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Biochemical regulation of pigment motility in vertebrate chromatophores: A review of physiological color change mechanisms

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Abstract The fundamental unit of rapid, physiological color change in vertebrates is the dermal chromatophore unit. This unit, comprised of cellular associations between different chromatophore types, is relatively conserved across the fish, amphibian, and reptilian species capable of physiological color change and numerous attempts have been made to understand the nature of the four major chromatophore types (melanophores, erythrophores, xanthophores, and iridophores) and their biochemical regulation. In this review, we attempt to describe the current state of knowledge regarding what classifies a pigment cell as a dynamic chromatophore, the unique characteristics of each chromatophore type, and how different hormones, neurotransmitters, or other signals direct pigment reorganization in a variety of vertebrate taxa [*Current Zoology* 62 (): – , 2016].

Keywords Chromatophore, Hormone, Neurotransmitter, Physiological color change, Pigment, Vertebrate

1 Introduction

In the animal kingdom, there are few phenomena as fascinating as the ability to quickly change color and pattern, an ability that can be used to hide from a predator or communicate with a conspecific. Rapid color change on the scale of seconds to hours, termed physiological color change, is typically accomplished via mobilization of pigments or nanostructures within specialized cells called chromatophores (Bagnara and Hadley, 1973; Aspögren et al., 2009). Although physiological color change has been observed in insects (Hinton and Jarman, 1973), arachnids (Insausti and Casas, 2008), crustaceans (Brown and Sandeen, 1948), and cephalopods (Florey, 1969; Hanlon and Messenger, 1988; Messenger, 2001), the structures and mechanisms of invertebrate color change are markedly different than those of vertebrates (Umbers et al., 2014). As such, this review focuses only on color-changing vertebrates (not including the birds and mammals which change color by altering blood flow to exposed skin; Rhodes et al., 1997) so that we might better describe the biochemical make-up and regulation of the motile chromatophores responsible for physiological color change in these taxa. Specifically, the objective of our review is to provide a detailed examination and description of the signaling molecules that influence the different types of chromatophore associated with physiological color change in vertebrates, complementing a recently

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published summary of several of these mechanisms (Sköld et al., 2013). We focus primarily on proximate mechanisms (i.e., biochemistry and cellular mechanisms) of rapid pigment translocation, and largely ignore the ultimate functions (i.e., the selective benefits that physiological color change confers) of physiological color change. Why physiological color change has evolved multiple times within vertebrates and how physiological color change confers selective advantages are equally interesting topics for discussion, but will not be addressed in detail.

Chromatophores are colorful cells that can be classified loosely by their overall color but are more accurately designated by their color-producing mechanisms or the types of pigments/structures that they contain (Aspengren et al., 2009). Pigmentary chromatophores impart color by selectively *absorbing* particular wavelengths of lights, whereas structural chromatophores produce color by *reflecting* and *scattering* particular wavelengths of light with cellular nanostructures. Among the pigmentary chromatophores that impart color via absorption of specific wavelengths of light, there are three predominant chromatophore types found in color-changing vertebrates: the black-brown melanophores (which contain melanin), red erythrophores, and yellow xanthophores (both of which can contain either carotenoids, pteridines, or some combination of the two). Additionally, there are two structural chromatophore types that imbue organisms with color via reflectance of light: the colorless iridophores and the white light-reflecting leucophores (both containing purines; Bagnara and Hadley, 1973; Bagnara and Matsumoto, 2006; Aspengren et al., 2009). All integumentary vertebrate chromatophore types originate from the embryonic neural crest (Bagnara and Hadley, 1973; Fujii, 1993; Bagnara and Matsumoto, 2006), a migratory, multipotent cell population (Le Douarin and Kalcheim, 1999). As chromatophores mature, they typically form functional associations with each other, termed “dermal chromatophore units”, just below the epidermis (Bagnara et al., 1968; Bagnara and Hadley, 1973).

Considered the fundamental unit of physiological color change in vertebrates, the dermal chromatophore unit incorporates melanophores, erythrophores, xanthophores, and iridophores into an isolated cellular system (Bagnara et al., 1968; Bagnara and Hadley, 1973; Fig. 1). This functional unit is capable of producing a wide range of colors by absorbing or reflecting specific wavelengths of light (Bagnara and Hadley, 1973; Grether et al., 2004). From surface of the integument to the base of the dermis, the chromatophore unit consists of the dendritic processes of the melanophore, a single layer of xantho- or erythrophores, a layer of iridophores, and the main cell body of the melanophore (Bagnara et al., 1968; Bagnara and Hadley, 1973; Fig. 1). It should be noted that, as conserved as this functional unit might be, the relative amounts of each chromatophore type and their relative arrangements vary from species to species and can even vary from one area to another on the same animal (Bagnara and Hadley, 1973).

While the dermal chromatophore unit can be considered to function as a single element with respect to color generation, the actual color reflected is the result of the four individual types of chromatophores serving different roles by undergoing dynamic, coordinated pigment reorganization (Bagnara et al., 1968; Bagnara and Hadley, 1973; Cooper and Greenberg, 1992; Grether et al., 2004; Aspengren et al., 2009). In general, the red-yellow erythrophores (or xanthophores) absorb the shorter wavelengths of light incident upon the dermal chromatophore unit, and the remaining wavelengths are either reflected back by the silvery iridophores or absorbed by the brown-black melanophores (Bagnara et al., 1968; Bagnara and Hadley, 1973; Nielsen, 1978; Aspengren et al., 2009; Fig. 2). It is the relative state of pigment dispersion and aggregation in each chromatophore, as well as their relative densities, that determines how much each contributes to the observed color of skin (Bagnara et al., 1968; Grether et al. 2004).

It is generally accepted that pigment mobilization must be carried out by motor proteins, such as kinesin, dynein, and myosin, which move along tracks provided by structural elements of the cytoskeleton (Bikle et al., 1966; Rodionov et al.,

1991; Nilsson and Wallin, 1997; Tuma and Gelfand, 1999; Aspengren et al., 2009). A model pathway proposed by Nery and Castrucci in their 1997 review describes the general pattern between receptor activation and pigment granule dispersal: First, a rise in intracellular second messenger (e.g., cyclic adenosine monophosphate = cAMP) concentration is induced by the binding of a signal molecule to its receptor on the plasma membrane (Nery and Castrucci, 1997). Next, the higher levels of cAMP activate protein kinase-A, which phosphorylates proteins bound to the pigment granules, causing these proteins to detach from the cytoskeleton and allowing the (recently freed) granule to be transported by kinesin (Fig. 3A) away from the perinuclear area (Nery and Castrucci, 1997). Conversely, pigment aggregation is typically achieved when protein kinase-A is inactivated, leading to protein dephosphorylation, which enables dynein to transport pigments back toward the center of the cell (Fig. 3B). This model, while simplified, allows for the generalization that extracellular signals can result in an intracellular cascade that causes pigment granules to associate with motor proteins to initiate color change.

Across vertebrate taxa, the greatest differences in the regulation of physiological color change depend on whether the integument is under neural control, endocrine control, or some combination of the two (Nery and Castrucci, 1997). However, given the broad range of color combinations that can be created by the dermal chromatophore unit (Grether et al., 2004), an appreciation of the complex regulatory pathways of each chromatophore type is essential for understanding both inter- and intraspecific differences in chromatophore control. In this review, we examine the effects of hormones, neurotransmitters, intracellular second messengers, and even ambient light on the chromatophores associated with physiological color change.

2 Melanophores

2.1 Content and structure

The melanophore's appearance is affected by the organization of its melanosomes (or melanin granules), which are vesicular organelles that exclusively contain melanin (Bagnara and Hadley, 1973). "Melanin" is a general term for all members of the tyrosine-derived class of pigments found in melanophores, which have high molecular weights and are chemically stable (Lerner and Fitzpatrick, 1950; Nicolaus and Piattelli, 1962; Ito and Wakamatsu, 2003). While all melanins exhibit these properties, there are several sub-classifications of melanin: neuromelanin, allomelanin, phaeomelanin, and eumelanin (Fedorow et al., 2005). Neuromelanin is specifically expressed in the central nervous systems of primates (Marsden, 1961; Fedorow et al., 2005), while allomelanin is found in the fungi, plant, and bacteria kingdoms (Fedorow et al., 2005). Phaeomelanin ranges from a muted yellow to red and is traditionally been recognized only in mammalian and avian melanocytes (Ito and Wakamatsu, 2003), though it has also recently been discovered in the shell of Hermann's tortoises *Eurotestudo boettgeri* (Roulin et al., 2013). Lastly, there is the brown-black eumelanin, or "true melanin," that is produced by birds, mammals, and all vertebrates capable of physiological color change (Aspengren et al., 2009) and this is the only melanin to which we will refer in this review.

Melanophores are capable of synthesizing their own melanin (Seiji et al., 1961, 1963) and, based on the final destination of the melanin produced, can be classified as either epidermal or dermal. Epidermal melanophores, responsible for the slower process of morphological color change, are commonly described as "spindle-shaped" with varying degrees of dendritic branching and are positioned at the base of the epidermis (Bagnara and Hadley, 1973). The cell body of the epidermal melanophore produces melanin granules and the processes, which weave around adjacent epidermal cells, are the sites of deposition of these granules into other cells (Bagnara and Hadley, 1973). Dermal melanophores, often associated with the dermal chromatophore unit, are found in the dermis and consist of a central cell body with dendritic processes that can be directed radially or upwards towards the epidermis, giving the cell a basket-like shape (Bagnara and Hadley, 1973;

Nielsen, 1978). Dermal melanophores retain all synthesized melanin and are, unlike the other types of chromatophores, exclusive to vertebrates capable of physiological color change (Bagnara and Hadley, 1973).

Given that color change takes time, even for organisms capable of very rapid color change, there was once a health debate about whether the dendritic melanophore processes were motile projections that simply carried melanosomes with them, or if the processes were static and only melanosomes translocated within them (Bagnara et al., 1968). However, Bagnara et al. (1968) observed that melanophores in the aggregated state (i.e., melanosomes clustered in the main cell body) possessed membranous layers where once melanophore processes had existed in the dispersed state. This evidence indicated that, in the absence of melanosomes, the processes had simply been emptied and collapsed in place rather than being drawn back into the cell body of the melanophore (Bagnara et al., 1968). Further support for the claim that melanophore processes are permanent structures is that the interactions between iridophore and melanophore plasma membranes are very intimate (Bagnara et al., 1968). For example, even when the melanophore is in the aggregated state, there are non-random indentations in the iridophore plasma membrane corresponding to the locations of known melanophore processes when dispersed (Bagnara et al., 1968). From this seminal work, the primary mechanism of physiological color change was determined to be the result of dynamic intracellular pigment transportation.

2.2 Mechanisms of color change

Though the specific factors that regulate melanosome motility frequently differ among taxa (see below), the mechanics of pigment translocation within melanophores appear to be relatively conserved among different groups. Exactly how are melanin granules transported so quickly within the melanophore, especially given their rather large size (up to several hundred microns in diameter; Bagnara and Hadley, 1973)? The process of melanosome translocation is thought to be coordinated by the microtubule and actin filament architecture of the cytoskeleton and the associated motors (Bikle et al., 1966; Rodionov et al., 1991, 2003; Nilsson and Wallin, 1997; Tuma and Gelfand, 1999; Fig. 3). Specifically, the molecular motor kinesin transports melanin granules towards the cell periphery during pigment dispersal (Rodionov et al., 1991), where melanosomes are subsequently transported along randomly oriented actin myofilaments (Tuma and Gelfand, 1999; Rodionov et al., 2003; Fig. 3A). When melanophores receive input producing melanosome aggregation, the tubulin-based molecular motor dynein hauls melanin granules back towards the nucleus (Nilsson and Wallin, 1997; Fig. 3B). For example, Logan et al. (2006) showed that microtubules are essential for melanosome transport in zebrafish *Danio rerio* by administering microtubule disrupting agents and observing that transport was diminished, slowed, or eliminated. These authors also investigated the role of actin-based microfilaments and found that they may have a role in tethering melanosomes to the periphery of the cell after dispersion. This 'tethering' could be a mechanism to reduce the cost of color change, by reducing the amount of cellular signalling and energy currency spent to keep the melanophores stellate.

2.3 Influence on color

In color changing vertebrates, the distribution of melanosomes within dermal melanophores (hereafter “melanophores”), especially in their dendritic processes, is a primary determinant of the lightness of the animal’s skin color. That is, as the area of melanosome distribution increases, more light is absorbed and a darker color is produced. Interestingly, however, melanophore aggregation and dispersal do not appear to be dose-dependent processes in the zebrafish *Danio rerio*. Instead, the melanophores of this species consistently demonstrate a translocation threshold where they completely aggregate or completely disperse if *at least* the minimum dose of translocation agonist necessary to stimulate pigment migration is administered (Logan et al., 2006). Hence, variation in the extent of dispersal would refer primarily to the rate of dispersal in

this species, as the pigment granules move through the intermediate stages of translocation from one extreme to the other (Logan et al., 2006). The binary nature of pigment dispersion observed in zebrafish may be species-specific, however, and more work is required to better understand how broadly this phenomenon extends among color changing vertebrates.

2.4 Regulation of melanosome motility within melanophores

The discovery of motile melanosomes triggered an abundance of investigations into the mechanisms and regulation of melanosome transport within melanophores, especially in species capable of rapid and possibly facultative color change (see reviews in Bagnara and Hadley, 1973; Nery and Castrucci, 1997; Aspengren et al., 2009; Stuart-Fox and Moussalli, 2009; Nilsson Sköld et al., 2013). Rapid, facultative color change has since been studied in a wide variety of vertebrate species including fish, amphibians, and reptiles (Wright and Lerner, 1960; Bagnara and Hadley, 1973; Nielsen, 1978; Hadley et al., 1985; Okelo, 1986; Maeno and Iga, 1992; Summers and Greenberg, 1994; Kotz et al., 1994; Osorio and Vorobyev, 1996; Larson and Summers, 2001; Mäthger et al., 2003; Logan et al., 2006). For melanophores, cyclic adenosine monophosphate (cAMP) appears to be one of the most prevalent and consistently agreed-upon cellular regulators of melanosome dispersal (reviewed in Tuma and Gelfand, 1999; see also Fujii, 2000). Numerous studies have found that an increase in the intracellular concentration of cAMP is associated with a dispersed melanosome distribution, whereas a decrease in cAMP causes the melanophore to reassume the aggregated state (Rozdzial and Haimo, 1986; Sammak et al., 1992; Fujii, 2000; Logan et al., 2006). In an experiment by Maeno and Iga (1992), forskolin, a potent activator of the cAMP synthesizing enzyme adenylate cyclase, was administered to melanophores and proved to be a powerful dispersant as well as an effective blocker of induced aggregation. Alpha-melanophore-stimulating hormone (α -MSH) and adrenocorticotrophic hormone (ACTH), collectively referred to as melanocortins, are also known to activate both the cAMP synthesizing enzyme adenylate cyclase and stimulate melanosome dispersal in reptiles, amphibians, and fish (Hadley and Goldman, 1969; Goldman and Hadley, 1970; Okelo, 1986; Maeno and Iga, 1992; Fujii, 2000).

Agouti proteins also function in color change processes by antagonizing melanocortin receptors and, consequently, counteracting the effects of α -MSH (Cerdá-Reverter et al., 2005; Zhang et al., 2010). Increased levels of agouti signaling protein are also associated with lighter colors and fewer melanosomes (Guillot et al., 2012). Up-regulation of specific agouti proteins even appears to underlie the background matching observed in zebrafish (Zhang et al., 2010), preventing α -MSH from elevating intracellular levels of cAMP. In addition to the effects of α -MSH and cAMP on color change, many studies provide evidence that calcium ions (Ca^{2+}) play an important role in physiological color change via their role as chemical messengers (Novales and Novales, 1965; Veerdonk and Brouwer, 1973; Graan et al., 1982a, 1982b; Iga and Takabatake, 1982). Despite these empirical results to the contrary, Kotz and McNiven (1994) assert that there is no evidence indicating a link between Ca^{2+} concentrations and melanosome motility. Therefore, it seems that the involvement of calcium ions in the regulation of pigment responses is still somewhat controversial (reviewed in Nery and Castrucci, 1997).

While α -MSH is considered a pigment dispersant across all taxa, two other hormones are associated with pigment cell motility: melatonin and melanin-concentrating hormone (MCH). These hormones can behave as either dispersants or aggregators at different concentrations in the melanophores of different species (Fujii, 2000; Aspengren et al., 2009). Melatonin is generally considered an aggregation inducer in fish, though melatonin sensitivity is highly variable among species (Nery and Castrucci, 1997; Fujii, 2000). While it has been suggested melatonin indirectly inhibits adenylate cyclase (Filadelfi and Castrucci, 1996), supporting its usual role as an aggregation agent (Rollag, 1988; Sugden, 1991; Fujii, 1993), melatonin can actually elicit pigment dispersion in some fish species (reviewed in Fujii, 2000) or even promote opposite responses in different areas of the same fish's skin (Nishi and Fujii, 1992). Similarly for MCH, which is typically an

aggregator (Oshima et al., 1986; Fujii, 2000; Logan et al., 2006; Aspengren et al., 2009), studies have found that high doses of MCH can induce dispersion (within teleosts). This duality of effect led to the hypothesis that there are two different types of MCH-receptors with either high or low MCH affinity (Oshima et al., 2001; Aspengren et al., 2009). Reptiles and amphibians are much less sensitive to MCH but, if given in high concentrations, MCH can stimulate melanosome dispersal (Wilkes et al., 1984; Fujii, 2000).

Primitive fishes are most susceptible to the influence of the hormones α -MSH, MCH, and melatonin because, unlike the bony teleost fishes, their physiological color change is mostly controlled by the endocrine system (Fujii, 2000). Physiological color change in teleost fish is also controlled by sympathetic enervation, making them sensitive to the catecholamine neurotransmitters epinephrine and norepinephrine (Fujii and Oshima, 1986; Maeno and Iga, 1992; Fujii, 2000). The sympathetic nervous system is responsible for the flight-or-fight response to stressful stimuli, which is brought about by the release of catecholamines that can act on either α - or β -adrenoceptors (Maeno and Iga, 1992). The roles of α - and β -adrenoceptors are conserved across all fish, reptile, and amphibian taxa, and these two types of receptors are often found within the same organism, either restricted locally or commingling in the plasma membrane of the same melanophore (Vaughan and Greenberg, 1987; Summers and Greenberg, 1994). Stimulation of α -adrenoceptors initiates a decrease in intracellular levels of cAMP concomitant with an aggregation response, while catecholamines can simultaneously activate β -adrenoceptors, which induce an increase in cAMP and causes melanosome dispersal (Maeno and Iga, 1992; Kotz et al., 1994; Nery and Castrucci, 1997; Fujii, 2000).

Competition between α - and β -adrenoceptors was first demonstrated in the green anole *Anolis carolinensis* by Vaughan & Greenberg in 1987. Because sympathetic nerves do not innervate anole skin, all physiological color change is mediated by circulating catecholamines binding to adrenoceptors and their respective effects on adenylate cyclase activity (Vaughan and Greenberg, 1987). These authors found that the eyespots of the green anole lack α -adrenoceptors, in contrast to the rest of the anole dermis (Vaughan and Greenberg, 1987). All other anole melanophores have α - and β -adrenoceptors, which are both stimulated by epinephrine (Vaughan and Greenberg, 1987). The β -receptors of the melanophores are activated by low levels of epinephrine and result in skin darkening, whereas high levels of epinephrine stimulate the α -adrenoceptors of those same melanophores to override the β -adrenoceptor response by inhibiting adenylate cyclase and thus the skin lightens (Vaughan and Greenberg, 1987). Therefore, an anole under duress will appear green everywhere except for its eyespots, which will be dark brown. Vaughan and Greenberg (1987) also found that anoles pretreated with a β -specific adrenergic blocker, propranolol, prior to treatment with epinephrine exhibited significantly retarded eyespot formation and were unable to attain the maximal dark brown body coloration. Vaughan and Greenberg (1987) suggest that both of these effects occurred as a result of epinephrine's ability to activate only α -adrenoceptors in propranolol-treated animals.

2.5 Summary

The melanophore is an important and dynamic contributor to physiological color change in fish, amphibians, and reptiles. There has been a great deal of research on the effects of particular compounds, hormones, and neurotransmitters (e.g., α -MSH, MCH, melatonin, catecholamines, and cAMP) on the translocation of the melanin granules from the perinuclear area of the melanophore cell body into the dendritic processes. Most of the aforementioned compounds act on receptors in the melanophore cell membrane and elicit a change in the activity of adenylate cyclase, and therefore a change in the intracellular concentration of cAMP. However, several classes of receptor are frequently present on individual melanophores, suggesting that there is a great deal of complexity in the biochemical regulation of melanosome organization and additional research remains to be done before we can fully clarify the range of interactions between different receptor

types and adenylate cyclase. Furthermore, considering the controversy surrounding the role of Ca^{2+} , its relationship to melanophore motility in fish, amphibians, and reptiles should be more specifically investigated. Additionally, the melanin pigment is ubiquitous across many more taxa than those discussed here, and further research has the potential to discover and clarify the additional functions it can serve in biological systems. Moving beyond melanophore-specific examinations, in-depth investigations of the dynamic interactions between melanophores and the other chromatophore types, which produce the remarkable color displays of fish, amphibians, and reptiles capable of physiological color change, should be a particularly fruitful area of future research.

3 Erythrophores and Xanthophores

3.1 Content, structure, and color

Erythrophores and xanthophores are two additional chromatophore types associated with the dermal chromatophore unit in vertebrates capable of physiological color change. These chromatophores are loosely classified by their predominant coloration, with xanthophores being principally yellow and erythrophores being principally red (Bagnara and Hadley, 1973). For some physiologically color changing vertebrates, including a variety of fish (Satake, 1980; Hadley et al., 1985; Oshima et al., 1986; Kotz et al., 1994; Sato et al., 2004) and amphibians (Bagnara et al., 1968; Nielsen, 1978), it has been determined that erythrophores and xanthophores are dynamic contributors to color change. However, there is no evidence that this is the case with all color changing vertebrates; there may be some species where the erythrophores or xanthophores serve only a passive role, and melano- and iridophores are the only dynamic chromatophores. For example, there is currently no definitive evidence of a dynamic contribution of the erythrophore/xanthophores to the rapid color change of any reptile species.

The yellow-to-red coloration of xanthophores and erythrophores is the result of the relative amounts, and types, of pteridine and carotenoid pigments within each chromatophore (Bagnara and Hadley, 1973). Traditionally, there are thought to be only two major pteridines involved in color change, drosopterin and sepiapterin (Obika et al., 1964), though drosopterin can exist in three forms (drosopterin, isodrosopterin, and neodrosopterin, all of which are found in the lizard *Sceloporus virgatus*; Weiss et al., 2012) and xanthopterin may underlie some orange skin coloration in reptiles and amphibians (Suga and Munesada, 1988; Morrison et al., 1995). In comparison, there are two entire families of carotenoids involved in physiological color change: the hydrocarbon carotenes and the oxygen-containing xanthophylls (Bagnara and Hadley, 1973). However, Chatzifotis et al. (2011) recognize two different classes of carotenoids based primarily on the color these molecules reflect: the yellows (e.g. beta-carotene, lutein, zeaxanthin) and the reds (e.g. astaxanthin esters). Because the wavelengths of light absorbed by carotenoid molecules (and hence, the wavelengths of light reflected which provide color) are dependent on the length of their conjugated pi-systems (Allen, 2008) and not the presence or absence of oxygen, the carotenoid classification suggested by Chatzifotis et al. (2011) represents a fundamentally different criterion for grouping carotenoid pigments.

The diversity of carotenoid types present in erythrophores and xanthophores is largely due to the fact that carotenoids can only be obtained through an animal's diet, as opposed to pteridines which can be synthesized *de novo* within the chromatophores (Bagnara and Hadley, 1973). Carotenoids are ingested rather indiscriminately, as different types of carotenoids often coexist within the same food source, and it is only after ingestion that animals can modify and metabolically convert the carotenoids according to established biochemical pathways (reviewed in Møller et al., 2000; McGraw, 2006; Svensson and Wong, 2011). On the other hand, only drosopterin (red) and sepiapterin (yellow) can be produced in abundance *de novo* in the vertebrate taxa examined in this review (Hama, 1963; Obika, 1963; Bagnara and

Hadley, 1973; Ichikawa et al., 1998). While the sepiapterin synthesis pathway has not yet been fully elucidated, it is known that once sepiapterin is formed from guanosine triphosphate (GTP), it often serves as a precursor of further pteridine differentiation (reviewed in Ziegler, 2003). However, sepiapterin is not a precursor to drosopterin; rather there is a branching point in the biochemical pathway from which both sepiapterin and drosopterin are synthesized from the same intermediate (Dorsett et al., 1979). Given the shared pathways of pteridine synthesis, it is surprising that individual chromatophores synthesize either drosopterin (erythrophores) or sepiapterin (xanthophores), to the near exclusion of the other type (Ichikawa et al., 1998).

Because xanthophores and erythrophores can both contain carotenoid vesicles, pterinosomes, or some combination of the two, the terms xanthophore and erythrophore are often used interchangeably. A commonly used subjective distinction between the two relies simply on whether the chromatophore appears more yellow (“xanthophore”) or red (“erythrophore”; Bagnara and Hadley 1973). Despite their overall similarity, xanthophores and erythrophores are distinct entities with subtle, but significant differences. One of these differences, exclusivity of pteridine production between chromatophore types (Ichikawa et al., 1998), has already been discussed. Other differences are related to the development and maturation of the chromatophores themselves. In a study of the metamorphosing brown frogs *Rana ornativentris*, Ichikawa et al. (1998) found that erythrophores were first observed in the hypodermis several stages after the first of the already formed xanthophores were finally moving out of the collagenous layer, where they were formed just above the hypodermis, into the subepidermal space that is the residence of mature dermal chromatophores (Ichikawa et al., 1998). As metamorphosis progressed, the xantho-, irido-, and melanophores assumed the arrangement of the typical dermal chromatophore unit, and were eventually joined by the erythrophores, which formed an unusual extra layer below the chromatophore unit (Ichikawa et al. 1998). This morphological evidence indicates that erythrophores and xanthophores originate independently of each other, at least in *R. ornativentris*.

In *R. ornativentris*, the major difference between xanthophores and erythrophores with regard to the carotenoid vesicles is in their respective quantities and distributions (Ichikawa et al., 1998). The carotenoid vesicles of erythrophores are generally restricted to the cytoplasmic periphery, whereas the more numerous carotenoid vesicles of xanthophores are found both near the nucleus and dispersed in the cytoplasm (Ichikawa et al., 1998). While the carotenoid vesicles were very similar in structure and development in the two chromatophores, there were stark differences in their pterinosome ontogeny (Ichikawa et al., 1998). In the xanthophore, development of the fibrous, concentric whorl (a characteristic property of pterinosomes) began at the center of the granule, whereas the whorl developed first at the periphery of erythrophore pterinosomes (Ichikawa et al., 1998). Also, pterinosomes in the erythrophore remained uniform in size throughout metamorphosis and on into the juvenile frog stages, whereas those of the xanthophore were diverse and grew in size during metamorphosis, but then shrunk when the frog completed the transition from tadpole to juvenile (Ichikawa et al., 1998). It is noteworthy to point out that, in addition to the pterinosomes of the respective chromatophores being significantly different in size, these two distinct types of pterinosomes were never observed to coexist within one chromatophore (Ichikawa et al., 1998). Given that this study was conducted on a single species, the general applicability of these conclusions is not known.

3.2 Mechanisms of color change

The mechanism of pigment granule reorganization is believed to be similar, if not the same, in erythrophores and melanophores in the sense that pigment granules in both cell-types associate with motor proteins for transport (Byers and Porter, 1977; Kotz et al., 1994; Nery and Castrucci, 1997). However, in contrast to melanophores, both the aggregation and dispersion processes in erythrophores were approximately twice as fast as melanosome translocation in melanophores (Kotz

et al., 1994). Furthermore, erythrophore pigments appear to aggregate quickly and with uniform velocity, while dispersing with saltatory motions at a slower rate (Byers and Porter, 1977; Beckerle and Porter, 1983; Kotz et al., 1994). The difference in dispersal and aggregation behavior of erythrophore pigments is likely due to the structural changes in the erythrophore during these different stages (Byers and Porter, 1977). Specifically, pigment cells aggregate by moving along microtubules within a three-dimensional lattice that is also withdrawn during aggregation. The restructuring of the collapsed lattice, required for pigments to be subsequently translocated away from the nucleus (i.e. dispersed), may account for the different rates of pigment movement between aggregation and dispersal (Byers and Porter, 1977). Additionally, the apparent differences in pigment granule motility between melanophores and erythrophores led Beckerle & Porter (1983) to investigate the effects of microtubule (MT) disruption and actin motility inhibition on pigment motility within erythrophores in squirrelfish *Holocentrus ascensionis* chromatophores. The MT depolymerizing agents colchicine and nocodazole were used to remove the radial MT cytoskeletal network, beginning at the cell periphery and depolymerizing towards the nucleus (Beckerle and Porter, 1983). In one treatment, the MTs were partially depolymerized while the pigment granules were retained in a dispersed state by administration of caffeine; after a sufficient amount of depolymerization had been allowed, epinephrine (EPI) was given to induce aggregation (Beckerle and Porter, 1983). Aggregation only occurred where MTs persisted closer to the nucleus and any pigment left farther out than the MTs extended remained dispersed (Beckerle and Porter, 1983). In a second experiment, MTs were completely depolymerized with nocodazole, and pigment granules lost their characteristic radial array and subsequent administration of EPI only induced localized clumping of pigment granules (Beckerle and Porter, 1983). Therefore, Beckerle and Porter (1983) concluded MTs are required for organized pigment translocation. In addition, an actin inhibitor, cytochalasin B, was administered to erythrophores, but no change to pigment motility was observed, which indicates that pigment dispersion and aggregation in erythrophores is independent of actin, unlike melanophores (Beckerle and Porter, 1983).

3.3 Regulation of pigment motility

In a parallel to melanophore responses to intracellular cAMP levels, calcium ions are the predominant signal for pigment aggregation and dispersion in erythrophores (Kotz et al., 1994). Kotz and McNiven (1994) investigated the effect of intracellular calcium ion concentrations (Ca^{2+}) in the intact, un-lysed erythrophores of squirrelfish *Holocentrus ascensionis* and found that an increase in Ca^{2+} was a concomitant, sufficient, and necessary requirement for pigment aggregation (Kotz et al., 1994). Interestingly, while the rise in Ca^{2+} is required for aggregation, the pigments remain aggregated even after Ca^{2+} has returned to basal levels (Kotz et al., 1994). This phenomenon led Kotz and McNiven (1994) to the conclusion that there must be another signaling molecule involved in pigment translocation and they turned their attention to the effect of changing cAMP levels on erythrophores, due to the importance of cAMP in melanophore motility. They found that a decrease in Ca^{2+} was necessary for pigment dispersion, but dispersal did not occur unless there was also simultaneous increase in cAMP (Kotz et al., 1994). While this response is analogous to cAMP's role in melanosome dispersion, a high concentration of cAMP in erythrophores can apparently be overridden by a sufficient increase in Ca^{2+} . In contrast, melanophore motility was completely unaffected by the absence or presence of intracellular Ca^{2+} (Kotz et al., 1994).

Under most conditions, erythrophores (hereafter used to describe both erythrophores and xanthophores, unless xanthophores were specifically studied) respond to extracellular signals with pigment migration patterns similar to the melanophore responses previously described (Oshima et al., 1986; Kotz et al., 1994). Erythrophores respond to α -MSH administration with pigment dispersion in teleosts and frogs, (Hadley et al., 1985; Oshima et al., 1986). In research

conducted by Hadley et al. (1985), a super-potent analogue of α -MSH, [Nle⁴, D-Phe⁷]- α -MSH or MSH*, was analyzed alongside α -MSH in the teleost *Lebistes reticulatus*, five species of amphibians (genera *Rana*, *Xenopus*, *Bufo*), the lizard *Anolis carolinensis*, and a rattlesnake *Crotalus atrox*. The analogue induced prolonged darkening in all species and, specifically, dispersion in the teleost erythrophores that lasted six days after saline rinse compared to the relatively fast re-aggregation time of four hours for melanophores (Hadley et al., 1985). These results indicate that not only is α -MSH able to directly act upon erythrophores in addition to melanophores, there also appears to be significantly different mechanism or potency of interaction between the hormone and the two chromatophores (Hadley et al., 1985).

As in melanophores, the hormones melatonin and MCH also induce pigment migration in erythrophores (Satake, 1980; Oshima et al., 1986); however, the catecholamine neurotransmitters epinephrine and norepinephrine have a more direct, one-sided relationship with erythrophores than melanophores (Beckerle and Porter, 1983; Oshima et al., 1986). Satake (1980) used melatonin as a control aggregation agent in the xanthophores of the common goldfish *Carassius auratus* when testing the effects and interactions of novel dispersants; thus, melatonin's role has so far only been observed to be an aggregation agonist in the xanthophores of fish species, as opposed to its bidirectional, dose-dependent influence in fish melanophores (reviewed in Fujii, 2000). Similarly, MCH also exclusively causes aggregation in both erythrophores and xanthophores of several teleost species (Oshima et al., 1986). While MCH and α -MSH are considered antagonizing hormones, MCH can induce aggregation in the absence of Ca²⁺, unlike the Ca²⁺-dependent dispersion agent, α -MSH (Oshima et al., 1986). Oshima et al. (1986) also tried to determine whether the effects of MCH are transduced by α - or β -adrenoceptors by administering MCH after treatment with either an α - or β -adrenoceptor blocking agent. Though erythrophore pigment motility induced by MCH was not found to make use of either adrenoceptor, the neurotransmitters epinephrine and norepinephrine certainly do (Oshima et al., 1986). As in melanophores, stimulation of α -adrenoceptors by catecholamines caused pigment aggregation in erythrophores (Oshima et al., 1986). However, despite the coincidence of α - and β -adrenoceptors on the erythrophores, there was no mention of catecholamines stimulating β -adrenoceptors and causing pigment dispersal (Oshima et al., 1986). Thus, information regarding the effects of β -adrenoceptor stimulation of erythrophores in teleosts is lacking. There is also a scarcity of research regarding the effects of melatonin, MCH, or catecholamines on the erythrophores of reptiles and amphibians; however, this is most likely due to the once prevalent belief that erythrophores only passively contributed to physiological color change in these taxa, acting as static filters to remove particular wavelengths of light (Bagnara and Hadley, 1973). Although light filtering is probably a primary function of erythrophores, these chromatophores also appear to provide dynamicity of physiological color change in some organisms by varying the extent of filtration (Satake, 1980; Hadley et al., 1985; Oshima et al., 1986; Kotz et al., 1994).

The dynamic filtration interaction between ambient light and erythrophores is perhaps best demonstrated not by chemical induction, but through pigment motility in response to different wavelengths of available light. In natural conditions, erythrophores are exposed to both the full spectrum of visible light and ultraviolet (UV) radiation; however, the results from a study by Sato et al. (2004) showed that exposure to different wavelengths of light, ranging from 365 (UV) to 600 nm (red end of visible spectrum), affected the direction of pigment motility in the Nile tilapia *Oreochromis niloticus*. Specifically, Sato et al. (2004) found that erythrophores dispersed their pigment at 440–550 nm (blue and green light) and aggregated their pigments at all other wavelengths (365–440 nm, 550–600 nm) as well as in darkness (Sato et al. 2004). These results make sense because the pigments of erythrophores (and xanthophores) absorb in the blue and green regions of the spectrum (Allen, 2008). The pigments within erythrophores disperse under these conditions because that is when they are capable of maximum absorption, and could provide a protective mechanism to prevent sun damage (Allen, 2008).

3.4 Summary

Despite reasonably complete knowledge regarding the mechanisms of pigment movement within the erythrophores and xanthophores of color-changing vertebrates, much remains to be uncovered. There is a great deal of support for analogous pigment mobilization in the erythrophores and melanophores of teleosts, yet there has been very little research on the responses of pterinosomes and carotenoid vesicle translocation in the erythrophores of reptiles, amphibians, and other fish species. Given the opposite effects of MCH on fish compared to reptile and amphibian melanophores, it is unknown whether MCH would aggregate pigments in reptile and amphibian erythrophores as it does in fish melanophores, or whether MCH would disperse erythrophore pigments as it does in reptile and amphibian melanophores. Additionally, very little research has been conducted exploring the effects of melatonin on erythrophores of any of the taxa discussed, and more in-depth investigations concerning stimulation of both α - and β -adrenoceptors of erythrophores are certainly needed.

4 Iridophores (with Brief Discussion of Leucophores)

4.1 Iridophores vs leucophores

Iridophores and leucophores are frequently lumped together in a catch-all, ‘light-reflecting chromatophore’ category. Though both provide color by reflecting light (as compared to pigmentary colors which imbue color primarily via selective absorption of particular wavelengths of light), iridophores and leucophores differ from one another in several key ways. First, leucophores reflect only white light, whereas iridophores can reflect specific spectra of light ranging from violet to red, including iridescent coloration (Fujii, 1993; Bagnara and Matsumoto, 2006). Second, iridophores tend to be located above melanophores within the dermal chromatophore unit (Bagnara and Hadley, 1973), while leucophores are typically found below the melanophore layer (Obika, 1988). Third, and perhaps most importantly, iridophores and leucophores differ with respect to the way that they effect color change. Leucophores are analogous in both form and function to melanophores in that they possess dendritic processes through which motile sub-units (‘leucosomes’ or ‘refractosomes’) travel (Fujii and Miyashita, 1979; Obika, 1988). In contrast, color change is effected within iridophores by changing the spacing and orientation of orderly distributed, light-reflecting platelets (Bagnara, 1966; Taylor, 1969; Fujii, 1993; *see below*). Lastly, leucophores are known to contribute to natural physiological color change in only a handful of vertebrates, located among the ray-finned fishes (killifish *Fundulus*, (Menter et al., 1979); medaka *Oryzias* (Obika, 1988); guppies *Poecilia*, formerly *Lebistes*, (Takeuchi, 1976)). However, albino individuals of the African clawed frog also possess leucophore-like cells (*Xenopus laevis*; Fukuzawa, 2004)). Because leucophores are present only in a restricted subset of color-changing vertebrates, we will focus the remainder of our discussion on the factors influencing color changing iridophores.

4.2 Content, structure, and mechanisms of color production

Iridophores, which contain stacks of colorless guanine platelets, effect physiological color change primarily via the alteration of brightness, or relative reflectance, of perceived colors (Bagnara et al., 1968; Bagnara and Hadley, 1973; Cooper and Greenberg, 1992). The organization of the guanine reflecting platelets into stacks causes iridophores to provide a highly reflective, often iridescent surface in the dermis of the skin (Bagnara et al., 1968; Cooper and Greenberg, 1992; Fujii, 1993; Nery and Castrucci, 1997). Iridophores do not contain an actual “pigment” molecule, rather the mutable arrangement of guanine platelets, in conjunction with their high refractive index, is a dynamic form of structural coloration (Fujii, 1993; Prum, 2006). Structural colors are produced by the interaction of light with specific, internal or intracellular structures of an organism (Fox, 1976; Prum, 2006); interactions that result in perceivable visual effects and colors (from

metallic or iridescent to yellow or blue) and thus contribute substantially to color changes in the skin (Fujii et al., 1991; Cooper and Greenberg, 1992; Morrison et al., 1996).

The color-producing mechanism within iridophores, guanine, is very different from the pigments of melanophores, erythrophores and xanthophores in two ways: it is inherently transparent and it is also a necessary biochemical component of every living cell (Nelson and Cox 2008). Unlike melanins, pteridines, and carotenoids, which serve, for the most part, an accessory role in pigment cells, guanine is an essential element of DNA and nucleotide-based intracellular energy currency (Nelson and Cox, 2008). In addition, there are other molecules that occur in iridophores in trace amounts relative to the abundant guanine, including another essential and ubiquitous purine, adenine, as well as hypoxanthine and uric acid (Stackhouse, 1966; Bagnara and Hadley, 1973; Ziegler, 2003). Though inherently colorless, the tight, but unorganized collection of free guanine sequestered into crystal-like structures imparts a huge refractive index of 1.83 (approximately 75% that of diamond, the most refractive material known) to the iridophore's reflecting platelets (Land, 1972; Fujii et al., 1991; Mäthger et al., 2003; Serway et al., 2009). The organization of these highly refractive reflecting platelets into stacks throughout the cytoplasm, a medium with a refractive index of 1.33, results in the iridophore having a very high reflective capacity (Land, 1972; Fujii et al., 1991). For the iridophores involved in physiological color change, this reflectivity is the result of multilayer thin-film interference created by the alternating layers of materials with high (guanine) and low (cytoplasm) refractive indexes (Denton and Land, 1971). In addition to differences in the refractive indexes between the guanine and cytoplasm layers, the size, shape, proximity, and orientation of the guanine reflecting platelets influence the observed colors. In most biological systems, including all vertebrate iridophores studied to date, the reflectivity of multilayer thin-film reflectors is 'non-ideal' if the optical thickness of all layers is not uniformly equal (Denton and Land, 1971).

4.3 Mechanisms of color change

Another distinctive feature of iridophores is that they have been observed to exhibit not one, but two kinds of platelet motion: a sliding, lateral dispersion (similar to dispersion in erythrophores) or an accordion-style stack expansion or compression. Whereas, lateral changes in platelet location have been observed only in gobiid fishes (Iga et al., 1990; Fujii et al., 1991), accordion-style changes in stack compression have been observed in the neon tetra (*Paracheirodon innesi*; Clothier and Lythgoe, 1987), blue damselfish *Chrysiptera cyanea* (Oshima and Fujii, 1987), ornate tree lizard *Urosaurus ornatus* (Morrison et al., 1996), and the panther chameleon *Furcifer pardalis* (Teyssier et al., 2015)). Regardless of the type of motion utilized by iridophores, the end result is a shift in the wavelengths of reflected light (Fujii et al., 1991; Goda and Fujii, 1998). With respect to reflecting platelet movement and concordant color changes, the terms “aggregation” and “dispersion” are not the most precise descriptors of platelet translocation in iridophores (though they are sufficient when describing pigment movement within melanophores, erythrophores, and leucophores: (Fujii, 1993; Goda and Fujii, 1998)). The different type of intracellular movement that occurs within iridophores prompted Goda and Fujii (1998) to propose the terms “LR response” and “SR response,” where LR and SR are abbreviations for the Longer-wavelength light-Reflecting response and the Shorter-wavelength light-Reflecting response. Loosely, LR corresponds to dispersion or increasing the distance between reflecting platelets (leading to longer-wavelength greens in the blue-green damselfish *Chromis viridis*; Oshima et al., 1989), and SR to aggregation or contraction of reflecting platelets (leading to shorter-wavelength blues in the blue-green damselfish, *C. viridis*; Oshima et al., 1989) so as to reduce the space between them (Goda and Fujii, 1998).

It is important to note, however, that whether the translocation of reflecting platelets is radial or lateral or the iridophores are dendritic or discoid, all reflecting platelet motility affects the distance between adjacent platelets and, consequently, the wavelength of light reflected by the iridophores (Mäthger et al., 2003). According to Mäthger et al. (2003), the reflection of

colored light in the paradise whiptail *Pentapodus paradiseus* depends on both the thickness of the guanine platelets and the distances between them in the platelet stack. Slight changes in the spacing between the platelets can result in a significant change in color (Teyssier et al., 2015) since the highest reflectivity is observed perpendicular to the surface, when the optical thicknesses (actual thickness * refractive index) of the platelets and spaces are one quarter of the wavelength reflected (Land, 1972; Mäthger et al., 2003). Fujii et al. (1991) noted in the dark sleeper (a species of fish *Odontobutis obscura*) that as reflecting platelets aggregated (SR response), the skin darkened and reflected color shifted from longer to short wavelengths (specifically, yellow to blue). Interestingly, Nagaishi et al. (1990) and Mäthger et al. (2003) also found that the colors reflected by iridophores transitioned hue as the angle of incident light changed from the normal (i.e. the colors were iridescent; Doucet and Meadows, 2009). This phenomenon, predicted by the physical properties of reflecting platelets (Huxley, 1968; Land, 1972) and observed in *ex situ* skin preparations (Nagaishi et al., 1990; Mäthger et al., 2003), coupled with the observed color changes in living fish suggests that motile reflecting platelets facilitate a secondary method of color change – changing reflecting platelet angle of incidence (Nagaishi et al., 1990). According to this "theory of blinds", named based on the similarity between changes in platelet angle and the movement of the slats of a Venetian blind, shifts in spectral peaks (i.e. hue and brightness) can also arise due to changes in the orientation of reflecting platelets within the iridophore layer (Nagaishi et al., 1990). In an impressive study, the Venetian blind model has recently been convincingly and empirically validated as a potentially significant force contributing to the rapid color change exhibited by organisms with motile iridophores (Yoshioka et al., 2011).

4.4 Relationships with other chromatophores

Since Bagnara et al. (1968) proposed the structure of the dermal chromatophore unit, there has been little deviation from this archetype across diverse taxa of vertebrates capable of physiological color change. The iridophores associated with color change are sequestered beneath a layer of erythrophores and within the upward-extending dendritic processes of melanophores (Bagnara et al., 1968). Though iridophores are usually part of the dermal chromatophore unit, there is no general model which can capture the ratio of iridophores to melanophores (or erythrophores) because the number of iridophores associated with an erythrophore or encased by a melanophore is highly variable (across taxa, within species, and among different regions of the same individual; Bagnara et al., 1968). Although there are many ways in which iridophores differ from melanophores and erythrophores, one peculiar difference is that there is no single description of an iridophore that is representative of them all (Iga et al., 1990; Fujii et al., 1991; Teyssier et al., 2015). That is to say, no one model of an iridophore can capture both the radial and lateral types of reflecting platelet motion, nor account for the observations of iridophores being either non-dendritic, like erythrophores, or dendritic, in which the reflecting platelets disperse into the processes as in melanophores (Iga et al., 1990; Fujii et al., 1991).

In addition to adjusting reflected light on their own, the iridophores have a dynamic and synergistic relationship with the erythrophores and melanophores of the dermal chromatophore unit (Bagnara et al., 1968). Bagnara et al. (1968) investigated the interactions between the iridophores and melanophores of several frog species and found that when melanophores were in the dispersed state, the processes of the melanophores, distended with melanosomes, extend over the top of its associated iridophores thereby preventing light from reaching the reflecting platelets' reflective surfaces. Conversely, under the influence of melanin aggregating agents (e.g. epinephrine, norepinephrine, MCH), melanosomes are concentrated (frequently below the iridophores) and contribute less to cell color, particularly because the same agents tend to produce

long-wavelength color shifts among iridophores (Kasukawa et al., 1986, 1987; Nagaishi and Oshima, 1989; Oshima and Kasai, 2002) and enlarge their reflecting area, preventing much light from reaching the melanin (Bagnara et al., 1968). In other words, the translocation of iridophore reflecting platelets is frequently reciprocal to that of melanosomes and the pigments of erythrophores (but not always; Bagnara and Hadley, 1969). Pale coloration in vertebrates that undergo physiological color change may, therefore, be dependent upon the combined effects of iridophore LR response coupled with melanosome aggregation (Nagaishi and Oshima, 1989), whereas skin darkening may primarily be the result of melanophore and erythrophore pigment dispersion (Bagnara et al., 1968; Fujii et al., 1991).

4.5 Regulation of reflecting platelet motility within iridophores

In exploring the factors that regulate iridophore reflecting platelet motility, many biochemical regulators have been examined based on their well-studied influence on melanophores. For example, one of the first investigations into the hormonal factors influencing platelet movement in amphibians discovered that reflecting platelets aggregated in response to the potent melanophore stimulating agent α -MSH, but were otherwise dispersed to provide the maximum surface area for reflectance (Bagnara et al., 1968). The relationship between α -MSH and reflecting platelet aggregation also appears in several fish species (dark sleeper *O. obscura*, Iga et al., 1990; Fujii et al., 1991); blue-green damselfish *Chromis viridis*, Oshima et al., 1989). However, this relationship is not universal, as the reflecting platelets of ornate tree lizard *Urosaurus ornatus* iridophores do not respond to exogenous administration of MSH (Morrison et al., 1996), nor do those of blue damselfish (*Chrysiptera cyanea*, Kasukawa et al., 1987) or common surgeonfish (*Paracanthurus hepatus*, Goda and Fujii, 1998). Additionally, research by Iga et al. (1991) showed that α -MSH-induced aggregation was inhibited in the absence of Ca^{2+} , indicating that platelet motility may depend, as with pterinosomes and carotenoid vesicles, on intracellular Ca^{2+} concentrations. Maeno and Iga (1992), who found that MSH caused dispersion in melanophores by activating adenylate cyclase and thereby increased intracellular amounts of cAMP, presented evidence that the iridophores of the dark sleeper *O. obscura* also respond to increasing concentrations of cAMP by aggregating reflecting platelets. Administration of the potent adenylate cyclase activator forskolin or a cAMP analogue (8-bromoadenosine 3',5'-cyclic monophosphate, or 8-Br-cAMP) resulted in the SR (platelet aggregation) response of reflecting platelets, but whether a decrease in cAMP levels was sufficient for dispersion was not examined (Maeno and Iga, 1992). However, Fujii et al. (2000) postulated that the overall result of α -MSH is aggregation in teleostean iridophores and that α -MSH does so by inhibiting adenylate cyclase activity. This claim, when compared with the findings of no effect for MSH on ornate tree lizards, blue damselfish, or common surgeonfish, makes generalizations regarding the effects of α -MSH or changes in cAMP concentrations on translocation of iridophore reflecting platelets impossible across all taxa.

Inconsistent findings regarding the effects of certain hormones or chemicals on reflecting platelet movement within iridophores are not restricted to investigations of α -MSH. There is just as little consistency in the investigations of melatonin and MCH in fish iridophores. Iga et al. (1990) and (Oshima et al., 1989) observed the LR (dispersed) response of iridophores after treatment with melatonin in the gobiid dark sleeper *O. obscura* and blue-green damselfish *Chromis viridis*, respectively. Similarly, Oshima et al. (1989) also observed the dispersal of reflecting platelets in response to MCH in *C. viridis*. However, MCH had no influence on iridophore motility in the blue damselfish *Chrysiptera cyanea* (Oshima et al., 1986), and neither melatonin nor MCH had any effect on reflecting platelet motility of the common surgeonfish *Paracanthurus hepatus* (Goda and Fujii, 1998). This inconsistency, just within teleost fish, illustrates that the effects of MCH, melatonin, and even MSH on iridophore reflecting platelet arrangement are currently indeterminate. Considering the variable effects of MCH, MSH, and melatonin upon the iridophores of different species, it is very likely that the

chromatophores of different species or taxa respond differently to various hormones because of inherent variation in hormone receptor types, locations, and abundances.

In stark contrast to the conflicting results concerning α -MSH, MCH, and melatonin, there is consistent agreement regarding the effects of catecholamine neurotransmitters upon fish iridophores. In multiple studies, norepinephrine was found to elicit the dispersive, LR response in the iridophores of teleosts by binding to α -adrenoceptors (Fujii, 2000), specifically in the following species: the dark sleeper (*Odontobutis obscura*, Fujii et al., 1991), the freshwater goby (*O. obscura*, Maeno and Iga, 1992), and the common surgeonfish (*Paracanthurus hepatus*, Goda and Fujii, 1998). Furthermore, Maeno and Iga (1992) determined that the freshwater goby iridophores only possess α -adrenoceptors and that α -adrenoceptor blockers effectively inhibited norepinephrine induced dispersion (Goda and Fujii, 1998).

As with melanophores and erythrophores, iridophores of several species also appear to dynamically respond to local environmental conditions. For example, the reflecting platelets of neon tetra *Paracheirodon innesi* respond to light by exhibiting the LR response (Lythgoe and Shand, 1982). Specifically, Lythgoe and Shand (1982) found that shining a white light on the lateral stripes of tetras caused an increase in the distance between adjacent reflecting platelets and an associated shift towards reflecting more long-wavelength light, even in decapitated fish. Subsequent investigation of neon tetra iridophores uncovered the presence of an opsin-based visual pigment located within the iridophores, suggesting a mechanism for the photoresponsiveness observed in these chromatophores (Lythgoe et al., 1984). In addition to light, the reflecting platelets in ornate tree lizard iridophores have been shown to respond to localized changes in temperature (Morrison et al., 1996). Specifically, heating the ventral, belly of skin of these lizards caused a reduction in the predominant wavelength of reflected light, causing an observed change in color from coppery/green to an intense blue (Morrison et al. 1996). Similar, temperature-dependent changes in structural coloration have been observed in the eastern fence lizard (*Sceloporus undulatus*, Langkilde and Boronow, 2012; Fig. 4). Though the specific mechanisms by which increases in temperature alter reflecting platelet spacing in these lizards are currently unknown, it has been postulated that these color changes serve as a signal of thermally-dependent performance capability which could be used during intrasexual contests (Langkilde and Boronow, 2012).

4.6 Summary

Evidently, there has been a plethora of research investigating the biochemical regulators of reflecting platelet translocation in fish iridophores, but relatively few investigations of reflecting platelet motility in amphibians or reptiles (with a notable recent exception; Teyssier et al., 2015). Additionally, the specific mechanisms of reflecting platelet translocation are well-understood in just a few species of fish. As in the previous discussions of erythrophores and melanophores, broad generalizations across taxa cannot be made with respect to hormones, neurotransmitters or other chemicals affecting reflecting platelet translocation. Even the abundant evidence describing fish iridophores is somewhat inconsistent and controversial at larger taxonomic levels, such that generalizations should be limited even within fish. An important exception to this rule is the effect of optical thickness on reflected wavelength (Land, 1972; Mäthger et al., 2003) because this relationship addresses physical properties of materials, which are constant despite the organism or cell in which they are found. Though there is potential for further research in many directions, there are three areas that would most clearly benefit from future study. The first would be determining the mechanism for platelet transport, especially given that there are two different kinds of motion, radial and lateral, which have been observed in various iridophores. Next, a more in-depth and consistent understanding of the effects of aggregation and dispersion agonists such as α -MSH, MCH, and melatonin as well as the roles of cAMP and Ca^{2+} as second messengers would allow clearer discourse when comparing

iridophores to other chromatophore types or when discussing the dermal chromatophore unit as a whole. Finally, much as with the erythrofore, there is a relative dearth of comparative information regarding iridophore motility in reptile and amphibian species capable of physiological color change.

5 Conclusions

Physiological color change is the result of dynamic pigment movement of multiple chromatophores and their interactions with each other within the dermal chromatophore unit. Regulated by hormones, neurotransmitters, and even environmental cues such as light and temperature, chromatophores undergo rapid pigment reorganization to produce a wide array of chromatic changes. Depending on the stimulus, different chromatophore types respond with either dispersion to maximize their relative contribution to the overall color produced, or aggregation to minimize their effect. Here, we have attempted to review the similarities and differences between melanophores, erythrofores, xanthophores, and iridophores, as well as how these different classes of chromatophores can rapidly confer specific colors to the dermis through a coordinated effort.

One important conclusion concerns the reciprocal relationship between iridophores and the other types of chromatophore. Iridophores, located within the basket-like projections of the melanophore, appear to frequently be dispersed to their maximum reflecting surface area when the overlying erythrofores are punctate and the melanophore processes are devoid of melanosomes. This relationship diminishes the colorful contribution of the melanophores and erythrofores and enhances the reflective capacity of the iridophores; this is the light or pale state. In the dark state, iridophores are aggregated and obscured by both the dispersed melanophores and erythrofores; therefore, color absorption is maximized and reflectivity diminished. The likelihood of a general, oppositional response for iridophores, coupled with the sparse literature regarding reptilian and amphibian iridophores, suggests that investigations of the iridophores of all the classes of vertebrates (and their intracellular transduction pathways) would greatly benefit the study of chromatophores and color change. There is also room for future inquiry into the physiological regulation of the erythrofores of reptile and amphibian species, as there is a similar lack of research of their physiological regulation compared to the abundance of information regarding fish erythrofores.

Although our primary focus in this paper was to review the current understanding of the cellular and biochemical processes underlying physiological color change, examining color change in an evolutionary context may yield new and exciting insights. Understanding how the ability to rapidly change color evolved, as well as how social and environmental factors might influence the evolution of color change (e.g. Stuart-Fox et al., 2007; Stuart-Fox and Moussalli, 2008), could offer key insights into certain mechanisms of chromatophore activity and function. Though we focused on chromatophore responses to particular neural or hormonal signals and excluded discussions of how or why chromatophores are stimulated, determining the reasons for organisms to undergo physiological color change is a crucial next step towards understanding the entire process of chromatophore control and function.

Stuart-Fox and Moussalli (2009) put forth an intriguing review of three of the most prominent functions of physiological color change: camouflage, communication, and thermoregulation. While color change is often used for background matching (Zoond and Eyre, 1934; Okelo, 1986; Vroonen et al., 2012; Stevens et al., 2014), animals with dynamic color change abilities could potentially employ multiple camouflage strategies in response to different predator types (Stuart-fox et al., 2008; Stuart-Fox and Moussalli, 2009). Such facultative, predator-specific crypsis was described by Stuart-Fox and Moussalli (2008) in Smith's dwarf chameleons *Bradypodion taeniabronchum*. Using model predators, these authors found that chameleons responded with better background matching for avian predators than for snakes, taking into account the

different visual systems of the predators. Whereas the objective of camouflage is to avoid detection or recognition by predators or prey, (Stevens and Merilaita, 2009), social selection frequently favors conspicuous coloration and rapidly changing color signals for use in intraspecific communication (Stuart-Fox and Moussalli, 2009). Animals capable of physiological color change have the benefit of communicating with transient, conspicuous color signals, but may experience reduced predation risk relative to species that display long-term, static coloration or patterns (Stuart-Fox and Moussalli, 2009). Therefore, rapid color change can facilitate courtship or be used to signal during aggressive interactions (e.g. Summers and Greenberg, 1994; O'Connor et al., 1999; Höglund et al., 2002; Ligon and McGraw, 2013; Ligon, 2014) without sacrificing the ability to remain inconspicuous after these brief intraspecific encounters (Stuart-Fox and Moussalli, 2009).

Color change can also serve in a homeostatic capacity by helping to regulate body temperature (Norris, 1967) and, for amphibians, possibly water balance (Stegen et al., 2004; Stuart-Fox and Moussalli, 2009). The vertebrates that undergo true physiological color change (i.e., through pigment translocation rather than increased or decreased blood flow) are typically ectothermic, and are thus dependent on external sources of heat for thermoregulation. Ectotherms can thermoregulate via behavior (e.g. shuttling between preferred habitats, Stevenson, 1985) or changes in physiology (Whitfield and Livezey, 1973), which suggests that another avenue of control over heat absorption may be critical to achieving higher levels of activity at lower temperatures or surviving climates with fluctuating temperatures (Silbiger and Munguia, 2008; Stuart-Fox and Moussalli, 2009). Darkening the skin increases the amount of light (and heat) absorbed, whereas a lightening of the skin increases reflected light and less heat will be absorbed (Norris, 1967; Sherbrooke, 1988; Walton and Bennett, 1993; Stuart-Fox and Moussalli, 2009). For amphibians like frogs, whose skin is freely permeable to water, increases in solar absorbance can also increase the rate of water loss (Stegen et al., 2004). Therefore, the ability to color change in frogs could not only allow them to regulate their body temperature to some degree, but also provide a measure of control over water balance (Stegen et al., 2004).

Though some animals may rely upon all of the aforementioned functions of physiological color change, given the markedly different environments typically inhabited by amphibians, reptiles, and fish, there is undoubtedly competition between the different faculties of color change and each species has likely evolved strategies to address and resolve color change unique to their selective pressures (Stuart-Fox and Moussalli 2009). While the origin of physiological color change in a distant vertebrate ancestor may be difficult to determine, the natural selection for camouflage to avoid predation and sexual selection for signaling capability are possibly the two most important factors in the evolution of color change (Stuart-Fox and Moussalli, 2009). Thermoregulation was probably influential for species whose environments demand it; however, compared to camouflage and communication, homeostatic regulation has only recently begun to be considered as a function of physiological color change (Stegen et al., 2004; Stuart-Fox and Moussalli, 2009). Therefore, the relative strength of selection exerted by thermoregulatory functions is even more speculative than others. Overall, the extent and specificity of color change with respect to signaling, camouflage, and homeostatic regulation, as well as the interactions and trade-offs between these three functions are currently unknown.

In this review, the physiological control of pigment translocation in the chromatophores and the effects upon the skin color of vertebrates capable of physiological color change were evaluated. In addition to the potential for expanding our knowledge of the regulatory biochemistry of the chromatophores of reptiles, amphibians, and fish, there exists a plethora of new, intriguing research directions related to physiological color change. The questions which remain to be answered involve fully understanding the mechanisms behind pigment organelle locomotion; the evolutionary pathways of color

change; determining the importance of and interactions between the various functions of color change; and whether there are previously unknown or unexamined receptors, stimuli, or biochemical pathways that affect chromatophores and physiological color change.

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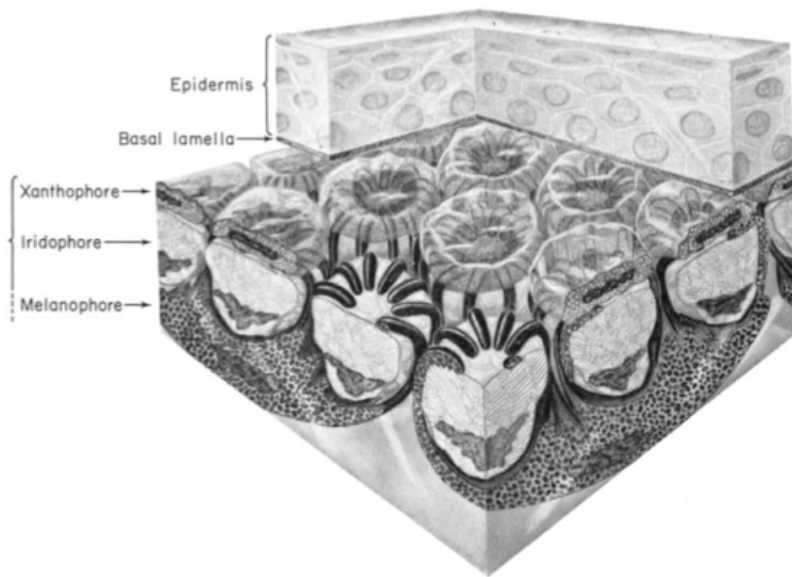


Fig. 1 Schematic representation of the dermal chromatophore unit, shown in the dispersed state

The dendritic processes of the basal melanophores, extending around and over the iridophores layer, are filled with melanosomes. Above the iridophores and the melanophore processes is the xanthophore (or erythrophore layer). Figure reproduced from Bagnara et al. (1968) under a Creative Commons License.

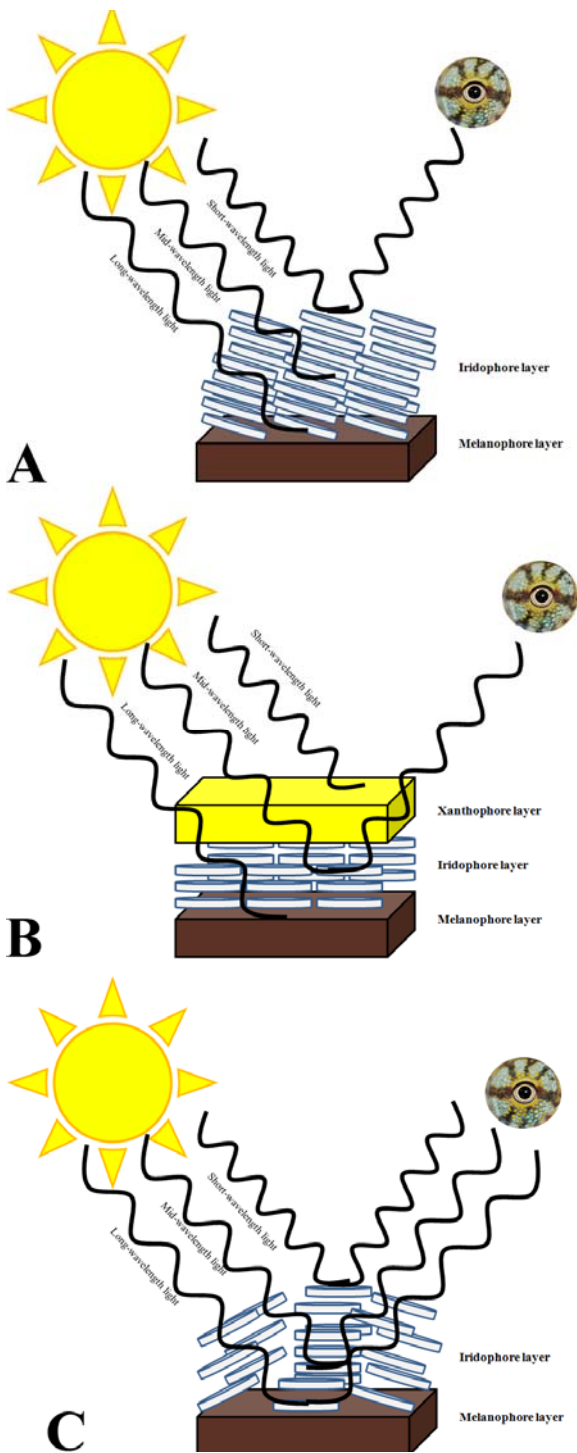


Fig. 2 Schematic representation of the potential contributions of the different elements of the dermal chromatophore unit to reflected light (i.e. color and brightness). Light from an external source (e.g. the sun) interacts with the dermal chromatophore, which influences the wavelengths of light transmitted from the body (and which become visible to external viewers).

A. Blue color can be attained when reflecting platelets in the iridophore layer selectively reflect short wavelengths of light and longer wavelengths of light are absorbed by the melanin-containing melanophore layer. **B.** Green or yellowish-green is reflected when the xanthophore layer absorbs short-wavelengths of light, the platelets in the iridophore layer reflect medium wavelengths of light, and the melanophore absorbs longer wavelengths of light. **C.** White light is reflected when iridophore platelets reflect or scatter all incoming wavelengths of light. Figure after Bagnara & Hadley 1973.

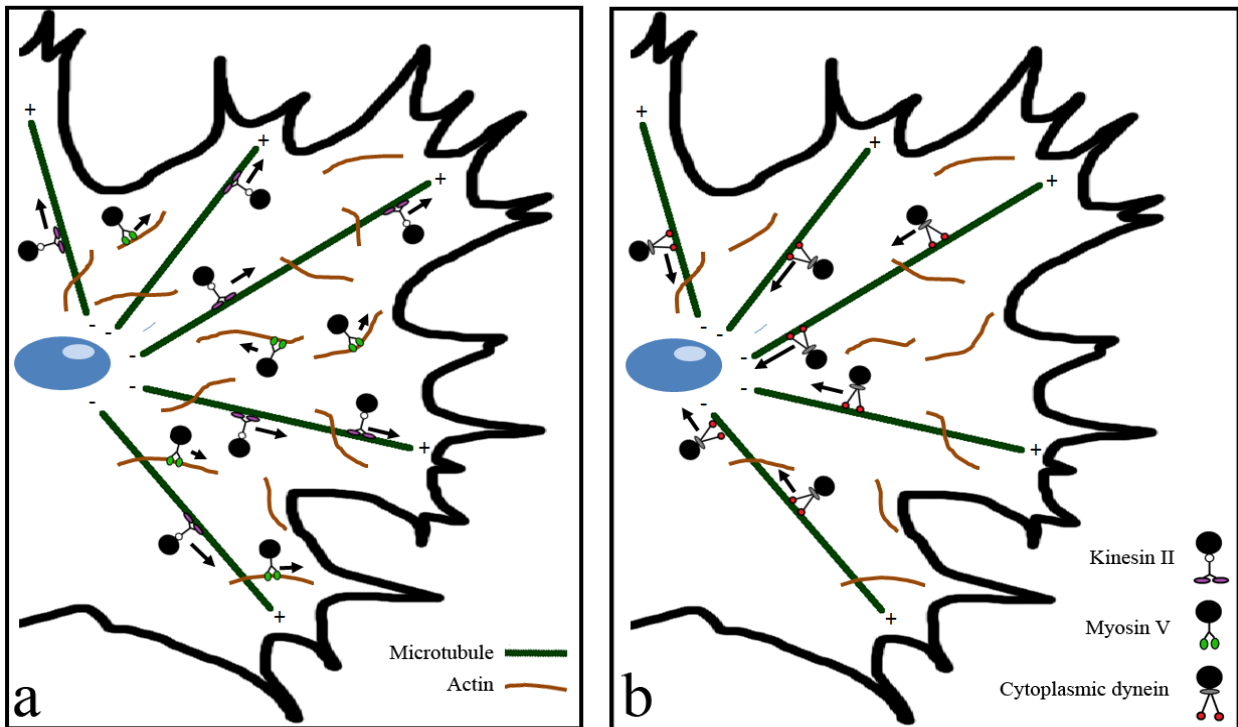


Fig. 3 Model of melanosome transport during dispersion and aggregation, redrawn from Tuma & Gelfand (1999)

A. Long-range melanosome dispersion occurs via transport by kinesin along microtubules. After reaching the cellular periphery, melanosomes are transported along randomly oriented actin filaments by myosin motor proteins. B. Melanosome aggregation occurs via microtubules but, in contrast to pigmentary dispersion, takes place with the aid of dynein molecular motors.

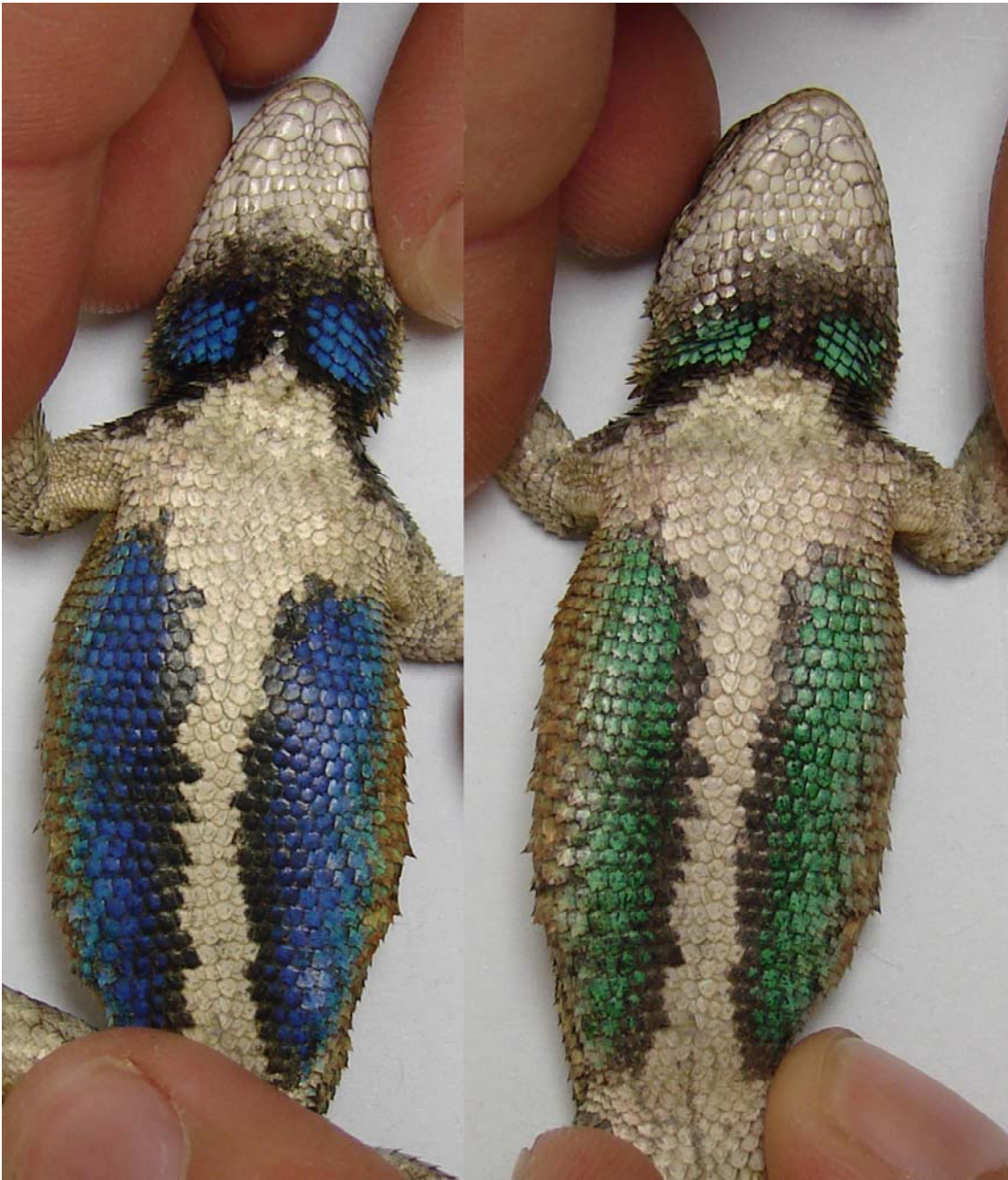


Fig. 4 Photographs of an individual male eastern fence lizard *Sceloporus undulates* exhibiting (A) blue coloration at warm temperature (28.8°C) and (B) green coloration at cool temperature (24.6°C)

Photographs courtesy of Tracy Langkilde.