

Chemical communication, sexual selection, and introgression in wall lizards

Hannah E. A. MacGregor,^{1,2,3} Rachel A. M. Lewandowsky,^{1,2} Patrizia d’Ettorre,⁴ Chloé Leroy,⁴ Noel W. Davies,⁵ Geoffrey M. While,^{1,2} and Tobias Uller^{2,6}

¹School of Biological Sciences, University of Tasmania, Hobart, Tasmania 7001, Australia

²Department of Zoology, Edward Grey Institute, University of Oxford, Oxford OX1 3PS, United Kingdom

³E-mail: hannah.macgregor@utas.edu.au

⁴Laboratory of Experimental and Comparative Ethology, University of Paris 13, Sorbonne Paris Cité 93430, Villetaneuse, Paris, France

⁵Central Science Laboratory, University of Tasmania, Hobart, Tasmania 7001, Australia

⁶Department of Biology, Lund University, Sölvegatan 37 223 62, Lund, Sweden

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Divergence in communication systems should influence the likelihood that individuals from different lineages interbreed, and consequently shape the direction and rate of hybridization. Here, we studied the role of chemical communication in hybridization, and its contribution to asymmetric and sexually selected introgression between two lineages of the common wall lizard (*Podarcis muralis*). Males of the two lineages differed in the chemical composition of their femoral secretions. Chemical profiles provided information regarding male secondary sexual characters, but the associations were variable and inconsistent between lineages. In experimental contact zones, chemical composition was weakly associated with male reproductive success, and did not predict the likelihood of hybridization. Consistent with these results, introgression of chemical profiles in a natural hybrid zone resembled that of neutral nuclear genetic markers overall, but one compound in particular (tocopherol methyl ether) matched closely the introgression of visual sexual characters. These results imply that associations among male chemical profiles, sexual characters, and reproductive success largely reflect transient and environmentally driven effects, and that genetic divergence in chemical composition is largely neutral. We therefore suggest that femoral secretions in wall lizards primarily provide information about residency and individual identity rather than function as sexual signals.

KEY WORDS: Femoral pores, hybrid zone, hybridization, olfaction, pheromones.

Population divergence in sexual characters used in communication shapes interactions upon secondary contact, with potential evolutionary consequences (West-Eberhard 1983; Panhuis et al. 2001). For instance, where one lineage has evolved exaggerated sexual characters favored by sexual selection, this can result in asymmetric patterns of introgression (e.g., Parsons et al. 1993; Stein and Uy 2006; Baldassarre and Webster 2013). The majority of research on sexually selected introgression has focused on the role of traits attributed to intersexual selection (e.g., female choice; Ryan and Wagner 1987). However, allopatric divergence in traits that primarily function in intrasexual communication, including colors and morphological features used in competition

between males, can also contribute to hybridization and introgression (see Moore 1987; Loehr et al. 2008 as examples). For example, if aggression toward divergent male phenotypes is biased or relaxed in males of one or both lineages (e.g., Pauers et al. 2008), certain male phenotypes could have an advantage in accessing high-quality resources and females. Alternatively, differences in signals may be used to discriminate and avoid males of the other lineage (e.g., Simeonovska-Nikolova 2006). In both cases, divergence in male communication should mediate spatial organization within hybrid zones and, as a consequence, encounter rates between males and females of different lineages.

Studies of vertebrates demonstrate that an evolutionary history of strong intrasexual selection can cause males of one lineage to be consistently dominant over males of the other lineage, contributing to asymmetric genetic and phenotypic introgression (Pearson and Rohwer 2000; McDonald et al. 2001; Owen-Ashley and Butler 2004; Teeter et al. 2008; While et al. 2015). Male–male contests are often resolved through communication (Searcy and Nowicki 2005), hence divergence in signals or cues associated with dominance and resource holding potential could reinforce or mitigate asymmetric introgression. Within this context, the literature on sexually selected introgression has thus far focused largely on visual and vocal characters. This is unsurprising given that colors and song are considered reliable signals with well-established roles in male–male competition as well as female choice (e.g., Alonso-Alvarez 2004; Abrahams et al. 2005; Zeil et al. 2006; Hamilton et al. 2013). In contrast, the role of chemical communication in mediating patterns of introgression is less clear, despite that chemical communication is taxonomically widespread and functionally important in reproductive behaviour (Wyatt 2014).

Chemical communication is particularly prevalent in lizards. In many species, males deposit femoral secretions over their home range (Mason and Parker 2010). These secretions are chemically complex and their composition may mediate social interactions, territoriality, and reproduction (e.g., López and Martín 2002; Carazo et al. 2007), and ultimately play a key role in determining mating success (Mayerl et al. 2015). Furthermore, it is widely believed that the composition or prevalence of particular compounds have evolved robust associations with other phenotypic characters and hence serve as signals of male health and competitive ability, that is, function as badges of status (Martín et al. 2007; López et al. 2009). In lacertid lizards, for example, the proportions of cholesterol and campesterol have been shown to correlate positively with body size (López et al. 2006; Martín and López 2007), and higher proportions of cholesta-5,7-dien-3-ol, ergosterol and waxy esters have been associated with lower parasite loads and higher immune responses (López et al. 2006; Martín et al. 2008). This has led to the suggestion that divergence in chemical composition is functional, and may contribute to reduced or biased hybridization upon secondary contact (Gabirot et al. 2012; Garcia-Roa et al. 2016). However, direct evidence for this hypothesis is limited. A role for chemical communication in hybridization and introgression has also been inferred from behavioral experiments, suggesting that males discriminate between con- and heterospecifics based on chemical cues (e.g., Cooper and Garstka 1987; Martín and López 2006a; Gabirot et al. 2010), and the observation that hybridization between chemically divergent but sympatric species is rare under natural conditions (Carretero 2008).

We studied the role of chemical communication in male dominance, spatial organization, and hybridization between two lineages of the common wall lizard, *Podarcis muralis* (Laurenti

1768). These lineages are native to north-central Italy and Western Europe, and have come together in several zones of secondary contact as a result of natural and human-mediated range expansion (While et al. 2015). Phenotypic divergence between the lineages is indicative of differences in the strength of sexual selection on morphology, coloration, and behavior (Heathcote et al. 2016; MacGregor et al. 2017). Hybridization is asymmetric, with evidence for adaptive introgression of visual sexual characters from the dominant Italian lineage into the Western European lineage (While et al. 2015). If chemical communication is also sexually selected then we predict (i) divergence in chemical characters between the lineages, (ii) consistent associations between chemical composition and male secondary sexual characters, and reproductive success, especially in the Italian lineage where sexual selection has been more intense, and (iii) clines in chemical profiles across the contact zone that resemble other sexually selected traits.

To test these predictions, we first established the extent of divergence in chemical profiles between lineages and associations with other male phenotypic traits. Second, we tested experimentally if the compositions of femoral secretions are associated with spatial organization, reproductive success, and hybridization in experimentally replicated zones of secondary contact. Finally, we examined the pattern of introgression of chemical profiles across a zone of secondary contact and tested if they corresponded to the patterns of sexually selected introgression previously demonstrated for morphology and coloration (While et al. 2015).

Methods

STUDY SPECIES

Common wall lizards, *P. muralis*, are small (45–75 mm snout–vent length [SVL]), diurnal lizards that inhabit a range of natural and human-modified habitats across southern and central Europe. Intraspecific diversity is high with several genetically and geographically distinct mitochondrial clades (Giovannotti et al. 2010; Schulte et al. 2012; Salvi et al. 2013). The lineages in this study represent two major mitochondrial clades that diverged in glacial refugia approximately two million years ago (Gassert et al. 2013; Salvi et al. 2013). Here, we refer to them as the Italian (ITA, here specifically corresponding to the Tuscan subclade *sensu* Schulte et al. 2012) and the Western European (WEUR) lineages. As well as being genetically differentiated, the populations of the Italian and Western European lineages studied here differ substantially in morphology and coloration, in particular in male secondary sexual characters (e.g., relative head size, bite force, testes mass, outer ventral scale UV-blue reflectance, While et al. 2015; MacGregor et al. 2017). Ventral color polymorphism (red/yellow/white) is present in some *P. muralis* populations, however, the influence of color polymorphism does not represent a target for our analyses because color morphs are at low frequencies in the Western

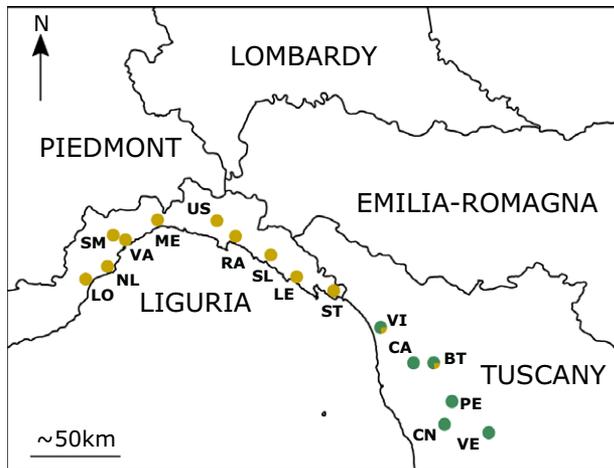


Figure 1. Map of the natural contact zone in northern Italy to show the locations of the sixteen populations sampled for cline analyses. Green and brown dots indicate association to the Italian and Western European mitochondrial lineages, respectively (data from While et al. 2015). Populations VI and BT have a mix of Italian and Western European haplotypes, and approximate the center of the contact zone.

European lineage and absent in Italian lineage (Uller et al. unpubl. data).

CHEMICAL SAMPLING AND ANALYSIS

Chemical sampling and phenotypic measurements

We captured 172 sexually mature males during their first seasonal reproductive episode (April–May) across three consecutive years (2013–2015). Sixty-four males were captured for use in our enclosure experiment (hereafter referred to as experimental males) from allopatric populations in Italy and Western Europe (to avoid the confounding effects of introgression; Table S1). One hundred and eight were captured from 16 populations in northern Italy (Fig. 1; Table S1) to test for patterns of chemical introgression (hereafter referred to as cline males). The 16 populations form a cline across a natural hybrid zone with an mtDNA center near Pisa in Tuscany (While et al. 2015).

We collected secretions from the femoral glands of all males by gently pressing around their femoral pores with sterilized forceps. For each male, secretions were collected directly into a glass vial (1.5 mL screw thread vials, Sigma-Aldrich, St. Louis, MO, USA). All samples from cline males were collected in the field immediately following capture. For experimental males, we collected two secretion samples from each individual to assess within-individual repeatability in chemical composition. The first sample was collected following their capture (April: half of individuals immediately upon capture, and the remaining prior to the release of males into the enclosures, see next), and the second between 49 and 75 days later (in June), immediately upon capture following the enclosure experiment. The secretion samples were

stored cold while in the field and then at -20°C until chemical extraction. In addition to femoral secretions, we also recorded a number of morphometric measurements and obtained tissue samples from each lizard for genetic analyses by removing the tip of the tail, which was preserved in 90% ethanol. Morphometric measurements included SVL (measured with a ruler to the nearest millimeter), body mass (measured to the nearest 0.01 g using digital scales), head length and head width (recorded to the nearest 0.1 mm with callipers), ventral blackness, and dorsal greenness. From the experimental males, we additionally measured testes mass, outer ventral scale color (OVS blue area, OVS hue, and OVS UV chroma), and a performance trait (maximum bite force) in the laboratory (see While et al. 2015; MacGregor et al. 2017 for full methods regarding morphology data).

Chemical extraction and identification

All secretion samples were dissolved in pentane and analyzed by gas chromatography mass spectrometry (GC-MS) with an Agilent Technologies 7890A gas chromatograph equipped an Agilent HP-5MS capillary column (30 m \times 0.25 mm \times 0.25 μm) with helium as carrier gas at 1 mL/min. The oven temperature was programmed at 50°C for 1 min, increased to 180°C at $30^{\circ}\text{C}/\text{min}$, then to 250°C at $10^{\circ}\text{C}/\text{min}$ and finally to 320°C at $5^{\circ}\text{C}/\text{min}$ and kept at 320°C for 30 min (total run time per sample = 33.3 min). The GC was coupled with an Agilent 5975 C mass spectrometer with 70 eV electron impact ionization.

Where possible, we identified chemical compounds within the samples on the basis of their mass spectra and retention times, which we verified using a computerized mass spectral library (National Institute for Standards and Technology 2008), and the assistance of an analytical chemist (NWD). Relative retention times were also used to assist in compound identification. When the identity of a compound was uncertain, we added the mass spectrum to an “in house” database for recognition across samples. As in previous reports on lizard secretions, including for *P. muralis* (Pellitteri-Rosa et al. 2014), many steroids could not be specifically identified and are reported by their characteristic retention times and ions. In total, we characterized 67 compounds in the femoral pore secretions of the males (Table S2).

To quantify the abundance of each compound, we integrated peak areas using MS Data Analysis software (Hewlett-Packard Chemstation Version C.00.07) with fixed integration parameters (Initial Threshold: 16, Initial Peak Width: 0.1, Initial Area Reject: 1.0). Several compounds had similar retention times, and thus co-eluted. To overcome this, we quantified the abundance of 14 compounds based on individual diagnostic ions (following McLean et al. 2012, the diagnostic quantitative ions used are reported in Table S2).

To reduce the number of variables to be used in multivariate statistical analyses and due to issues associated with

accommodating large numbers of zero values (Martin and Dri-jfhout 2009; Ranganathan and Borges 2011), only compounds that were consistently detected across secretion samples were selected for our analyses. Thus, the number of compounds to be used in the enclosures and cline analyses was reduced to the 21 compounds that were detectable in >98% of samples (Table 1). None of the initial 67 compounds were specific to either lineage; therefore, our removal of compounds from the enclosures and cline analyses that had low detectability was unlikely to exclude potential targets for sexually selected introgression.

For both the experimental and cline males, we generated a relative measure of abundance for each compound by log-normal transforming the peak area according to the formula: $Z_{i,j} = \ln[Y_{i,j}/g(Y_j)]$, where $Z_{i,j}$ is the standardized peak area i for male j , $Y_{i,j}$ is the peak area i for male j , and $g(Y_j)$ is the geometric mean of all peaks for male j (Aitchison 1986). To apply the transformation formula on profiles with nondetectable compounds, we replaced zero values ($n = 10$ within enclosure male samples and $n = 1$ within cline male samples) with the proportion of the total ion current that represented the minimum percentage that was detected for a single compound within a sample considering all samples. Secretion samples showing signs of contamination were excluded ($n = 6$ enclosure male samples).

REPEATABILITY AND DIVERGENCE IN CHEMICAL COMPOSITION

From the experimental males, we estimated within-individual repeatability in the relative abundances of the 21 chemical compounds between the first and second secretion sample. Intraclass correlation coefficients (ICC values) and their confidence intervals were calculated using the ANOVA-based method (i.e., Lessells and Boag 1987) implemented in R package ‘ICC’ (Wolak et al. 2012).

We assessed the extent of divergence in chemical profiles between the Italian and Western European lineages using a permutational multivariate analysis of variance (MANOVA; ‘adonis’ function, R package ‘vegan’; Oksanen et al. 2016) with lineage as a fixed effect and a Euclidean distance matrix of the relative abundances of all 21 compounds as a response. This was run on the experimental males only to avoid confounding effects of introgression (see above). To confirm that there were consistent differences between the lineages independent of sampling date during the breeding season, we performed the MANOVAs separately for the relative abundances of compounds in the first and second secretion sample of the males. Because half of the first samples were collected in the laboratory, we included a fixed factor (captive/noncaptive) in the former analysis to account for the effects of conditions experienced in captivity. Chemical variation within and between the lineages was visualized by principal coordinates analysis of the Euclidean distance matrix.

ASSOCIATIONS WITH SEXUAL MORPHOLOGY, SPATIAL ORGANIZATION AND REPRODUCTIVE SUCCESS

Using seminatural enclosures, we tested experimentally if the relative chemical composition of femoral secretions could function as sexual signals via covariance with male phenotype, dominance, and within- or between-lineage reproductive success. In April 2013, we transported 128 sexually mature lizards (the 64 experimental males and 64 females) captured from the allopatric Italian and Western European localities (Table S1) to laboratory facilities at the Department of Zoology, University of Oxford, United Kingdom. The lizards were transported from the field in cloth bags (kept below 10°C) and, once in the laboratory, they were housed in plastic terraria (590 × 390 × 415 mm³) under a 12-h light:12-h dark cycle, and provided with 6 h of UV lighting per day prior to the experiment.

Seminatural enclosures setup

In May 2013, we simulated the initial stage of secondary contact between the Italian and Western European lineages by releasing lizards into eight (~ 7 × 7 m²) experimental enclosures at the John Krebs Field Station, University of Oxford. Full details of the experiment are described elsewhere (MacGregor et al. 2017). In brief, we released male lizards into one of eight enclosures such that there were four Italian and four Western European males per enclosure. The males were allowed at least nine days to establish territories prior to the release of four Italian and four Western European females per enclosure. We monitored the eight enclosures during the lizards’ second seasonal reproductive episode (May and June 2013) to collect positional and social interaction data (based on a previously published ethogram, Heathcote et al. 2016). To distinguish territorial interactions from nonterritorial male–male behavior, we only classified interactions as male–male competition if they also included a submissive behavior (i.e., a retreat) by one male in the presence of another. Submissive behaviour determined which male was recorded as the winner of the encounter, and these data were used to generate within-enclosure dominance scores for each male (David 1988; Gammell et al. 2003).

The core home range areas for each individual were estimated from positional point-data in Ranges 8 (Kenward et al. 2008). We deemed the area of the 50% isopleth, generated using a fixed-kernel contour analysis with a fixed smoothing parameter of 0.75 (a balance between under and over smoothing; see Kie 2013), to represent a lizard’s core home range. For each male, we calculated the sum of his percentage core home range overlap with the core home ranges of same lineage males, other lineage males, same lineage females, and other lineage females. These overlap scores were used as response variables in tests for associations between male chemical profiles and spatial overlap.

Table 1. Details of the mean relative abundances and repeatability for 21 chemical compounds in the secretions collected from the experimental males.

Compound class	Detection % All samples	Mean relative abundance (CI _{95%})				ICC value (CI _{95%})					
		All males		WEUR		All males		ITA		WEUR	
		All males	ITA	All males	ITA	All males	ITA	All males	ITA	All males	ITA
<u>Heptadecene_RT8.25</u>	100	-0.60 (-0.86,-0.34)	-1.26 (-1.51,-1.01)	0.13 (-0.16,0.41)	0.49 (0.26,0.67)	0.31 (-0.06,0.60)	0.31 (-0.09,0.63)	0.31 (-0.06,0.60)	0.31 (-0.09,0.63)	0.31 (-0.09,0.63)	
<u>2-Heptadecanone_RT9.85</u>	100	-1.20 (-1.38,-1.01)	-1.45 (-1.64,-1.26)	-0.91 (-1.21,-0.61)	0.40 (0.15,0.60)	0.29 (-0.08,0.59)	0.45 (0.07,0.72)	0.29 (-0.08,0.59)	0.45 (0.07,0.72)	0.45 (0.07,0.72)	
Squalene_RT18.34	100	0.34 (0.09, 0.59)	0.51 (0.09,0.93)	0.15 (-0.11,0.41)	0.22 (-0.05,0.46)	0.43 (0.07,0.68)	0.00 (-0.62,0.11)	0.43 (0.07,0.68)	0.00 (-0.62,0.11)	0.00 (-0.62,0.11)	
<u>Unidentified steroid_RT19.10</u>	99	-1.37 (-1.49,-1.25)	-1.56 (-1.72,-1.39)	-1.17 (-1.32,-1.01)	0.30 (0.03, 0.53)	0.29 (-0.08, 0.59)	0.00 (-0.45, 0.33)	0.29 (-0.08, 0.59)	0.00 (-0.45, 0.33)	0.00 (-0.45, 0.33)	
<u>Cholesta-3,5-diene_RT19.34</u>	98	-1.60 (-1.86,-1.35)	-0.22 (-0.50,0.06)	0.17 (0.00,0.35)	0.31 (0.05,0.54)	0.53 (0.21,0.75)	0.01 (-0.38,0.40)	0.31 (0.05,0.54)	0.01 (-0.38,0.40)	0.01 (-0.38,0.40)	
<u>Unidentified steroid_RT19.57</u>	99	-0.03 (-0.20,0.14)	-0.22 (-0.50,0.06)	0.17 (0.00,0.35)	0.16 (-0.11,0.41)	0.33 (-0.04,0.62)	0.00 (-0.40,0.39)	0.33 (-0.04,0.62)	0.00 (-0.40,0.39)	0.00 (-0.40,0.39)	
<u>Unidentified steroid_RT19.75</u>	98	-0.87 (-0.98,-0.75)	-0.85 (-1.00,-0.70)	-0.89 (-1.08,-0.70)	0.37 (0.11,0.58)	0.60 (0.30,0.79)	0.25 (-0.15,0.59)	0.60 (0.30,0.79)	0.25 (-0.15,0.59)	0.25 (-0.15,0.59)	
<u>Unidentified steroid_RT20.76</u>	100	-1.06 (-1.19,-0.94)	-1.27 (-1.47,-1.07)	-0.83 (-0.93,-0.73)	0.31 (0.04,0.54)	0.00 (-0.38,0.34)	0.14 (-0.27,0.50)	0.31 (0.04,0.54)	0.14 (-0.27,0.50)	0.14 (-0.27,0.50)	
<u>Tocopherol¹ methyl ether_RT20.78</u>	98	-0.75 (-1.28,-0.22)	0.92 (0.61,1.23)	-2.61 (-3.00,-2.23)	0.95 (0.92,0.97)	0.75 (0.53,0.87)	0.82 (0.62,0.92)	0.95 (0.92,0.97)	0.82 (0.62,0.92)	0.82 (0.62,0.92)	
<u>Unidentified steroid_RT20.99</u>	99	-1.73 (-1.89,-1.57)	-2.07 (-2.26,-1.89)	-1.34 (-1.53,-1.16)	0.40 (0.15,0.60)	0.21 (-0.17,0.53)	0.36 (-0.03,0.66)	0.40 (0.15,0.60)	0.36 (-0.03,0.66)	0.36 (-0.03,0.66)	
<u>Alpha¹-Tocopherol_RT21.95</u>	100	0.50 (-0.02,1.02)	2.10 (1.70,2.51)	-1.28 (-1.61,-0.95)	0.81 (0.70,0.89)	0.34 (-0.03,0.63)	0.38 (-0.02,0.67)	0.34 (-0.03,0.63)	0.38 (-0.02,0.67)	0.38 (-0.02,0.67)	
<u>Cholesterol¹_RT21.95</u>	100	4.19 (4.07,4.31)	3.87 (3.77,3.98)	4.54 (4.41,4.68)	0.48 (0.24,0.66)	0.00 (-0.38,0.35)	0.23 (-0.17,0.57)	0.48 (0.24,0.66)	0.23 (-0.17,0.57)	0.23 (-0.17,0.57)	
<u>Cholesta-5,7-dien-3-ol_RT22.27</u>	100	2.07 (1.83,2.32)	2.58 (2.36,2.79)	1.51 (1.15,1.88)	0.39 (0.13,0.60)	0.00 (-0.36,0.37)	0.08 (-0.32,0.46)	0.39 (0.13,0.60)	0.08 (-0.32,0.46)	0.08 (-0.32,0.46)	

(Continued)

Table 1. Continued.

Compound class	Detection % All samples	Mean relative abundance (CI _{95%})			ICC value (CI _{95%})		
		All males	ITA	WEUR	All males	ITA	WEUR
Ergosterol¹ (Ergosta-5,7,22-trien-3-ol)_RT22.79	100	-1.01 (-1.18,-0.84)	-0.71 (-0.91,-0.51)	-1.36 (-1.58,-1.11)	0.27 (0.00,0.50)	0.26 (-0.11,0.57)	0.00 (-0.51,0.26)
Unidentified¹ steroid_RT22.95	100	-0.69 (-0.91,-0.47)	-1.17 (-1.37,-0.96)	-0.15 (-0.46,0.15)	0.46 (0.22,0.65)	0.43 (0.08,0.69)	0.08 (-0.32,0.46)
Campesterol¹ (Ergost-5-en-3β-ol)_RT23.01	100	0.05 (-0.29,0.38)	-0.79 (-1.12,-0.46)	0.98 (0.61,1.34)	0.47 (0.22,0.65)	0.20 (-0.17,0.52)	0.00 (-0.43,0.35)
Cholesta¹-4-en-3-one_RT23.26	100	-0.28 (-0.58,0.02)	-0.68 (-1.08,-0.27)	0.16 (-0.25,0.58)	0.47 (0.23,0.66)	0.40 (0.04,0.67)	0.38 (-0.01,0.67)
Ergosta¹-5,8-dien-3-ol_RT23.48	100	1.25 (1.04,1.46)	1.39 (1.19,1.60)	1.10 (0.72,1.47)	0.00 (-0.28,0.26)	0.07 (-0.30,0.42)	0.00 (-0.45,0.33)
Unidentified¹ steroid_RT24.24	99	0.46 (0.32,0.59)	0.41 (0.27,0.54)	0.51 (0.25,0.76)	0.17 (-0.10,0.42)	0.25 (-0.12,0.57)	0.14 (-0.26,0.51)
Unidentified¹ steroid_RT24.48	99	1.19 (0.92,1.46)	1.43 (1.21,1.64)	0.93 (0.41,1.45)	0.26 (-0.01,0.49)	0.35 (-0.02,0.63)	0.22 (-0.18,0.56)
Unidentified waxy ester_RT25.45	100	1.14 (0.91,1.37)	0.74 (0.42,1.05)	1.59 (1.31,1.87)	0.20 (-0.07,0.45)	0.00 (-0.37,0.35)	0.00 (-0.44,0.34)

¹Compounds quantified based on the scaling of individual quantitative ions rather than the integration of compound peak areas.

Compounds are listed in order of their characteristic retention time in minutes (RT), appended to each compound ID. Detection percentage and across and within-lineage mean relative abundances (based on secretions collected in the second sampling period) are reported for each compound, as well as within-individual repeatability (ICC) estimates between the first and second sampling periods based on all males and separately by lineage. Negative ICC estimates are interpreted and reported as evidence for zero repeatability (Nakagawa and Schielzeth 2010). The 95% confidence intervals for values are given in brackets. Compounds in bold differed between the lineages in their relative abundance based on nonoverlapping confidence intervals. Compounds underlined were repeatable based on overall ICC value confidence intervals that did not include zero.

At the end of female gestation, we recaptured all the experimental lizards bar two males (ITA and WEUR) and four females (two ITA, two WEUR) that presumably died during the experiment and one female (ITA) that we could not recapture until after the breeding season. The remaining females were housed in terraria until they laid, at which point we removed the clutches and incubated them at a constant 28°C and humidity (5:1 vermiculite:water volume) until hatching. At hatching, we obtained tail tissue samples from all juveniles for paternity analysis. We isolated DNA from 203 offspring (hatchlings: 191, embryos: 12) and 128 adults using the DNeasy 96 Blood & Tissue Kit (Qiagen, Valencia, CA, USA), following manufacturer's instructions (with overnight lysis). Given the limited number of potential fathers (eight per enclosure), we genotyped individuals at six microsatellite loci (Heathcote et al. 2015) and assigned offspring paternity in Cervus 3.0 (Marshall et al. 1998). This resulted in the retainment of 183 offspring for further analyses (see MacGregor et al. 2017 for further details).

Associations of chemical profiles with morphology, behaviour and reproductive success

Because ICC values were highly variable among compounds (see next), we based the descriptions of the chemical composition of secretions and the enclosure analyses on the relative abundances of the 21 compounds in the second secretion samples from males, collected immediately following the enclosures experiment ($n = 57$ after the removal of contaminated samples and accounting for two males that were not recaptured). To enable tests for associations between chemical profiles, male morphology, behavior and reproductive success, and to assess the putative function of secretions as sexual signals, we performed principal components analyses on the relative abundances of the compounds separately by lineage. For each lineage, PC1–PC5 were retained for further analyses (Table S3).

We ran several models to examine the extent to which these chemical profiles predicted male morphology, behaviour, and reproductive success. First, to establish the extent to which chemical profiles could function as signals of dominance status and their association with phenotypic traits linked to male competitive ability, we assessed the strength of correlations between within-lineage chemical PC scores (Table S3) and male dominance scores and morphological trait values (all variables standardized within lineage: mean = 0, SD = 1). To test for statistical associations between chemical profiles and dominance status, we ran a linear mixed model (LMM) for each lineage with male dominance score as the response variable and PC1–PC5 as predictors. Because dominance depends upon social environment, we controlled for enclosure as a random effect. Second, to examine whether chemical profiles predicted male–male and male–female spatial overlap, we generated candidate LMMs within each lineage, with all pos-

sible linear combinations of PC1–PC5 as putative predictors of overlap (owing to a lack of a priori hypotheses), and enclosure as a random effect. Pairwise interactions between components were not included due to difficulties in their interpretation. We ran and evaluated all candidate models based on second-order Akaike's information criterion (AICc). In addition to the top-supported models ($\Delta\text{AICc} \leq 2$), we report model-averaged parameter estimates generated from full-model averaging due to the absence of strongly supported best performing models (e.g., $\text{AICcWt} > 90\%$; Symonds and Moussalli 2011). Multimodal inferences were applied using R package 'glmulti' (Calcagno and de Mazancourt 2010). Finally, following the same method, we examined associations between chemical profiles and relative within- and between-lineage fertilization success (the latter for Italian males only owing to differences in the incidence of hybridization). Relative fertilization success was calculated by dividing the absolute number of sired offspring for a male by the mean of all males within his enclosure. We also tested if the associations of chemical profiles with morphology, behavior, and reproductive success remained consistent when considering only those compounds with moderate to high repeatability within individuals (see below and Table S4).

PATTERNS OF CHEMICAL PROFILE INTROGRESSION ACROSS A ZONE OF SECONDARY CONTACT

Cline analyses

We tested predictions regarding the direction of chemical introgression across our 16 populations in northern Italy using a geographic cline approach (e.g., Szymura and Barton 1986; Gay et al. 2008). We first performed a principal component analysis on transformed relative abundances of the 21 compounds in the 108 cline samples. We retained the first six principal components for further analyses (accounting for 76% of the total variance; Table S5). To test the extent to which geographic variation among populations was a function of isolation-by-distance, we performed a Mantel test between a matrix of chemical distances and geographic distances (based on 10,000 permutations). Chemical distances were defined as the mean Euclidean distances among populations based on PC1–PC6 and geographic distances as linear terrestrial distances. In addition, we examined the correlation between a chemical index score (see below) and a hybrid index score (available for $n = 66$ individuals, generated based on neutral nuclear microsatellite markers for a previous study, While et al. 2015).

Compounds that differ significantly in their relative abundance between lineages and are repeatable within individuals are the most likely targets for selective divergence or selective introgression following secondary contact. Consequently, we analyzed patterns of chemical introgression for the 12 compounds that met this criteria (see Table 1). To test for patterns of overall chemical introgression, we generated a chemical index from

PC1 to PC3 (accounting for 73% of the total variance, Table S6) according to the formula: $S = (1 + (D_{ITA}/D_{WEUR}))^{-1}$, where D_{ITA} is the Euclidean distance of PCs from an origin defined by the mean PCs of reference Italian individuals (populations VE and PE; Table S1), and D_{WEUR} is the Euclidean distance from an origin defined by the mean PCs of reference Western European individuals (populations LO, NL, and VA; Table S1), such that $S > 0.5$ reflects more Italian-like profiles and $S < 0.5$ reflects more Western European-like profiles. Clines were fitted for the chemical index, the 12 individual compounds, mtDNA haplotype frequencies (for comparison with mitochondrial genetic background, data generated by While et al. 2015), a hybrid index (for comparison with neutral expectation, While et al. 2015), and male dorsal greenness (for comparison of patterns of selected introgression, While et al. 2015) using the Metropolis-Hastings Markov chain Monte Carlo algorithm implemented in the R package 'hzar' (Derryberry et al. 2014). We later excluded population LO from our analyses of patterns of chemical introgression due to low sample size. For the phenotypic characters, we evaluated five candidate models (fitted tails [none, left, right, mirror, or both] all with estimated trait mean and variance [right, left, and center]), and for mtDNA frequencies and the hybrid index, we evaluated 10 candidate models (all possible combinations of fitted tails [none, left, right, mirror, or both] and scaling [fixed or free] Derryberry et al. 2014). Estimated cline center and width are reported from the best-fitting models based on AICc (see Table S7). The coincidence of cline centers for the chemical index versus the hybrid index, the chemical index versus greenness, and for the individual compounds versus the hybrid index and greenness were assessed using the maximum-likelihood derived confidence intervals.

Results

CHEMICAL COMPOSITION AND CONSISTENCY

The lipophilic chemical composition of the femoral secretions (Table S2) was consistent with that previously reported for this species (Martín and López 2006b; Martín et al. 2008; Pellitteri-Rosa et al. 2014). Considering those 21 compounds selected from the samples on the basis of their common occurrence (Table 1), the lizard secretions consisted primarily of steroids (85.7%), but also contained waxy esters (3.7%), tocopherols (7.6%), terpenoids (2.1%), alkenes (0.7%), and ketones (0.3%). On average, the five most abundant compounds across both lineages were cholesterol (59.6%), cholesta-5,7-dien-3-ol (10.3%), alpha-tocopherol (5.8%), unidentified steroid_24.48 (3.9%), and ergosta-5,8-dien-3-ol (3.9%). However, the relative quantities of the 21 compounds varied considerably in their within-individual repeatability (range of ICC: 0–0.95; Table 1).

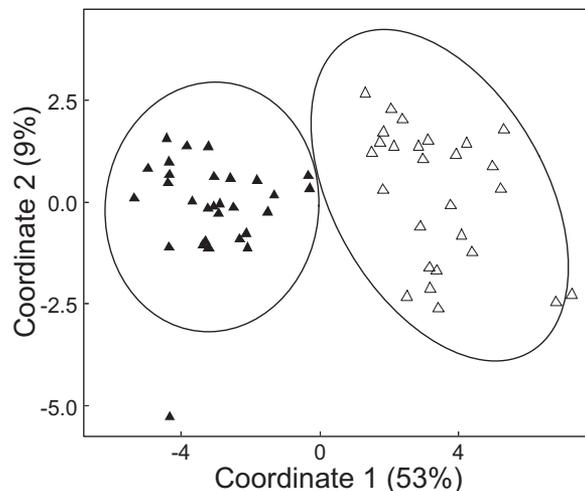


Figure 2. Principal coordinates analysis plot to show separation of the lineages based on the relative abundances of 21 compounds in the femoral secretions of males. Filled triangles are Italian males and unfilled triangles are Western European males. Ellipses represent the 95% confidence for each lineage. Percentage variation explained by each coordinate is reported in brackets.

EVIDENCE FOR DIVERGENCE BETWEEN THE LINEAGES

The overall chemical profile of secretions differed between the lineages at both sampling times (First secretion samples – Lineage: $F_{1,55} = 30.53$, $P < 0.001$, $R^2 = 0.35$, Captivity: $F_{1,55} = 1.89$, $P = 0.10$, $R^2 = 0.02$; second secretion samples – Lineage: $F_{1,56} = 44.95$, $P < 0.001$, $R^2 = 0.45$). The lineages differed in the relative abundance of all six chemical classes represented by the 21 compounds, specifically, in tocopherols and a terpenoid, which were higher in abundance in the Italian lineage, and steroids, a waxy ester, an alkene, and a ketone, which were higher in abundance in the secretions of Western European males (Tables 1 and S8). Consequently, principal coordinates analysis resulted in clear clustering by lineage (Fig. 2). Of the 21 compounds, 14 showed significant differences in their relative abundance between Italian and Western European secretion samples, and of these, 12 compounds had moderate to high repeatability within individuals (defined as ICC values with confidence intervals excluding zero; Table 1).

ASSOCIATIONS WITH SEXUAL CHARACTERS, SPATIAL ORGANIZATION AND REPRODUCTIVE SUCCESS

We examined the associations between chemicals and morphological and color characters that have established (i.e., outer ventral scale ornamentation) and putative (i.e., dorsal greenness and ventral blackness) signal function. Within-lineage chemical variation was correlated with dominance, body size, and color characters in the Italian and Western European lineages, however, the

significance of these correlations dropped out after correcting for false discovery rate (Table S9). From an LMM, chemical variation captured by PC4 was marginally significantly associated with dominance in the Italian lineage; however, there were no significant associations between chemical profiles and dominance in either lineage when considering only the repeatable compounds (Table S10).

The core territories of Italian males overlapped significantly more with the core territories of both Italian and Western European females than did the core territories of Western European males. Italian males also overlapped less with males of their own lineage than did Western European males with males of their own lineage (Fig. S1). For Italian males, PC3, PC4, and PC5 predicted overlap with same lineage males (Table 2). Chemical components also occurred within the best performing models predicting overlap between Italian males and Western European males as well as male–female spatial overlap, however, the null model was the top-supported model in all cases (Table 2). For Western European males, the null model occurred within the best performing models predicting male overlap with same lineage and other lineage males and with females of the same lineage. However, PC1, PC3, PC4, and PC5 predicted overlap with Italian females and the null model was not equally well supported (Table 3).

Hybridization was highly asymmetric and occurred mostly between Italian males and Western European females (35% of Western European female offspring sired by an Italian father vs. 6% in the opposite direction, detailed results reported in MacGregor et al. 2017). We found limited support for a relationship between chemical profiles and reproductive success in males of the Italian lineage. Specifically, while there was some evidence for chemical associations with within-and between-lineage (i.e., hybridization) reproductive success, the null models were equally well supported in both cases (Table 4). In contrast, for Western European males, a single model for within-lineage reproductive success was supported with PC1 as a negative predictor. We were unable to examine the corresponding effects on between-lineage reproductive success in Western European males due to a lack of incidence of hybridization. When we reran the above analyses of the predictors of spatial overlap and reproductive success using principal components generated from only compounds that were repeatable ($n = 14$; Table S4), the null model occurred within the best performing models in all cases (see Tables S11–S13).

PATTERNS OF CHEMICAL INTROGRESSION ACROSS A ZONE OF SECONDARY CONTACT

Geographic distance between pairs of populations was positively correlated with chemical distance (Mantel Test [10,000 perm]: $r = 0.52$, $P < 0.001$; Fig. S2). Chemical index score was highly correlated with a hybrid index score based on nuclear microsatellite markers generated by While et al. (2015) ($r = 0.74$; Fig. S3).

Consistent with this, cline fitting of the chemical index suggested geographic patterns of chemical variation across the contact zone are similar to that of introgressed microsatellite markers (Figs. 3 and S4 and Table S14). However, from the clines fitted individually to the 12 compounds, we found that three compounds, unidentified steroid_RT20.76, tocopherol methyl ether, and cholesterol, did not support the patterns of neutral introgression, and instead suggested a geographic pattern of introgression similar to dorsal greenness (Fig. S5; Table S14).

Discussion

Under sexual selection, divergence in chemical signals should mediate patterns of hybridization during secondary contact and lead to asymmetric patterns of introgression. In this study, we identified characteristics of chemical profiles in two lineages of the common wall lizard. We found that the chemical profiles of wall lizards were variable between lineages, but were only weakly associated with male secondary sexual characters with no consistent sexual selection on individual compounds. Furthermore, we found limited evidence for selective introgression of chemical profiles across a natural contact zone where sexually selected introgression of color and morphology has previously been documented (While et al. 2015). Nevertheless, three compounds were identified as candidates for asymmetric introgression via direct selection or genetic linkage with visual or behavioral characters. Combined, our results suggest that divergence in the chemical composition of femoral secretions in wall lizards is largely neutral, and that associations with male phenotypes and reproductive success may be transient or environment-dependent and play a minor role in the evolution of reproductive isolation and introgression. This implies that the likely function of wall lizard scent marks may be to mediate individual recognition and territory residency rather than to convey physical or behavioral attributes.

The causes of divergence in the chemical composition of lizard secretions are contentious (Font et al. 2012). Divergence may be driven by differences in the direction and intensity of intra- or intersexual selection on males (López and Martín 2004), local adaptation through, for instance, selection for transmission efficiency under differing climates (Alberts 1992; Martín et al. 2015); or through stochastic change (e.g. Runemark et al. 2011). Our study goes some way toward testing the sexual selection hypothesis. The two lineages used here have evolved distinct differences in morphology and visual traits that function in male–male competition, which give a competitive advantage to Italian males. This drives the asymmetric introgression of suites of sexually selected characters from the Italian lineage into the Western European lineage (While et al. 2015). If male chemical profiles have similarly diverged under sexual selection, we would predict that some chemical characteristics (i) associate with male

Table 2. The top-supported models ($<2 \Delta\text{AICc}$) from analyses to identify associations between chemical profiles (defined as PC1–PC5; Table S3) and male–male and male–female overlap in the Italian lineage.

Overlap	Category	Model	k	AICc	ΔAICc	AICcWt	Predictor	β	$\text{CI}_{95\%}$	Model-averaged β	Unconditional $\text{CI}_{95\%}$
Male–male	Same lineage	PC3 + PC5	5	300.2	0	0.27	PC3	10.85	1.68, 20.38	8.23	–3.46, 19.92
		PC3 + PC4 + PC5	6	300.59	0.39	0.22	PC5	13.73	4.51, 22.99	13.27	2.97, 23.56
							PC3	10.66	1.94, 19.65		
							PC4	–6.88	–15.20, 1.33	–2.97	–11.13, 5.19
							PC5	14.20	5.45, 22.97		
							Null	3	307.56	7.36	0.01
	Other lineage	Null					Null	3	326.96	0	0.22
							PC1	4	327.77	0.81	0.15
Male–female	Same lineage	Null	3	356.67	0.00	0.28					
		PC4	4	358.29	1.63	0.12	PC4	15.53	–14.86, 45.93	4.48	–12.31, 21.27
	Other lineage	Null					Null	3	341.59	0	0.28
							PC3	4	343.41	1.82	0.11

For each model, the number of parameters (k), second-order Akaike's information criterion (AICc), difference in AICc from the top-performing model (ΔAICc), and the relative likelihood of the model (AICcWt) are reported. Regression coefficients (β) and 95% confidence intervals ($\text{CI}_{95\%}$) are presented for each predictor. Model-averaged parameter estimates (model-averaged β) generated via full-model averaging and unconditional 95% confidence intervals (unconditional $\text{CI}_{95\%}$) are also reported, adjacent to predictors on their first appearance in the table. Predictor effect sizes with 95% confidence intervals that do not include zero are in bold. When not included in the top-performing models AICc values for the null model are presented in italics for comparison.

Table 3. The top-supported models (<2 ΔAICc) from analyses to identify associations between chemical profiles (defined as PC1–PC5; Table S3) and male–male and male–female overlap in the Western European lineage.

Overlap	Category	Model	k	AICc	ΔAICc	AICcWt	Predictor	β	CI _{95%}	Model averaged β	Unconditional CI _{95%}
Male–male	Same lineage	PC3	4	293.82	0.00	0.18	PC3	16.56	0.90, 36.92	10.54	–4.61, 25.68
		Null	3	294.25	0.44	0.15					
		PC3 + PC5	5	294.77	0.96	0.11	PC3	20.55	4.42, 40.53		
Male–female	Other Lineage	Null	3	286.83	0.00	0.33	PC5	13.01	–4.48, 31.64	4.73	–17.6, 27.06
	Same lineage	Null	3	286.08	0.00	0.21					
		PC4	4	286.66	0.57	0.16	PC4	12.09	–4.20, 27.04	4.84	–8.93, 18.61
Other lineage		PC4 + PC5	5	295.16	0	0.16	PC4	19.42	0.85, 37.33	15.05	–8.77, 38.87
		PC5	4	296.29	1.13	0.09	PC5	–21.58	–38.88, –4.81	–11.69	–35.83, 12.44
		PC1 + PC4	5	296.53	1.38	0.08	PC1	–18.69	–35.88, –1.92	–4.83	–20.39, 10.72
PC3 + PC4 + PC5		PC4	4	296.74	1.59	0.07	PC4	23.32	4.88, 49.96		
		PC3	3	296.74	1.59	0.07	PC3	11.61	–1.15, 30.29	4.95	–10.14, 20.04
		PC4	4	296.74	1.59	0.07	PC4	20.25	2.36, 37.79		
PC1 + PC3 + PC4		PC5	4	297.11	1.95	0.06	PC5	–18.17	–35.77 –0.80		
		PC1	3	297.11	1.95	0.06	PC1	–16.38	–33.08, 0.08		
		PC3	4	297.11	1.95	0.06	PC3	14.29	2.41, 34.62		
Null		PC4	4	297.11	1.95	0.06	PC4	23.81	6.34, 41.26		
		Null	3	300.01	–4.85	0.01					

For each model, the number of parameters (k), second-order Akaike's information criterion (AICc), difference in AICc from the top-performing model (ΔAICc), and the relative likelihood of the model (AICcWt) are reported. Regression coefficients (β) and 95% confidence intervals (CI_{95%}) are presented for each predictor. Model-averaged parameter estimates (model-averaged β) generated via full-model averaging and unconditional 95% confidence intervals (unconditional CI_{95%}) are also reported, adjacent to predictors on their first appearance in the table. Predictor effect sizes with 95% confidence intervals that do not include zero are in bold. When not included in the top-performing models AICc values for the null model are presented in italics for comparison.

Table 4. The top-supported models (<2 Δ AICc) from analyses to identify associations between chemical profiles (defined as PC1 to PC5; Table S3) and relative fertilization success within or between lineage (Italian males only).

Lineage	Relative fertilization success	Model predictors	<i>k</i>	AICc	Δ AICc	AICcWt	Predictor	β	CI _{95%}	Model-averaged β	Unconditional CI _{95%}	
ITA	Within lineage	PC4	4	96.06	0.00	0.15	PC4	0.32	-0.07, 0.70	0.15	-0.24, 0.54	
		Null	3	96.08	0.02	0.15						
		PC3	4	97.43	1.37	0.08	PC3	0.23	0.23	-0.17, 0.62	0.07	-0.17, 0.31
		PC3 + PC4	5	97.50	1.44	0.07	PC3	0.23	0.23	-0.15, 0.60		
							PC4	0.32	0.32	-0.06, 0.69		
	Between lineage	PC5	4	97.77	1.71	0.06	PC5	0.20	0.20	-0.20, 0.59		
		PC4 + PC5	5	97.88	1.82	0.06	PC4	0.32	0.32	-0.06, 0.70		
		Null	3	99.67	0.00	0.20	PC5	0.20	0.20	-0.18, 0.57	0.05	-0.15, 0.26
WEUR	Within lineage	PC3	4	100.03	0.36	0.16	PC3	-0.32	-0.73, 0.10	-0.14	-0.52, 0.24	
		PC5	4	101.51	1.84	0.08	PC5	-0.19	-0.61, 0.23	-0.05	-0.25, 0.15	
		PC1	4	76.79	0.00	0.35	PC1	-0.46	-0.79, -0.12	-0.40	-0.87, 0.07	
		Null	3	80.80	4.01	0.05						

For each model, the number of parameters (*k*), second-order Akaike's information criterion (AICc), difference in AICc from the top-performing model (Δ AICc), and the relative likelihood of the model (AICcWt) are reported. Regression coefficients (β) and 95% confidence intervals (CI_{95%}) are presented for each predictor. Regression coefficients (β) and 95% confidence intervals (CI_{95%}) are presented for each predictor. Model-averaged parameter estimates (model-averaged β) generated via full-model averaging and unconditional 95% confidence intervals (unconditional CI_{95%}) are also reported, adjacent to predictors on their first appearance in the table. Predictor effect sizes with 95% confidence intervals that do not include zero are in bold. When not included in the top-performing models, AICc values for the null model are presented in italics for comparison.

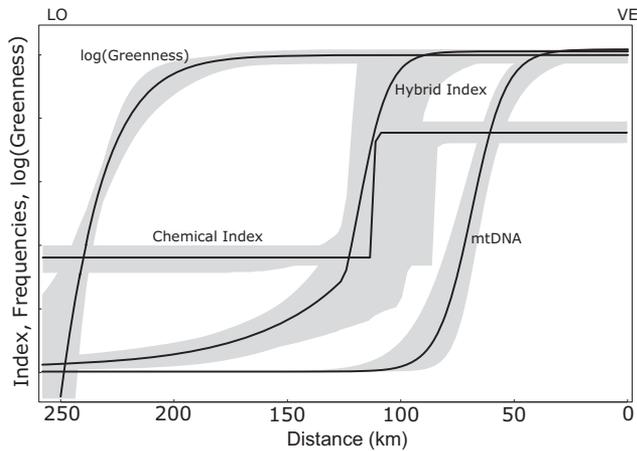


Figure 3. The maximum-likelihood cline and the 95% credible cline region for the best-fitting models (Table S14) for the chemical index, mtDNA haplotype frequencies, the hybrid index, and dorsal greenness (scored on a scale of 1–10 and log-transformed to improve fit to model assumptions). Distance is the cumulative distance from the south-easternmost population Colle di Val D’Elsa (VE) in Tuscany with increasing distance westwards toward the westernmost population Loano (LO) in Liguria. The chemical index was calculated based on the 12 compounds that differed between the lineages in their relative abundance and were repeatable within individuals (see Table 1), and excludes data from population LO due to low sample size.

secondary sexual characters, (ii) influence success in male–male competition for territory and fertilizations, (iii) predict reproductive success and hybridization; and (iv) show evidence of adaptive introgression from the Italian to the Western European lineage. In this study, we found evidence for some but not all of these predictions.

The relative abundances of several compounds were associated with sexual characters, spatial overlap, and reproductive success. However, these associations were not always consistent between the lineages and in some instances were even reversed. One potential explanation for this is that different chemical characters function in intrasexual versus intersexual communication. Indeed, behavioral studies of *P.muralis* and closely related species suggest females can discriminate between males based on the composition of their femoral secretions (e.g., Lopez et al. 2003; Martín and López 2006b, Heathcote et al. 2014). However, a role for intersexual selection in shaping the chemosensory traits of wall lizards is not empirically supported by the literature (Font et al. 2012), and, overall, our results are consistent with the latter conclusion. For example, we found little evidence that females associate with males with a particular chemical composition. Furthermore, previous work suggests that neither Italian nor Western European females discriminate based on male quantitative traits or lineage (Heathcote et al. 2016).

Based on the overall weakness of associations between chemical profiles and within- and between-lineage fertilization success, we infer that heritable chemical characters that have diverged between the Italian and Western European lineages are unlikely to be under consistent ongoing sexual selection (although PC1 was negatively associated with within-lineage reproductive success in Western-European males). In support of this, and in contrast to morphological and visual traits, the pattern of introgression of the overall chemical profiles followed the pattern for microsatellite markers, and hence, conformed to neutral expectations in the presence of asymmetries in hybridization. This result is consistent with the limited evidence for a relationship between chemical profiles and hybridization success in Italian males. Combined this suggests that divergence in chemical profiles has played a limited role in mediating the asymmetric introgression observed in zones of secondary contact (While et al. 2015). The clear exception to this is tocopherol methyl ether, whose geographic cline closely resembled geographic variation in dorsal greenness across the contact zone. Interestingly, this compound was negatively associated with Italian male dominance, and dominance was the best predictor of Italian male reproductive success in the enclosures (MacGregor et al. 2017). Thus, this does not suggest that direct selection would cause the introgression of tocopherol methyl ether, but instead supports that genetic linkage with genomic regions contributing to sexually selected color and morphology is a more likely explanation. Thus, we believe the data are consistent with the interpretation that overall chemical variation across the contact zone is largely driven by neutral processes.

Presuming that the chemical profile of a male wall lizard’s femoral gland secretions is not under consistent inter- or intrasexual selection, what then is the function of chemical communication? One possibility is that the chemical profiles primarily function as a signature mixture, a variable set of compounds, which is learnt by other males, allowing them to distinguish individuals (Wyatt 2010, 2014). Indeed, due to their chemical complexity, femoral gland secretions may be better suited than any other cue for use in individual recognition because a very high level of specificity is possible. This explanation is consistent with our observation of only weak associations between male chemical profiles and fertilization success, and is supported by a wealth of empirical studies on lizards demonstrating differential male behavioral responses to the scents of familiar and unfamiliar individuals (e.g., Aragón et al. 2001; Font and Desfilis 2002), and even recognition of individual identity based on chemical cues (Carazo et al. 2008). If primarily functioning as signature mixtures, the correlations between chemical characters and male sexual characters presented here more likely reflect transient associations with weakly heritable chemical traits; associations that may easily break down during hybridization, thereby leading to effectively neutral patterns of chemical introgression.

Conclusions

Combined with previous studies (While et al. 2015; MacGregor et al. 2017), our experimental and field data highlight the potentially differing functions for visual and chemical communication systems in lizards, with consequences for patterns of character introgression between two lineages (see Greig et al. 2015 for similar discordant patterns between plumage color and song in birds). In contrast to comparative evidence invoking intrasexual selection as a mechanism for the evolution of visual traits used for communication in lacertid lizards (Pérez i de Lanuza et al. 2013), our study suggests that chemical traits may not be subjected to the same selection pressures. In fact, we suggest that the chemical profiles of femoral gland secretions in wall lizards may not reliably function as sexual signals as is commonly assumed. Instead, the utility of chemical profiles may be because they allow recognition of competitors based on experience, thereby playing little role in the evolution of reproductive isolation or adaptive introgression.

AUTHOR CONTRIBUTIONS

TU, HEAM and GMW conceived and designed the study. HEAM, TU, GMW and RAML conducted fieldwork. HEAM carried out the enclosure experiment. Pd'E and CL processed the secretion samples. HEAM and RAML extracted the chemical data assisted by NWD, Pd'E and CL. HEAM analysed the data. HEAM, TU and GMW wrote the manuscript with input from all authors.

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DATA ARCHIVING

The doi for our data is <https://doi.org/10.5061/dryad.4j8pq>.

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Supporting Information

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Figure S1. Bar plots depicting differences in spatial overlap between the lineages.

Figure S2. The linear relationship between geographic and chemical distances between populations sampled from across the secondary contact zone.

Figure S3. The relationship between the chemical index score and hybrid index score for individuals sampled from across the secondary contact zone.

Figure S4. The maximum-likelihood cline for best-fitting models (Table S7) for (1) a chemical index based on the relative abundances of 12 compounds that differed significantly between the lineages and were repeatable within individuals excluding samples from population LO, (2) a chemical index based on the relative abundances of 21 compounds, and (3) a chemical index based on the relative abundances of 21 compounds excluding samples from population LO.

Figure S5. The maximum-likelihood cline and the 95% credible cline region for best-fitting models (Table S7) for (a) heptadecene, (b) 2-heptadecanone, (c) unidentified steroid_RT19.10, (d) unidentified steroid_RT20.76, (e) tocopherol methyl ether, (f) unidentified steroid_RT20.99, (g) alpha-tocopherol, (h) cholesterol, (i) cholesta-5,7-dien-3-ol, (j) unidentified steroid_RT22.95, (k) campesterol, and (l) cholesta-4-en-3-one (data were scaled 0–1 prior to analysis and exclude samples from population LO due to low sample size).

Table S1. Population sampling details.

Table S2. Details of the 67 lipophilic chemical compounds characterized within the femoral gland secretions of wall lizards.

Table S3. Rotated factor loadings for PC1–PC5 from within-lineage principal components analyses performed on the relative abundances of 21 compounds in the second secretion sample of the enclosure males.

Table S4. Rotated factor loadings for PC1–PC5 from within-lineage principal components analyses performed on the relative abundances of the 14 compounds that were repeatable within individuals based on ICC values (see Table 1).

Table S5. Rotated factor loadings for PC1–PC6 from a principal components analysis performed on the relative abundances of 21 compounds in the secretion samples of the cline males.

Table S6. Rotated factor loadings for PC1–PC3 from a principal components analysis performed on the relative abundances of 12 compounds in the secretion samples of the cline males.

Table S7. AICc values for genetic and phenotypic cline models (mtDNA and hybrid index: Model I = none, fixed; Model II = left, fixed; Model III = right, fixed; Model IV = mirror, fixed; Model V = both, fixed; Model VI = none, free; Model VII = left, free; Model VIII = right, free; Model IX = mirror, free; Model X, both, free; phenotypic characters: Model I = none, Model II = left, Model III = right, Model IV = mirror, Model V = both).

Table S8. Differences between the lineages in the percentage relative abundances of six chemical classes of compound.

Table S9. Within-lineage Pearson's correlation coefficients between the phenotypic traits of experimental males and the chemical profiles of their femoral gland secretions (defined as PC1–PC5; Table S3).

Table S10. Results from LMMs run within each lineage to examine chemical associations with male dominance.

Table S11. The top supported models ($<2 \Delta AICc$) from analyses to identify associations between chemical profiles based on those compounds found to be repeatable within individuals in their relative abundance (defined as PC1–PC5; Table S4) and male–male and male–female overlap in the Italian lineage.

Table S12. The top-supported models ($<2 \Delta AICc$) from analyses to identify associations between chemical profiles based on those compounds found to be repeatable within individuals in their relative abundance (defined as PC1–PC5; Table S4) and male–male and male–female overlap in the Western European lineage.

Table S13. The top-supported models ($<2 \Delta AICc$) from analyses to identify associations between chemical profiles based on those compounds found to be repeatable within-individuals in their relative abundance (defined as PC1–PC5; Table S4) and relative fertilization success within or between lineage (Italian males only).

Table S14. Estimates of the cline center (c : the distance from sampling location VE in Tuscany) and cline width (ω : $1/\text{maximum slope}$) for the best-fitting cline models for the chemical index, mtDNA haplotype frequencies, the hybrid index, greenness score, and 12 individual chemical compounds.

Table S15. Rotated factor loadings for PC1 (body size) from a principal components analysis performed on three body size related traits in the experimental males: snout-vent length (SVL), head length and mass.