

Research



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Author for correspondence:

Cristina Romero-Díaz

e-mail: cromerod@asu.edu

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Evolutionary loss of a signalling colour is linked to increased response to conspecific chemicals

Cristina Romero-Díaz¹, Jake A. Pruett^{2,3}, Stephanie M. Campos⁴, Alison G. Ossip-Drahos⁵, J. Jaime Zúñiga-Vega⁶, Cuauhchuatl Vital-García⁷, Diana K. Hews² and Emília P. Martins¹

¹School of Life Sciences, Arizona State University, Tempe, AZ, USA

²Department of Biology, Indiana State University, Terre Haute, IN, USA

³Department of Biological Sciences, Southeastern Oklahoma State University, Durant, OK, USA

⁴Center for Behavioral Neuroscience, Neuroscience Institute, Georgia State University, Atlanta, GA, USA

⁵Department of Chemistry and Physical Sciences, Marian University, Indianapolis, IN, USA

⁶Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico City, México

⁷Departamento de Ciencias Veterinarias, Instituto de Ciencias Biomédicas, Universidad Autónoma de Ciudad Juárez, Ciudad Juárez, México

CR-D, 0000-0002-0718-4055; AGO-D, 0000-0002-6019-173X; JJZ-V, 0000-0002-9661-1521; CV-G, 0000-0002-3634-2964; DKH, 0000-0002-2252-6711; EPM, 0000-0002-8952-3240

Behavioural responses to communicative signals combine input from multiple sensory modalities and signal compensation theory predicts that evolutionary shifts in one sensory modality could impact the response to signals in other sensory modalities. Here, we conducted two types of field experiments with 11 species spread across the lizard genus *Sceloporus* to test the hypothesis that the loss of visual signal elements affects behavioural responses to a chemical signal (conspecific scents) or to a predominantly visual signal (a conspecific lizard), both of which are used in intraspecific communication. We found that three species that have independently lost a visual signal trait, a colourful belly patch, responded to conspecific scents with increased chemosensory behaviour compared to a chemical control, while species with the belly patch did not. However, most species, with and without the belly patch, responded to live conspecifics with increased visual displays of similar magnitude. While aggressive responses to visual stimuli are taxonomically widespread in *Sceloporus*, our results suggest that increased chemosensory response behaviour is linked to colour patch loss. Thus, interactions across sensory modalities could constrain the evolution of complex signalling phenotypes, thereby influencing signal diversity.

1. Introduction

Functional interactions between signal elements across sensory modalities (e.g. visual, acoustic, and chemical) occur in many extant species [1]. For example, the inflating vocal sac of male túngara frogs modulates the attractiveness of the advertisement call [2]. The intensity of male brown-headed cowbird visual displays modifies the attractiveness of the song [3], and the presence of a vibratory courtship signal makes female wolf spiders more receptive to more visually ornamented males [4]. However, evidence that functional interactions may reflect evolutionary links between signals and/or sensory systems across different modalities remains scarce and conflicting (see below).

Some hypotheses on the evolution of multimodal signals predict positive correlations across sensory modalities, such as the ‘redundant’ or ‘backup’ signals hypothesis, which proposes that different signal elements convey the same information about the signaller [1,5]. For example, in many wolf spiders (genus *Schizocosa*), visual courtship elements (e.g. leg taps) have a seismic counterpart.

The multimodal signal is suggested to be redundant and there is a positive evolutionary correlation between visual and seismic signal elaboration [6]. Negative correlations are predicted by the ‘signal compensation’ or ‘trade-off’ hypothesis. Signal compensation is expected when signal transmission and reception is influenced by environmental conditions and the efficacy of each signal element is higher in a specific environment [5]. Zebrafish (*Danio rerio*) respond more strongly to visual cues in a foraging context, but when maintained in turbid water, they exhibit a sensory preference for chemical over visual cues [7]. A permanent, rather than transient, increase in water turbidity should favour an evolutionary shift towards the more efficient sensory modality [8,9]. Alternatively, common condition-dependence of two or more signalling traits on different sensory modalities can lead to a trade-off determined by the balance between costs of elaboration/maintenance and information content for each trait [9,10]. For example, song complexity and plumage ornamentation are sexually selected traits broadly considered honest signals of the male condition in birds [11–13]. A comparative study across carduelin finches (subfamily Carduelinae) revealed that song complexity and carotenoid-based plumage colouration are negatively correlated, suggesting that over evolutionary time, selection leads to the displacement of one condition-dependent trait by another [10,14]. Other hypotheses, such as the ‘multiple messages’ hypothesis, predict the independent evolution of different sensory modalities [1,14]. In contrast with carduelines, song and plumage colouration in estrildid finches (family Estrildidae) evolve independently, and are each associated with different socio-ecological traits, suggesting that these sexual signals convey different information and/or respond to different selection pressures [14,15]. These two finch studies also illustrate how evolutionary patterns for functionally equivalent multimodal signals are inconsistent across taxa.

Multimodal research has traditionally focused on the evolution of signal design (production), rather than on receiver responses to the signal. However, receiver sensory systems are a key component in driving the evolution of signal form and function [8,16]. Evolutionary loss in one of two or more integrated or interactive modalities of a complex signal could impact signal receivers in different ways. Receivers may have to compensate for loss of information by relying more strongly on alternative signal components in the same or in different sensory modalities [9,17]. Some three-spine stickleback (*Gasterosteus aculeatus*) populations have replaced an ancestral red badge used in male–male competition by a black badge. Black males, smaller than red males, respond more aggressively to red compared to black males, suggesting that black males no longer rely on red cues as honest signals of fighting ability and have shifted reliance onto black cues [18]. Signal compensation across sensory modalities, however, has seldom been assessed. In perhaps the best example to date, Gray *et al.* [19] failed to detect an increase of female reliance on male chemosensory cues after the loss of male calling song in Pacific field crickets (*Teleogryllus oceanicus*). Here, we used 11 species from the lizard genus *Sceloporus* to test whether and how an evolutionary loss of a signalling colour patch affects behavioural responses to a chemical (conspecific scents) or to a predominantly visual signal (a conspecific lizard).

The genus *Sceloporus* is a large North American lizard clade in which species often co-occur [20]. They are

characterized by the use of multimodal and multicomponent social signals that combine visual (motion and colour) and chemical elements for communication [21]. Ritualized push-up and headbob motion displays are employed for informing nearby conspecifics of individual, sex, and species identity, usually at individual territorial borders [22–24]. Motion displays also expose ventrolateral and throat colour patches, most frequently found in males and typically blue, which are used during territorial disputes between males to signal aggression and fighting ability [22,23,25,26]. In addition, lizards actively and/or passively deposit scent marks secreted from femoral glands onto the substrate that serve as cues of physiological condition [27], and potentially convey individual and sex identity information [28,29]. Lizards respond to conspecific scents by adjusting aggression [30,31], altering their activity levels and space use within territories [32]. *Sceloporus* species vary considerably in their dorsal and ventral colour scheme, patterning and conspicuousness [17], motion displays [33], and chemical composition of femoral gland secretions [31,34]. Behavioural responses to conspecific signals (visual and chemical) are not only context-dependent (i.e. influenced by intrinsic and extrinsic factors), but also species-specific [30,32,35–37], thus comparative approaches are needed to identify general response patterns.

Ancestral character reconstruction infers that ventral colour patches have been lost over evolutionary time at least seven independent times within the genus *Sceloporus* [38]. This transition from coloured to plain (patchless) has affected patch conspicuousness to the lizard’s visual system [17]. In a series of field experiments, we assessed behavioural responses of lizards from 11 *Sceloporus* species, seven coloured and four patchless, to either conspecific scents (a chemical signal) or conspecific lizards (a visual signal). By design and mode of presentation (see Methods), conspecific lizards primarily acted as a visual signal, although we cannot categorically discard the involvement of other minor sensory components (e.g. sound, odour). Comparative and experimental studies in *Sceloporus* have revealed a potential compensation between the loss of visual elements (colour and/or motion) and chemosensory behaviour [31,36,37,39], suggesting an evolutionary link between colour patch production and chemical signal perception. Here, we explicitly test this hypothesis by adopting a comparative multimodal framework across several more species. If signal compensation exists, we predicted that the lack of colour patches would increase behavioural responses to conspecific scents. By contrast, if there is no signal compensation, coloured and patchless *Sceloporus* species should have similar behavioural responses to conspecific scents. In addition, *Sceloporus* species in which males have lost the colourful belly patch still respond to the blue belly colour on an intruder lizard, suggesting a lack of a tight evolutionary link between colour patch production and visual signal perception [26,40]. If colour patch production and visual signal perception are evolutionarily decoupled, as these earlier studies suggest, we predicted that the lack of a colour patch would not affect responses to a visual signal. That is, coloured and patchless species would show similar responses to a conspecific lizard. By contrast, if an evolutionary link exists between colour patch production and visual signal perception, we predicted that patchless species would decrease display response to a conspecific lizard.

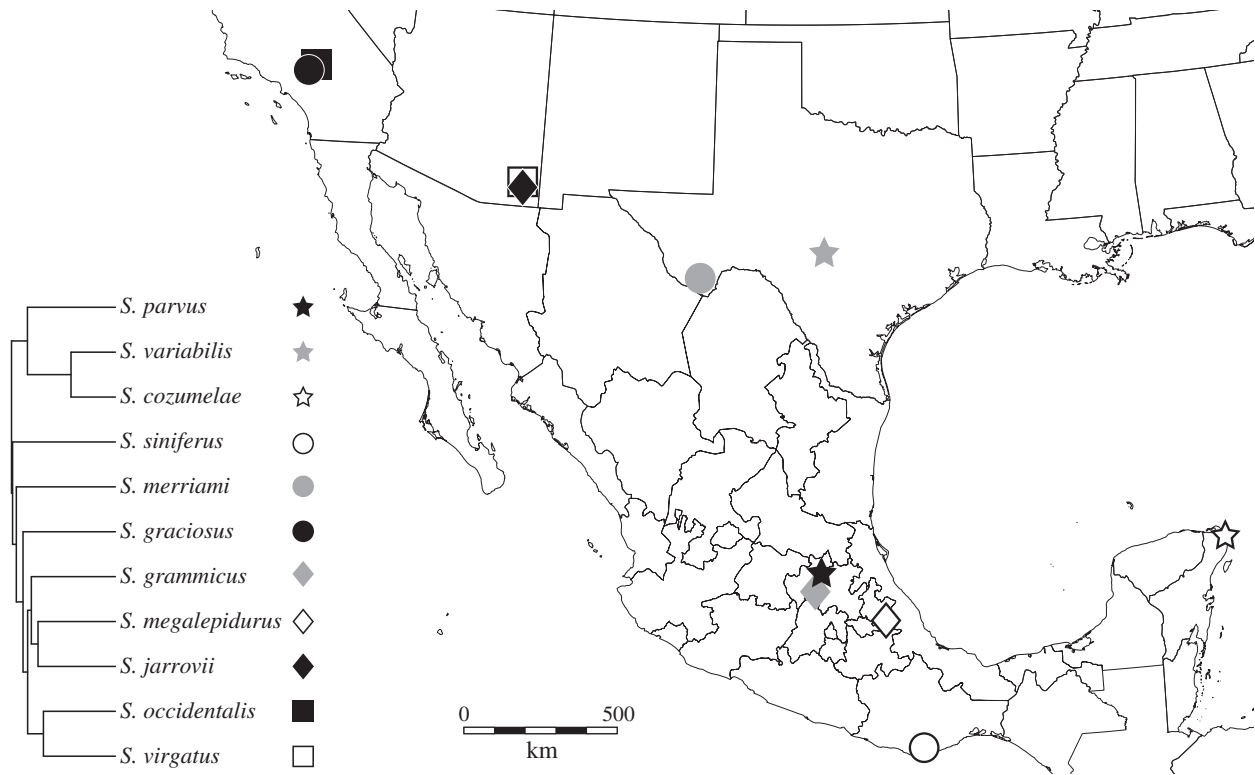


Figure 1. Phylogenetic relationships (as per [41]) and geographical site location of the 11 *Sceloporus* species studied in the USA and Mexico. *Sceloporus virgatus* and *S. jarrovii* co-occur in one study site, whereas *S. graciosus* and *S. occidentalis* co-occur in another. Species grouped in the same 'clade' (three clades and one paraphyletic group) share the same symbol. Open symbols indicate species with evolutionary loss of belly colour patches and filled symbols indicate species that retain the colour patches. Grey and black colours identify different species within clades.

2. Methods

(a) Field trials

Between 2012 and 2016, we studied behavioural responses of 11 species spread across the lizard genus *Sceloporus* to field presentations of either a chemical signal (conspecific scents) or a predominantly visual signal (a live conspecific), both used in intraspecific communication [21]. Chemical and visual field presentations were conducted as two separate experiments due to seasonal constraints and the challenging logistics of studying multiple species at different locations. Based on a well-supported molecular phylogeny by Wiens *et al.* [41], we identified four distinct groups of species (clades or paraphyletic groups): three groups containing three species each and one group of two more closely related species (figure 1). The exception is the trio *S. siniferus*, *S. merriami*, and *S. graciosus*, which are comparatively more distantly related, and we only group them here for statistical convenience. In addition, 4 of these 11 species represent four independent evolutionary transitions from ancestral coloured belly patch to patchless (*S. cozumelae*, *S. siniferus*, *S. megalepidurus*, and *S. virgatus*, figure 1; see also [38]) so that each of our four defined 'clades' included one 'patchless' species (i.e. lacking colourful ventral patches) and one or two 'coloured' species (i.e. having colourful ventral patches). We tested clades/species in random order through the years.

(i) Chemical experiment

We performed chemical cue trials in the wild during times of daily peak activity with 9–22 males of each species (electronic supplementary material, table S1). Procedure details can be found in [30,31]. Briefly, while wearing vinyl gloves, we cut 2 × 2 cm, X-shaped pieces from clean brown filter paper to use as swabs. We tied swabs to a small (approx. 5 cm) dark string. Swab papers were either scented ('chemical cue') or unscented

('chemical control'). We wiped scented swabs along the cloacal area and femoral pore row of a conspecific, sexually mature male that acted as a chemical donor. Femoral pores are openings of subjacent femoral glands that secrete waxy substances used in lizard chemical communication [42]. Unscented swabs were left as is. We used all swabs within 24 h. If not used immediately, we stored swabs individually in clean, small, zip-lock plastic bags and kept them in a freezer at -20°C . Upon use, we attached the other end of the swab string to a fishing pole and a blind observer placed the cue paper less than one body length from the head of the focal male. If the focal male did not move away for at least 30 s, we recorded his behaviour for approximately 5 min from a distance of 3 m or more. In cases in which the focal male moved away from the cue during the first 10–30 s of the trial, the observer slowly repositioned the stimulus swab less than one body length from the male's head. We would only reposition the swab once per trial, and we aborted trials in which another lizard approached the focal subject or if the focal subject moved away, out of sight from the observer, before 3 min had passed after the start of the trial. We alternated presentations of scented and unscented cues throughout the day, and captured focal males after trials, measuring body size, confirming sex and sexual maturity, and using them as chemical donors for subsequent trials. We avoided using near neighbours (greater than one home-range distance away) as donors. We marked lizards with a single dot of non-toxic paint on the dorsal surface to avoid repetitions. All lizards were exposed to only one of the two treatment groups (chemical cue or chemical control). Behavioural data from chemical cue presentation trials for 4 of the 11 species (*S. parvus*, *S. cozumelae*, *S. merriami*, and *S. siniferus*) are also reported in [31].

(ii) Visual experiment

As a behavioural control, we recorded the undisturbed behaviour ('baseline' behaviour) of 18–50 additional adult males per

species (electronic supplementary material, table S1), in the same populations where we conducted the chemical experiment. We approached individuals to an average distance of 4–5 m, and filmed them for 10 min using Canon Elura 100 camcorders at 29.97 frames per second (fps). All recordings took place during peak breeding season and within each species' daily activity period [34]. We also conducted male–male staged territorial intrusions ('STIs') broadly following the procedure already described in [35]. In short, we captured and tied a male stimulus lizard from a non-neighbouring territory to a 10 cm string attached to a fishing pole and placed the stimulus male on the substrate at a distance of 2 m from the focal subject. We then recorded the focal subject's behaviour for up to 30 min per trial, one trial per individual. If after 5 min, the focal subject did not appear to have noted the stimulus, we moved the tethered animal closer, to within 1 m of the focal subject, and kept recording. Often, the focal subject would move out of sight or engage with the stimulus male by charging towards him repeatedly, in which case we would cut the trial short. By contrast, tethered individuals usually froze or attempted to flee. Given the lack of physical contact between conspecific intruders and focal subjects, the absence of scent-marking behaviour from intruders and the short-lived presence of the stimulus male in the focal lizard's territory, we consider conspecific intruders a predominantly visual stimulus. The trial duration ranged from 1 to 30 min with the median 5–12 min across all species (electronic supplementary material, table S1). After the end of each trial, we attempted to catch the focal lizard to collect mass and snout-to-vent length (SVL) data. Because we did not know the focal lizard's body size until its capture, stimulus lizards were *a priori* not size-matched to focal subjects or otherwise controlled. Nevertheless, SVL of the intruder was on average within 5 mm of that of the resident male, with a median difference of 3.5 mm across species, and we found no significant differences on focal-resident SVL difference between patchless and coloured species ($F_{1,166} = 0.8$, $p = 0.37$). Baseline and STI behavioural data from 4 of the 11 species (*S. parvus*, *S. cozumelae*, *S. merriami*, and *S. siniferus*) are also presented in [35,36]. Baseline behavioural data for all 11 species are also reported in [34].

(b) Scoring

From observations of field trials and video recordings, we scored the number of standard 'chemical acts', as the combined counts of tongue flicks, lip smacks, jaw rubs, and nose touches [43] per trial. We scored the number of visual display behaviours ('visual displays') in the response of focal males to stimuli as the total count of individual headbob displays (stereotyped series of up-and-down movements of the head and/or torso), whether or not accompanied by 'full-shows' (static postural displays of stretched legs, arched back, lateral flattening, and gular extension), per trial. Chemical acts reflect the reception and perception of chemical cues by the vomeronasal and main olfactory system in reptiles [42], whereas visual displays are frequent in the context of broadcasting individual identity, in the absence of a direct audience, and in agonistic, territorial interactions [23]. Chemical acts and visual displays are typical *Sceloporus* responses in playback studies to chemical (e.g. [28,29]) and visual stimuli (e.g. [35]), respectively.

(c) Statistics

We conducted data analyses in R Statistical Software v. 3.5.0 [44] by pooling the data from the two field experiments ('chemical experiment' and 'visual experiment') together. Each lizard was measured in only a single trial with a single treatment. We fitted two negative binomial generalized linear models (GLM) using the package MASS [45], to determine overall behaviour differences between coloured and patchless species, while

accounting for species relatedness, using the raw counts of 'chemical acts' and 'visual displays' as dependent variables and the log trial duration as an offset variable. We included in these two models the factors: treatment (with four levels: baseline/STI/chemical control/chemical cue), male belly colour for each species (coloured/patchless), 'clade' (A: *S. parvus*, *S. variabilis*, and *S. cozumelae*/B: *S. siniferus*, *S. merriami*, and *S. graciosus*/C: *S. grammicus*, *S. megalepidurus*, and *S. jarrovi*/D: *S. virgatus* and *S. occidentalis*) and their first- and second-order interactions. These four 'clades' diverged approximately 36 Mya within a very short period of time [41], thus tree topology consists of long branches leading to extant taxa, making the relationships across clades roughly like a star phylogeny where each clade (except for B) has a common ancestor not shared with any member of another clade. This makes the use of more complex phylogenetic comparative methods unnecessary [46,47], and instead we incorporate phylogenetic information using a factor. We then fitted separate negative binomial GLMs per species to test for treatment differences in chemical and visual display behaviour separately. We calculated *post hoc* contrasts using the `glht` function of the package `multcomp` [48] and applied a Holm–Bonferroni correction to correct for multiple testing. We verified model assumptions on the residuals. When assumptions were not met (due to the presence of outliers or violation of the assumed mean–variance relationship), we confirmed results using non-parametric statistics, Kruskal–Wallis tests, and Dunn's test of multiple comparisons with Holm adjustment on estimated frequencies of chemical acts and visual displays per 5 min. We also used these standardized behavioural frequencies in figures to facilitate visual comparisons across treatments.

3. Results

(a) Males of patchless species increased chemical behaviour in response to conspecific scents

Males of patchless, but not coloured, *Sceloporus* species responded to the chemical cue with increased chemical behaviour compared to the chemical control, leading to a significant overall interaction between species belly colour and treatment (table 1 and figure 2a). Separate analyses per species revealed that males from three of the four patchless species produced more tongue flicks, lip smacks, jaw rubs, and nose touches in the presence of conspecific scents than they did during chemical control, baseline, or STI trials (all $Z > 2.1$, all $p < 0.05$; figure 3, bottom row). The exception was *S. siniferus*, whose males responded with the same level of chemical behaviour in all four treatments. In contrast with these three patchless species, males of coloured species did not perform more chemical acts in response to a conspecific scent relative to an unscented control (figure 2b), showing equally high levels of chemical response behaviour towards both scented and unscented filter paper (figure 3, top 2 rows). However, five out of seven coloured species also performed more chemical acts in response to conspecific scents compared to baseline and/or STI trials (figure 3, top 2 rows).

Males of patchless species did not produce more chemical behaviour than did males of coloured species in baseline observations (figure 2). Among patchless species, *S. virgatus* was the most chemically active, significantly more so than *S. siniferus*, *S. cozumelae*, and *S. megalepidurus*, whereas *S. siniferus* was the least chemically active (electronic supplementary material, figure S1a). Similar to the patchless

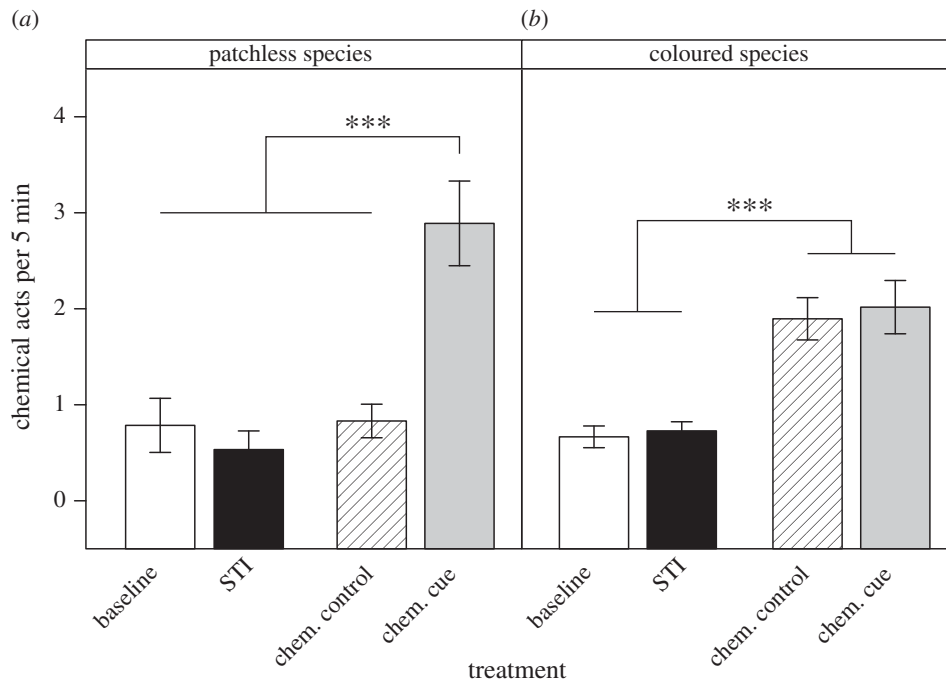


Figure 2. Overall effect of treatment on chemical acts. (a) Males of the patchless *Sceloporus* species increased chemical acts (number of tongue flicks + lip smacks + jaw rubs + nose touches) in the presence of male conspecific scents (chemical cue) compared to any other treatment (baseline, undisturbed lizard behaviour; STI, staged territorial intrusion of a male conspecific; chem. control, unscented chemical control). (b) Males of the coloured species increased chemical acts in chemical presentation trials compared to baseline or STI trials. Shown are means \pm 1 s.e. of chemical acts standardized per 5 min and asterisks denote the significance level of *post hoc* contrasts (***) ($p < 0.001$).

Table 1. Results of negative binomial GLMs on male number of chemical acts and visual displays per trial in response to field presentations of visual or chemical stimuli. Treatment (baseline/STI/chemical control/chemical cue), belly colour (coloured/patchless), and clade (A: *S. parvus*, *S. variabilis*, and *S. cozumelae*/B: *S. siniferus*, *S. merriami*, and *S. graciosus*/C: *S. grammicus*, *S. megalepidurus*, and *S. jarrovi*/D: *S. virgatus* and *S. occidentalis*) were modelled as factors. Significant terms are depicted in bold.

| model term | d.f. | chemical acts | | visual displays | |
|--|------|---------------|------------------|-----------------|------------------|
| | | X^2 | <i>p</i> -value | X^2 | <i>p</i> -value |
| treatment | 3 | 102.80 | <0.001 | 337.83 | <0.001 |
| belly colour | 1 | 6.89 | 0.008 | 0.52 | 0.471 |
| clade | 3 | 51.35 | <0.001 | 71.14 | <0.001 |
| belly colour \times treatment | 3 | 10.46 | 0.015 | 4.55 | 0.208 |
| clade \times belly colour | 3 | 25.61 | <0.001 | 14.87 | 0.002 |
| clade \times treatment | 9 | 18.15 | 0.033 | 64.00 | <0.001 |
| clade \times belly colour \times treatment | 9 | 18.52 | 0.030 | 36.25 | <0.001 |

S. siniferus, the coloured species in clade B (i.e. *S. merriami* and *S. graciosus*) also produced the fewest chemical acts, significantly fewer than *S. grammicus* and *S. jarrovi* in clade C ($X^2_1 = 16.42$, $p < 0.001$, electronic supplementary material, figure S1b). Moreover, we found variation in the response of different clades to treatments, leading to a significant triple interaction between clade, belly colour, and treatment (table 1).

(b) Loss of colour patch does not affect responses to a conspecific lizard

No distinct pattern emerged between coloured and patchless species with respect to visual display behaviour (electronic supplementary material, figure S2). We found that males of

some coloured (e.g. *S. graciosus*), but also some patchless species (e.g. *S. megalepidurus*), increased visual displays in the presence of a conspecific intruder (STI) compared to baseline behaviour (figure 4; electronic supplementary material, figure S3a), leading to a triple interaction between clade, belly colour, and treatment (table 1). With the exception of *S. cozumelae* males, which produced a high frequency of visual displays in the presence of scented cues, visual displays were more common in response to a male conspecific (STI; a mostly visual stimulus) than in response to scented cues. For example, *S. merriami* and *S. grammicus* performed more head-bob displays in STI compared to cue trials (figure 4 top row).

In general, an increase in receiver visual displays was more likely in response to a live conspecific than an increase of chemical acts, and an increase of chemical acts was more

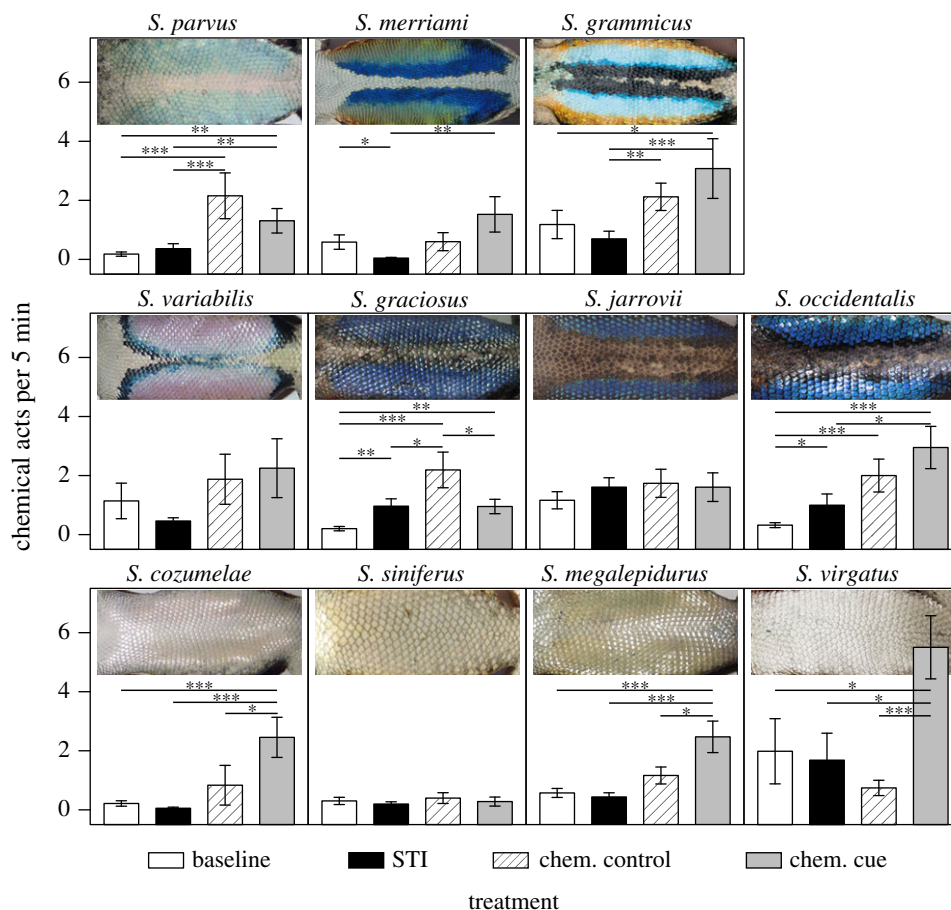


Figure 3. Frequency of chemical acts (number of tongue flicks + lip smacks + jaw rubs + nose touches in 5 min) for 11 *Sceloporus* species, seven coloured (top 2 rows) and four patchless (bottom row), in response to treatments (figure 2). We presented male lizards in the field with either a chemical signal, or a visual signal, to test for multimodal compensation in patchless versus coloured species. Shown are means \pm 1 s.e. and results from pairwise contrasts (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Species plots are grouped vertically by clade and photos depict the ventral surface (head is to the left) of a typical male of each species. (Online version in colour.)

common in response to a chemical cue (electronic supplementary material, figure S3). However, because patchless species responded comparatively more chemically to conspecific scents than coloured species did, we found a significant interaction between belly colour and treatment on the difference between visual displays and chemical acts (visual displays–chemical acts, electronic supplementary material, figure S3) in chemical, but not visual, trials (visual experiment: $X^2_1 = 0.24$, $p = 0.62$; chemical experiment: $X^2_1 = 4.95$, $p = 0.03$).

4. Discussion

(a) Compensation between colour patch production and chemical signal response

Evolutionary changes in one sensory modality can affect other modalities and thus the evolution of multimodal signals. Here, we demonstrate a link between an evolutionary change in a colour patch and chemical signal responses in *Sceloporus* lizards. Species with an evolutionary loss of a belly colour patch increased chemosensory responses to a chemical signal (figures 2 and 3), while their behavioural responses to a multicomponent (colour, motion) visual signal seemed unaffected (figures 2–4). This suggests an evolutionary interaction between visual and chemical signalling modalities that can result in compensation for a component

change in a sensory modality through increased reliance on alternative sensory modalities [5,31,36,38].

In line with previous studies [30,31], patchless males responded more vigorously to field presentations of conspecific chemical secretions than to a blank control swab. The exception was *S. siniferus*, which represents a more ancient loss of the colour patch (*ca* 40 Mya [38]), exhibits a reduced number of femoral pores and, despite the lack of colour patch, remains more chromatically conspicuous to conspecifics in its habitat compared to its patchless congeners [17,38]. Although *S. siniferus* were not particularly responsive to conspecific intruders (STI, figures 3 and 4), their increased conspicuousness could partly explain why they do not exhibit cross-modal compensation. In Pacific field crickets (*T. oceanicus*), the contemporary loss of calling song by some, but not all, males does not seem to increase female reliance on chemosensory cues during mate location [19]. Instead, both male and female crickets from populations with low frequency of callers plastically increase phonotaxis behaviour towards calling song, likely facilitating mate encounters for both callers and non-callers [49]. This pre-existing behavioural plasticity may be replacing the need to rely more heavily on additional sensory modalities to find mates. Similarly, some swordtail fish (genus *Xiphophorus*) that have lost ancestral vertical bars and swords used in sexual selection have also replaced courtship behaviour with a sneak-chase mating strategy that circumvents male–male competition and female

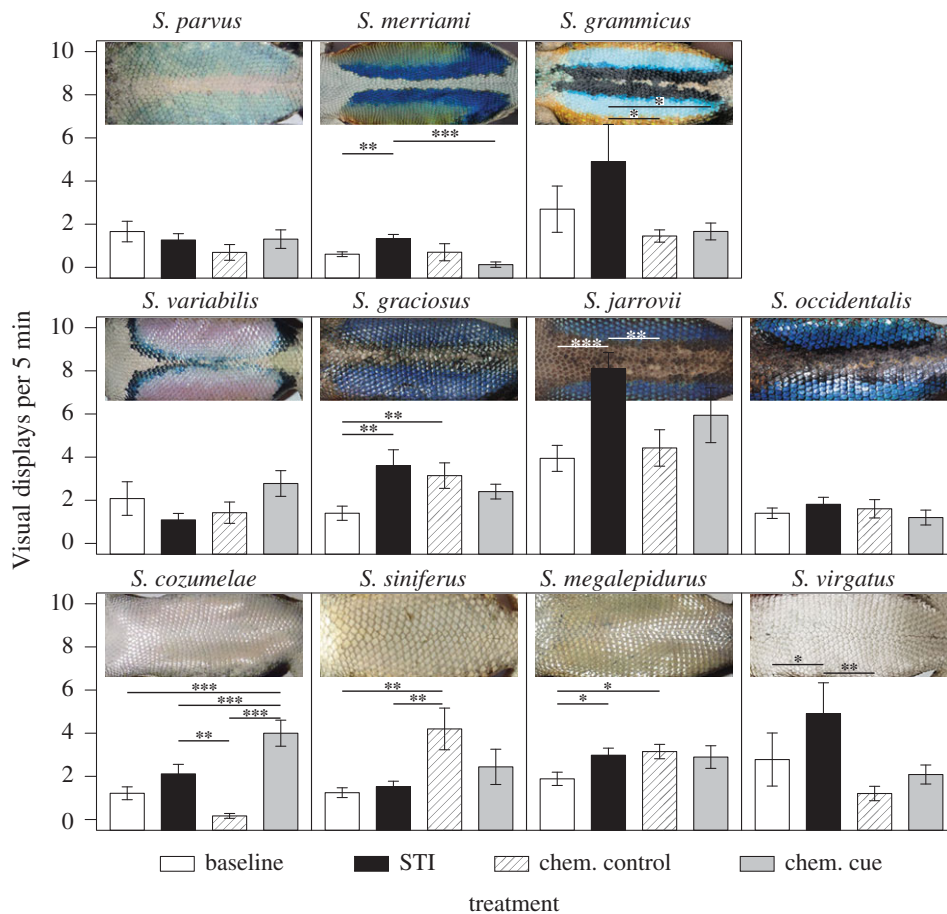


Figure 4. Frequency of visual displays (headbob displays, with or without ‘full-show’ in 5 min) for 11 *Sceloporus* species, seven coloured (top 2 rows) and four patchless (bottom row), in response to treatments (figure 2). Male lizards were presented in the field with either a chemical signal or a visual signal to test for multimodal compensation in patchless versus coloured species. Shown are means \pm 1 s.e. and results from *post hoc* pairwise contrasts (* p = 0.05; ** p = 0.01; *** p < 0.001). Species plots are grouped vertically by clade and photos depict the ventral surface (head is to the left) of a typical male of each species. (Online version in colour.)

choice [50]. In this way, behaviour may buffer cross-modal compensation of recent signal losses (*T. oceanicus*, *Xiphophorus*) and alternative signalling elements in the same sensory modality may be introduced over much longer periods of evolutionary time (*S. siniferus*) [17,38].

In contrast with patchless species, no coloured species performed more chemical acts in the presence of conspecific scents. This result agrees with two previous studies, one based on data from 4 out of these 11 *Sceloporus* species [31], and another comparing *S. virgatus* with a lizard of the sister genus *Urosaurus* that also has coloured ventral patches (*U. ornatus*) [39]. In this latter study, *U. ornatus* were less likely to perform chemical acts and performed them less frequently than *S. virgatus* did after being introduced in a resident conspecific’s home terrarium. Finally, in baseline and chemical control trials, lizards from all patchless and most coloured species, except for *S. parvus*, *S. graciosus*, and *S. occidentalis*, showed similar chemosensory responses (figure 3). Presentation of a filter paper during chemical trials was enough to elicit a reaction from lizards of these three species, suggesting that comparisons across our two field experiments in coloured species should be done with caution.

(b) Responses to visual stimuli are common and conserved across taxa

Increased display responses of similar magnitude to a predominantly visual stimulus occurred in both coloured and

patchless species (figure 4; electronic supplementary material, figure S2). Since the presence of colour patches appears to be ancestral in this group [38], this supports that the evolution of display behaviour is decoupled from the evolution of the colour patch [26,40] and display behaviour seems to be conserved across the genus. In other words, the loss of a colour patch has not compromised the response to a visual signal [26], suggesting that there is some redundancy in this multicomponent trait, which has conferred evolutionary resilience to the display [33,51]. Alternatively, in species that lack belly patches, colour may no longer offer essential information, the information may have become unreliable, and/or receiver attention may have shifted to different elements of the multicomponent signal [9]. Comparing only two *Sceloporus* species, one coloured (*S. consobrinus*) and one patchless (*S. virgatus*), a previous study reported that the loss of coloured abdominal patches coincided with a decline in the receiver’s response to a ‘full-show’ (a stereotyped visual display) performed by another male [52], which somewhat contradicts the pattern we found for 11 species. This illustrates how larger comparative studies can offer unique insight into evolutionary relationships between different sensory modalities and are desirable to identify broad patterns.

The pervasiveness of visual display responses to visual stimuli further suggest that display behaviour and colour patch are under different selective regimes and/or evolving at different rates [40]. Ventral patch colouration, in particular, exhibits no phylogenetic signal across this group of species and is

presumably under sexual selection, with patchless and coloured species evolving towards different trait optima [17]. The loss of signal components presumably under sexual selection suggests that costs imposed by opposing forces (e.g. natural selection) outweigh sexual selection, or that sexual selection relaxes or changes direction over time [9]. The loss of calling song in Pacific field crickets has been favoured by the negative impacts of a parasitoid fly that uses acoustic signals to find its hosts [49], and loss of sexually selected traits in swordtail fish have resulted from relaxed male competition and female preferences [50]. The rapid colonization of *Sceloporus* lizards across North and Central America [20] may have increased selection for improved transmission efficacy and detectability of sexually selected signal components in new habitats. At the same time, the contextual use of the push-up display [23] may provide a semi-private channel of intraspecific communication, limiting the costs imposed by visual predators and other unwanted eavesdroppers. We thus hypothesize that pressure for maintaining optimal signalling functionality in new environments under a selection regime dominated by sexual selection forces (male–male competition) favoured the evolution of cross-modal signal compensation after the loss of a ventral colour patch in *Sceloporus* lizards. Signal compensation may be further favoured if underlying the cross-modal link was a pleiotropic process (shared genetic control or tight genetic linkage across signalling traits and sensory systems in different modalities) [53,54].

Overall, we found higher rates of visual display behaviour than chemosensory behaviour, suggesting that *Sceloporus* lizards are more visually oriented. Visual displays occurred more frequently in response to tethered intruders while chemical acts were more frequent in chemical cue presentations. This agrees with previous findings in *Sceloporus*, where headbob displays were more common in response to a visual-only stimulus, whereas tongue flicks were more likely when lizards were presented with chemical secretions only [24,31]. Multimodal (visual + chemical) signallers may not always preferentially rely on visual cues, like mantis shrimp (*Neogonodactylus oerstedii*) [55] or wolf spiders (*Pardosa milvina*) [56], which are more chemically reliant during male contests and courtship, respectively. Staged territorial intrusions were designed to act mostly as a visual stimulus. However, it is possible that STI trials included a minor chemical component (e.g. in cases when focal subjects approached intruders very closely). Because we found increased frequency of chemical acts in response to the scented cue compared to a conspecific intruder (figure 3), this seems unlikely to be the case. Future studies combining visual and chemical stimuli simultaneously will provide further insight on cross-modal links between signals.

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(c) Conclusions

Using a comparative behavioural framework, here, we demonstrate an evolutionary link between loss of a colour patch and chemosensory response (signal compensation), suggesting indirect cross-modal effects may result from changes in one sensory modality in functionally interacting or integrated phenotypes [51]. Thus, interactions across sensory modalities can influence the evolution of complex signalling phenotypes and signal diversity along with deterministic and stochastic forces [51,57]. Signal loss is a major source of phenotypic diversity and because signalling traits mediate fitness-related interactions like male–male competition and mate choice, it can play an important role in species diversification [53,57]. The conditions under which signal compensation in sexually selected traits evolves likely involve several factors. These include some form of major cost on the lost signalling trait/element, relatively strong sexual selection for maintenance of signalling functionality, the availability of suitable, alternative signalling components/modalities, and the absence of severe constraints preventing the shift [9,18,19], such as evolutionary history [57], elapsed evolutionary time [19], biomechanical constraints [58], or pleiotropic effects [54]. In this light, holistic approaches to the study of animal communication seem appropriate to understand how multimodal animal signals evolve.

Ethics. All experiments were approved by the Indiana University Bloomington Institutional Animal Care and Use Committee (Protocol 10-013 to E.P.M.), and the Indiana State University IACUC (Protocol 492636 to D.K.H.). Permission to collect and work with lizards was granted by Carolyn Ohls at Christmas Mountain Oasis (TX), the Indiana State Department of Natural Resources, the United States Forest Service, the Arizona Department of Game and Wildlife, the California Department of Fish and Wildlife, the Texas Parks and Wildlife Department and the Secretaría de Medio Ambiente y Recursos Naturales Oficio Núm. SGPA/DGVS/04386/12.

Data accessibility. Data used in this study are available as part of the electronic supplementary material.

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Competing interests. We declare we have no competing interests.

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