability in density change across replicate islets may partly explain the complex changes in the proportions of the three socially relevant signalling compounds.

These potential proximate mechanisms of temporal dynamics in scent mark composition remain speculative and require further research to ultimately determine whether the observed variability is functionally significant (e.g. providing information on age status) or merely a nonadaptive response to environmental variation. Regardless of the mechanisms, understanding the flexibility of phenotypic traits, as examined in this study, is key for uncovering how individuals respond to novel or changing environments (Merilä & Hendry, 2014). Such flexibility may promote the evolution of functional traits by enabling flexible individuals to colonize environments that would be inaccessible for less flexible individuals (Price et al., 2003). Conversely, it may slow down trait evolution by sparing individuals from the forces of selection, as flexibility allows them to express the phenotype required in given environment (Price et al., 2003). In addition to conducting behavioural chemosensory assays, we plan to continue monitoring the experimental populations to assess the long-term dynamics of lizard chemical signals and their social relevance within the population.

## AUTHOR CONTRIBUTIONS

Simon Baeckens and Colin M. Donihue designed the study; Anthony Herrel, Panayiotis Pafilis and Colin M. Donihue collected the data; José Martín conducted chemical analyses; Simon Baeckens analysed the data and wrote the manuscript. All authors aided in interpreting the results and contributed to editing the final paper.

## ACKNOWLEDGEMENTS

We thank K Brock, M Vasilopoulou-Kampitsi, A-C Fabre and C Santoro for help in the field. We also thank Oru Kayak who donated two boats for this project and to Giannis Bizas for generously allowing us to work on his islands. Finally, we thank the anonymous reviewers and associate editor Pau Carazo for the valuable input.

## FUNDING INFORMATION

This work was funded by an FWO (12I8822N) and Belgian American Educational Foundation Postdoctoral Fellowship (S.B.), a National Geographic Waitt Grant (C.M.D.), a grant from the Yale Institute of Biospheric Studies (C.M.D.), an NSF Postdoctoral Fellowship 1609284 (C.M.D.), financial support from MINECO CGL2014-53523-P (J.M.), and an ATM MNHN grant (A.H.). In addition, 65 donors contributed to a crowdfunding campaign by C.M.D. on www.experiment.com; without their support, this project would not have been possible.

## CONFLICT OF INTEREST STATEMENT

Anthony Herrel is an Associate Editor of *Functional Ecology* but took no part in the peer review and decision-making processes for this paper.

## DATA AVAILABILITY STATEMENT

Data accessible on Figshare https://doi.org/10.6084/m9.figshare.16586315.v1 (Baeckens et al., 2024).

## ORCID

*Simon Baeckens* https://orcid.org/0000-0003-2189-9548
*José Martín* https://orcid.org/0000-0001-6648-3998
*Panayiotis Pafilis* https://orcid.org/0000-0002-1832-4906
*Anthony Herrel* https://orcid.org/0000-0003-0991-4434
*Colin M. Donihue* https://orcid.org/0000-0003-1096-8536

## REFERENCES

Adamopoulou, C., Valakos, E. D., & Pafilis, P. (1999). Summer diet of *Podarcis milensis*, *P. gaigeae* and *P. erhardii* (Sauria: Lacertidae). *Bonner Zoologische Beiträge*, *48*, 275–282.

Aebischer, N., Robertson, P., & Kenward, R. (1993). Compositional analysis of habitat use from animal radio-tracking data. *Ecology*, *74*, 131–1325.

Aragón, P., López, P., & Martín, J. (2008). Increased predation risk modifies lizard scent-mark chemicals. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *309*, 427–433.

Arnold, E. N. (1987). Resource partition among lacertid lizards in southern Europe. *Journal of Zoology*, *1*, 739–782.

Arslan, M., Bint Akhtar, F., Zaidi, P., & Qazi, M. H. (1980). Age-related changes in testicular histology and androgen levels of the immature spiny-tailed lizard, *Uromastix hardwicki*. *General and Comparative Endocrinology*, *42*, 63–70.

Baeckens, S. (2019). Evolution of animal chemical communication: Insights from non-model species and phylogenetic comparative methods. *Belgian Journal of Zoology*, *149*, 63–93.

Baeckens, S., García-Roa, R., Martín, J., & Van Damme, R. (2017). The role of diet in shaping the chemical signal design of lacertid lizards. *Journal of Chemical Ecology*, *43*, 902–910.

Baeckens, S., Huyghe, K., Palme, R., & Van Damme, R. (2017). Chemical communication in the lacertid lizard *Podarcis muralis*: The functional significance of testosterone. *Acta Zoologica*, *98*, 94–103.

Baeckens, S., Martín, J., García-Roa, R., Pafilis, P., Huyghe, K., & Van Damme, R. (2018). Environmental conditions shape the chemical signal design of lizards. *Functional Ecology*, *32*, 566–580.

Baeckens, S., Martín, J., García-Roa, R., & Van Damme, R. (2018). Sexual selection and the chemical signal design of lacertid lizards. *Zoological Journal of the Linnean Society*, *183*, 445–457.

Baeckens, S., Martín, J., Pafilis, P., Herrel, A., & Donihue, C. M. (2024). Data from: Temporal dynamics of scent mark composition in field-experimental lizard populations. *Figshare*, https://doi.org/10.6084/m9.figshare.16586315.v1

Baeckens, S., & Whiting, M. J. (2021). Investment in chemical signalling glands facilitates the evolution of sociality in lizards. *Proceedings of the Royal Society B: Biological Sciences*, *288*, 20202438.

Ballinger, R. E., Newlin, M. E., & Newlin, S. J. (1977). Age-specific shift in the diet of the crevice spiny lizard, *Sceloporus poinsetti* in southwestern New Mexico. *American Midland Naturalist*, *97*, 482.

Bartoń, K. (2023). *MuMIn: Multi-model inference*. CRAN.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48.

Beckers, O. M., & Wagner, W. E. (2011). Male field crickets infested by parasitoid flies express phenotypes that may benefit the parasitoids. *Animal Behaviour*, *82*, 1151–1157.

Best, T. L., & Pfaffenberger, G. S. (1987). Age and sexual variation in the diet of collared lizards (*Crotaphytus collaris*). *The Southwestern Naturalist*, *32*, 415.

Boullis, A., Detrain, C., Francis, F., & Verheggen, F. J. (2016). Will climate change affect insect pheromonal communication? *Current Opinion in Insect Science*, *17*, 87–91.

Brock, K. M., Baeckens, S., Donihue, C. M., Martín, J., Pafilis, P., & Edwards, D. L. (2020). Trait differences among discrete morphs of a color polymorphic lizard, *Podarcis erhardii*. *PeerJ*, *8*, e10284.

Brock, K. M., Donihue, C. M., & Pafilis, P. (2014). New records of frugivory and ovophagy in *Podarcis* (Lacertidae) lizards from East Mediterranean Islands. *North-Western Journal of Zoology*, *10*, 223–225.

Campos, S. M., Strauss, C., & Martins, E. P. (2017). In space and time: Territorial animals are attracted to conspecific chemical cues. *Ethology*, *123*, 136–144.

Cole, C. (1966). Femoral glands in lizards: A review. *Herpetologica*, *22*, 199–205.

Cooper, W. E., Pérez-Mellado, V., & Vitt, L. J. (2002). Lingual and biting responses to selected lipids by the lizard *Podarcis lilfordi*. *Physiology & Behavior*, *75*, 237–241.

Cox, R. M., Skelly, S. L., Leo, A., & John-Alder, H. B. (2005). Testosterone regulates sexually dimorphic coloration in the eastern fence lizard, *Sceloporus undulatus*. *Copeia*, *2005*, 597–608.

Dalton, R. M., Underwood, N. C., Inouye, D. W., Soulé, M. E., & Inouye, B. D. (2023). Long-term declines in insect abundance and biomass in a subalpine habitat. *Ecosphere*, *14*, e4620.

De Pasqual, C., Groot, A. T., Mappes, J., & Burdfield-Steel, E. (2021). Evolutionary importance of intraspecific variation in sex pheromones. *Trends in Ecology & Evolution*, *36*, 848–859.

Diamond, M. (1986). Overview: Laboratory experiments, field experiments and natural experiments. In J. Diamond & T. J. Case (Eds.), *Community ecology* (pp. 3–22). Harper and Row.

Donihue, C. M., Herrel, A., Foufopoulos, J., & Pafilis, P. (2022). Body condition and jumping predict initial survival in a replicated Island introduction experiment. *Biological Journal of the Linnean Society*, *135*, 490–498.

Donihue, C. M., Herrel, A., Martín, J., Foufopoulos, J., Pafilis, P., & Baeckens, S. (2020). Rapid and repeated divergence of animal chemical signals in an Island introduction experiment. *Journal of Animal Ecology*, *89*, 1458–1467.

Donihue, C. M., Herrel, A., Taverne, M., Foufopoulos, J., & Pafilis, P. (2023). The evolution of diet and morphology in insular lizards: Insights from a replicated Island introduction experiment. *Animals*, *13*, 1788.

Dunlap, K. D. (1995). Hormonal and behavioral responses to food and water deprivation in a lizard (*Sceloporus occidentalis*): Implications for assessing stress in a natural population. *Journal of Herpetology*, *29*, 345.

Durtsche, R. D. (2000). Ontogenetic plasticity of food habits in the Mexican spiny-tailed iguana, *Ctenosaura pectinata*. *Oecologia*, *124*, 185–195.

Evans, S. R., Gustafsson, L., & Sheldon, B. C. (2011). Divergent patterns of age-dependence in ornamental and reproductive traits in the collared flycatcher: Aging of ornaments and reproductive traits. *Evolution*, *65*, 1623–1636.

Ferkin, M. H. (2010). Age affects over-marking of opposite-sex scent Marks in meadow voles, *Microtus pennsylvanicus*. *Ethology*, *116*, 24–31.

Fögen, T. (2014). Animal communication. In G. L. Campbell (Ed.), *The Oxford handbook of animals in classical thought and life* (pp. 216–232). Oxford University Press.

Fox, R. J., Donelson, J. M., Schunter, C., Ravasi, T., & Gaitán-Espitia, J. D. (2019). Beyond buying time: The role of plasticity in phenotypic adaptation to rapid environmental change. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *374*, 20180174.

García-Roa, R., Jara, M., Baeckens, S., López, P., Van Damme, R., Martín, J., & Pincheira-Donoso, D. (2017). Macroevolutionary diversification of glands for chemical communication in squamate reptiles. *Scientific Reports*, *7*, 9288.

García-Roa, R., Sáiz, J., Gómara, B., López, P., & Martín, J. (2017). Dietary constraints can preclude the expression of an honest chemical sexual signal. *Scientific Reports*, *7*, 6073.

García-Roa, R., Sáiz, J., Gómara, B., López, P., & Martín, J. (2018). How to tackle chemical communication? Relative proportions versus semi-quantitative determination of compounds in lizard chemical secretions. *Ecology and Evolution*, *8*, 2032–2040.

Groot, A. T., Blankers, T., Halfwerk, W., & Burdfield Steel, E. (2024). The evolutionary importance of intraspecific variation in sexual communication across sensory modalities. *Annual Review of Entomology*, *69*, 21–40.

Guo, X., Yu, Q., Chen, D., Wei, J., Yang, P., Yu, J., Wang, X., & Kang, L. (2020). 4-Vinylanisole is an aggregation pheromone in locusts. *Nature*, *584*, 584–588.

Heathcote, R. J. P., Bell, E., d'Ettorre, P., While, G. M., & Uller, T. (2014). The scent of sun worship: Basking experience alters scent mark composition in male lizards. *Behavioral Ecology and Sociobiology*, *68*, 861–870.

Hendry, A. P. (2017). *Eco-evolutionary dynamics*. Princeton University Press.

Khannoon, E. R., El-Gendy, A., & Hardege, J. D. (2011). Scent marking pheromones in lizards: Cholesterol and long chain alcohols elicit avoidance and aggression in male *Acanthodactylus boskianus* (Squamata: Lacertidae). *Chemoecology*, *21*, 143–149.

Knapp, R., & Moore, M. (1995). Hormonal responses to aggression vary in different types of agonistic encounters in male tree lizards, *Urosaurus ornatus*. *Hormones and Behavior*, *29*, 85–105.

Knapp, R., & Moore, M. C. (1996). Male morphs in tree lizards, *Urosaurus ornatus*, have different delayed hormonal responses to aggressive encounters. *Animal Behaviour*, *52*, 1045–1055.

Kokko, H. (1998). Good genes, old age and life-history trade-offs. *Evolutionary Ecology*, *12*, 739–750.

Kopena, R., López, P., & Martín, J. (2014). Relative contribution of dietary carotenoids and vitamin E to visual and chemical sexual signals of male Iberian green lizards: An experimental test. *Behavioral Ecology and Sociobiology*, *68*, 571–581.

Kopena, R., Martín, J., López, P., & Herczeg, G. (2011). Vitamin E supplementation increases the attractiveness of males' scent for female European green lizards. *PLoS One*, *6*, e19410.

López, P., Amo, L., & Martín, J. (2006). Reliable signaling by chemical cues of male traits and health state in male lizards, *Lacerta monticola*. *Journal of Chemical Ecology*, *32*, 473–488.

López, P., Aragón, P., & Martín, J. (2003). Responses of female lizards, *Lacerta monticola*, to males' chemical cues reflect their mating preference for older males. *Behavioral Ecology and Sociobiology*, *55*, 73–79.

López, P., Gabirot, M., & Martín, J. (2009). Immune activation affects chemical sexual ornaments of male Iberian wall lizards. *Naturwissenschaften*, *96*, 65–69.

López, P., & Martín, J. (2005). Age related differences in lipophilic compounds found in femoral gland secretions of male spiny-footed lizards, *Acanthodactylus erythrurus*. *Zeitschrift fur Naturforschung—Section C, Journal of Biosciences*, *60*, 915–920.

López, P., & Martín, J. (2012). Chemosensory exploration of male scent by female rock lizards result from multiple chemical signals of males. *Chemical Senses*, *37*, 47–54.

Lymberakis, P., Pafilis, P., Poulakakis, N., Sotiropoulos, K., & Valakos, E. D. (2018). The amphibians and reptiles of the Aegean Sea. In S. Sfenthourakis, P. Pafilis, A. Parmakelis, N. Poulakakis, & K. A. Triantis (Eds.), *Biogeography and biodiversity of the Aegean. In honour of prof. Moysis Mylonas* (pp. 169–189). Broken Hill Publishers Ltd.

MacGregor, H. E. A., Lewandowsky, R. A. M., D'Ettorre, P., Leroy, C., Davies, N. W., While, G. M., & Uller, T. (2017). Chemical communication, sexual selection, and introgression in wall lizards. *Evolution*, *71*, 2327–2343.

Madden, I. E., & Brock, K. M. (2018). An extreme case of cannibalism in *Podarcis erhardii mykonensis* (Reptilia: Lacertidae) from Siros Island, Cyclades, Greece. *Herpetology Notes*, *11*, 291–292.

Mangiacotti, M., Baeckens, S., Fumagalli, M., Martín, J., Scali, S., & Sacchi, R. (2023). Protein–lipid association in lizard chemical signals. *Integrative Organismal Biology*, *5*, obad016.

Mangiacotti, M., Baeckens, S., Scali, S., Martín, J., Van Damme, R., & Sacchi, R. (2021). Evolutionary and biogeographical support for species-specific proteins in lizard chemical signals. *Biological Journal of the Linnean Society*, *134*, 912–928.

Martín, J., Amo, L., & López, P. (2008). Parasites and health affect multiple sexual signals in male common wall lizards, *Podarcis muralis*. *Naturwissenschaften*, *95*, 293–300.

Martín, J., Civantos, E., Amo, L., & López, P. (2007). Chemical ornaments of male lizards *Psammodromus algirus* may reveal their parasite load and health state to females. *Behavioral Ecology and Sociobiology*, *62*, 173–179.

Martín, J., & López, P. (2000). Chemoreception, symmetry and mate choice in lizards. *Proceedings of the Royal Society B: Biological Sciences*, *267*, 1265–1269.

Martín, J., & López, P. (2006a). Age-related variation in lipophilic chemical compounds from femoral gland secretions of male lizards *Psammodromus algirus*. *Biochemical Systematics and Ecology*, *34*, 691–697.

Martín, J., & López, P. (2006b). Links between male quality, male chemical signals, and female mate choice in Iberian rock lizards. *Functional Ecology*, *20*, 1087–1096.

Martín, J., & López, P. (2006c). Vitamin D supplementation increases the attractiveness of males' scent for female Iberian rock lizards. *Proceedings of the Royal Society B: Biological Sciences*, *273*, 2619–2624.

Martín, J., & López, P. (2007). Scent may signal fighting ability in male Iberian rock lizards. *Biology Letters*, *3*, 125–127.

Martín, J., & López, P. (2010a). Condition-dependent pheromone signaling by male rock lizards: More oily scents are more attractive. *Chemical Senses*, *35*, 253–262.

Martín, J., & López, P. (2010b). Multimodal sexual signals in male ocellated lizards *Lacerta lepida*: Vitamin E in scent and green coloration may signal male quality in different sensory channels. *Naturwissenschaften*, *97*, 545–553.

Martín, J., & López, P. (2013). Responses of female rock lizards to multiple scent marks of males: Effects of male age, male density and scent over-marking. *Behavioural Processes*, *94*, 109–114.

Martín, J., & Lopez, P. (2014). Pheromones and chemical communication in lizards. In J. L. Rheubert, D. S. Siegel, & S. E. Trauth (Eds.), *Reproductive biology and phylogeny of lizards and tuatara* (pp. 43–77). CRC Press.

Martín, J., & López, P. (2015). Condition-dependent chemosignals in reproductive behavior of lizards. *Hormones and Behavior*, *68*, 14–24.

Martín, J., López, P., Gabirot, M., & Pilz, K. M. (2007). Effects of testosterone supplementation on chemical signals of male Iberian wall lizards: Consequences for female mate choice. *Behavioral Ecology and Sociobiology*, *61*, 1275–1282.

Mason, R. T., & Parker, M. R. (2010). Social behavior and pheromonal communication in reptiles. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology*, *196*, 729–749.

Massot, M., Clobert, J., Montes-Poloni, L., Haussy, C., Cubo, J., & Meylan, S. (2011). An integrative study of ageing in a wild population of common lizards: Integrative study of ageing. *Functional Ecology*, *25*, 848–858.

Mayerl, C., Baeckens, S., & Van Damme, R. (2015). Evolution and role of the follicular epidermal gland system in non-ophidian squamates. *Amphibia-Reptilia*, *36*, 185–206.

McGraw, K. J., & Ardia, D. R. (2003). Carotenoids, immunocompetence, and the information content of sexual colors: An experimental test. *The American Naturalist*, *162*, 704–712.

Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. *Evolutionary Applications*, *7*, 1–14.

Morton, F. B., & Altschul, D. (2019). Data reduction analyses of animal behaviour: Avoiding Kaiser's criterion and adopting more robust automated methods. *Animal Behaviour*, *149*, 89–95.

Müller-Schwarze, D. (1971). Pheromones in black-tailed deer (*Odocoileus hemionus columbianus*). *Animal Behaviour*, *19*, 141–152.

Nieberding, C. M., Fischer, K., Saastamoinen, M., Allen, C. E., Wallin, E. A., Hedenström, E., & Brakefield, P. M. (2012). Cracking the olfactory code of a butterfly: The scent of ageing. *Ecology Letters*, *15*, 415–424.

Nisa Ramiro, C., Rodríguez-Ruiz, G., López, P., Da Silva Junior, P. I., Trefaut Rodrigues, M., & Martín, J. (2019). Chemosensory discrimination of male age by female *Psammodromus algirus* lizards based on femoral secretions and feces. *Ethology*, *125*, 802–809.

Ord, T. J., Peters, R. A., Clucas, B., & Stamps, J. A. (2007). Lizards speed up visual displays in noisy motion habitats. *Proceedings of the Royal Society B: Biological Sciences*, *274*, 1057–1062.

Osada, K., Tashiro, T., Mori, K., & Izumi, H. (2008). The identification of attractive volatiles in aged male mouse urine. *Chemical Senses*, *33*, 815–823.

Osada, K., Yamazaki, K., Curran, M., Bard, J., Smith, B. P. C., & Beauchamp, G. K. (2003). The scent of age. *Proceedings of the Royal Society of London B*, *270*, 929–933.

Pafilis, P., Herrel, A., Kapsalas, G., Vasilopoulou-Kampitsi, M., Fabre, A.-C., Foufopoulos, J., & Donihue, C. M. (2019). Habitat shapes the thermoregulation of Mediterranean lizards introduced to replicate experimental islets. *Journal of Thermal Biology*, *84*, 368–374.

Patnaik, B. (1994). Aging in reptiles. *Gerontology*, *40*, 200–220.

Patricelli, G. L., Krakauer, A. H., & Taff, C. C. (2016). Variable signals in a complex world: Shifting views of within-individual variability in sexual display traits. *Advances in the Study of Behavior*, *48*, 319–386.

Plasman, M., Sandoval-Zapotitla, E., & Torres, R. (2019). Immune response declines with age in a wild lizard. *Biological Journal of the Linnean Society*, *128*, 936–943.

Price, T. D., Qvarnström, A., & Irwin, D. E. (2003). The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London B*, *270*, 1433–1440.

Pruett, J. A., Zúñiga-Vega, J. J., Campos, S. M., Soini, H. A., Novotny, M. V., Vital-García, C., Martins, E. P., & Hews, D. K. (2016). Evolutionary interactions between visual and chemical signals: Chemosignals compensate for the loss of a visual signal in male Sceloporus lizards. *Journal of Chemical Ecology*, *42*, 1164–1174.

Pyke, G. H. (1984). Optimal foraging theory: A critical review. *Annual Review of Ecology and Systematics*, *15*, 523–575.

R Core Team. (2024). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Rantala, M. J., Jokinen, I., Kortet, R., Vainikka, A., & Suhonen, J. (2002). Do pheromones reveal male immunocompetence? *Proceedings of the Royal Society B: Biological Sciences*, *269*, 1681–1685.

Romero-Diaz, C., Campos, S. M., Herrmann, M. A., Lewis, K. N., Williams, D. R., Soini, H. A., Novotny, M. V., Hews, D. K., & Martins, E. P. (2020). Structural identification, synthesis and biological activity of two volatile cyclic dipeptides in a terrestrial vertebrate. *Scientific Reports*, *10*, 4303.

Romero-Diaz, C., Campos, S. M., Herrmann, M. A., Soini, H. A., Novotny, M. V., Hews, D. K., & Martins, E. P. (2021). Composition and compound proportions affect the response to complex chemical signals in a spiny lizard. *Behavioral Ecology and Sociobiology*, *75*, 42.

Roughgarden, J. (1972). Evolution of niche width. *The American Naturalist*, *106*, 683–718.

Schoener, T. W. (1971). Theory of feeding strategies. *Annual Review of Ecology and Systematics*, *2*, 369–404.

Shannon, C. E. (1948). A mathematical theory of communication. *The Bell System Technical Journal*, *27*, 379–423, 623–656.

Siepielski, A. M., DiBattista, J. D., & Carlson, S. M. (2009). It's about time: The temporal dynamics of phenotypic selection in the wild. *Ecology Letters*, *12*, 1261–1276.

Soroye, P., Newbold, T., & Kerr, J. (2020). Climate change contributes to widespread declines among bumble bees across continents. *Science*, *367*, 685–688.

Stahr, C., Svatoš, A., & Seidelmann, K. (2013). Chemical identification, emission pattern and function of male-specific pheromones released by a rarely swarming locust, *Schistocerca americana*. *Journal of Chemical Ecology*, *39*, 15–27.

Symonds, M. R. E., & Elgar, M. A. (2008). The evolution of pheromone diversity. *Trends in Ecology & Evolution*, *23*, 220–228.

Thaker, M., Lima, S. L., & Hews, D. K. (2009). Alternative antipredator tactics in tree lizard morphs: Hormonal and behavioural responses to a predator encounter. *Animal Behaviour*, *77*, 395–401.

Trivers, R. (1972). Parental investment and sexual selection. In B. Campbell (Ed.), *Sexual selection and the descent of man* (pp. 136–179). Routledge.

Uhl, B., Wölfling, M., & Bässler, C. (2022). Mediterranean moth diversity is sensitive to increasing temperatures and drought under climate change. *Scientific Reports*, *12*, 14473.

Umbers, K. D. L., Symonds, M. R. E., & Kokko, H. (2015). The Mothematics of female pheromone signaling: Strategies for aging virgins. *The American Naturalist*, *185*, 417–432.

Valakos, E. (1986). The feeding ecology of *Podarcis erhardii* (Reptilia-Lacertidae) in a main insular ecosystem. *Herpetological Journal*, *1*, 118–121.

Verheggen, F. J., Haubruge, E., & Mescher, M. C. (2010). Alarm pheromones—Chemical signaling in response to danger. In G. Litwack (Ed.), *Vitamins and hormones* (pp. 215–239). Elsevier.

Weldon, P. J., Flachsbarth, B., & Schulz, S. (2008). Natural products from the integument of nonavian reptiles. *Natural Product Reports*, *25*, 738–756.

Wyatt, T. D. (2003). *Pheromones and animal behavior*. Cambridge University Press.

Zozaya, S. M., Higgie, M., Moritz, C., & Hoskin, C. J. (2019). Are pheromones key to unlocking cryptic lizard diversity? *The American Naturalist*, *194*, 168–182.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Figure S1:** Scree plot visualizing the percentage of variation explained by each principal component.

**Figure S2:** Illustration of how the relationship between lizard body size and the proportion of octadecanoic acid in lizard chemical secretions changes over time.

**Figure S3:** Historical climate data (2016–2023) for spring from Paros, the large island located <1-km from the experimental islets (excluding Petalida, with a distance of ~5-km).

**Figure S4:** Lizard capture efforts.

**Table S1:** The ten main classes of lipophilic compounds identified in the femoral gland secretions of adult male *Podarcis erhardii* lizards, analyzed using gas chromatography-mass spectrometry (GC-MS).

**Table S2:** Statistical output of the linear mixed-effect model (LMM) with PC1 or PC2 as response variable and ‘year’ and ‘population’ as interaction fixed effect, and ‘individual’ as random effect.

**Table S3:** Among-population variation in temporal change in PC2.

**Table S4:** Statistical output of the linear mixed-effect model (LMM) with chemical diversity or generalized LMM with chemical richness as response variable and ‘year’ and ‘population’ as interaction fixed effect, and ‘individual’ as random effect.

**Table S5:** Among-population variation in temporal change in chemical diversity.

**How to cite this article:** Baeckens, S., Martín, J., Pafilis, P., Herrel, A., & Donihue, C. M. (2024). Temporal dynamics of scent mark composition in field-experimental lizard populations. *Functional Ecology*, *00*, 1–11. https://doi.org/10.1111/1365-2435.14687