CHAPTER 8

Endocrinology of Species Differences in Sexually Dichromatic Signals

Using the Organization and Activation Model in a Phylogenetic Framework

Diana K. Hews and Vanessa S. Quinn

Many animals have conspicuous social signals. Often these signals are expressed in one sex and function in the context of mate choice, intrasexual competition, or both (Andersson 1994; Bradbury and Vehrencamp 1998). A more complete understanding of sex-specific signals will come from integrative studies within a phylogenetic context (Ryan, Autumn, and Wake 1998). Integrative studies document the action of natural and sexual selection on signalers and receivers; determine the mechanistic basis of signals, signal perception and processing; and use historical perspectives to ask how sensory systems evolve (Endler 1992; Ryan 1997). Although much is known about ecological and evolutionary aspects of sexually selected traits (Andersson 1994), considerably less is known about their developmental basis and how selection has acted on these developmental mechanisms.

This chapter focuses on our proximate physiological work. Specifically, we examine sex steroids and how they control both sexual signaling morphology and signaling behavior. We focus on endocrine mechanisms because sex steroid hormones play fundamental roles in the development and expression of sexual differences in vertebrates (Becker, Breedlove, and Crews 1992). Evolutionary biologists increasingly recognize the key role endocrine systems can play in the expression of correlated suites of life history traits (e.g., Moore 1991; Moore 1995; Mousseau and Fox 1998; Sinervo and Svensson 1998). In particular, the endocrine system can result in the coupling of display morphology and display behavior.

Currently, we are assessing the roles of sex steroid hormones, their receptors, and their cellular actions in mediating expression of a sexual color signal and of the behavior involved in signaling. Our long-term

goal is to study key species pairs in the lizard genus *Sceloporus* that vary in the degree of sexual dichromatism in a signaling trait. The signaling trait we study is a pair of large, brilliant, blue patches of abdominal skin on an otherwise white background. The patches are exposed to conspecifics in stereotyped postural displays during aggression and courtship in *Sceloporus* and in some species of its sister genus, *Urosaurus* (tree lizards) (Fig. 8-1). Differences in expression of abdominal patches in *Sceloporus* appear to covary with differences in aggression (Vinegar 1975; Quinn and Hews 2000; Hews and Benard, in press). We have begun our endocrine work by focusing on two closely related species and a third more distantly related species of *Sceloporus*. When results are available for multiple species within a clade it will be possible to apply statistically rigorous phylogenetic analyses, such as independent contrasts (Martins and Hansen 1996). The goal will be to determine whether there are common endocrine mechanisms underlying independent evolutionary state transitions of sexual dichromatism.

There is great potential with *Sceloporus* lizards for using explicit comparative methods to examine evolutionary patterns in the mechanistic bases of sexually dimorphic signaling traits. The group has a well-corroborated phylogeny (Reeder and Wiens 1996; Wiens and Reeder 1997), with a number of independent losses of sexual dichromatism in abdominal patches, due to either loss of the patches in males or gains of them in females (Wiens 1999). Thus, our work highlights a poorly studied but potentially important aspect of sexual selection: the evolutionary *loss* of sexual signals. Phylogenetic studies suggest that such losses may be common in a number of vertebrate clades (Peterson 1996; Price and Birch 1996; Omland 1997; Burns 1998; Wiens 1999). Determining the evolutionary forces producing losses and the various physiological bases of such evolutionary change will increase our general understanding of the evolution of sexually dimorphic signals (Emerson 1994, 1996; Reynolds and Harvey 1994).

Sexual Dimorphism in Color Signals and Aggressive Behavior in *Sceloporus* Lizards

The blue abdominal patches are involved in sex recognition and in aggression (Cooper and Burns 1987; Quinn and Hews 2000), develop with sexual maturation, and show little seasonal variation in their expression. Phylogenetic analyses (Wiens 1999) strongly support the hypotheses that (1) sexual dichromatism in the abdominal patches is the ancestral state in this genus, and (2) that monochromatism represents evolutionary loss

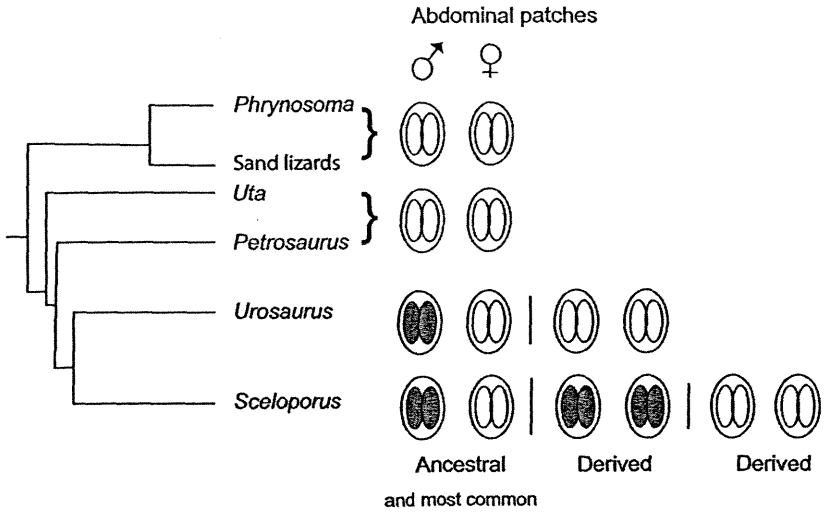

Figure 8-1 Simplified phylogeny of *Phrynosomatidae* (based on Reeder and Wiens [1996]). For each genus (or the clade of *Phrynosoma* and the sand lizards: *Uma, Callisaurus, Cophosaurus, Holbrookia*) the adjacent illustrations indicate character states for abdominal coloration of males and females (based on Wiens [1999]). Variation within a genus, such as in *Sceloporus*, is indicated by the presence of more than one set of male-female character states. Solid ovals = blue abdominal patches present; open ovals = blue abdominal patches absent.

of sexual dichromatism in the abdominal patches, either by trait gain in females or by trait loss in males. There are at least 10 independent evolutionary losses of abdominal patches in *Sceloporus* males, and 8 independent gains of them in females. *Sceloporus* males exhibit high levels of territorial aggression compared with females (reviews in Stamps [1977b, 1983b], Carpenter [1978a], and Martins [1994]). Thus, territorial aggression is typically sexually dimorphic in this genus. The behavior patterns involved in aggressive displays are well described for many *Sceloporus* species (e.g., Carpenter 1978a; Ruby 1978; Moore 1987). Growing evidence (discussed later) suggests that increased aggression and occurrence of the abdominal patches covary in this genus.

Our research focuses on three *Sceloporus* species: the eastern fence lizard (*S. undulatus consobrinus*), the striped plateau lizard (*S. virgatus*), and the mountain spiny lizard (*S. jarrovii*). Each represents one of three possible patterns of sexual dichromatism in signaling traits in *Sceloporus* (Wiens 1999). The most common pattern is *sexual dichromatism* in abdominal coloration (Plate 11; *S. undulatus*). Only males exhibit

territorial aggression, and only males have the blue abdominal patches. In a second pattern, *monochromatic feminized*, neither sex has the blue abdominal patches, and for at least one species (*S. virgatus*), rates and intensities of territorial aggressive behavior patterns are reduced in males (Vinegar 1975; Quinn and Hews 2000; Hews and Benard, in press), and male-male home range overlap is the highest documented (74%) for any *Sceloporus* species (Abell 1999b). The sister species to the clade that contains only *S. virgatus* and *S. exsul* (both of which are species with loss of blue abdominal patches in males) is *S. undulatus* (Wiens and Reeder 1997). Thus, the monochromatic-feminized species and the sexually dichromatic species on which we focus our research are very closely related. Finally, in the third pattern, *monochromatic masculinized*, both males and females have blue abdominal patches, and for at least one species (*S. jarrovii*), both sexes exhibit territorial aggressive display behavior. Females use the patches during aggressive female-female territorial encounters that involve the same stereotyped display postures used by males (Ruby 1978; Moore 1987; Woodley and Moore 1999a, b).

Sceloporus species vary in other aspects of ventral coloring. In addition to the abdominal patches, there are throat patches that also exhibit sex and species differences. Throat patches vary in color intensity and size (Stebbins 1985). Even within a species, populations of *S. occidentalis* exhibit extensive variation in the intensity and amount of blue color, both in abdominal patches and in throat patches, and in the degree to which sexes differ in these traits (Camp 1916). Thus, many *Sceloporus* species differ interspecifically as well as intraspecifically in other aspects of dorsal coloration and throat patterning (see also Wiens and Reeder [1997] and Wiens, Reeder, and Montes de Oca [1999]). It is important to recognize that our terminology *monochromatic* or *sexually dichromatic* refers only to the presence or absence of the blue abdominal patches. Variation in the size and intensity of coloration of the abdominal patches and of the throat patches (including presence/absence) also deserves careful study.

In addition to this evolutionary diversity in the occurrence of sexual signal dichromatism and the well-corroborated phylogeny, other features recommend *Sceloporus* as a group for integrative comparative studies of sexual signals. In many phrynosomatid lizards, territoriality and courtship are central to male reproductive success (e.g., Hews 1990, 1993; Abell 1997; see also chapters 1, 3, and 9). Lizards are excellent species for field studies of territorial aggression (e.g., Hews 1993; see

also chapters 1, 5, 7, 9, and 10). Lizards are also excellent subjects for hormonal studies in the laboratory (reviews in Crews and Greenberg [1981] and Moore and Lindzey [1992]; Hews, Knapp, and Moore 1994) and field (e.g., Marler and Moore 1988; DeNardo and Sinervo 1994; Moore, Hews, and Knapp 1998). Unpublished electroretinograms and microspectrophotometry studies (E. Loew and L. Fleishman, personal communication) suggest that *Sceloporus* lizards have four cone types and good hue discrimination well into the blue end of the wavelength spectra. Finally, there is a wealth of behavioral, ecological and evolutionary research on this genus (Sites et al. 1992). In particular, a variety of functional studies have focused specifically on blue coloration in *Sceloporus* (e.g., Vinegar 1975; Cooper and Burns 1987; Cooper and Greenberg 1992; Dixon 1993; Abell 1997, 1998a, b, c, 1999b; Quinn and Hews 2000; Wiens 2000; Hews and Benard, in press).

In sum, *Sceloporus* offers an excellent array of features for a comparative neuroendocrine study of sexual dimorphism. Some key features are as follows:

1. Loss in males or gain in females of sexually dichromatic signaling traits
2. Reduction of sexual dimorphism in signaling behavior (via increased aggression in females or reduced aggression in males)
3. A well-corroborated phylogeny
4. A substantial body of ecological research providing a rich context for interpreting comparative studies
5. The ability to conduct detailed adult and neonatal endocrine studies including those that manipulate steroid hormone profiles in adults or hatchlings

Endocrine Studies of Species Variation in Sexual Dimorphism of Signaling Morphology and Signaling Behavior

In the following sections, we present an overview of the approaches we are using to study how hormonal controlling mechanisms have evolved in this signaling system as well as an overview of the endocrine regulation of sexual differentiation in vertebrates with a particular focus on reptiles. Then, after providing a brief background on reproduction in *Sceloporus,* we present a series of questions we are asking, as well as some of our findings, about the effects of sex steroid hormones on the development and expression of the signaling trait in *Sceloporus.* We then turn to a discussion of brain regions mediating aggression in

vertebrates. We overview our first endocrine studies related to the signaling behavior—aggressive display—focusing on describing the distribution of androgen receptors (ARs) in the brains of males and females. Our endocrine work is only in the beginning stages, but we feel that this overview of our preliminary results provides a useful illustration of a research program that seeks to integrate endocrine approaches in an evolutionary context.

Organization and Activation: The Endocrine Basis of Sexual Differentiation

Sex steroid hormones in vertebrates are central to the development of sexual differences, a process known as sexual differentiation (Becker, Breedlove, and Crews 1992). The organization and activation hypothesis has provided a successful construct in elucidating the role of sex steroid hormones in sexual differentiation (Phoenix et al. 1959; Arnold and Breedlove 1985; Kelley 1988). This hypothesis proposes that sex steroid hormones affect sexual differentiation by *organizational* effects, which are permanent and occur early in life during a discrete critical period, and by *activational* effects, which are temporary and occur in adults. These two modes of hormone action can be thought of as representing extremes on a continuum (Arnold and Breedlove 1985). Traits may require only one or the other type of action, but often traits require both organization and then later activation for complete sexual differentiation. Although the generality of this paradigm is being reassessed (e.g., Crews 1993; Arnold 1996; Kendrick and Schlinger 1996; Wade 1999), it remains a powerful guide for endocrine research on sexual differences.

Sexual differentiation in reptiles appears to follow the basic paradigm for tetrapod vertebrates, involving both organizational and activational effects with resulting dimorphism in brain, behavior, and morphological traits (Crews 1985; Crews and Silver 1985; Adkins-Regan 1987; Moore and Lindzey 1992; Hews and Moore 1995; Godwin and Crews 1997; O'Bryant and Wade 1999; Rhen and Crews 2000). There is a body of endocrine work on reptiles with temperature-dependent sex determination such as the leopard gecko (*Eublepharis macularius*) (Tousignant and Crews 1994, 1995; Rhen and Crews 2000) and on bisexual and unisexual whiptail lizards (*Cnemidophorus* species; reviewed in Godwin and Crews [1997]). However, fewer studies have examined early organizational effects of sex steroid hormones

on sexual differentiation for the more typical species with genetic sex determination, such as *Sceloporus.* Nevertheless, studies on tree lizards (*Urosaurus ornatus*) (Hews and Moore 1995) and sexual whiptails (*Cnemidophorus inornatus*) (Wade, Huang, and Crews 1993) indicate that expression of male-typical traits requires androgen exposure during development. Our *Sceloporus* work provides a detailed examination of sexual differentiation of a perhaps more typical bisexual species with genetic sex determination.

Several fundamental endocrine mechanisms that have been found to contribute to sexual differences are likely to contribute also to interspecific variation in sexual differentiation of color and aggression. First, different species could vary in sexual differences in levels of circulating hormone, or in the timing of elevations in plasma hormone concentrations at critical periods in ontogeny, thus affecting organizational actions of the hormone (or hormones). Second, species could vary in sex-specific sensitivity to circulating steroid hormones. Such differences can be owing to tissue-specific differences in the hormone receptors (e.g., distribution, abundance, or regulation) or in the genetic regulation of trait expression (e.g., the trait is no longer under hormonal control). Third, species could vary in sex-specific activity of key metabolic enzymes (aromatase, 5α-reductase) that convert testosterone to other biologically active hormones (17 β-estradiol, 5α-dihydrotestosterone, respectively). Besides mediating differentiation of the color signal, these differences in receptors and metabolic enzymes could themselves result from earlier sexual differences in steroid hormone profiles. Our research examines these endocrine attributes, all of which could contribute to the sex and species differences observed in *Sceloporus* lizards.

Evolutionary Endocrine Studies of Sexual Differences

A growing body of work examines the endocrine mechanisms underlying species differences in sexual signals. Pioneering neuroendocrine work identified anatomical brain dimorphism in vocal control regions that correlated with sexual differences in singing behavior among bird species (Brenowitz and Arnold 1985; Arnold et al. 1987). Brain sexual dimorphism in the distribution of steroid hormone receptors and in the metabolic activities of key brain regions correlates with species differences in pseudocopulation and copulation behavior in the unisexual whiptail lizard and its sexual congener (*Cnemidophorus uniparens* and *C. inornatus,* respectively) (Crews, Wade, and Wilczynski 1990; Wade

and Crews 1991a, b, 1992; Godwin and Crews 1997). Seasonal variation among some bird species in sexual dimorphism in sexual behavior correlates with male-female differences in plasma testosterone levels of adults (Wingfield 1994) or differences in other hormones (Kimball and Ligon 1999). Evolutionary alterations in placental endocrine enzymes apparently contribute to the extraordinary masculinization in morphology and behavior of female spotted hyenas (*Crocuta crocuta*) (Glickman et al. 1992). Variation in behavior patterns that define mating systems (e.g., pair-bond behavior; parental behavior) is correlated with species differences in the abundance of receptors for the peptide hormone arginine vasopressin in several vole species (*Microtus* spp.) (reviewed in Young, Wang, and Insel [1997]). Transgenic mice with the vasopressin receptor from a monogamous vole species exhibited increased vasopressin-induced affiliative behavior, which characterizes the monogamous pair-bonded voles (Young et al. 1999). These studies collectively suggest that endocrine mechanisms underlie much of the evolutionary variation in sexual differences (but see also Arnold [1996]).

However, little of this work on naturally occurring variation among species in sex differentiation has used an explicit phylogenetic context, and most other studies have not focused on sexually selected behavior and signaling traits. Emerson and colleagues provide a notable exception (Emerson, Rowsemitt, and Hess 1993; Emerson 1996), examining acoustic signaling, combat behavior, and morphological traits used in fighting within a clade of the frog genus *Rana*. The loss or diminution of these seasonally activated male traits correlates with reduced plasma androgen levels. Similarly, Staub and coworkers (Dempsey, Reilly, and Staub 1996) have initiated a hormonal study of sexual differences in aggression and morphology in plethodontid salamanders (*Aneides*), and measure levels of several hormonal parameters that could mediate these differences, including plasma concentrations of sex steroid hormones and the abundance of hormone receptors in the dimorphic target tissue (jaw musculature). Sexual dimorphism in the distribution of brain receptors and metabolic capabilities may explain sex and species differences in behavior in bisexual and unisexual *Cnemidophorus* (reviewed in Godwin and Crews [1997]). Although it is not known for anurans if color functions in sexual signaling, Hayes (1997) provides an example of how hormonal mechanisms may constrain the evolution of sexual differences. He details specific endocrine mechanisms regulating pigmentation and proposes that they might limit the evolution of sexual

dichromatism in anurans (Hayes 1997). Birds, however, are known to recognize and respond to sexual differences in coloration (Andersson 1994), and substantial endocrine work has examined plumage dichromatism. Kimball and Ligon (1999) surveyed studies on the hormonal control of sexually dimorphic plumage in four major avian orders (Galliformes, Anseriformes, Charadriiformes, and Passeriformes). They found that three major endocrine mechanisms (estrogen dependence, testosterone dependence, luteinizing hormone dependence) and one nonendocrine mechanism (strict genetic control) were responsible for the plumage dichromatism, with little species variation in the mechanism within each order (studies on a total of 26 species were analyzed). Note that sexual differences in color signals often are *not* mediated by endocrine mechanisms (e.g., birds, Owens and Short [1995]). Thus, although there are comparative studies of species differences in sexual signals, there is clearly a need for more studies with the explicit aim of examining the evolution of endocrine mechanisms of sexually dimorphic signaling traits in a phylogenetic context.

Androgen Dependence of Aggression in *Sceloporus*

One of the common themes resulting from endocrine work in many vertebrates is that aggressive behavior is often mediated by androgens. Thus, a starting point for the endocrine study of species differences in aggression in *Sceloporus* is an examination of the evidence that such behavior is androgen mediated. Such work includes documenting seasonal expression of the behavior, which suggests a correlation with seasonal elevation in plasma androgen levels that is often associated with spermatogenesis.

There has been considerable work on the neuroendocrine basis of behavior of selected reptiles (reviews in Moore and Lindzey [1992] and Godwin and Crews [1997]), and on *Sceloporus* lizards in particular. Territorial aggressive behavior is seasonally expressed in most, if not all, of the North American *Sceloporus* species. These *Sceloporus* lizards are seasonal breeders, and seasonal variation in intensity of aggression has been well described for males of all three species that we are studying, and for females of the masculinized species, *S. jarrovii.* Here is what we know about *Sceloperus* species:

TYPICAL DICHROMATIC SPECIES

An association between seasonal changes in aggression and plasma androgens occurs in many populations of the "typical" species, *S. undulatus*

(McKinney and Marion 1985; Klukowski and Nelson 1998; Smith and John-Alder 1999).

MONOCHROMATIC SPECIES WITH MASCULINIZED FEMALES

Considerable work in *S. jarrovii* has documented seasonal steroid hormone profiles and correlations with elevated levels of territorial aggression in adult males (Ruby 1978; reviews in Moore and Lindzey [1992]) and females (Woodley and Moore 1999a, b). Experimental work with male *S. jarrovii* has identified androgen-dependent and androgen-independent components of territorial aggression in males (reviewed in Moore and Lindzey [1992]). Correlative and experimental work (Woodley and Moore 1999a, b) suggests a potential role for androgens in aggression of these masculinized females. Because this and several other monochromatic *Sceloporus* species with masculinized females are ovoviviparous, masculinization could result from exposure to sex steroid hormones in the maternal environment (e.g., vom Saal 1979). This hypothesis is currently being explored for *S. jarrovii* (D. Painter and M. C. Moore, personal communication). However, other monochromatic *Sceloporus* species with masculinized females also include oviparous forms, such as *S. occidentalis taylori* (California) and *S. undulatus tristichus* (New Mexico, Colorado). Thus, there may be substantial evolutionary variation in the endocrine mechanisms underlying such masculinization of females within *Sceloporus.*

MONOCHROMATIC SPECIES WITH FEMINIZED MALES

Only one of the monochromatic species with feminized males, *S. virgatus,* has been studied. In this species, seasonal elevations in plasma androgen concentrations in males (Abell 1998c) correlate with the single bout of breeding activity and territoriality in late May and early June (Vinegar 1975; Rose 1981; Abell 1998b).

Species Variation in Actions of Sex Steroid Hormones in *Sceloporus*

For some vertebrates, adult sexual differences in circulating androgen levels are involved in adult differences in trait expression. Often such adult activational actions of hormones involve sexually dimorphic substrates that also require early organizational effects of sex steroid hormones in perinatal stages of development. For example, in many male passerine birds, seasonal increases in plasma androgens in males mediate breeding-season territorial singing, and the brain regions involved in song production require early masculinization (Nelson 2000).

Examination of the relative importance of organizational versus activational effects of sex steroid hormones requires studies that measure plasma concentrations of hormones in adults and in hatchlings, as well as experiments that manipulate hormone levels in both.

DO SEX AND SPECIES DIFFERENCES IN EXPRESSION OF ABDOMINAL COLOR PATCHES CORRELATE WITH ADULT DIFFERENCES IN SEX STEROID HORMONE PROFILES?

One endocrine hypothesis to explain sex and species differences in abdominal patches in *Sceloporus* is that, as adults, individuals with abdominal blue patches have higher androgen levels. That is, the expression of these traits differs because of differences in activational actions of androgens. However, unlike plumage in many passerine birds, the occurrence of abdominal blue patches in *Sceloporus* lizards does not decline in the nonbreeding season; the patches are expressed year-round. There may be some seasonal variation in the intensity of the blue hue and/or the size of the blue patch, but there are few studies directly examining this possibility.

The lack of seasonal change in this male-typical trait suggests that plasma androgen levels might not correlate with occurrence of the color patches, when comparing males of species with and without the trait. Indeed, published studies on hormones of various *Sceloporus* species provide provisional support for this conclusion. For example, during the breeding season, male *S. virgatus,* the species with male loss of the abdominal blue patches, have androgen levels similar to breeding levels of androgens in congeners (Abell 1998c; Abell and Hews 1999). Hormone manipulations in adults are also ineffective in altering the sexually dichromatic expression of the trait. The sexual difference in abdominal blue patches is not altered by castration of adult males or by androgen implants in adult females (Kimball and Erpino 1971; Moore 1987; Rand 1992; John-Alder et al. 1996). Thus, data on circulating hormone concentrations and results of adult hormone manipulations both are consistent with the hypothesis that sexual differences in the patches do not result from differences in activational actions of sex steroid hormones.

IS SEXUAL DICHROMATISM IN THE ABDOMINAL SIGNAL IN *S. UNDULATUS* MEDIATED BY EARLY ORGANIZATIONAL ACTIONS OF ANDROGENS?

The sexual differences in abdominal blue patches could arise from differences not in adult plasma levels of sex steroid hormones, but in levels in the hatchlings. Thus, there could be differences in the organizational

actions of hormones. Sexual differentiation of peripheral traits (i.e., outside the central nervous system [CNS]) in male vertebrates is often due to the 5α-reduction of testosterone (T) to dihydrotestosterone (DHT) (McEwen, Luine, and Fischette 1988; Becker, Breedlove, and Crews 1992). DHT is a biologically active metabolite of T in many vertebrates, and the enzyme that converts T to DHT is 5α-reductase. Female-typical plumage in males of the domestic Sebright breed of chickens involves a mutation altering expression of this enzyme (George, Nobel, and Wilson 1981).

This enzyme that converts T to DHT is implicated in the sexual differentiation of blue abdominal patches in male tree lizards, *Urosaurus ornatus,* a species in the sister genus to *Sceloporus.* Castration of hatchling males abolishes expression of the trait, indicating the organizing role of androgens (Hews, Knapp, and Moore 1994). Females will express the male-typical abdominal patches only if given DHT as intact hatchlings but will not express the trait if given T at this age or given either androgen as adults (Hews and Moore 1995). Thus, given the role of DHT in the expression of blue abdominal patches in tree lizards, we focused on the hypothesis that DHT is also necessary for hormonal organization of blue abdominal patches in *Sceloporus.*

The endocrine mechanisms underlying sexual differentiation of the abdominal blue patches may be the same for sexually dichromatic taxa in the sister genera *Urosaurus* and *Sceloporus.* Specifically, our results (Quinn and Hews, unpublished data) indicate that organizational effects of DHT are also central to the expression of this sexually dichromatic trait in *S. undulatus.* Both DHT and T influenced the expression of traits that are activated by androgens in adult lizards (Fig. 8-2A, cloacal gland secretions in the tail base and femoral gland secretions). In addition, as was found for tree lizards, only hatchling *S. undulatus* females given intraperitoneal DHT implants expressed the male-typical abdominal patches (Fig. 8-2B).

Blood levels of androgens in hormone-implanted animals must be assayed to confirm that plasma DHT levels due to the implants were physiological and not pharmacological. Similarly, a study determining the effects of castration on male hatchlings remains to be conducted. However, these data support the hypothesis that the organizational role of DHT in the expression of the male-typical signaling morphology in these two sister genera, *Urosaurus* and *Sceloporus,* are the same.

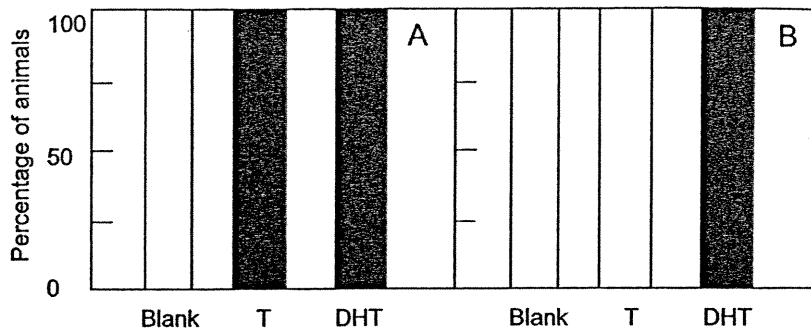

Figure 8-2 Effects of long-lasting androgen implants on expression of male-typical traits in hatchling *Sceloporus undulatus* females when implanted 20 d posthatching. (*A*) Cloacal glands and femoral pores. These are activated by androgens in several lizard species. (*B*) Blue abdominal patches. Open bars = female-typical trait expression; solid bars = male-typical trait expression; blank = empty control implant; T = testosterone implant; DHT = 5α-dihydrotestosterone implant. n = 4–6 per treatment group.

ARE DIFFERENCES IN THE SEXUAL DICHROMATISM OF ABDOMINAL COLORATION BETWEEN THE SISTER SPECIES *S. UNDULATUS* AND *S. VIRGATUS* MEDIATED BY SPECIES DIFFERENCES IN THE ORGANIZATIONAL ACTIONS OF ANDROGENS?

One hypothesis for the lack of abdominal patches in *S. virgatus* involves differences in organizational effects of androgens. This hypothesis proposes that blue patches are not organized by DHT in *S. virgatus* because levels of circulating androgens in hatchling *S. virgatus* differ from those in young males of the dichromatic *S. undulatus* during the time period when androgens affect sexual differentiation of the trait. There are currently no data on hormone profiles of hatchlings for any *Sceloporus* species during this stage of ontogeny. However, some data indirectly support the hypothesis that the *timing* of a critical period appears similar, at least when it ends. Implants of T given to intact male *S. virgatus* at 40 d posthatching had no effect on abdominal coloration (Abell 1998a). This posthatching age is when abdominal blue coloration just begins to be expressed in some males in two other dichromatic species (*U. ornatus,* Hews and Moore [1995; 1996]; *S. undulatus,* Hews, unpublished data). This would suggest that, like *U. ornatus,* the critical period for this trait (if it were to occur in *S. virgatus*) is earlier than day 40. Specifically, it is possible to have a tissue's fate (blue, white) determined

earlier in ontogeny by a hormonal effect but have the differentiation take place later in ontogeny. For example, the organizational effect of the hormone may be to increase the number or type of hormone receptors in target cells. Then, when higher levels of the hormone (or hormones) arise later, perhaps during puberty as in *U. ornatus,* the hormone (or hormones) act and the skin cells are altered, causing the permanent expression of factors that produce the blue coloration. Thus, if the process of sexual differentiation seen in *U. ornatus* also occurs in *Sceloporus,* then intact male *S. virgatus* given long-lasting T implants early after hatching (during the time when the fate of the abdominal skin patch is determined) should develop the abdominal patches. We are currently testing this hypothesis with such a manipulation. If this manipulation produces blue abdominal patches in *S. virgatus* males, it would suggest that the evolutionary loss is the result of decreased circulating levels of T during the critical period.

Another endocrine hypothesis about the evolutionary patch loss in *S. virgatus* concerns 5α-reductase activity in the abdominal skin. Recall that this enzyme converts T into DHT, and that in *U. ornatus* expression of blue abdominal patches requires organization by DHT. We are currently rearing hatchling *S. virgatus* to test this reductase hypothesis. If hatchlings given DHT implants develop blue abdominal patches, and those given T implants do not, it would suggest that the evolutionary loss of the patches is due to decreased activity of 5α-reductase in the skin during the critical period.

Endocrine manipulations can reveal if hormones affect sexual differentiation. To confirm that this naturally occurs, one must verify that hatchlings of the sexes and species with different abdominal patches naturally have different levels of the endocrine attributes (hormone levels, enzymes that metabolize hormones, hormone receptors). For example, our preliminary results indicate that DHT implants given to female *S. undulatus* hatchlings result in expression of blue abdominal patches, explaining the sex difference in the expression of this trait in this dichromatic species. It is therefore important to establish that there are indeed endogenous endocrine differences. If androgen manipulations alter the expression of abdominal patches, then one would predict that there are naturally occurring differences in circulating androgens at the ontogenetic stage when androgen manipulations were successful. Several major endocrine differences could contribute to endogenous differences in exposure to DHT. For example, hatchlings could differ in

circulating levels of T and/or in the activity of 5α-reductase, and thus in levels of DHT.

Species Variation in Target Tissue Sensitivity to Hormones: ARs

Another attribute of the endocrine system that could also contribute to these sex and species differences among *Sceloporus*, both in signal morphology and in signaling behavior (aggression), is sensitivity to androgens. Sensitivity to a hormone could vary because of differences in either the abundance or nature of the AR, or because expression of the trait is no longer hormone dependent. Mutations in ARs are associated with a variety of clinical disorders in humans (McPhaul et al. 1993), and they are also known for strains of mice (e.g., Freeman et al. 1995). Mutations in the ARs affect levels of aggression in rodent strains (e.g., Simon and Whalen 1986).

Similarly, ARs could also vary among species and affect trait expression. For example, ARs could differ in occurrence and abundance in particular targets (e.g., Kelley et al. 1989; Boyd et al. 1999). Alternatively, species that differ in trait expression may have mutations in the AR gene that result in differences in receptor function, altering the binding specificity or affinity. In addition, work on other steroid hormone receptors reveals that, within a species, there can be multiple forms of a steroid hormone receptor. For example, in the rough skinned newt (*Taricha granulosa,* Orchinik, Murray, and Moore [1991]) and in the house sparrow (*Passer domesticus,* Breuner and Orchinik [1999]), there are at least two glucocorticoid receptors. In each species, one form is a fast-acting membrane-associated receptor and one is a slower-acting intracellular receptor. Thus, differences between species in selection acting on such variation in receptor populations could produce differences in trait expression.

Our research group is beginning to describe the distribution of ARs in the brain of *Sceloporus.* In the near future, we will begin work to examine the abundance of ARs in abdominal skin. In the following section, we provide a brief review of brain regions mediating aggression in vertebrates in general, and the evidence supporting this role for these regions in reptiles, specifically. In the next section, we present some of our initial results from a study determining the distribution of ARs in the brain of *S. undulatus.* Describing the brain distribution of ARs in this sexually dichromatic species is the first step in exploring the differences in aggression among *Sceloporus* species.

WHAT BRAIN REGIONS MEDIATE AGGRESSION?

In tetrapod vertebrates, several brain areas appear to be key mediators of aggression, and these areas are being examined in our research. In a variety of birds and mammals, studies using lesions or electrical stimulation have implicated the hypothalamus and parts of the limbic system (hippocampus, septum, amygdala) as important brain regions involved in aggression (Albert and Walsh 1984; Crews and Silver 1985; Albert et al. 1990; McGregor and Herbert 1992). Androgens often act in these particular brain regions and affect aggression. For example, in castrated male ring doves (*Streptopelia risoria,* Barfield [1971]) and quail (*Coturnix c. japonica,* Watson and Adkins-Regan [1989]), androgen implants in the preoptic area of the anterior hypothalamus stimulate aggression.

In reptiles, the basal portion of the dorsoventricular ridge is considered to be homologous to the mammalian amygdala (although the terminology is somewhat contradictory when comparing neuroanatomical studies of various lizards (cf. Peterson 1980; Propper, Jones, and López 1992; Wade 1997). This view has recently been upheld and clarified by a detailed phylogenetic analysis of the connections of amygdalar subnuclei in representative tetrapod taxa (Bruce and Neary 1995). Comparison among representatives of several major vertebrate groups revealed that there are similarities in homeobox genes that are expressed during brain development in specific brain regions (Fernandez et al. 1998). These comparisons clarified relationships among vertebrates in their respective telencephalic subdivisions (Fernandez et al. 1998), and these telencephalic relationships concord with the homologies proposed by Bruce and Neary (1995).

Functional experiments in reptiles support the role of the amygdalar region (basal dorsoventricular ridge) in the expression of aggression (Peterson 1980), a role that is a conserved functional homology with other tetrapod vertebrates. Lesions in the amygdalar area in caimans (*Caiman crocodilus*) decreased aggression (Keating, Korman, and Horel 1970). Bilateral lesions of the amygdalar area in western fence lizards (*S. occidentalis*) abolished the aggressive responses of dominant males to conspecifics (Tarr 1977). In the collared lizard (*Crotaphytus collaris*), aggressive and defensive postures were elicited with electrical stimulation of the anterior dorsoventricular ridge, amygdaloid complex, septal and preoptic areas, hypothalamus, thalamus, regions adjacent to the nucleus profundus mesencephali, and the reticular formation (Sugerman and Demski 1978). Bilateral lesions of the ventromedial nucleus of the

amygdalar area of green anoles (*Anolis carolinensis*) resulted in unimpaired assertion and challenge displays, but a reduction in courtship behavior. By contrast, lesions in the paleostriatum reduced assertion and challenge displays but had no effect on courtship behavior (Greenberg, Scott, and Crews 1985). Studies involving lesions in another telencephalic region, the anterior two-thirds of the dorsal ventricular ridge (the striatum) (Peterson 1980), showed no change in social behavior and animals resumed their presurgical positions in the dominance hierarchy (reviewed in Peterson [1980]).

DO DIFFERENCES IN DISTRIBUTION OF ARs CORRELATE WITH SEXUAL DIFFERENCES IN SIGNALING BEHAVIOR?

Using a technique called immunohistochemistry, brain cells containing ARs can be labeled with an antibody that is AR specific. Cells thus labeled by the antibody are considered "AR positive." Visualization of the location of AR-positive cells on histological sections of the brain then allows one to describe the distribution of AR-positive cells in different brain regions, and densitometry allows quantification of the abundance of AR-positive cells in each brain region.

We have documented the distribution of AR-positive cells in the brains of adults of the sexually dichromatic *S. undulatus,* using the polyclonal AR antibody PG21 (Hews, Moga, and Prins 1999) (Fig. 8-3). We found AR-positive cells in many identified brain nuclei (dense clusters of neuronal cell bodies, as distinguished from a cell nucleus). Such nuclei in and near the hypothalamus, for example, play important roles in mediating sex-typical behavior in many vertebrates (Becker, Breedlove, and Crews 1992; Nelson 2000). Specifically, we found AR-positive cell nuclei in males ($n = 6$) in several regions, including the external nucleus of the amygdala; the arcuate, ventromedial, and periventricular nuclei of the hypothalamus; and the basal forebrain. In contrast with males, we did not find AR-positive cells in the amygdala or in hypothalamic regions in females ($n = 3$).

We also found dense concentrations of AR-positive fibers in male *S. undulatus* in the medial cortex, periventricular hypothalamus, and lateral forebrain bundle. AR-positive neuronal fibers were also present in the preoptic area of the hypothalamus, habenula, and deep layers of the optic tectum. In females, AR-positive fibers were relatively sparse but showed a similar distribution to that in males.

What is known about the distribution of ARs in the brains of other vertebrates with sexual differences in aggression? In the well-studied rat

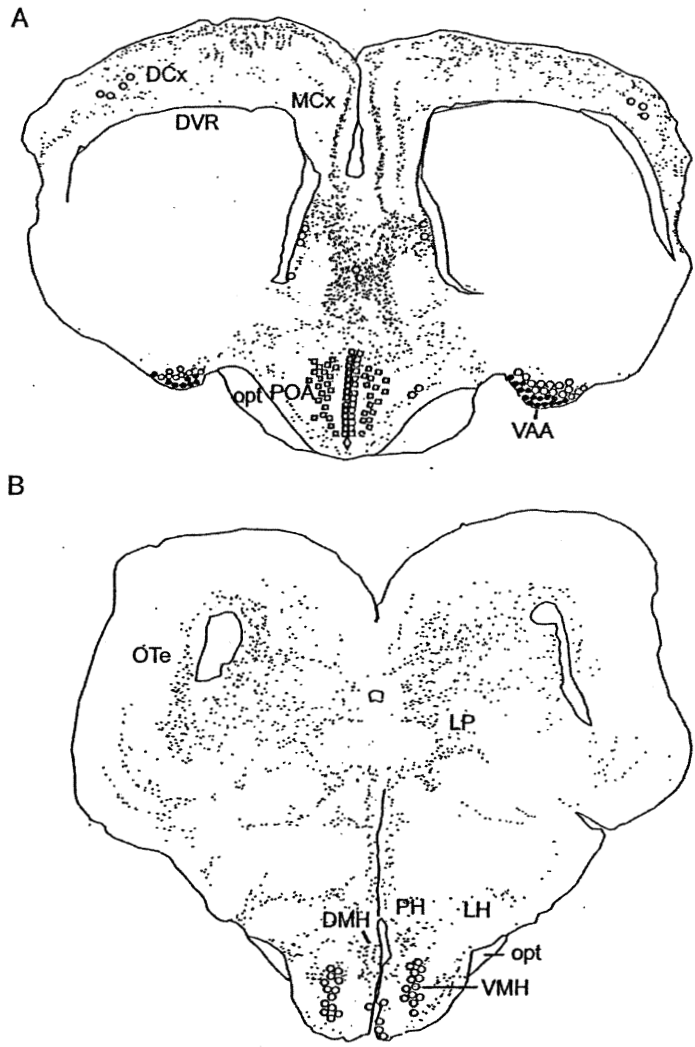

Figure 8-3. Drawings from an immunohistochemical study indicating locations of AR-positive fibers, cell nuclei, and cell soma for (*A*) rostral and (*B*) midlevel sections of brain in a male of sexually dichromatic *Sceloporus undulatus*. Small dots = fibers (possible axonal staining); large solid circles = darkly stained cell nuclei; large open circles or open squares = lightly stained cell nuclei and/or cytoplasmic staining. DCx = dorsal cortex; DMH = dorsomedial hypothalamic nucleus; DVR = dorsal ventricular ridge; LH = lateral hypothalamus; LP = lateral posterior thalamic nucleus; MCx = medial cortex; opt = optic tract; OTe = optic tectum; PH = posterior hypothalamus; POA = preoptic area; VAA = ventral anterior amygdalar nucleus; VMH = ventromedial hypothalamic nucleus (following Bruce and Neary [1995]).

(*Rattus norvegicus*), areas of the brain with the highest density of ARs were the amygdala, septum, hippocampus, medial preoptic area, anterior and lateral hypothalamus, median eminence, and adenohypophysis (Simerly et al. 1990). Similar work involving in situ hybridization in *Cnemidophorus* lizards revealed concentrations of AR mRNA and estrogen receptor (ER) mRNA in septal, amygdaloid, cortical, preopotic, and several hypothalamic brain nuclei (Young et al. 1994). In the amygdalar area, the medial external nucleus was labeled only with the AR probe (and not the ER probe), parallel to AR labeling seen in the medial nucleus of the amygdala of the rat (Simerly et al. 1990). Our preliminary results for the typical sexually dichromatic *S. undulatus* are, in general, consistent with results from other sexually dimorphic vertebrates. Our current studies are now comparing these distributions of AR-positive cells observed in *S. undulatus* with the distributions in males and females of the two monochromatic species (both sexes blue and aggressive, both sexes white and with decreased aggression).

Histological Study of Sexually Dichromatic Color Signals

As part of a study of aggression and color signals, one can also seek to understand the cellular basis of the color signal, and possible roles of sex steroid hormones in the sexual differentiation of these differences in the skin that comprise the signaling trait. In general, very little is known about the endocrine processes involved in sexual differentiation of the cells involved in the production of sexual color signals in vertebrates. There is some endocrine work examining cellular targets, and examples include studies of carotenoid-based signals of adult guppies (reviewed in Houde [1997]), and seasonal avian plumages (see citations in Kimball and Ligon [1999]).

We are examining the cells in the skin that are targets of the sex steroid hormones. The cellular basis of hue and intensity of color patterns has been studied in only a handful of lizard species (Taylor and Hadley 1970; Bagnara and Hadley 1973; Sherbrooke and Frost 1989; Morrison and Frost-Mason 1991; Cooper and Greenberg 1992; Morrison, Sherbrooke, and Frost-Mason 1996). Color patterns in animal skin result from differences in the abundance and relative locations of several types of skin pigment cells, or chromatophores (Cooper and Greenberg 1992; Morrison 1995). Melanophores have melanin-containing organelles called melanosomes. Melanin is a pigment that absorbs all wavelengths, usually imparting a black appearance to the skin (or brown, if chromatophores containing other pigments lie above the

melanophore). Melanophores are found deep in the dermal layer but can have finger-like projections extending up into the more superficial layers of the dermis. Another chromatophore type, the iridophore, is located superficial to (above) the deeper melanophores and can (along with other chromatophores) alter which wavelengths are reflected from the skin, and thus the appearance of the skin. Iridophores do not contain pigments but instead have intracellular platelets (often of guanine) that can selectively reflect blue wavelengths via interference. Other chromatophores (e.g., xanthophores, erythrophores) occur in lizard skin.

The model for production of blue versus white skin that we are testing involves only iridophores and melanophores (Cooper and Greenberg 1992). This model proposes that blue wavelengths are reflected off the skin by the iridophore layer. The remaining wavelengths are transmitted through the iridophore and either are absorbed by underlying melanin in the melanophores, yielding a blue appearance to the skin, or are reflected back by underlying layers such as collagen if melanin is absent, yielding a white appearance (Fig. 8-4).

Does the Abundance of Melanin Differ in Blue and White Skin?

We tested the hypothesis that sexual differences in the abundance of dermal melanin correlate with sexual differences in the occurrence of abdominal blue patches in *S. u. consobrinus.* We sampled abdominal skin from blue patches or from the "patch location" in females and prepared the tissues using standard dehydration, paraffin embedding, and staining procedures. We quantified the area of darkly stained melanin in sets of transects across the samples. Male and female *S. undulatus* differed significantly in the density of dermal melanin (Fig. 8-5).

We also prepared histological samples from the two sexually monochromatic species, *S. virgatus* and *S. jarrovii,* and the differences were as predicated by the melanin hypothesis. White abdominal skin (taken from where the blue patch would be located in other *Sceloporus* males) of *S. virgatus* males almost entirely lacked a melanin layer. Conversely, the density of dermal melanin in samples of skin taken from the blue abdominal patches of female *S. jarrovii* was relatively high and did not differ statistically from the melanin density in abdominal patch skin samples from males (Quinn and Hews, unpublished data).

The melanin hypothesis predicts that abundance of dermal melanin should be higher in the blue versus white skin in hormone-manipulated animals that are induced to express blue abdominal patches. Thus, the

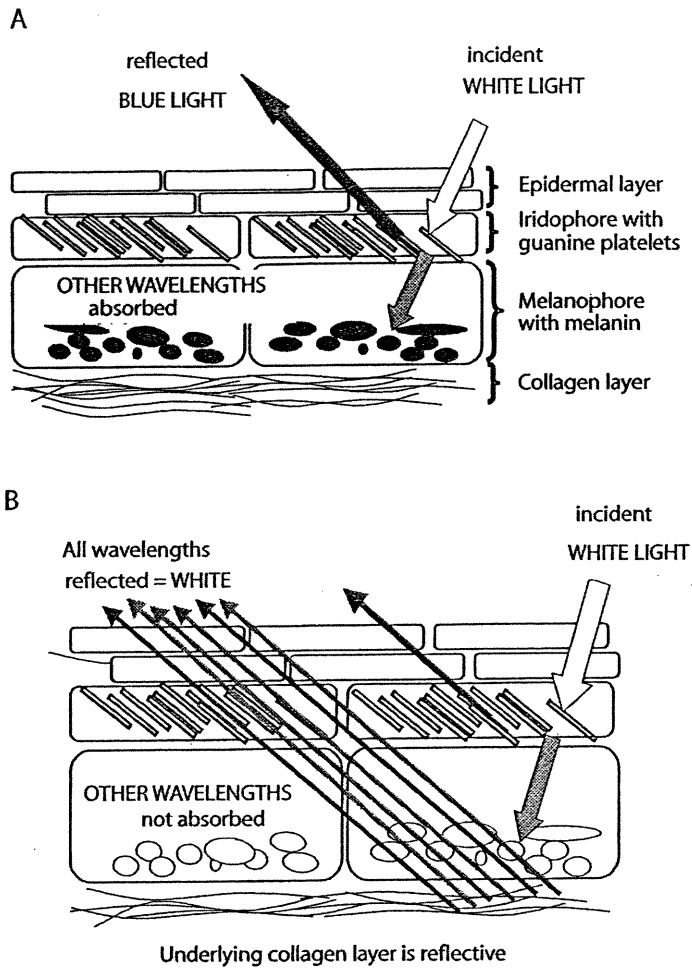

Figure 8-4 Illustration detailing model proposed by Cooper and Greenberg (1992) for production of (*A*) blue versus (*B*) white skin in lizards. For blue skin, iridophore cells have intracellular guanine platelets (open rectangles) that selectively scatter blue wavelengths; other wavelengths are transmitted through the iridophore and absorbed by melanin (solid ovals) in the underlying layer of melanophore cells, resulting in the appearance of blue skin. For white skin, melanin is absent, and the remaining wavelengths are reflected back by an underlying reflective layer (e.g., collagen), along with the blue from the guanine platelets, resulting in the appearance of white skin.

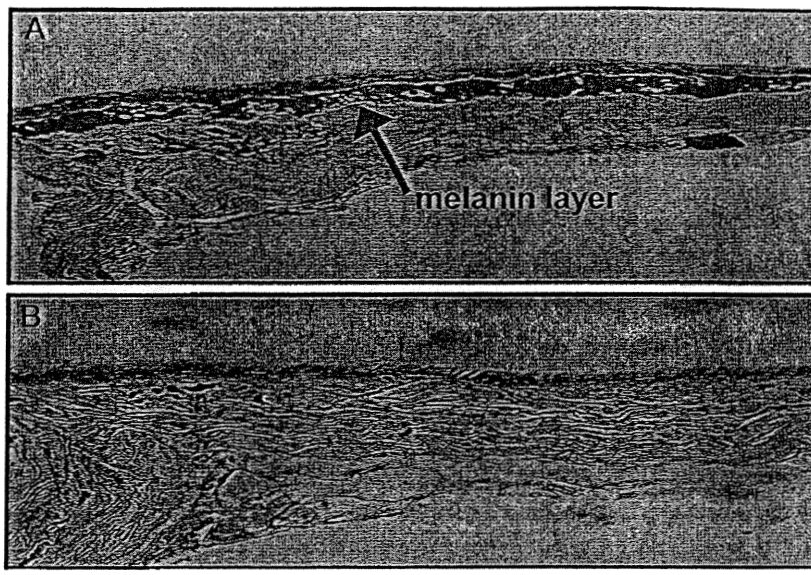

Figure 8-5 Histological section through skin taken from patch location in a male ([*A*] blue skin) and a female ([*B*] white skin), in a typical sexually dichromatic species, *Sceloporus undulatus consobrinus.*

hatchling female *S. undulatus* that we have successfully manipulated to express the male-typical blue of this species (Fig. 8-2B) should have greater dermal melanin density compared with that in white abdominal skin of control female hatchlings. This analysis is under way.

Does the Ultrustructure of Iridophores Differ in Blue and White Skin?

Another direction to explore involves the ultrastructure of the dermal chromatophores. In particular, the arrangement of the reflecting guanine platelets in the iridophores (Fig. 8-4) deserves attention. These platelets are present in the skin from both a white-bellied horned lizard (*Phrynosoma modestum,* Sherbrooke and Frost [1989]) and the blue abdominal patches of tree lizards (*U. ornatus,* Morrisson, Sherbrooke, and Frost-Mason 1996) and sagebrush lizards (*S. graciosus,* Morrison and Frost-Mason [1991]). However, the guanine platelets are highly organized in the blue abdominal skin of *S. graciosus,* with a regular brick-like arrangement within the cell. By contrast, in the white abdominal skin of *P. modestum,* the reflecting platelets lack an organized layered arrangement and reflect white light rather than blue wavelengths

produced by interference phenomena. Differences in techniques are not a likely explanation for these species differences in platelet organization because these studies were all conducted in the same laboratory.

Differences among *Sceloporus* in occurrence of blue abdominal patches might result, at least in part, from species differences in the arrangement of platelets in the iridophores. Besides the arrangement of the platelets, the individual size of the platelets (Fox 1976) may be key to whether the skin appears blue or not. Thus, transmission electron microscopic (TEM) analysis of the ultrastucture of iridophores will be necessary for a more complete understanding of the variation in the production of this visual signal. Initial TEM work on adult males of the white-bellied *S. virgatus* indicates that iridophores are present (Fig. 8-6) (K. Yanek and D. Hews, unpublished data). We currently are quantifying variables that can affect whether blue wavelengths of light are reflected, including platelet size, interplatelet spacing, and average number of platelet layers.

Conclusion

To understand fully the evolution of communication systems, and the selective forces that act on these systems, one must explore the many components of the communication system. These components include the production and control of the signal, as well as how it is transmitted in the environment, received by other individuals and processed in the CNS, and responded to by the receiver. The functional study of conspicuous male secondary sex characteristics, and conspecific responses to these traits, has led to a better understanding of the process of sexual selection (Andersson 1994), especially when such studies are carried out in a phylogenetic context. For example, analysis of signals and receivers has provided support for the sensory bias mechanisms of sexual selection (Basolo 1990, 1995; Ryan and Rand 1990, 1995; Endler 1992). Our work on functional aspects of the signal, which we do not describe in this chapter, currently involves documenting male and female responses to the presence and absence of abdominal blue patches (e.g., Quinn and Hews 2000).

Studying the roles that hormones play in the development of sexually dimorphic traits leads to a more complete understanding of these traits. In addition, studying these aspects of sexual signals in a phylogenetic context allows comparisons to be made among species, rather than simply between males and females, and the tracking of changes in endocrine mechanisms that underlie phylogenetic character state transi-

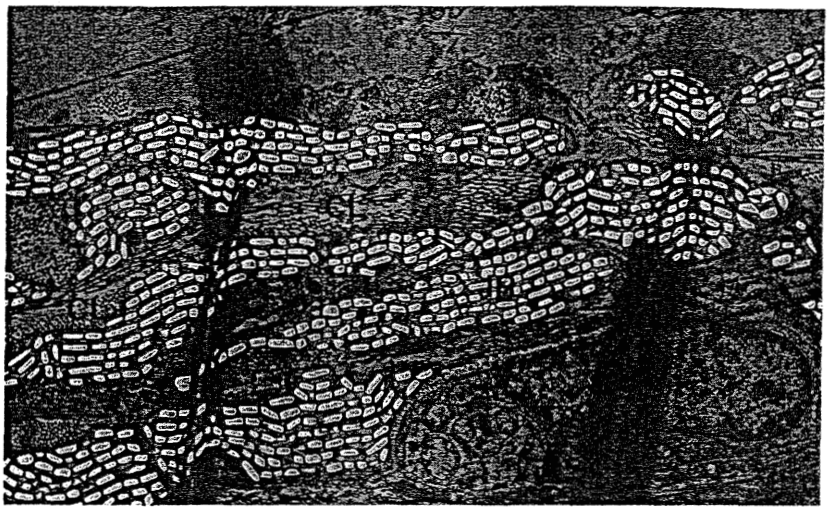

Figure 8-6 Transmission electron micrograph of abdominal skin from a male of white-bellied *Sceloporus virgatus*. The skin was taken from the same location as where the blue patches occur in other species. IR = iridophore; RP = reflecting platelet; CL = collagen layer.

tions in sexual differences in aggression and associated color signals. In this chapter, we focused on (1) aspects of signal production, (2) how hormones are involved in the development of a sexually dichromatic signaling trait, and (3) how hormones may be acting on the target tissues to result in species differences in the expression of the signaling trait. We discussed our work on three species of *Sceloporus* that vary both in the expression of aggression and in the development of an associated color signal. Manipulating pre- or posthatching hormone levels in the context of the organization and activation hypothesis in male and female *S. undulatus* and *S. virgatus* will reveal whether abdominal coloration is controlled by the sex steroid hormone, DHT, as it is in *Urosaurus,* the sister genus to *Sceloporus.* Future studies on the receptors in the skin and the presence and activity of enzymes that convert T into DHT are planned and should reveal the relative contributions of these components to the sex and species differences in signal morphology. Sex and species differences in the occurrence of the signal (abdominal coloration) are likely due, at least in part, to differences in the abundance of melanin in the layers of dermal melanophores that underlie the

iridophore layer. Other elements of the dermal chromatophore unit require additional attention.

Intimately related to this work on the signaling trait is the study of the endocrine regulation of the development and expression of signaling behavior. Woodley and Moore (1999a, b) have begun work on the masculinized females of *S. jarrovii,* exploring the activational roles of sex steroid hormones. Our work in progress is determining the distributions of ARs in the brain regions known to mediate aggression in vertebrates, including reptiles, and we will be comparing between the sexes and among the three *Sceloporus* study species. Determining the relative importance of organizational and activational effects of sex steroid hormones in contributing to sex and species differences in aggressive behavior will also need attention, as will assessing the relative importance of endocrine and nonendocrine mechanisms.

The genus *Sceloporus* provides an excellent opportunity for conducting such comparative endocrine and functional studies. Because of the multiple independent events of both loss in males and gain in females of the color patches (Wiens 1999), we can ask, Do similar endocrine mechanisms underlie similar but independent character state transitions?