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Integumental Chromatophores of a Color-Change, Thermoregulating Lizard, *Phrynosoma modestum* (Iguanidae; Reptilia)

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ABSTRACT

A horned lizard inhabiting the Chihuahuan Desert has a dermal chromatophore architecture (arrangement) significantly different from that of the only other lizard, *Anolis carolinensis,* whose dermal chromatophore unit was previously described. In *Phrynosoma modestum,* only two cell types (rather than three) are involved in physiological color change. One type, iridophores, are organized into a thick layer overlying and engulfing the second type, melanophores, which have processes that penetrate through the iridophore layer to the outer surface of the dermis. Iridophore reflecting platelets lack an organized layered arrangement, reflecting white light rather than colors produced by interference phenomena. These two cell types are the major effectors of thermoregulatory color change.

Xanthophores and erythrophores, uninvolved in physiological color change for the most part, are both widely scattered at low densities and aggregated into elaborate patterns, thus contributing to background color matching and camouflage. The chromatophore arrangement in *P. modestum* may be typical of desert lizards that utilize physiological color change mainly for thermoregulation.

Other findings of interest include (1) the observation of mosaic chromatophores, wherein a single cell contains organelles representative of three chromatophore types; (2) the unreported ontogenetic sequence of appearance of melanophores, followed by iridophores, and lastly by xanthophores, in embryonic *P. modestum*; and (3) electron dense material organized in concentric lamellae of the pterinosomes.

INTRODUCTION

Chromatophores of poikilotherm vertebrates have captured the attention of biologists for many years. Interest in these cells has focused on developmental origin, classification of cell types by structure, types of organelles (development, chemistry, and reflective properties), architectural arrangements of cells within the epidermis and dermis, hormonal responses and receptors, neural innervations and receptors, and role

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in morphological and physiological color change (Parker, 1938, 1948; Fingerman, 1963; Waring, 1963; Taylor and Bagnara, 1972; Bagnara and Hadley, 1973).

Among reptiles, saurians exhibit the most dramatic physiological color change. Three genera of lizards have received considerable attention from investigators interested in the mechanisms of such change: *Chamaeleo, Anolis,* and *Phrynosoma* (Parker, 1938, 1948; Fingerman, 1963; Waring, 1963; Taylor and Hadley, 1970; Bagnara and Hadley, 1973). Previous studies on color change in horned lizards were done many years ago (Parker, 1906, 1938, 1948; Redfield, 1916, 1918) and were not accompanied by detailed examination of the architectural arrangement of integumental chromatophores. Our current knowledge of lizard skin chromatophores is based almost entirely on a single species, *Anolis carolinensis* (von Geldern, 1921; Forsdahl, 1959; Alexander and Fahrenbach, 1969; Taylor and Hadley, 1970; Bagnara and Hadley, 1973).

In this paper we consider the architectural arrangement of the dermal chromatophores of *Phrynosoma modestum,* their color-generating organelles, mosaic chromatophores, and the sequential appearance of the various pigment cell types during embryonic development. A model of the integumental chromatophore architecture for desert lizards that use color change mainly for thermoregulation is proposed and compared to the dermal chromatophore unit of *A. carolinensis* (Taylor and Hadley, 1970) and to that of anurans (Bagnara et al., 1968).

MATERIALS AND METHODS

Adult specimens of *P. modestum* were collected near Portal, Cochise Co., Arizona, in 1983 and 1984. Lizards were maintained in captivity (Sherbrooke, 1987) until sacrificed for skin samples from various locations on the dorsal surface of the abdomen. Individuals were of various colors; this population is polymorphic (Sherbrooke, 1981).

Eggs laid by gravid females were incubated in vermiculite (Zweifel, 1961; Sherbrooke, 1987). At various times during development, the integumental surface of single embryos was examined under a dissecting microscope for evidence of developing pigment cells.

The outer surface of skin on the dorsal abdomen of living lizards, excised pieces of skin in physiological saline, and skin whole mounts (in Karo syrup) were examined and photographed under various magnifications of a dissecting microscope. In a few cases, the epidermis was removed, using a solution of 2 M NaBr, in order to more clearly expose the surface of the underlying pigment cells of the upper dermis and to examine the epidermal chromatophore pattern.

Fixation and electron microscopic examination followed the procedures of Frost and Robinson (1984). Skin samples were fixed in 2.5 percent glutaraldehyde in 0.2 M cacodylate buffer (pH 7.3) for 12 hours at 4–6°C. Samples were postfixed in 2 percent osmium tetroxide for 1.5–2.0 hours, rinsed, and stored in 0.2 M cacodylate buffer. These skin samples were then dehydrated in a graded ethanol series. Skin was embedded in Epon and sections were cut with a diamond knife on a Sorvall MT-1 ultramicrotome. Sections were collected on Formvar-coated and carbon-stabilized grids, stained with uranyl acetate-lead citrate, and viewed in a Philips 300 transmission electron microscope. Several skin samples were placed in 1.6×10^{-8} M melanocyte-stimulating hormone (α-MSH), a concentration that is physiologically effective for color change (Sherbrooke, 1988), for 60 minutes prior to fixation.

RESULTS

Ontogenetic Appearance of Chromatophore Types

Eggs from three clutches laid by different *P. modestum* females were periodically opened and the embryos were examined for evidence of chromatophores. The types of pigment cells observed were recorded. In all cases, melanophores developed first, by the 26th, 27th, and 31st day of incubation of each clutch. In all clutches iridophores were the second chromatophore type to develop, by the 26th, 34th, and 44th day of incubation. In the first clutch, melanophores and iridophores were first noted on the same day (26th), but the former were well established and the

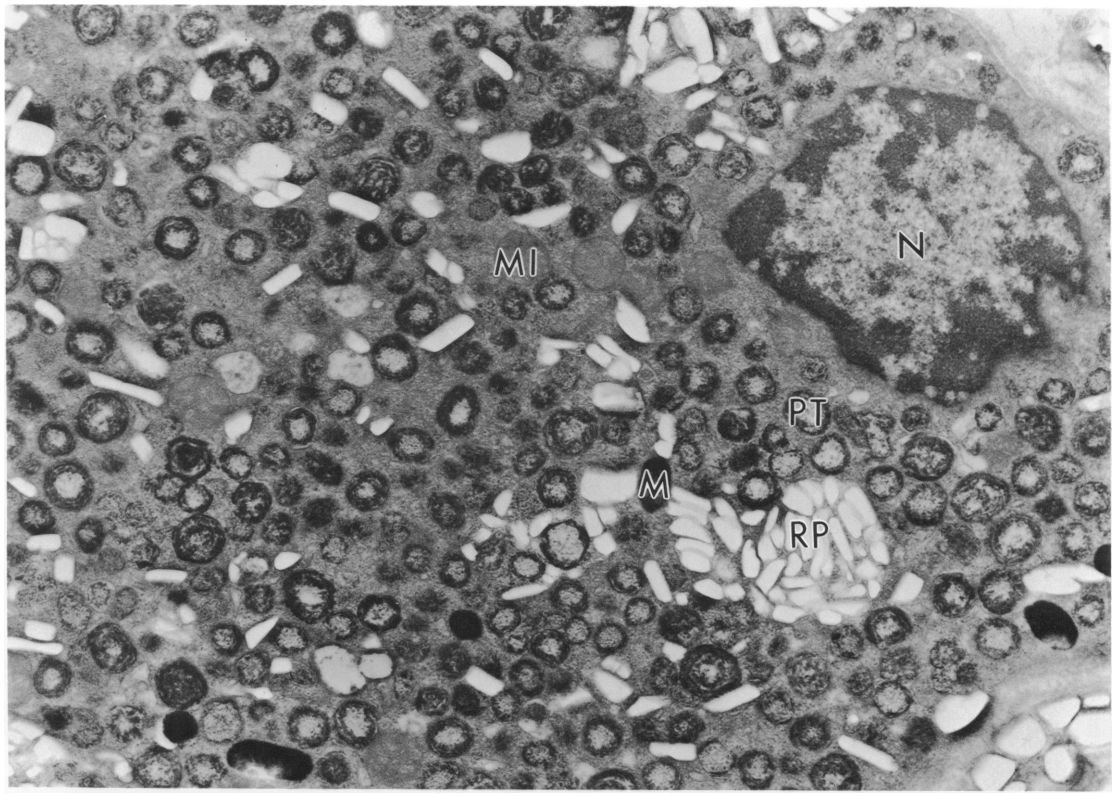

Fig. 1. Transmission electron micrograph of a mosaic dermal chromatophore from *Phrynosoma modestum* dorsal integument treated with 1.6×10^{-8} M α-MSH for 60 min prior to fixation. To the left of the nucleus (N), the cell contains three types of color-producing organelles, pterinosomes (PT), reflecting platelets (RP), and a few melanosomes (M). Interspersed throughout the cytoplasm are mitochondria (MI). ×12,240.

latter were just beginning to appear. Iridophores developed in groups, forming white "islands" on the skin. In the first two clutches, xanthophores developed last, by the 40th and 47th days. In the third clutch, xanthophores had not appeared by the time the last egg was opened on the 50th day.

Mosaic (Polychromatic) Cells

A few mosaic or polychromatic cells were observed. These cells contain multiple types of color-generating organelles, each of which is normally restricted to a specific type of chromatophore. One such mosaic cell contained organelles characteristic of all three types of dermal chromatophores—melanophores, iridophores, and xanthophores. In this cell pterinosomes were most numerous, followed by reflecting platelets, and melanosomes were least abundant (fig. 1). Mosaic cells were more frequently observed in α-MSH-treated skin samples.

Melanophores

The cell bodies of dermal melanophores lie deep within the dermis (figs. 2, 3). These cell bodies may be surrounded both above and below by iridophores that can be identified under polarized light (figs. 2, 3). Elongate processes extend through the overlying iridophore layer to positions below the epidermis (figs. 2, 4A) where they extend laterally along the upper level of the dermis (figs. 2A, 4B). During skin darkening, melanin-containing melanosomes move into the processes, thus positioning black pigment granules above

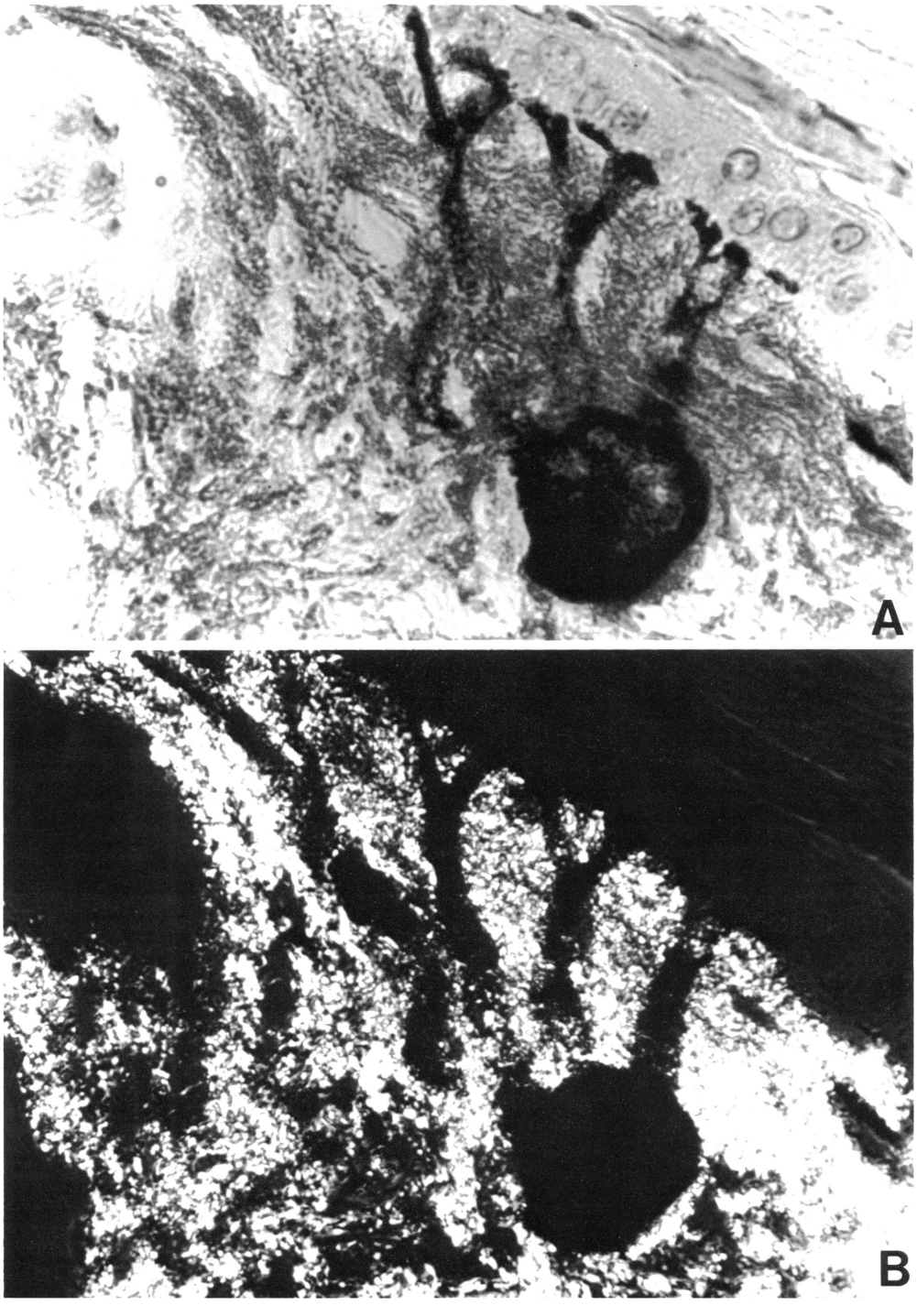

Fig. 2. Light micrographs of *Phrynosoma modestum* dermis during skin darkening. **A.** Dermal melanophore of *P. modestum*. Note that melanosomes occupy the cell processes that extend from the deep perinuclear portion of the cell to the surface of the dermis. **B.** Dermal iridophore (reflecting platelet) zone of cells appears as bright areas under polarized light: same view as fig. 2A. The iridophore layer extends from below the melanophores to the surface of the dermis.

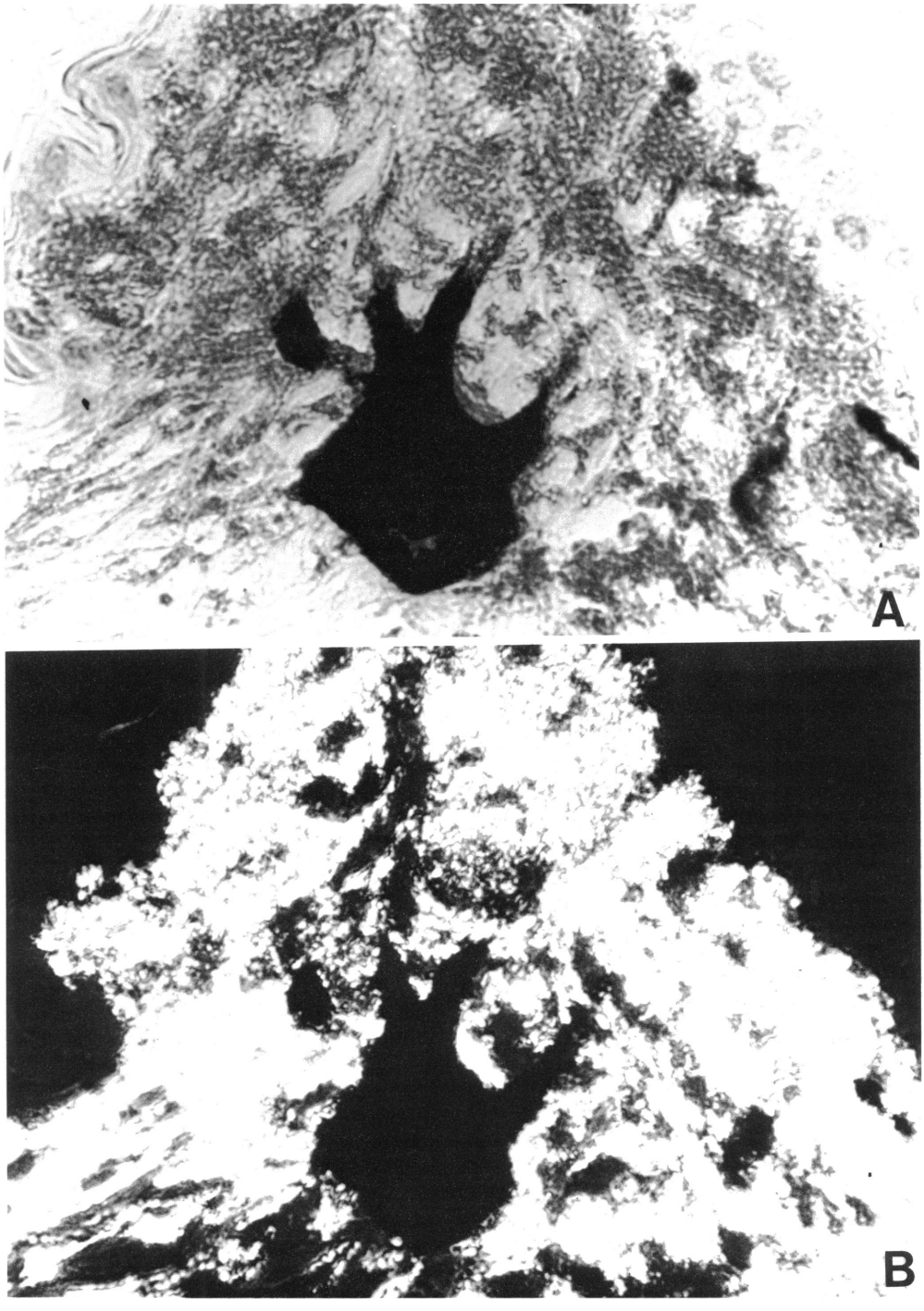

Fig. 3. Light micrographs of *Phrynosoma modestum* dermis during skin lightening. **A.** Dermal melanophore of *P. modestum.* Note that melanosomes are withdrawn from the cell process and are concentrated in the deep perinuclear portion of the cell. **B.** Dermal iridophore (reflecting platelet) zone of cells appears as bright areas under polarized light: same view as fig. 3A. The iridophore layer extends from below the melanophores to the surface of the dermis.

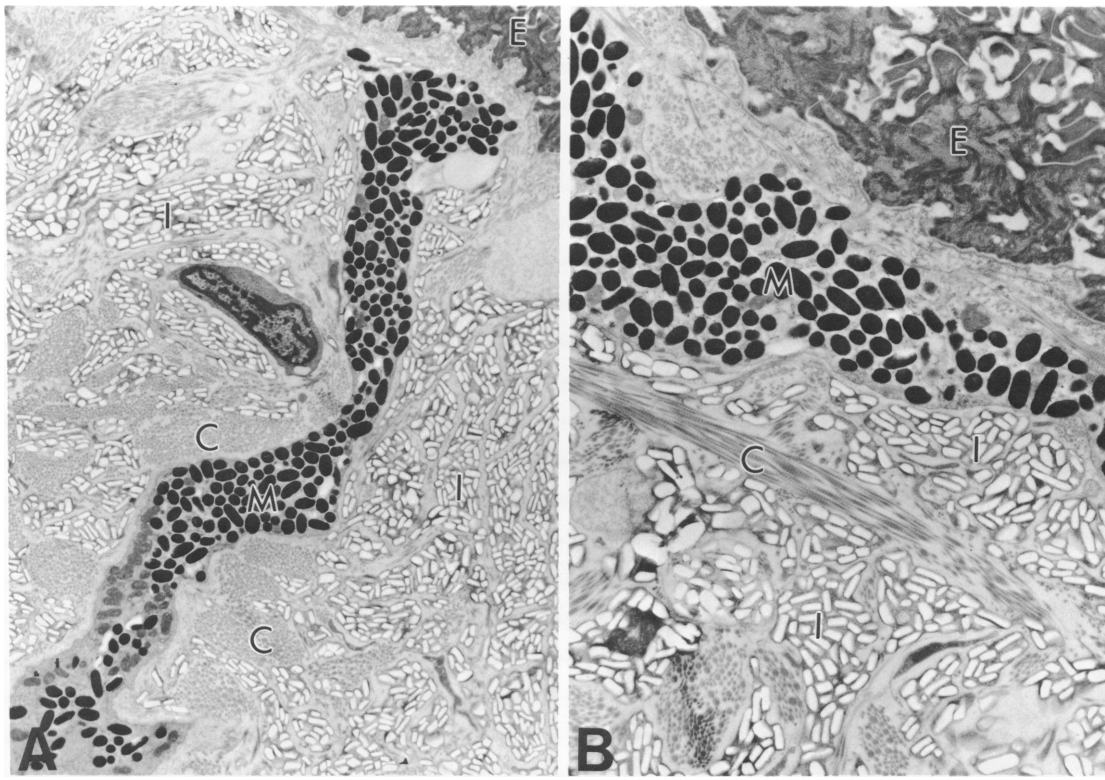

Fig. 4. Transmission electron micrographs of melanophore processes in the dermis. **A.** Melanophore (M) process extending up through iridophores (I) and collagen bundles (C) to the upper edge of the dermal-epidermal (E) border. ×3630. **B.** Melanophore (M) process extending along the upper edge of the dermis, above the iridophores (I) and collagen bundles (C), and below the epidermis (E). ×6160.

iridophores (fig. 2). When this happens there is a reduction of melanosome numbers in the perinuclear portion of the melanophore. When the integument lightens in color, melanosomes have been withdrawn from the melanophore processes and are concentrated in the perinuclear area (fig. 3).

Iridophores

Clearly, these are the dominant chromatophores of the dorsal (as well as ventral) skin, occurring in abundance from just below the basement membrane to the underlying connective tissue layer (figs. 2, 3, 4A). Iridophore reflecting platelets are purported to contain crystalline guanine as a pigment (Bagnara et al., 1988). Although the guanine content of the reflecting platelets is lost during tissue preparation, the size, form, and orientation of the platelets are retained by virtue of fixation of the limiting membrane of these organelles (fig. 4). Generally, platelets are rectangular, oblong, or ovoid in shape. Some platelets are observed to be arranged end-to-end in lines, and lines of platelets may occur in several layers (fig. 5). Although this suggests a degree of structural ordering between adjacent organelles, there appears to be no overall organized arrangement of platelets within iridophores. Likewise, iridophores themselves are scattered throughout the dermis and are thus not regularly arranged with respect to one another (figs. 4, 5).

Xanthophores

Xanthophores lie at the uppermost level of the dermis, above even the outer iridophores and somewhat interspersed with them (fig. 6). Thus, they are found just below the epidermal/dermal boundary. Internally, xan-

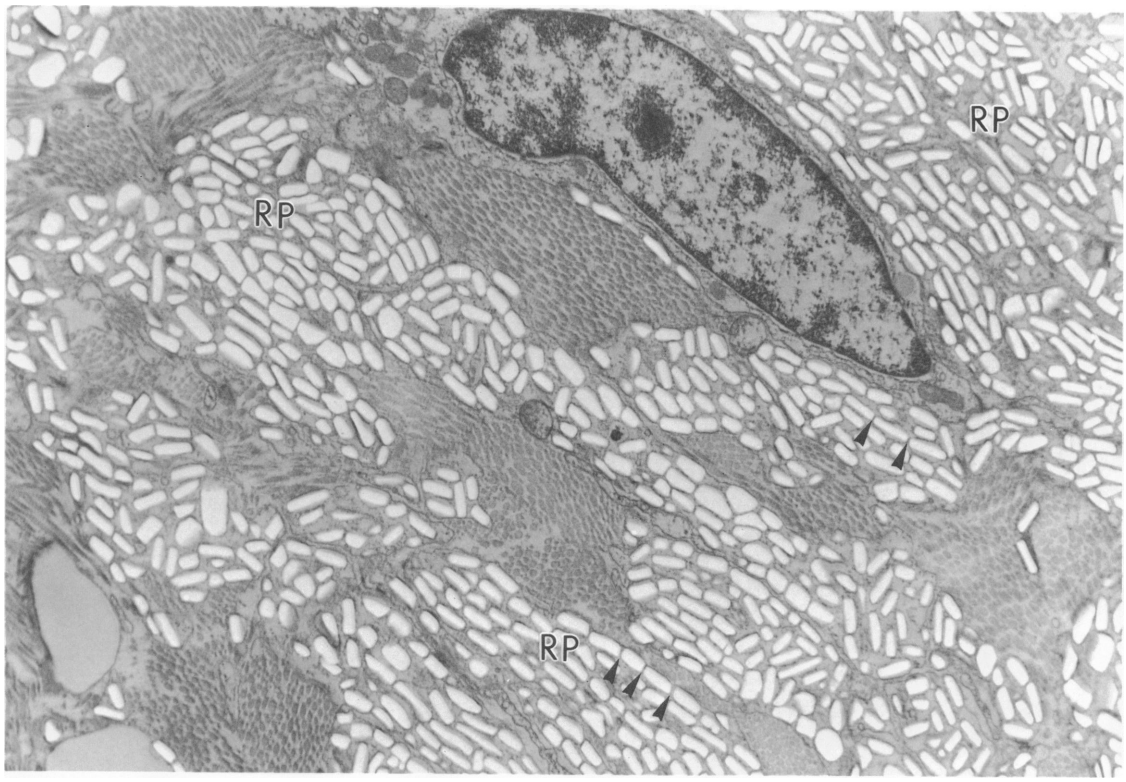

Fig. 5. Transmission electron micrograph of the extensive "network" of iridophore processes in *Phrynosoma modestum* dorsal skin. Note the end-to-end alignment (arrows) of many of the reflecting platelets (RP) and the occasional stacking of end-to-end aligned platelets. ×9900.

thophores contain primarily pterinosomes, and occasionally carotenoid vesicles, both of which are characteristic organelles of xanthophores. Pterinosomes of the individuals illustrated in figure 6 are unusually dense, which may be indicative of the biochemical composition of the pigments within the organelles (see Discussion).

Examination of the skin surface under a dissecting microscope showed that xanthophore distribution varies greatly over the dorsum. In most areas of dorsal skin these cells are widely spaced; however in areas that are distinctly patterned, xanthophores are present in much higher densities. Often, xanthophores have processes that extend out to cover the surface of the iridophore layer.

Collagen Fibers

Bundles of collagen fibers are abundant in the dermis; this correlates well with the thick elastic qualities of the skin. The fibers occur immediately below the basement membrane and are also interspersed among the dermal chromatophores (figs. 4–6). When sectioned longitudinally, their banding is apparent, whereas in cross section, they appear as groupings of solid, roundish structures lacking cellular membranes (figs. 4–6).

DISCUSSION

Bagnara and Hadley (1973) have standardized the terminology associated with vertebrate chromatophore types and their color-generating organelles: (1) epidermal melanophores (cytes) contain melanosomes; (2) dermal melanophores also contain melanosomes; (3) iridophores contain reflecting platelets; and (4) xanthophores or erythrophores contain pterinosomes and/or carotenoid vesicles and, as a result, are brightly colored (yellow, red, orange).

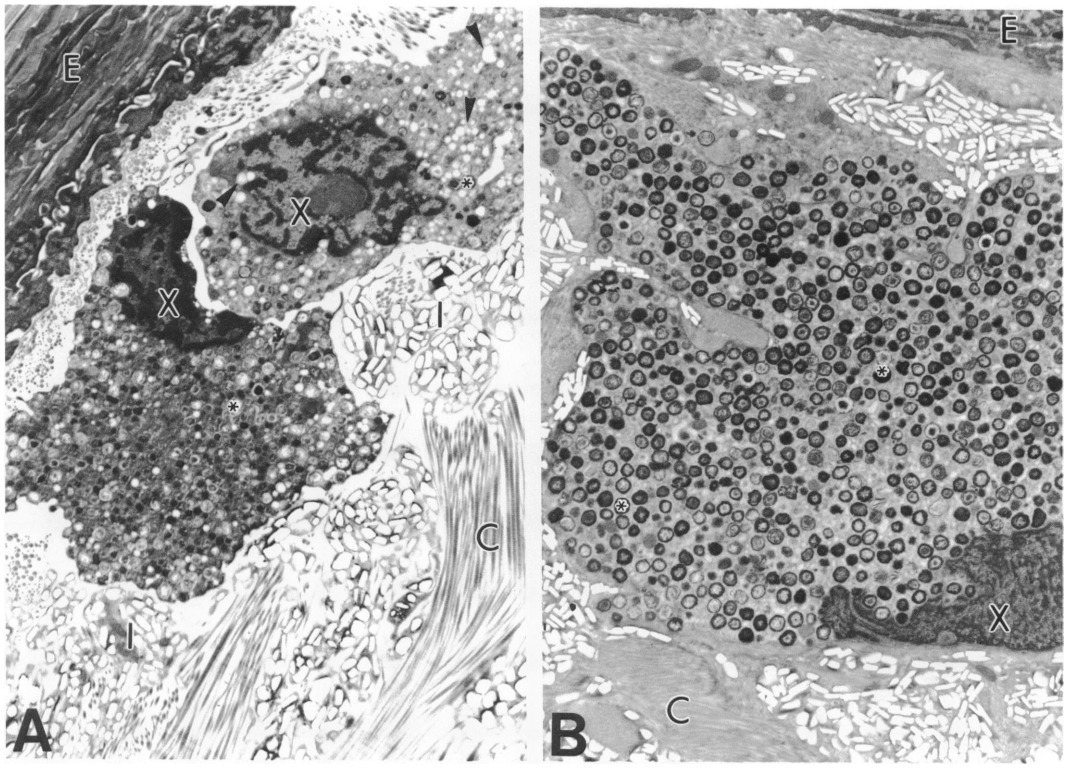

Fig. 6. Transmission electron micrographs of xanthophores from *Phrynosoma modestum* dorsal skin. **A.** Xanthophore (X) closely apposed to the basement membrane of the epidermis (E) with iridophore (I) processes and collagen (C) fibers below. Within the cytoplasm of these xanthophores are numerous electron-dense pterinosomes (*) and carotenoid vesicles (arrows) as well as prominent nuclei (the X denoting "xanthophore" is within the nucleus). ×3960. **B.** Another example of a xanthophore illustrating the electron-dense, concentrically organized fibrous material (presumably pteridine pigment) within the pterinosomes (PT). ×3630.

Ontogenetic Appearance of Chromatophore Types

It has been suggested that the ontogenetic appearance of chromatophore types in poikilotherm vertebrates occurs in a definite sequence. In amphibians, dermal melanophores occur first, followed by xanthophores, and then iridophores (Collins, 1961; Bagnara and Hadley, 1973; Frost et al., 1984). In the three clutches of *P. modestum* eggs examined herein, a different sequence of chromatophore ontogeny was observed with melanophores appearing first, followed by iridophores, and lastly by xanthophores.

The shell enclosed nature of the reptilian egg makes it likely that such a sequence simply has not been observed before. Moreover, because of the small number of animals examined herein, we suggest that our findings are preliminary and need to be subjected to further verification by histological study of chromatophore appearance not only in *P. modestum,* but in other groups of reptiles as well. Further observations may provide insight into the development and differentiation of chromatophore types and their respective color-generating organelles.

Mosaic (Polychromatic) Cells

All integumental chromatophores are derived from neural crest cells that migrate from the developing neural tube to locations throughout the embryo during development (DuShane, 1935; Bagnara and Hadley, 1973; LeDouarin, 1982, 1984; Bagnara, 1987). Because of this common embryonic origin, the

idea that pigment cell types all form from a common "chromatoblast" has received much support (Bagnara et al., 1979a, 1979b; Bagnara, 1981, 1983). The "signal" to differentiate into a particular pigment cell type is, at present, not well understood, but the existence of mosaic chromatophores and the observation that chromatophore types can interconvert in vitro (Ide, 1978) suggest that there is plasticity in the differentiative capabilities of these cell types.

Mosaic pigment cells have been observed in a variety of vertebrates, including reptiles (Bagnara and Taylor, 1970; Bagnara and Ferris, 1971; Bagnara, 1972; Taylor and Bagnara, 1972; Ferris and Bagnara, 1972; Bagnara et al., 1978a, 1978b, 1979a, 1979b; Frost and Malacinski, 1980; Bagnara, 1981, 1983). The application of α-MSH to some skins previous to fixation appears to increase the incidence of mosaic cells. Taylor (1969) reported melanization of amphibian iridophores in response to intermedin. The significance of mosaic chromatophores in the dermis of *Phrynosoma* is unclear. It may reflect an artifact produced by hormonal stimulus or this may be a bona fide "cell in transition."

Pterinosome Ultrastructure

It is also significant that pterinosomes in the xanthophores of *P. modestum* all contain moderate to heavy amounts of fibrous, electron-dense material organized in concentric lamellae. The electron-dense fibrous material has been observed by numerous investigators and is justifiably assumed to reflect the presence of pteridine pigments. Frost et al. (1984, 1986) demonstrated (in axolotls) that the more brightly colored an animal was (in this case a golden albino axolotl), the more densely pigmented were its pterinosomes. Moreover, axolotls with enhanced yellow background pigmentation due to guanosine treatment have pterinosomes with significantly more electron-dense pigment than normal (Frost et al., 1987). In both cases, the enhanced yellow coloration of these axolotls was due to the presence of the yellow pteridine pigment, sepriapterin.

The appearance of the pterinosomes in *P. modestum* (see fig. 6) suggests a similar phenomenon. The intense electron density of these organelles, together with the yellow, red, and/or pink color of the animals themselves, suggests the presence of sepriapterin and/or drosopterins (red pteridine pigments) in the integument. Whether this speculation is accurate awaits further biochemical testing.

Architectural Arrangement of Integumental Chromatophores

The integumental architecture of color-changing poikilotherm vertebrates is characterized by two complexes of chromatophores, one epidermal and one dermal. The vertebrate epidermal melanin unit consists of melanin-synthesizing melanophores and adjacent, associated Malpighian cells that are receptor cells for melanin elaborated in the epidermal melanophores (Hadley and Quevedo, 1966). In *P. modestum,* epidermal melanophores (cytes), with typical elliptical melanosomes, occur within the α- and β-keratin layers of the outer epidermis (Sherbrooke, 1988), where they play a supplementary role in pattern formation, but no role in physiological color change. A few melanophores (apparently dermal) appear to have processes that extend into epidermal portions of mechanoreceptors (Sherbrooke, 1988).

Rapid color changes are reportedly effected by the dermal chromatophore unit (Bagnara et al., 1968). During skin darkening this involves intracellular transport (Schliwa and Euteneuer, 1983) of melanosomes from the melanophore cell body deep in the dermis into melanophore processes that extend upward toward the surface of the dermis. Here the melanosomes come to lie between an upper layer of xanthophores and an underlying layer of iridophores (anurans), or melanophore processes may overlap both layers (*Anolis*) (Taylor and Hadley, 1970; Bagnara and Hadley, 1973). Iridophores and xanthophores may also exhibit changes in organelle distribution (Bagnara, 1969; Bagnara and Hadley, 1969, 1973; Bagnara et al., 1969). Chromatophore cell membrane receptors respond to melanotropic peptides (Hadley, 1984; papers in Hadley, 1988; Sherbrooke, 1988), catecholamines of the autonomic nervous system (Nilsson, 1983; Hadley, 1984;

Sherbrooke, 1988), and other hormones (Bagnara and Hadley, 1973).

The architectural arrangement of dermal chromatophores of *Phrynosoma* has not been studied previously. Bagnara et al. (1968) based their concept and description of the dermal chromatophore unit on studies of amphibians. Von Geldern (1921) described the chromatophore structure and arrangement of cells in the lizard *A. carolinensis,* as have later investigators (Alexander and Fahrenbach, 1969; Taylor and Hadley, 1970). Taylor and Hadley (1970) schematically interpreted the dermal chromatophore unit of *A. carolinensis,* and thus postulated wider taxonomic applicability of the concept of a multicellular chromatophore unit, consisting of three cell type layers, to color-changing poikilotherms (Bagnara and Hadley, 1973). In two snakes lacking the ability for physiological color change, *Natrix natrix* and *Vipera ammodytes,* this arrangement of chromatophores into three cell-type units is absent (Miscalencu and Ionescu, 1972, 1973).

Striking color differences are obvious between *A. carolinensis* and *P. modestum. Anolis carolinensis* is uniformly green, changing to uniform brown during darkening. Dorsal skin of *Phrynosoma modestum* is darkly patterned on a pale background (Sherbrooke, 1988; Sherbrooke and Montanucci, 1988). The dorsal pattern may contain a variety of yellow, red, pink, or other colors (Sherbrooke, 1981, 1988; Sherbrooke and Montanucci, 1988). *Phrynosoma modestum* has been called the bleached horned lizard (Sherbrooke, 1981) because of its ability to turn nearly white over most of its dorsum.

Surface examination of living skin or whole mounts shows the overwhelming predominance of white light reflected from the scales. Clearly scattered over this background are black processes of melanophores, and yellow or red xanthophores that may have lateral extending processes. Brightly colored chromatophores vary greatly in density from one scale to another; many scales have only a few isolated bright-colored cells. Melanophore processes are more numerous on the surface of the white iridophore layer in regions that are more darkly patterned; likewise, brightly colored chromatophores increase in number in areas of colored pattern. Xanthophores and melanophores are often observed to be intermingled in pattern-forming areas.

Thermoregulation and Chromatophore Arrangement

Studies on integumental chromatophore architecture in reptilian species that undergo physiological color change have focused on *Anolis carolinensis.* This species utilizes chromic adaptation mainly for cryptic background matching that requires the attainment of green coloration to match surrounding foliage (von Geldern, 1921; Alexander and Fahrenbach, 1969; Taylor and Hadley, 1970).

Color change in *P. modestum* appears to be largely associated with thermoregulation, not background color matching (Sherbrooke, 1988). Darkening and lightening of the skin are due to translocation of melanosomes within dermal melanophores. During skin lightening melanosomes vacate melanophore processes lying on the surface of the dermis and move into deeper-lying portions of the cell; this is reversed during skin darkening. Possible movement of organelles within other types of chromatophores, known in some other vertebrates (Bagnara, 1969; Bagnara et al., 1969; Bagnara and Hadley, 1969, 1973) but not in *A. carolinensis* (Taylor and Hadley, 1970), was not studied.

The white color of *P. modestum* scales over much of the lizard's integument is attributable to the thick layers of dermal iridophores that extend upward nearly to the basement membrane of the epidermis. The organization of reflecting platelets in *P. modestum* contrasts sharply with that found in *A. carolinensis* iridophores, where apparently the crystal arrangement and spacing are critical for the production of blue-green color by thin-film interference (Land, 1972; Rohrlich and Porter, 1972; Frost and Robinson, 1984). In *A. carolinensis,* the platelets are highly organized in rows and layers, whereas in *P. modestum,* they approach a random arrangement. This near random arrangement of platelets may be responsible for the near total reflectance of white light (Rohrlich and Porter, 1972; Menter et al., 1979). Kleese (1981) found (in snake skin) that species with layered iridophores have a higher dorsal integument reflectance than do species with scattered

iridophores. Thus the thick iridophore layer of cells in *P. modestum* presumably serves an important function in a lizard utilizing color change for thermoregulation. When not covered by overlying melanosomes, iridophores reduce heat gain by reflecting visible-spectrum radiation.

Thus, the functional dermal chromatophore unit in *P. modestum* is clearly distinct from that found in *A. carolinensis,* although the basic proximal-to-distal relationship of cell types is the same. In order to achieve green camouflage coloration, *A. carolinensis* utilizes the combined light of two cell types, yellow reflected light from xanthophores and blue-green refracted light from the thin-film interference system of iridophore organelles. Color change darkening to brown involves movement of melanosomes to positions lying above the xanthophores and the iridophores. In *P. modestum,* there are basically only two chromatophores involved in color change. The iridophores reflect out all wavelengths of visible light and play no part in mixing wavelengths with light reflected off the layer of overlying xanthophores to form a cryptic color. A similar difference in chromatophore architecture was found by Bagnara et al. (1968) between green/brown color-change frogs (*Hyla cinerea* and *Agalychnis dachnicolor*) and a nongreen color-change frog (*Hyla arenicolor*).

Thermoregulation-Crypticity Compromise

In *P. modestum,* the xanthophores and erythrophores do play an important role in crypticity, a second consideration in dorsal coloration. Their distribution over the animal's back creates patterns useful for camouflage (Cott, 1940; Sherbrooke, 1988; Sherbrooke and Montanucci, 1988) and for blending into the colors of the surrounding terrain (Norris and Lowe, 1964). The melanophores function in relation to the iridophores as the regulators of skin darkening and lightening. In effect, these two cell types function as a dermal chromatophore unit for changing the radiation balance of the skin, thus facilitating thermoregulation. Iridophores containing reflecting platelets, whose arrangement produces reflectance of most wavelengths of visible light, in combination with melanophores that extend from deep within the iridophore layer onto its surface, may be characteristic of desert and other saurian species utilizing color change for thermoregulation. Such physiological considerations for color changes may be compromised by background-matching considerations leading to an adaptive compromise in the coloration of a lizard (Norris and Lowe, 1964; Norris, 1967).

This compromise can be visualized in the various components of chromatophore architecture. Considerations for the role of integumental pigment cells in influencing thermoregulation are addressed through two interacting cell types: the iridophore/melanophore cell complex. Needs of an animal for crypticity are addressed by the distribution and density (color and pattern formation) of various static chromatophores located above the iridophore layer—xanthophores and melanophores in this case. Thus, the structure of the entire chromatophore complex graphically illustrates the adaptive compromises associated with these two roles of coloration.

Striking similarities and differences can be seen by comparing the dermal chromatophore architecture of anurans (Bagnara et al., 1968), *A. carolinensis* (Taylor and Hadley, 1970), and *P. modestum.* Taylor and Hadley (1970) have discussed the difference between the dermal chromatophore unit of anurans and *A. carolinensis. Phrynosoma modestum* is similar to *A. carolinensis* in its overall arrangement of the three chromatophore cell types, surface xanthophores over iridophores, which are underlain by melanophores having processes that extend to the iridophore upper surface and through which melanosomes may be translocated. Apparently in both species, iridophores and xanthophores do not change organelle distribution during physiological color change. *Phrynosoma modestum* differs from *A. carolinensis* in that: (1) the chromatophore layers are not as distinctly separated, with the outer xanthophores extending into a thick, irregularly arranged layer of iridophores; (2) the arrangement of reflecting platelets within iridophores is near random, promoting wide-spectrum reflectance; (3) the melanophore cell

body is surrounded above and below by the iridophore layer; and (4) the xanthophore (or erythrophore) layer is very sparse or absent over portions of the skin, leaving a two-layer chromatophore unit as the basic structure effecting color change. It seems likely that this two-layer chromatophore unit is the basic structure for effecting color change in a variety of lizards that utilize this ability mainly for thermoregulation. The bright-colored pigment cells, on the other hand, play a distinctly separate role, that of pattern formation and background color matching.

ACKNOWLEDGMENTS

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