Chapter 7

VISUAL AND CHEMICAL SIGNALS OF SOCIAL COMMUNICATION: PROVIDING THE LINK TO HABITAT AND ENVIRONMENT

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ABSTRACT

Social communication in most reptiles involves primarily visual and chemical signals. Components of visual signals include movement (frequency, motor pattern), colors and color patterns, which can convey different information. Chemical signals also can be complex, containing a variety of components conveying information on species identity, sex, reproductive condition, body size, social status, feeding status, and immune status. Fence lizards (*Sceloporus*) show fascinating variation in color patterning and in motion display patterning, and behavioral responses to conspecific chemicals. *Sceloporus* are found in an array of habitats in North and Central America, as diverse as shrub-steppe habitat of the Great Basin desert, arid deserts of the American southwest and Mexico, pine-oak woodlands, and semi-deciduous tropical forests. Working at the species level and in a phylogenetic context, we study the evolution of multimodal and multicomponent communicative signals. Centered on behavior, our work examines physiological mechanisms involved in signal production and in signal reception, and the potential costs and benefits of signals and signal components. We are especially interested in endocrine mechanisms and evolutionary variation in these mechanisms. Endocrine mechanisms have the potential to constrain or to facilitate evolution, depending on the degree to which suites of traits are closely coupled to a given endocrine mechanism.

Keywords: *Sceloporus*, androgens, melanin, femoral glands, color patches, display rate, crypsis, phylogenetic methods, spectral reflectance

INTRODUCTION

Lizards exhibit multimodal signals in which color, motion and chemicals are key signal components (Cooper and Greenberg 1992, Halpern 1992, Ord et al. 2001, Johnson et al. 2010, Mason and Parker 2010). We focus on species in the North American lizard genus *Sceloporus* because there is repeated evolutionary variation in the occurrence of the color component of an aggressive signal (blue belly patches, Figure 1). These lizards are commonly referred to as fence lizards and have blue belly patches that are expressed in males but not females of most *Sceloporus* (Wiens 1999). In some derived species, the sexes are monochromatic and either both sexes have the blue patches or neither sex does (Weins 1999). These patches appear to be used primarily in intrasexual signaling. Males typically reveal the belly patches during stereotyped aggressive postural displays involving dorsolateral flattening, and males often position themselves laterally to an opponent (e.g., Carpenter 1978 and Ferguson 1977, Cooper and Burns 1987, Martins 1993, Quinn and Hews 2000, Hews and Quinn 2003, Robbins et al. 2010). In *Sceloporus* species in which females also express the blue belly patches, work suggests the patches play roles in female-female aggression (e.g., Martins 1993, Woodley and Moore 1999). The abdominal patches are not typically revealed during courtship, and research suggests there is little direct role for the abdominal blue patches in female mate choice (Swierk et al. 2012). In both a blue-bellied (*S. undulatus*) and a white-bellied (*S. virgatus*) species, paint manipulations reveal that male abdominal coloration cannot explain female mating decisions (Quinn and Hews *in preparation*). Female choice for specific male traits is also uncommon in other territorial polygamous lizards (Tokarz 1995; Sullivan and Kwiatkowski 2007, Uller and Olsson 2008), although recent work suggests that multiple traits together may influence female association and even mating preferences (Hamilton and Sullivan 2005, Bleay and Sinervo 2007, Swierk et al. 2012) in the absence of territory cues, which can be primary in importance (e.g., M'Closkey et al. 1987, Hews 1990, Hews 1993).

Motion is also an important part of the visual display in *Sceloporus*. A commonly used motion display, the headbob (Carpenter and Ferguson 1977), can be used by male and female lizards to broadcast individual, sex, and species identity, and individuals typically do so at territorial or home range borders (e.g., Martins 1991, 1993). Exposure of the abdominal patches is commonly done while performing the up-down pattern of a head-bob; this combination of posture (arched back and dorsolateral flattening to reveal the abdominal skin) and motion (the up-down pattern and cadence of the headbob) are referred to as a "full-show" display. The full show is an aggressive display given during male-male interactions. The posture of a full show may also be performed while static; we refer to this display as a "full show hold". Full show hold displays are highly escalated displays seen in male-male interactions. They often are done in close proximity (several body lengths) to the opponent male, and the display often preceeds a charge and bite. Frequently both males will be engaged in full show hold display simultaneously.
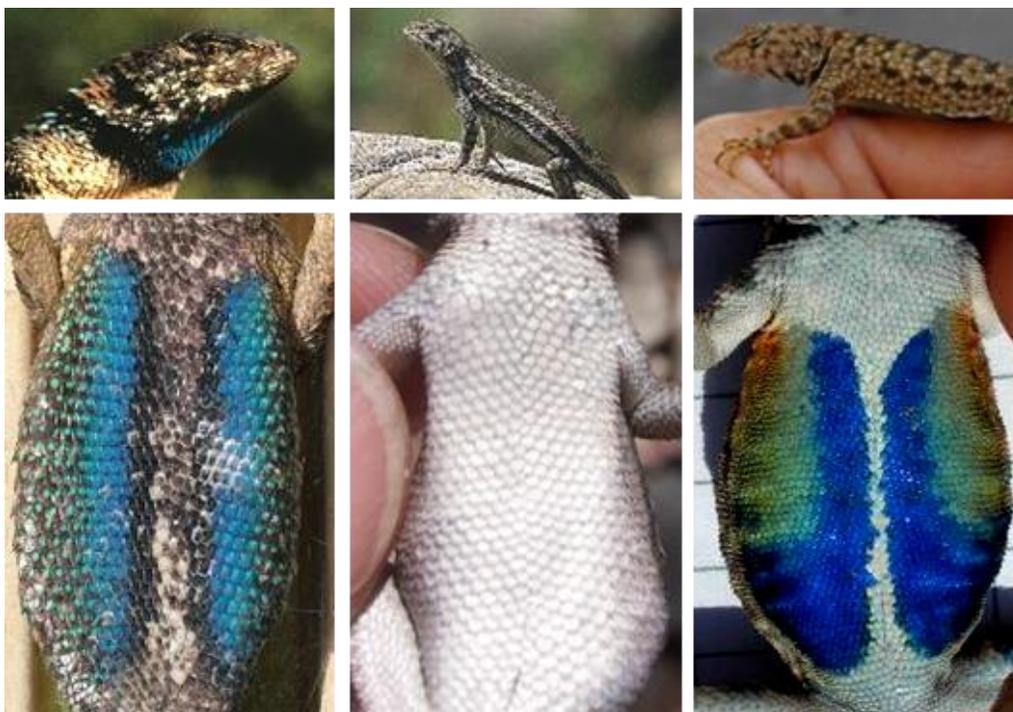
Figure 1. Species in the lizard genus *Sceloporus* vary in abdominal coloring. In most species, males have a pair of bright blue belly patches and females do not (male *S. undulatus* shown, left above and below). In a handful of species both sexes lack abdominal patches (*S. virgatus* male shown, middle, above and below). In still other species, the abdominal blue of males is partially replaced with other colors (*S. merrami* male shown, right, above and below).

Lizards in the *Sceloporus* genus also use chemical displays to defend territories and to attract mates (Duvall 1979, Martins et al. 2006). Many squamate reptiles communicate chemically (Mason 1992, Mason and Parker 2010). Typically researchers assess the ability to detect and to discriminate among different potential chemical cues by determining differences in tongue-flick rates and/or association preferences. A growing number of studies have linked phenotypic attributes to specific components of chemical secretions in vertebrates (Wyatt 2003). Using femoral gland secretions alone, lizards can discriminate male attributes associated with dominance status, color, and body size for example (Moreira et al. 2006, Carazo et al. 2007, López et al. 2006, Martín and López 2006, 2007). Like many other lizards, *Sceloporus* use chemical signaling (Martins et al. 2006). Indeed, the genus name refers to the large femoral pores that exude chemical secretions from a row of femoral glands (Cole 1966).

Habitats of *Sceloporus* Lizards and Potential Effects on Signal Traits

Sceloporus are found in an array of habitats in North and Central America (Figure 2). These habitats are as diverse as the scrub-steppe habitat of the Great Basin desert, arid deserts of the American southwest and Mexico, pine-oak woodlands, and semi-deciduous tropical forests (see photos also in Kohler and Heimes 2002). In most species, males are territorial and

individuals use rocks, logs, or trees as elevated perches to view their territory and for performing broadcast displays. Some species are primarily arboreal, others are saxicolous (rock-dwelling).

Figure 2. Habitats of *Sceloporus* lizards vary tremendously. (Top left): Semi-deciduous tropical forest on the Pacific coast of Oaxaca, Mexico is one habitat that the white-bellied *S. siniferus* can be found. (Top right): Rock areas and rock faces of the Chihuahuan desert are the preferred habitat of Merriam's canyon lizard, *S. merriami*. (Bottom left): Riparian corridors in mixed coniferous-oak woodlands of the Sierra Madre Occidental are a favored habitat of the striped plateau lizard, *S. virgatus*. (Bottom right): exposed sunny sites with logs and rocks are typical of the diverse habitats in which the widely-distributed Eastern fence lizard, *S. undulatus* can be found.

The striped plateau lizard, *Seloporus virgatus*, is the best-studied example of a "white-white" species (both sexes without the belly patches). In this species we have documented a number of changes in various components of the social signaling system, in comparison to the closely related *S. undulatus*. We have studied *S. virgatus* in the Chiricahua Mountains of southeast Arizona, USA (and see Figure 2). This species occurs in Sierra Madrean habitat, characterized by sycamore (*Platanus*), oak (*Quercus*), and conifers (e.g., *Pinus, Juniperus),* and a variety of shrubs such as yucca (*Agave*), acacia (*Acacia*) and sumac (*Rhus*).

Perhaps the best-studied "blue belly" species is the eastern fence lizard, *S. undulatus*. This widely-distrubuted species occupies a wide range of habitats in eastern North America (Conant and Collins 1998). They are typically found in areas with exposed rock or in open-canopy forests such as the Pine Barrens in the US state of New Jersey. In the central US state of Missouri, *Sceloporus* can be found on exposed limestone, dolomite, and chert in the Southwestern Ozark Mountains, on sandstone and shale in the northern Ozark Mountains, and on igneous rocks of volcanic origin in the Missouri Glades in the eastern portion of the state. In the southeasteern United States (New Jersey to Florida) *S. undulatus* are common in dry, open forests, and are found using the bases of trees, logs and stumps for perches and refuges.

Communicative signals can be under multiple sources of selection, many of which are related to habitat. For example, selection may favor signals that are conspicuous to conspecifics, yet animals must trade off potential benefits of detection by conspecific receivers with the potential risk of being detected by predators (Ryan 1985, Stuart-Fox et al. 2003, 2004). One of our goals is to delineate tradeoffs among signal components in *Sceloporus* species. Further, these tradeoffs may be affected differently by different habitats. For example, variation in predation intensity may select for the loss of abdominal blue and reduction of signaling. Another arena of tradeoffs involves physiological factors (Hill and McGraw 2006). For example, color pigments involved in the signaling traits, such as carotenoids or melanin, may also play key roles in immune function, including in lizards (e.g., López et al. 2009) and investment in coloration versus immune function may be an important tradeoff (e.g., Ruiz et al. 2011). Habitats and populations may vary in exposures to pathogens, thus potentially altering the tradeoff. And androgens, which may influecne coloration, also may directly affect parasite loads (Cox and John-Alder 2007). Aspects of habitat quality, such as resource availability or parasite abundance, can affect body condition of animals which in turn may affect investment strategies in components of signaling traits.

A key habitat factor for visual signals is ambient lighting, which can influence hue perception (Endler 1991, Fleishman et al. 1997). Habitats may also vary in the average background motion against which displays are viewed by conspecific (Fleishman 1992). For chemical signals, relative humidity and other weather factors may influence signal persistence (Alberts 1991), as might the nature of the substrate on which the chemicals are deposited.

Complex Signaling Traits

Chemical and visual signals in lizards are examples of signals that differ in sensory modality (how a stimulus, chemical versus visual, is transduced to the nervous system of the lizard). Multimodal signals in animal communication are receiving increasing study (Partan and Marler 1995, Rowe 1999, Uetz and Roberts 2002, Candolin 2003), although more work is needed to understand complex interactions among signal components (Partan and Marler

2005). When one aspect of a complex signal evolves, a host of other behavioral, hormonal, sensory, and other aspects of both the sender and receiver are also potentially influenced. Consistent and strong correlations among traits, mediated perhaps by wide-ranging actions of hormones, can potentially constrain phenotypic evolution, making changes less likely and limiting the types of changes that can occur (Hau 2007, Zera et al. 2007, Adkins-Regan 2008, McGlothlin and Ketterson 2008). Alternatively, phenotypic integration might speed evolution, making it happen in bursts of correlated changes rather than gradually (Adkins-Regan 2008, Ketterson et al. 2009, Kopp 2009). Kopp (2009) particularly calls for examining the genetic basis of similar phenotypic changes in multiple evolutionary lineages. In our work on *Sceloporus* lizards, we are asking whether endocrine integration of the components of a multimodal communicative signal has constrained shifts in the evolution of a complex lizard display.

We are especially interested in evolutionary patterns of both visual and chemical signaling traits. Negative association between visual display and chemosensory behavior has been documented (Hews and Benard 2001, Thompson et al. 2008, Hews et al. 2011). Hence changes in chemical signal composition might be expected as compensation for blue-loss in *Sceloporus virgatus*, for example.

Many elements of social signaling in *Sceloporous* are mediated by hormones, as in many vertebrates (Adkins-Regan 2005). The blue belly patches develop with sexual maturation, are permanently expressed in adults, and show relatively little seasonal variation (Kimball and Erpino 1971, Hews and Quinn 2003, Cox et al. 2008). In several lizard species (in *Sceloporus* and in the sister genus *Urosaurus*), androgen manipulations suggest that expression of blue belly patches is mediated by elevated plasma androgens in hatchlings (Kimball and Erpino 1972, Rand 1992, Hews et al. 1994, Hews and Moore 1995, Hews and Quinn 2003, Cox et al. 2005) and, to some extent, plasma androgens in adults (Cox et al. 2008). Chemical signaling traits are also androgen-mediated. Secretion of the femoral glands can be activated in adults of both sexes by testosterone (reviewed in Hews and Quinn 2003). Finally, in at least two *Sceloporus* species, adult differences in plasma androgens also correlate with sex- and species-differences in the aggressive behaviors that display the abdomen in two species (blue males *S. undulatus,* white males *S. virgatus,* Hews et al. 2012).

Previous work on *Sceloporus* lizards and other vertebrates also suggests linkages between social signaling and a second hormonal system, the melanocortin system. Melanocortins play diverse roles (reviewed in Ducrest et al. 2008), including having direct or indirect effects on coloration patterns based on eumelanin, aggressive behavior, chemical signaling, and immune function. For example, the melanocortin melanocyte stimulating hormone (MSH) acts directly on cells in the skin (melanocytes) to increase melanogenesis in those cells. Melanocytes are fascinating cells and may be far more than simple pigment-containing cells (Slominski and Wortsman 2000, Cone 2006, Slominski 2009). Melanocytes produce a number of hormones and signaling molecules that are more-typically associated with the brain (e.g., pro-opiomelanocortin, POMC, and POMC peptides; corticotropin releasing hormone, CRH) and receptors for a diverse array of hormones. Sequence variation in melanocortin receptor genes (e.g., *Mc1r*) is associated with variation in melanin-based color signals, chemical signals, and aggression in a variety of vertebrates (Ducrest et al. 2008, Hubbard et al. 2010) including *Sceloporus* (Rosenblum et al. 2004, Robertson & Rosenblum 2009). Ducrest et al. (2008) suggest that pleiotropic effects of the melanocortins might contribute to the covariance between melanin-based coloration and other signaling traits and behaviors in diverse

vertebrates, including salmonid fish, lizards, birds and mammals. The review by Ducrest and colleagues emphasizes behavioral syndromes and within population variation. Population and species also vary in genes coding for melanocortin receptors (e.g., *Mc1r*) and associations with eumelanin signals, reveal sex and population differences in these signals and aggression (e.g., Kopp 2009, Uy et al. 2009, Roulin and Salamin 2010, Malfi et al. 2011). Finally, elements within the melanocortin signaling system are up-regulated by androgens (Mo et al. 2009), suggesting linkages between these two hormonal systems.

This brief overview suggests that actions of androgens and of the melanocortin system may contribute to the covariation seen in signaling traits in *Sceloporus.* Below, we describe work in which we have begun to detail associated differences in traits involved in signaling in a white species (*S. virgatus*) and several blue species, and the traits all have either demonstrated or potential linkages to at least one of these two hormonal systems. Specifically, white males (*S. virgatus*) exhibit less aggressive visual signaling, respond to visual signals less and to chemical signals more, have lower plasma androgen levels, and have lower numbers of androgen receptors in two key hypothalamic areas compared to males of a closely-related blue species. In addition to exposing the abdominal skin less (i.e., lower rates of the full show display), this white species also has little melanin in the abdominal skin location where the blue patch occurs in the blue species. Finally, preliminary evidence suggests that males in the white species have a larger sensory structure, the vomeronasal organ, involved in detecting conspecific chemicals, compared to *S. undulatus* (blue) males.

Evolutionary Changes in Signals

Our work currently focuses on the species with *male loss* of the secondary sexual trait, a poorly studied aspect of sexual selection (Wiens 2001, Morris et al. 2005, Ord and Stuart-Fox 2006, Tinghitella and Zuk 2009). Overlaying the presence/absence of blue belly patches on the most recent phylogenies (Leaché 2010, Wiens et al. 2010), we found 6-10 evolutionary episodes in which the blue belly patch appears to have been lost in *Sceloporus* (assuming the ancestral character state is blue males, white females; Weins 2001). We have selected four of these episodes (Figure 3) in which the losses are clearly independent of each other and that offer examples of both recent and longer-term divergences. Within each of the four clades, we have selected three species for closer study: one species that has lost the blue belly patches, a recently-diverged sister taxon, and a more distant, blue-bellied, out-group. Note that in the two older clades, the sister taxon has also lost some, if not all, of their belly blue.

The phylogeny is sufficiently robust for our studies, despite some disagreement about methodology and resulting fine structure (Wiens et al. 2010, Leaché 2010). Parsimony analysis suggests that male-limited blue patches are likely ancestral to the *Sceloporus* lineage, and that monochromatism in *Sceloporus* species represent evolutionary loss of sexual dimorphism, either by female gain or by male loss of the blue belly patches (Wiens 1999). However, this parsimony analysis may be biased by a relatively large radiation of viviparous species basal in the *Sceloporus* tree. Viviparity and blue abdominal patches are closely coupled, with viviparous species being less likely to lose the blue belly patches over evolutionary time (Ossip-Klein et al. 2013a). If the large viviparous clade is treated as a
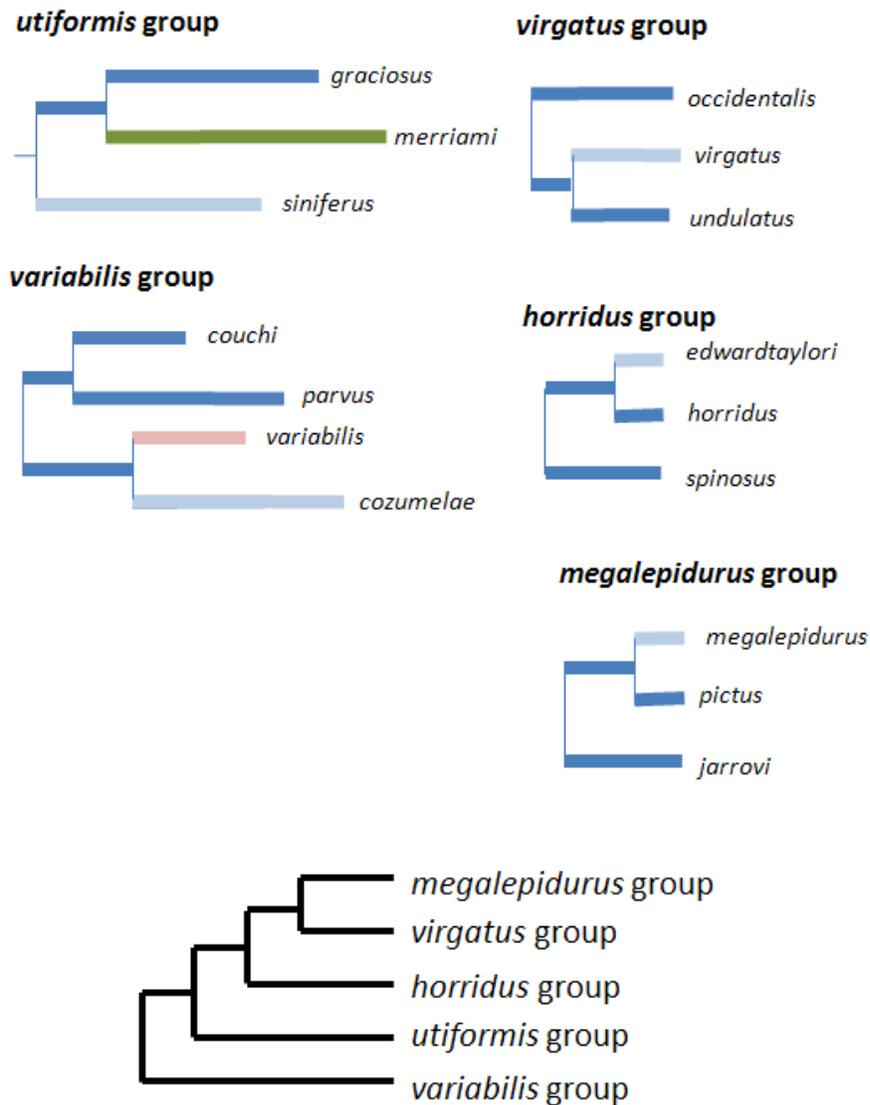
Figure 3. Five clades within *Sceloporus* showing the target species in which we have selected to study multimodal signal evolution. Darker blue branches represent species with blue belly patches; pale blue branches represent species without abdominal blue patches; pink branch represents a species that has bicolored blue and pink patches; green branch represents species with bicolored blue and pale green patches. Thus, within each clade there are independently-evolved species in which the males do not have the abdominal blue patches, and close relatives with abdominal blue. Note that several of the lineages are relatively older and white species in these lineages may represent the ancestral character state, whereas white species in the more recently derived clades represent male loss of abdominal blue. The phylogenies (and branch lengths) are adapted from maximum likelihood analysis of nuclear and mtDNA, based on Wiens et al. 2010.

single taxon, older (more basal) species in the genus in which both sexes are white ("white-white") may represent the ancestral character state, whereas more recently evolved "white-white" *Sceloporus* may represent species with male loss of blue patches. Species in which females have abdominal blue but males lack them do not occur.

RESEARCH METHODS

We are using a multi-faceted approach to studying the evolution of visual and chemical signaling in *Sceloporus* lizards. Our studies include measuring the quality and rates of display behaviors in the field, and measuring a number of traits that are associated with signal production or signal reception, for both visual and chemical signals. For some data, we are applying phylogenetic comparative methods to address questions about the pattern of evolutionary change.

Ancestral Habitat Reconstructions

We began by using the current geographic distributions of *Sceloporus* lizards to infer the ancestral habitats of this genus (Lawing 2012). First, we used global distribution databases to identify more than 60,000 unique geographic locations at which *Sceloporus* lizards have been found. We then obtained detailed measures of the climate (19 variables including temperature, seasonality, rainfall, etc.) at each of these locations from global databases, using this information to create "climate envelopes" that describe the existing habitat of each species. We used also phylogenetic information to infer the ancestral climate envelopes of *Sceloporus* lizards extending from the beginning of the genus (about 20 million years ago) to the present. Geologists have used similar climatic measures to reconstruct the habitats available throughout the history of our planet. Using methods outlined in Lawing and Polly (2011) and calibrating the reconstruction with the handful of available *Sceloporus* fossils, we then overlaid our climate envelope reconstructions onto the maps based on historical geology. The result is a reconstruction of the geographic distribution, habitats and climates experienced by *Sceloporus* during the roughly 20 million years during which this genus has been evolving, based on the best available geological, geographic and phenotypic data (Lawing 2012, Lawing et al. *in preparation*).

Visual Signals: Aggressive Display Behavior

Aggression in territorial vertebrates is often assessed by recording responses to a staged territorial intrusion (STI), in which a tethered or caged, unfamiliar, same-sex individual is placed near a free-ranging male. We use STIs to measure aggression in free-ranging males. Because territorial males move conspicuously, sit on prominent perches, and engage in broadcast display, we can determine status in a 5-min observation period without having to map territories. We first videotape and record undisturbed rates of behavior for 10 min, followed by a 10-min STI with a conspecific adult male. In each STI, we record the level of

escalation, rates of each display, and number of movements and direct aggressive behaivors (charge, bite). For a STI, a stimulus male is tethered at the end of a 2-m pole and placed at 2 m from the focal male. We then capture the focal male to measure his body mass and length from snout to vent (length-vent length or SVL). Because size differences can influence the outcome of aggressive encounters, we discard STIs in which males are more than 3 mm different in SVL.

Visual Signals: Conspicuousness, Spectral Reflectance and Background

Historically, studies of animal coloration and conspicuousness have relied upon human vision, which may underestimate the spectral variation in animal coloration (Fleishman et al. 1993, Stuart-Fox et al. 2004). Recently developed visual models take into account the visual properties of the receiver to calculate the chromatic (hue) contrasts between the animal and the background (Vorobyev and Osorio 1998, Stuart-Fox et al. 2003). Thus, to understand the potential costs associated with conspicuous ornamentation, we model signal conspicuousness to potential avian predators by explicitly accounting for properties of their visual systems. To calculate chromatic contrasts of these potentially conspicuous colors, we measured the spectral reflectance of the lizards' dorsal surfaces, the ventral surfaces, and the substrate where the lizards were found, using an Ocean Optics reflectance spectrophotometer. We use published data for daylight illumination (Endler 1993), and filtered these data through the blue tit (*Parus caeruleus*) mathematical visual model, to approximate how an avian predator would view lizards against their habitats. This currently is the only published complete visual model for a bird.

Visual Signals: Signaling Patches and Potential Information Content

The information about the sender that color patches and other signaling traits may communicate is a major area of research. One manner researchers use to evaluate the potential information content of traits is to determine if other phenotypic attributes correlate with particular signaling traits. One example of how habitats can affect signaling is illustrated by a study with our colleagues (Cain et al. *in preparation*). We examined whether and how anthropogenic disturbance, specifically forestry logging activity, influences trait correlations and hence the potential for altering the reliability of signaling traits. Studying male *Sceloporus undulatus hyacinthinus* in Kentucky, we photographed the ventral surface of individuals in a standardized manner, and then used image analysis software to measure the size (area) of three paired color patches: abdominal blue, abdominal black, and throat blue. Furthermore, body condition may be useful trait to assess, both for potential mates and for potential competitors. Variation in body mass or body condition (body mass relative to a given body length) may result from comparatively "shorter-term" environmental effects on the phenotype. We addressed the signaling potential of all of these traits.

Proximate Mechanisms Underlying Visual Signals:
Histological Analysis of Color

To analyze for melanin we use histology to visual melanin in the abdominal skin. We preserve skin samples taken from the patch locations in 10% buffered formalin, then use a decalcification fluid to soften the scales. After paraffin embedding we cut cross sections (7 um thick), mount the sections, deparaffinize, rehydrate and stain sections with Harris-modified hematoxylin-eosin stain (Quinn and Hews 2003).

Endocrine methods use plasma levels of androgens in the lizard's blood which are measured using radioimmunoassay, and, more recently, enzyme-linked immunosorbent assays (EIA kits). We also use immunohistochemistry on thin sections of frozen brains to visualize cells containing androgen receptors (Hews et al. 2012).

Chemical Signals: Chemical Signal Production

We focus on secretions from the femoral glands, a series of exocrine structures located on the medial surface of the thigh in many lizards (Cole 1966). Although males generally produce more of these secretions than do females, the amount of secretion produced appears to be an indication of body size and reproductive condition rather than a signal conveying individual or sexual identity (Martins et al. 2007). To date, femoral gland secretions in lizards have been found to consist of both proteins (Alberts et al.1993) and lipids (Weldon et al. 1990, Alberts 1990, 1993, Escobar et al. 200, Martín and López 2005, 2006, Gabirot et al. 2008). The lipid fraction primarily is comprised of carboxylic acids and t heir esters, steroids and alcohols. For example, cholesterol and cholesta-5,7-dien-3-ol are some of the main components of the lipid fraction in the wall lizard, *Podarcis hispanica* (Martín and López 2006). In the green lizard (*Lacerta vivipara*) lipophilic compounds include ten steroids (mainly cholesterol) and four carboxylic acids between n-C-12 and n-C-18, and minor components such as squalene, cc-tocopherol, and two waxy esters (Gabirot et al. 2008).

Connections between endocrine status and specific chemical components of signals have received little attention in reptiles. In garter snakes, steroids and skin secretions are correlated (Mason et al. 1987), but such correlations of femoral gland secretions and steroid hormones are rarely examined in lizards (Martín et al. 2007). Lizard femoral glands are more active in the breeding season and femoral glands are typically much more active in males than females (Mason 1992). Manipulative studies reveal that femoral gland activity is an androgen-mediated trait in many lizards (Kimball and Erpino 1971, Hews et al. 1994, Hews and Moore 1995, Abell 1998, Hews and Quinn 2003, Cox et al. 2008). Cholesterol, a precursor for *de novo* synthesis of all steroid hormones, is a major component of femoral gland secretions in a variety of lizards (Escobar 2001, López and Martin 2005, López et al. 2006, Martín and López 2006 a,b, Gabirot et al. 2008), suggesting that steroid hormones and components of femoral gland secretions may be commonly linked.

The femoral gland secretions vary importantly in lipid content and proportions. Steroid hormones and lipid-soluble metabolites of steroid hormones may occur in the femoral gland secretions via passive diffusion and/or via active secretory processes in the gland. Hence steroid hormones may be one component that can signal dominance status and reproductive condition, for example. In mammals, sebaceous glands are affected by the melanocortin

system. We are currently testing the hypothesis that melanocortins (e.g., α-MSH) may be involved in regulating femoral gland secretions in *Sceloporus* (R Seddon and D Hews).

Evolutionary Analyses

To study evolutionary patterns we use modern phylogenetic comparative tools. To date, we have used data for 39 *Sceloporus* lizard species that we have gathered from published studies and online databases or have collected ourselves (Ossip-Klein et al. 2013a). We then use linear model approaches to answer specific questions about ancestral habitats, evolutionary relationships between color, motion, and chemical signals, the physiological mechanisms underlying those signals, and the behavioral consequences of evolutionary shifts in those signals. We begin with a focus on the phenotype and apply generalized least squares to incorporate phylogeny as part of a weighting of the error term (Martins and Hansen 1997). This approach is an extension of the popular Felsenstein (1985) contrasts method to allow some flexibility in the underlying evolutionary assumptions. In addition, we apply methods that explicitly ask which aspects of the environment most influence particular aspects of phenotypic evolution. For example, we use Hansen's method (1997, see also Butler and King 2004, Hansen et al. 2008) to ask which aspects of the selective regime best predict signal evolution, and the phylogenetic mixed model (Housworth et al. 2004) to ask whether evolutionary changes are better predicted by recent environmental shifts or by forces that track the phylogeny over longer periods of time.

RESULTS AND DISCUSSION

Ancestral Habitats

Twenty million years ago, when the first *Sceloporus* lizards appeared, the world was a much warmer, drier place. Mexico, the current center of radiation for *Sceloporus* lizards, was a hot, dry desert – a much harsher environment than currently used by any *Sceloporus* species. Our reconstructions (Lawing 2012, Lawing et al. *in preparation*) suggest that *Sceloporus* most likely arose in the ancient grasslands of central North America (the oldest fossil was found in Nebraska), and that over the next 5 million years, those early *Sceloporus* moved westward to cooler montane areas as global temperatures increased even further. The first burst of speciation and radiation in *Sceloporus* occurred about 14 million years ago, when the earth began a long, cooling cycle (Figure 4). During this cooling period (from 14 to 9 mya), thorn scrub habitats began to appear at lower elevations. *Sceloporus* migrated into that thorn scrub habitat, and radiated into the basal forms of the main species groups we see within *Sceloporus* today. About 6 million years ago a second long cooling cycle began, and *Sceloporus* also began moving south into their current center of radiation in Baja California and mainland Mexico. At this point, the genus also underwent a second burst of speciation that further subdivided each of those basal lineages into multiple, independent species (Figure 4). As shown by this reconstruction (Lawing 2012, Lawing et al. *in preparation*), the

beginning of global cooling cycles seem to have been important periods of diversification and adaptive radiation for *Sceloporus* lizards.
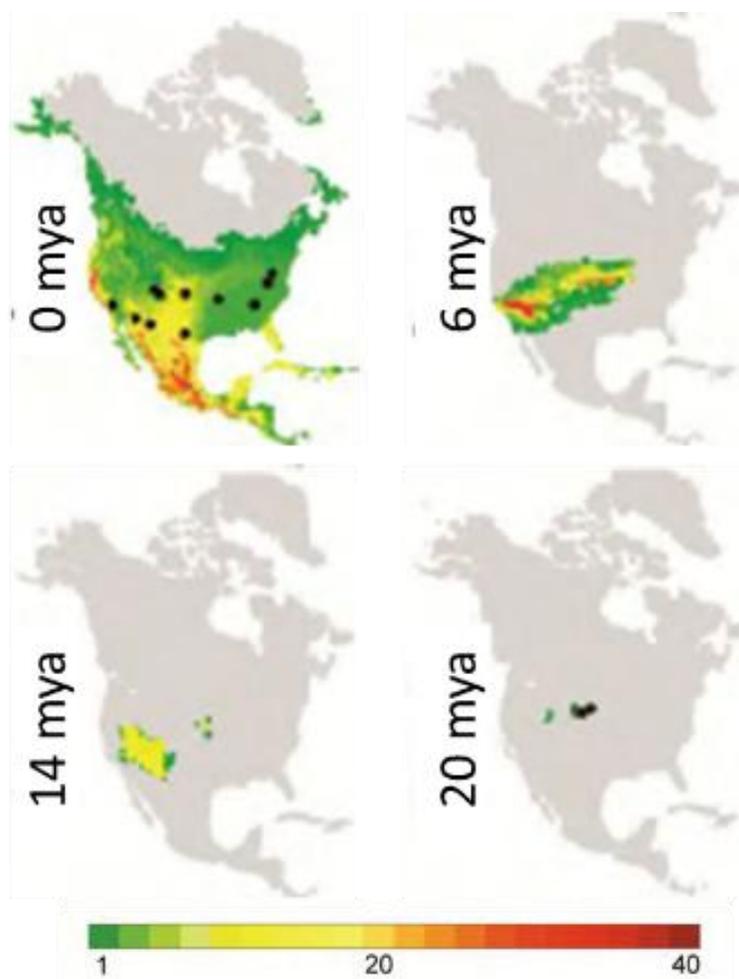

Figure 4. Absolute species richness (the number of species) for *Sceloporus* at four historical time points, as inferred by reconstruction of species climate envelopes on habitat available at that time. Black points represent the location of known fossils. Brighter colors represent more species. Modified from Lawing (2012).

Visual Signals: Aggressive Display Behavior

One of our first questions is whether other *Sceloporus* species in which males are "white" (lacking abdominal blue patches) also have lower levels of territorial display behaviors and less aggressive responses to conspecific males. Recently we used the staged territorial intrusion (STI) procedure to measure display and aggression response of males to a conspecific male intruder in two species, *S. siniferus* and *S. merriami*, both in the older

Utiformis group (see Figures 1 and 3). One is white bellied (*siniferus*) and the other has abdominal patches that are part blue and part sage-green (*merriami*).
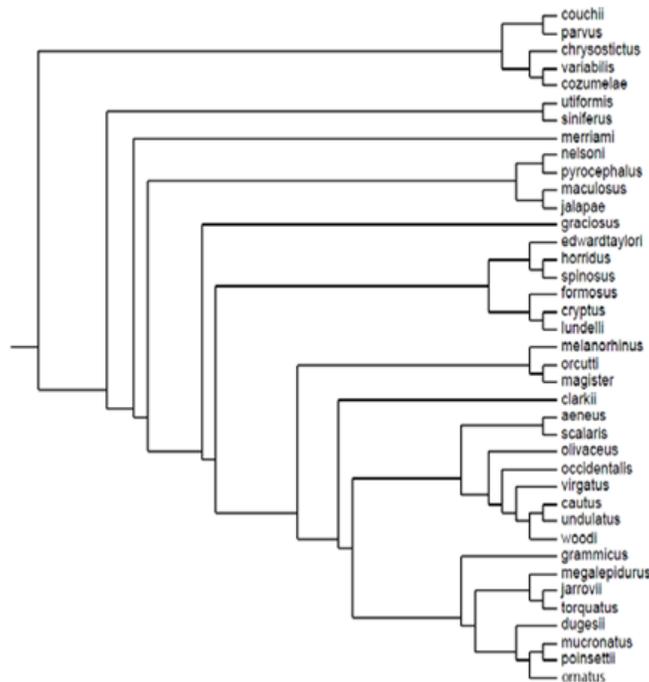

Figure 5. One of two pruned trees used in Ossip-Klein et al. (2013a), to examine patterns of evolution in motion, color and chemical (femoral pore number) traits. The phylogeny and branch lengths are based on maximum likelihood analysis of nuclear and mtDNA by Wiens et al. (2010).

Contrary to our prediction based on the comparisons between *virgatus* and *undulatus*, males of the older white species, *siniferus* exhibited farily high rates of aggressive display to the introduced intruder (Figure 6). However, *merriami* with blue in the abdominal patches exhibited lower rates of aggressive display ("fullshow" which involves headbobs performed while posture is dorsolaterally flatened and back is arched), but higher rates of direct aggression (charge and bite, Figure 6). One factor that may account for this difference is habitat. Individuals in the white *siniferus* species live in light gaps and clearings in the forest, and we often found them on small exposed twigs and logs a meter off the ground. To escape they often simply lept into the underbrush. By contrast, the blue *merrami* favors more exposed open areas (rock faces of canyon walls), where retreat sites appeared more limited. Hence, it may be less risky to simply charge and attempt to bite the intruder, instead of to engage in lengthy bouts of signaling that may increase the risk of detection by predators.

Signaling Patches and Potential Information Content

The information about the sender that color patches and other signaling traits may communicate is a major area of research. One manner researchers use to evaluate the potential information content of traits is to determine if other phenotypic attributes correlate

with particular signaling traits. Different habitats might influence the information content of signals. Habitats may differ in a number of key parameters that may alter cost-benefit tradeoffs, and hence shift investment strategies. Resource availability, predator density, parasite levels, thermal opportunities are examples of some important factors that can vary between populations and species in different habitats. Hence studying trait correlations in different populations and different species may suggest factors that affect trait correlations and signal information content.
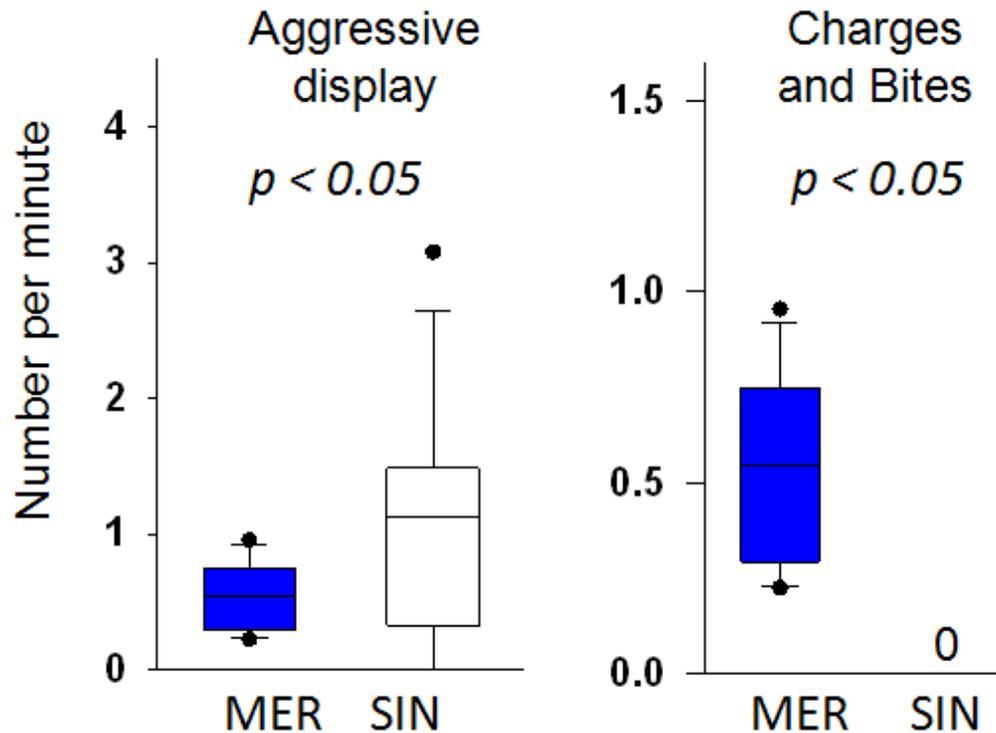

Figure 6. Median rates of aggressive display behavior and direct aggressive behaviors (charges and bites) in male *S. merriami* (MER, blue in abdominal patch) and *S. siniferus* (SIN, white abdomen) during staged territorial intrusions. The p-values are from Kolmogorov-Smirnov tests for differences in distributions, n = 13 pairs for each species. Box plots are presented.

Hormones, Parasites, and Color Patches

In the western fence lizard (*S. occidentalis*) infection with the malarial parasite *Plasmodium* altered ventral blue coloration and plamsa testosterone levels in breeding adult males (Dunlap and Schall 1995). The Mexican red-headed lizard, *S. pyrocephalus* has a complex set of colored bars and patches on both the abdomen and the throat in adults of both sexes. Comparisons of hue, value and chroma of black belly bars, blue belly regions, black throat strips and red or yellow throat patches revealed complex correlations of certain color attributes of certain body regions with plasma levels of reproductive sex steroid hormones, whereas other attributes of coloration correlated with endoparasite load and plasma levels of

the stress hormone corticosterone (Calisi and Hews 2007, Calisi et al. 2008). Hence some components of ventral coloration could convey information about reproductive state and other components can indicate aspects of health status and stress hormones. We currently are conducting similar studies on associations between spectral attributes of abdominal coloration and plasma levels of testosterone and corticosterone in other *Sceloporus* species.

Body Condition, Fluctuating Asymmetry (FA) and Relative Color-Patch Sizes

In our analysis of *S. undulatus* males from a population in Kentucky, we found that forest logging disturbance significantly altered some aspects of traits that may be important in male-male interactions in adult *S. undulatus*. Because patch size varied with body size, we first calculated residual patch size for each trait by regressing the average patch size for each male on body size (snout-vent length, SVL). We then examined correlations between these patch size residuals and body mass residuals, our measure of "condition", for each site and trait. On the undisturbed site, Belly Blue patch size residuals correlated significantly with body mass residuals, but did not on the disturbed site. Hence, males in better condition had relatively larger Belly Blue patches for their body length, but only on the undisturbed site. No other correlations between residual patch size and residual body mass were significant (Cain et al. *in preparation*).

We also measured fluctuating asymmetry (FA) in the size of each of the three paired patches. This measure (Palmer and Strobeck 2003) is the random deviation from perfect bilateral symmetry and may reflect developmental instability. There is evidence that conspecifics assess FA in traits in a number of species (Espmark et al. 2000), including humans, and an animal that has near equal left-right symmetry may be preferred as a mate. Individual differences in FA may result from differences in "longer-term" environmental effects, such as stressors experienced throughout ontogeny. We detected signicant FA in all three patch traits (and no directional asymmetry), following analysis procedures detailed in Palmer and Strobeck (2003). Hence FA in these traits may potentially be used by conspecifics (Folstad et al. 1996, Espmark et al. 2000), especially since these lizards often position their body laterally to their opponent when displaying the patches. We detected a site difference in FA, for only one color patch trait, the blue throat patches. Throat blue showed a significant site difference, with higher mean FA in patch size on the disturbed site than the undisturbed site (effect size for the difference in Throat Blue was 0.87). Tull and Brussard (2007) saw similar effects of anthropogenic disturbance on morphological traits (scale counts) in *S. occidentalis*; individuals had higher FA on sites with heavy off-road vehicle use than on sites with no off-road vehicle access. Importantly, our results indicate that anthropogenic disturbance, and potentially other types of environmental stressors, may alter signaling traits.

Within-population variation in color patches and the potential information content needs more study in *Sceloporus*. In other lizards, color patches can correlate with fitness traits, such as bite force, sprint performance, or dominance (e.g., Meyers et al. 2006). Quinn and Hews (2000) found that male *S. undulatus* with their blue patches painted white are less likely to win in staged encounters with control males whose patches are painted blue. In staged male-male encounters Robertson and Rosenblum (2010) found that male *S. undulatus* were more aggressive towards opponents with larger abdominal patches. In some *S. undulatus*

populations, relative sizes of the black portions of ventral badges (throat, chest, abdomen) correlated with body size and condition, tail and hind-limb length, and head size, but these relationships were not consistent in all populations (Langkilde and Boronow 2010).

Signaling Patches and Conspicuousness

We determined conspicuousness of male and females for two species with male abdominal blue, using measures of lizards's dorsal and ventral reflectance, measures of substrate reflectance, and an empirically-determined mathematical model of the avian visual system. For *S. undulatus* (males blue, Figure 1), we found that both males and females are more conspicuous ventrally than dorsally, and that males' blue abdominal patches are more conspicuous than the white abdomen of females (Ossip-Klein et al. 2013b). We also found that for *S. merriami*, a species where males have blue and pale green portions to the abdominal patches (Figure 1), the green is less conspicuous than the blue and more conspicuous than white. Thus, when males display their ventral coloration while engaging in aggressive male-male encounters, they increase their conspicuousness to avian predators, and this behavior likely is costly (Ossip-Klein et al. 2013b).

Proximate Mechanisms Underlying Visual Signaling

At the proximate level, there are a number of aspects of visual signals that we are studying to explain species differences in blue coloration. These include how the color is produced in the skin at the cellular level, what endocrine mechanisms may be involved, what genetic variation may be involved in the species differences in abdominal color, and the visual perceptual abilities for both color and motion.

Color Production in Abdominal Skin

In lizards as in other vertebrates, color patterns in skin result from the abundance and relative locations of various chromatophore cells (Bagnara and Hadley 1973, Cooper and Greenberg 1992). Melanophores (melanocytes in mammals) are chromatophores that contain melanin, a pigment that absorbs all wavelengths and thus imparts a black hue. These cells are typically in the dermal layer, and variation in melanin density explains the presence or absence of blue belly patches in *Sceloporus* (Quinn and Hews 2003). Specifically, in blue skin, blue wavelengths are refracted back by a layer of iridophores (cells containing an ordered array of reflecting guanine platelets), while all other wavelengths are absorbed by the underlying melanin. In white skin, all wavelengths apparently are reflected back by a highly reflective layer of collagen under melanophores, which have little or no melanin.. This difference has been found in three *Sceloporus*, including one species in which both sexes are white abdominally (*virgatus*), one in which both sexes have abdominal blue patches (*jarrovii*), and a sexually dichromatic species, *undulatus* in addition to a species in the sister genus, *Urosaurus ornatus*, that also has male-only expression of abdominal blue patches (Quinn and Hews 2003). In addition to this correlational evidence, we also have manipulative

support for the key role of the melanin layer. Using hormone implants to elevate hatchling levels of androgens in female *S. undulatus*, a species in which females lack abdominal blue, we were able to produce females with abdominal blue patches. Histological evaluation of patch skin in these females revealed dramatically increased melanin over control females (who lacked abdominal blue; Hews and Quinn 2003).

Other cellular factors may contribute to the lack of abdominal blue. For example, species could differ in the iridophore layer, as in white-bellied *Phrynosoma* compared to blue-bellied *Sceloporus undulatus* (Sherbrooke and Frost 1988). This layer is important in producing the blue reflectance. However results of a study by Hews and colleagues (*in preparation*) reveal only minor difference in the nanostructure of the iridophores in an analysis of transmission electron micrographs of abdominal skin from *S. undulatus* (blue) and from *S. virgatus* (white). Hence, variation in iridophores is not likely to be contributing to the presence or absence of abdominal blue across *Sceloporus*.

Aggression and Androgens

As in many vertebrates, a relatively rich body of work in *Sceloporus* lizards indicates that androgens mediate aggression (e.g., reviewed in Moore and Lindzey 1992, Wade 2011). With our colleagues, we have shown that species differences in adult plasma testosterone in *Sceloporus* lizards can contribute to explaining species differences in aggression. Specifically, in *S. undulatus* (blue species) males have higher plasma testosterone than male *virgatus* (white species), during the peak of the breeding season when maximal levels of territorial behaviors are expressed, but that males and females do not differ in plasma testosterone in a white species (*virgatus*). Thus, sex and species differences in aggressive signaling correlate with sex and species differences in plasma testosterone levels in these two *Sceloporus* species that differ in abdominal coloration (Hews et al. 2012).

Genetic Variation

We have shown that melanin plays a key role in explaining the presence or absence of abdominal blue patches. Melanocortins, such a melanocytes stimulation hormone, are involved in melanin deposition in vertebrates (Cone 2006). Sequence variation in *Mcr1*, the gene for the melanocortin-1 receptor, is associated with variation in skin color in *S. undulatus* populations, both in dorsal patterning (Rosenblum et al. 2004) and in the blue belly signaling patches (Robertson and Rosenblum 2009), supporting the role of melanin in abdominal blue variation. With our colleague Erica Rosenblum, we are testing the hypothesis that species differences in the occurrence of the abdominal blue patches correlate with amounts of dermal melanin, and with sequence variation in the *Mcr1* gene and/or expression levels of the gene.

Visual Perception of Color and of Motion Displays

We have used life-like robots to manipulate and present visual display or visual display with checmicals to lizards. These rubber robots are made from plaster molds of a museum

specimen and then painted dorsally to resemble a conspecific. A motor pushes dowel attached to the forebody up and down, and this created a specific-specific pattern of headbob display. A video of this robot for *Sceloporus graciosus* is available at the following URL address: http://link.springer.com/article/10.1007%2Fs00265-005-0954-2.

Robotic playback studies suggest that the *Sceloporus* pushup display combines the blue color of the belly patches with the up-and-down motion of the headbobs because males pay more attention to the blue belly signal, whereas females attend primarily to the motion (Martins et al. 2005). In *S. undulatus* (blue), this sex difference is explained, in part, by a difference in the underlying visual physiology. Nava (2009) showed that male *S. undulatus* are better able to detect blue than are females. The sexes also differ in motion detection. Female *S. graciosus* (Nava et al. 2009) and *S. undulatus* (Nava et al. 2012) are better able to detect fast motions than are males, explaining in part why female lizards appear also to pay more attention than do males to the motion component of pushup displays (Martins et al. 2005). Males are better able to see and pay more attention to blue and to slow motions, suggesting there may be a trade-off between color and sensitivity to different aspects of the motion display. If such trade-offs exist, white species may lose the sex difference in fast motion detection as well as the sex difference in spectral sensitivity when selection to perceive blue is relaxed. We currently are exploring these ideas.

Evolutionary Patterns in Visual Signals

Sex differences in color traits are often due to different selective pressures acting on males and females, and can be achieved through single sex shifts in ornamentation (e.g., Hofmann et al. 2008, Oliver and Monteiro 2011). Sex differences in color can increase over an evolutionary timescale, often through single-sex shifts in color (Hofmann et al. 2008). In *Sceloporus*, there appears to be several, independent losses of sex differences in color, due to male shifts from blue to white ventral coloration. However, there are several limitations to the human eye (including the inability to detect ultraviolet light), and we thus often underestimate the spectral variation present in animal color patterns (Vorobyev and Osorio 1998, Stuart-Fox et al. 2003).

We thus tested for cryptic (i.e., not visible to the human eye) sex differences in color across white-bellied lizards, and also asked how sex differences in color changes over evolutionary time across four morphologically diverse *Sceloporus* species (*S. siniferus, S. merriami, S. undulatus, S. virgatus*). With our colleagues (Ossip-Klein et al. 2013b), we found that two white-bellied lizards that appear to be the same color, actually exhibit cryptic sex differences in color, with the older lineage (*S. siniferus*) exhibiting a greater degree of sexual dimorphism than the younger lineage (*S. virgatus*). Specifically, we found that *S. virgatus* is sexually dimorphic across the short-wavelengths of light, and that *S. siniferus* exhibits statistically significant sex differences across the short-wavelengths, and the ultraviolet and the middle-wavelengths of light.

In this same study we also found that sex differences in color increase over an evolutionary timescale, comparing these four species, two more-recent species (one blue and one white) and two older species (one blue and one white). Specifically we saw increases in the ultraviolet and the middle-wavelengths of light, for both white-bellied species and blue-bellied species. The younger white lineage, *S. virgatus*, perhaps can be considered an early

version of the older white lineage, *S. siniferus*, which has been evolving as a white lineage for a longer time. Over evolutionary time we see gains of sex differences in the ultraviolet and in the middle-wavelengths of light, leading to increased sexual dimorphism in color in the older lineages. These same relationships hold true for the two blue lineages as well. The younger blue lineage, *S. undulatus*, exhibits sex differences in hue, brightness, and chroma in the short and in the long wavelengths of light. Again, thinking of the younger blue lineage as an early version of the older blue lineage, we see a gain of sex differences in the ultraviolet, and a gain of sex differences in the mid-wavelengths of light, which leads to increased sex differences in color over evolutionary time. Thus we find that white-bellied lizards exhibit cryptic sex differences in color, and sex differences in color increase over evolutionary time for both blue and white lineages.

Chemical Signaling Traits: Signal Production

The rate and the amount of chemicals deposited on substrates is a topic of interest to us. We know that individual lizards can vary in the number of marks deposited, and that there is also sexual and seasonal variation (Martins et al. 2006). We also know that androgens stimulate secretion rates (reviewed in Hews and Quinn 2003). Below, we describe comparative work in which we use the average number of femoral pores, which varies across species, as a proxy for importance of chemical signaling. This however remains to be determined and future work will explore whether there is an association between the number of femoral pores and the total amount of secretions that are produced and deposited.

We are analyzing femoral gland secretions using gas chromatography and mass spectrophotometry. So far we have identified major peaks associated with cholesterol and squalene. Among other roles, squalene is a precursor to cholesterol and has been identified in snake pheromones (Mason and Parker 2010). Cholesterol is the precursor to all sex steroid hormones. Signal persistence, a key feature, may vary with habitat (Alberts 1992). Evaporation rates of volatiles increase with higher temperature and humidity (Wyatt 2003), and in mammals, tropical species have putative marking pheromones that have a higher molecular weight than those of species from temperate forests (Alberts 1992). In the green iguana (*Iguana iguana*), from tropical habitats, scent mark composition has a higher percentage of and greater variation in the types of lipids, than in desert iguana (*Dipsosaurus dorsali*s) of the US deserts (Alberts 1993). Hence, we will need to take into consideration these climatic differences in the species we compare, when we determine the chemical compositions of the FG secretions in the 12 species of *Sceolporus* we are studying now.

Behavioral Responses to Conspecific Chemicals

We found behavioral differencs in responses to conspecific chemicals. In males of a white *Sceloporus* species (*virgatus*), a field experiment showed that conspecific chemicals are indeed signals: males exposed male cues increased rates of two visual display behaviors but males in a blue species (*undulatus*) did not (Hews et al. 2011). Based on this response from the white species, which is an example of an evolutionarily more recent "male-loss" of abdominal blue, we predict that males in other white *Sceloporus* species will also respond

more to conspecific chemicals compared to a closely-related species with adbominal blue patches.

Sensory Structures for Detecting Conspecific Chemicals

In vertebrates, sensory neurons from the olfactory epithelia (Main Olfactory organ) project to the main olfactory bulb, and neurons from the vomeronasal sensory epithelia (vomeronasal organ, VNO) project to the accessary olfactory bulb (Houck 2009). Perception of many pheromones is mediated by the VNO, a cartilage-encased elongated organ associated with the vomer bone in the rostral nasal cavity. In many species, signals and sensory responses to signals differ between the sexes (Godwin and Crews 1997, Adkins-Regan 2005). Sex and species differences in olfactory systems have been documented for a number of vertebrates (e.g., Eisthen 1992, Segovia and Guillamon 1993, Dawley 1998, Xiao et al. 2004, Woodley 2007), and can be driven by early organizational actions of sex steroid hormones during sexual differentiation (Adkins-Regan 2005). Elevated androgens also can increase rates of chemosensory behaviors in adults (e.g., Schubert et al. 2006). In reptiles, chemosensory behaviors (e.g., tongue-flick) deliver chemicals to the sensory epithelium of the VNO (Graves 1993).

We found species differences in behavioral responses to conspecific chemicals. In males of a white *Sceloporus* species (*virgatus*), a field experiment showed that conspecific chemicals are indeed signals: males exposed male cues increased rates of two visual display behaviors but males in a blue species (*undulatus*) did not (Hews et al. 2011). Hence, processing of the cues differs at some level, between these two species. Preliminary analysis of VNO epithelium data for male *virgatus* and male *undulatus* support this hypothesis: mean volume in male *virgatus* is greater than that in *undulatus*, both for absolute and relative volume.

In males of some lizards, a combination of behavioral and neural data suggest that the primary role of scent marks is in male-male interactions (Font et al. 2012). In the lacertid *Podarcis liolepis,* females do not respond differently to conspecific and congeneric male scent marks, nor do females prefer territories marked by larger males. By contrast, males can evaluate the competitive ability of other males via their scent marks. The accessory olfactory bulbs, which receive inputs from the VNO, are larger in males than in females. Font and colleagues argue that their data are most consistent with a primary role of intrasexual selection in male chemical communication (Font et al. 2012). Our VNO data will also allow us to track the evolutionary patterns in size of this sensory epithelium, and also the patterns of sexual size dimorphism in the VNO epithelium.

Evolutionary Patterns among Visual and Chemical Signaling Traits

Long-term signal evolution is shaped by a variety of selective pressures. Selection may favor signal components that convey additional information, or components that improve message transfer to a conspecific receivers. Selection on these components may also involve populations that occupy multiple environments. To date we have tested the relative importance of information and sensory modality in shaping the long-term evolution of

multimodal signals in 39 *Sceloporus* species by assessing associations between components of visual and chemical signaling, and habitat categories.

With our colleagues (Ossip-Klein et al. 2013a) we tested for pairwise evolutionary relationships among three types of signals (chemical, color and motion). For this study we pruned the phylogenetic tree (the genus has over 90 species) and included 39 species in our analysis, because we had data on display structure, femoral pore number, and abdominal coloration only for these 39 species. To broadcast identity at territorial boundaries, male *Sceloporus* use both visual motion (headbob) and chemical signals, whereas to signal aggression they use the colorful blue belly patches (Martins 1991, Thompson et al. 2008). If information content (e.g., the identity of the signaler) is the primary driver of long-term diversification, we expected to see a tight evolutionary link between chemical cues and the species-typical headbob displays, such that evolutionary changes in one would be strongly associated with evolutionary changes in the other. In contrast, if signal mode (chemical versus visual) is the primary driver of long-term diversification, we expected to see a tight evolutionary link between the motion and color components of the visual signal. A third possibility suggested by empirical studies is an evolutionary trade-off between color and chemical cues. Loss of the color signal in *S. virgatus* is associated with an increase in chemosensory behavior (Hews and Benard 2001, Hews et al. 2011), but no change in the use of the motion display (Quinn and Hews 2010). The relationship between color and chemicals may be because both features function primarily at short distances. Male lizards make the blue belly patches visible by flattening themselves laterally, but tend to do so primarily after moving into close proximity of a territorial intruder (Martins 1993). Similarly, lizards sample femoral pore secretions by tongue-flicking at close distances. In contrast, headbob displays are most often broadcast from open perches to potential receivers at longer distances. Thus physical distance could drive an evolutionary trade-off between color and chemical cues that does not directly impact motion.

Using modern phylogenetic comparative methods we found a negative correlation between evolutionary changes in visual-motion (headbob) and chemical (femoral pore number) signals. However, we found only indirect ties between the evolution of color and motion signals (both of which are perceived visually); this linkage was through viviparity, for which we found a strong association with blue. We found no evidence of an evolutionary link between color and chemical signals. We did find a negative correlation between arboreality and chemical signals (number of femoral pores), suggesting that substrate may affect the efficacy of chemical signaling. Overall, our results suggest that information content, in this case, broadcasting individual identity versus signaling aggression, appears to play a more important role than sensory modality or physical distance in guiding long-term signal evolution (Ossip-Klein et al. 2013a).

CONCLUSION

The *Sceloporus* genus provides an outstanding study system for addressing many questions about signal evolution. This is a relatively speciose genus with a number of independent origins of males with white abdomen. One of our goals in this chapter was to illustrate the array of approaches that can be brought to bear on the questions of signal

evolution. This will provide us with more statistical power than often is available for phylogenetic comparative studies. Further, although we are focussing on males with and without the abdominal blue, there are also a number of independent origins of female gain of the abdominal blue that could be similarly studied.

In the future, we will have more detailed data sets for many of the traits described in this chapter. With these data we will assess the evolutionary associations between signaling traits, and whether endocrine mechanisms produce suites of tightly linked traits. One possibility is that, in the older lineages with white males, we will see that selection may have pulled apart components of the suites that are seen in the more recently evolved white-male species. This result would suggest that endocrine mechanisms may facilitate evolutionary transitions where suites of correlated traits may be favored, but that over evolutionary time with subsequent diversification, these suites of traits maybe become uncoupled.

This group of species has been enjoyable and rewarding to work on. As herpetologists, it has been wonderful to get to learn about a number of different species and the habitats that they occupy. It is also intriguing to develop hypotheses that relate the diversity in the traits that we study to habitat features that the lizards occupy. As scientists, it is satisfying to be able to address questions from multiple perspectives, such as physiology, genetics, development, behavior, ecology, and evolution. The fence lizard group will continue to provide us much to work with.

ACKNOWLEDGMENTS

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