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Zebrafish Pigment Pattern Formation: Insights into the Development and Evolution of Adult Form

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Abstract

Vertebrate pigment patterns are diverse and fascinating adult traits that allow animals to recognize conspecifics, attract mates, and avoid predators. Pigment patterns in fish are among the most amenable traits for studying the cellular basis of adult form, as the cells that produce diverse patterns are readily visible in the skin during development. The genetic basis of pigment pattern development has been most studied in the zebrafish, *Danio rerio*. Zebrafish adults have alternating dark and light horizontal stripes, resulting from the precise arrangement of three main classes of pigment cells: black melanophores, yellow xanthophores, and iridescent iridophores. The coordination of adult pigment cell lineage specification and differentiation with specific cellular interactions and morphogenetic behaviors is necessary for stripe development. Besides providing a nice example of pattern formation responsible for an adult trait of zebrafish,

stripe-forming mechanisms also provide a conceptual framework for posing testable hypotheses about pattern diversification more broadly. Here, we summarize what is known about lineages and molecular interactions required for pattern formation in zebrafish, we review some of what is known about pattern diversification in *Danio*, and we speculate on how patterns in more distant teleosts may have evolved to produce a stunningly diverse array of patterns in nature.

1. INTRODUCTION

Nature is filled with patterns: leopard spots, seashell swirls, the branches of a deciduous tree. Patterns also lurk beneath the skin of animals in precise rows of vertebrae, arrangements of kidney tubules, and convolutions of the cerebral cortex. Biological pattern formation has been studied most extensively during early embryogenesis, and significant questions remain about the inductive signals and morphogenetic behaviors that generate particular arrangements of cells and tissues. Perhaps even more unanswered questions concern patterning during the lesser-studied periods of late embryonic, fetal, and neonatal development of amniotes and the larval and juvenile stages of anamniotes. Despite the relative lack of attention, elucidating gene functions and appreciating cell behaviors and interactions at postembryonic stages are important for understanding how adult traits are patterned. Identifying differences in gene activities—and their cellular consequences—will be essential to understanding directions and rates of evolutionary change.

Among adult traits, pigment patterns are some of the most amenable for mechanistic analyses because they are readily visible in skin, fur, and feathers. Pigment patterns can differ markedly within and between species and are subject to both natural and sexual selection, offering camouflage and UV protection and functioning in species recognition, mate choice, and other aspects of organismal behavior and physiology (3–5, 20, 44, 45, 47, 80, 118). Pigment patterns also have received theoretical attention, and their broader features sometimes can be recapitulated by simple and elegant dynamics in silico (14, 144, 149). Turing mechanisms that produce self-organizing stable patterns through interactions involving local activation and long-range inhibition have received the most attention (98, 147, 154), though recent analyses indicate more complexity in participating cell types than originally envisaged (see Section 4). Putting genetic and cellular mechanisms to these patterns—and their models—is a challenge but one that has seen considerable progress in recent years.

In vertebrates, skin pigment cells are derived from the embryonic neural crest, a population of pluripotent cells that forms during embryogenesis and contributes to many of the most spectacular and diverse adult traits, including teeth and jaws, beaks, the peripheral nervous system, and pigmentation (19, 35, 42, 66, 132, 140). Birds and mammals have a single pigment cell type, the melanocyte, that transfers melanin granules to developing skin, feathers, and hair or fur. Ectothermic vertebrates including fish, amphibians, and reptiles develop multiple pigment cell types, or chromatophores, including black melanophores, which are homologous to melanocytes, yellow-orange xanthophores, red erythrophores, iridescent iridophores, and white leucophores. Chromatophores retain their pigments intracellularly and are visible throughout development, making pigment patterns of ectotherms particularly accessible for studying cellular interactions and morphogenetic behaviors during pattern formation (2, 79, 95, 104, 142, 153); the experimental and genetic tractability of teleosts, in particular, have proven useful for bringing together genetics with studies of cell behavior and even phenotype variation in nature.

Pigmentation mechanisms have been examined in fishes including cichlids, guppies, stickleback, cavefish, platyfish, medaka, and clownfish (12, 59–61, 87, 96, 97, 117, 122, 125, 126, 129).

Thus far, however, genetic and cellular mechanisms of lineage specification, differentiation, and morphogenesis have been studied most extensively during pigment pattern formation in zebrafish, *Danio rerio* (**Figure 1**). Zebrafish adults have dark stripes and light interstripes, resulting from the spatial organization of three major classes of pigment cell types in the skin hypodermis, between the musculature and epidermis (39–41, 67). Stripes consist of black melanophores beneath sparsely distributed iridescent iridophores. Interstripes comprise yellow-orange xanthophores above densely packed iridophores. Zebrafish also have more superficial pigment cells on their scales, and melanophores at this location confer a dark cast to the dorsum. There are, additionally, stripes and light patches on the median fins. The utility of zebrafish for studying pigmentation was evident from its early days as a developmental genetic model organism: The first zebrafish mutants (*golden*^{b1}, *brass*^{b2}, *albino*^{b4}, *sparse*^{b5}, *leopard*^{t1}) recovered from pet stores or laboratory stocks had pigment cell defects and were viable in aquaria (53, 137), despite the presumed importance of coloration in the wild.

In the first part of this review, we attempt to summarize more than two and a half decades of research on the genetic and cellular bases of stripe formation in adult zebrafish, including development of the three major adult pigment cell types and cell–cell interactions required for pattern formation. Then we discuss how some patterns of zebrafish relatives can be understood with reference to stripe-forming mechanisms identified in zebrafish. Finally, we conclude by speculating on future directions of research in adult pigment patterning mechanisms.

2. ONTOGENY OF ZEBRAFISH ADULT STRIPE DEVELOPMENT

During embryogenesis, pigment cell precursors migrate from the neural crest to generate an embryonic/early larval (EL) pigment pattern by approximately day 4 consisting of yellow xanthophores dispersed over the flank and melanophores and iridophores along the dorsal and ventral edges of the body and over the yolk, and a few melanophores in a lateral stripe along the horizontal myoseptum (56) (**Figure 1*a***). The pattern changes little over the next several days, though a small number of melanophores are added to the lateral stripe (46, 89–91).

Like many organisms, postembryonic development of zebrafish involves the acquisition of new features and the remodeling or even loss of features that arose at earlier stages (85, 86, 105). For the pigment pattern, postembryonic stages witness the appearance of new pigment cells, the disappearance of some old pigment cells, and rearrangements of both new and old pigment cells to generate stripes and interstripes. The onset of the transformation is marked by two changes beginning approximately on the eighth day of development: Yellow EL xanthophores gradually fade from view, entering a cryptic state (84) (**Figure 2*a***, **subpanel *i***), and adult iridophores develop near the horizontal myoseptum, where some EL melanophores persist (**Figure 2*a***, **subpanel *ii***). Though initially limited to anterior and posterior regions, the new iridophores soon occupy the middle flank from just behind the head to the tail, thereby marking the first, or primary, interstripe (105, 114). As this pattern element becomes more prominent, new adult melanophores start to appear: Lightly melanized cells can be seen scattered over the flank, both within and adjacent to the nascent interstripe (**Figure 2*a***, **subpanel *iii***). These melanophores move as they differentiate, and soon, dark stripes of well-melanized cells are evident dorsal and ventral to the primary interstripe. Meanwhile, some fully melanized EL melanophores—initially present in the lateral stripe of the EL pattern but now within the developing adult interstripe—move short distances dorsally to join the adult stripes (**Figure 2*a***, **subpanels *iii,iv***) or are lost from the pattern altogether (108, 112, 114, 141). As these events unfold, iridophores develop not just in the interstripe but also at low density among the stripe melanophores (113, 135, 136) and orange xanthophores appear within the interstripe (78, 84) (**Figure 2*a***, **subpanel *iv***). As the fish continues to grow, the pattern

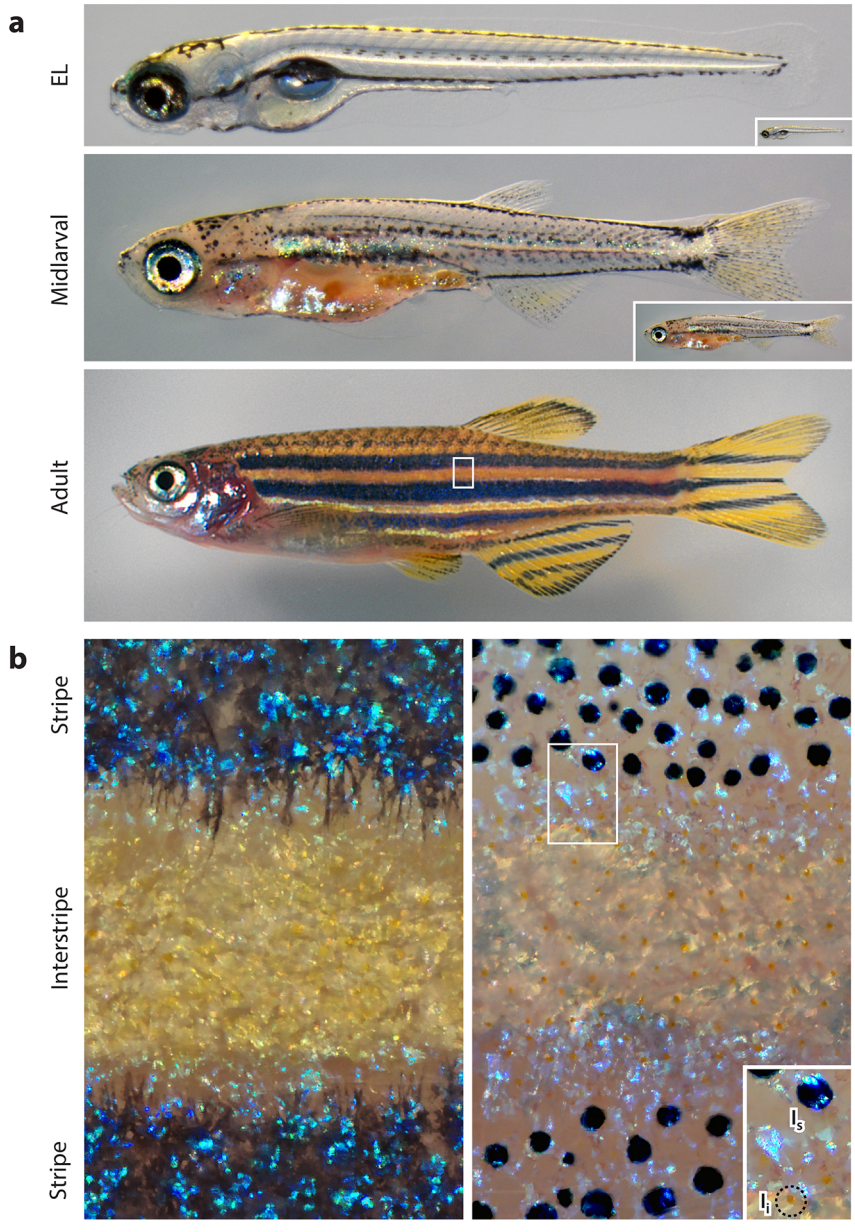
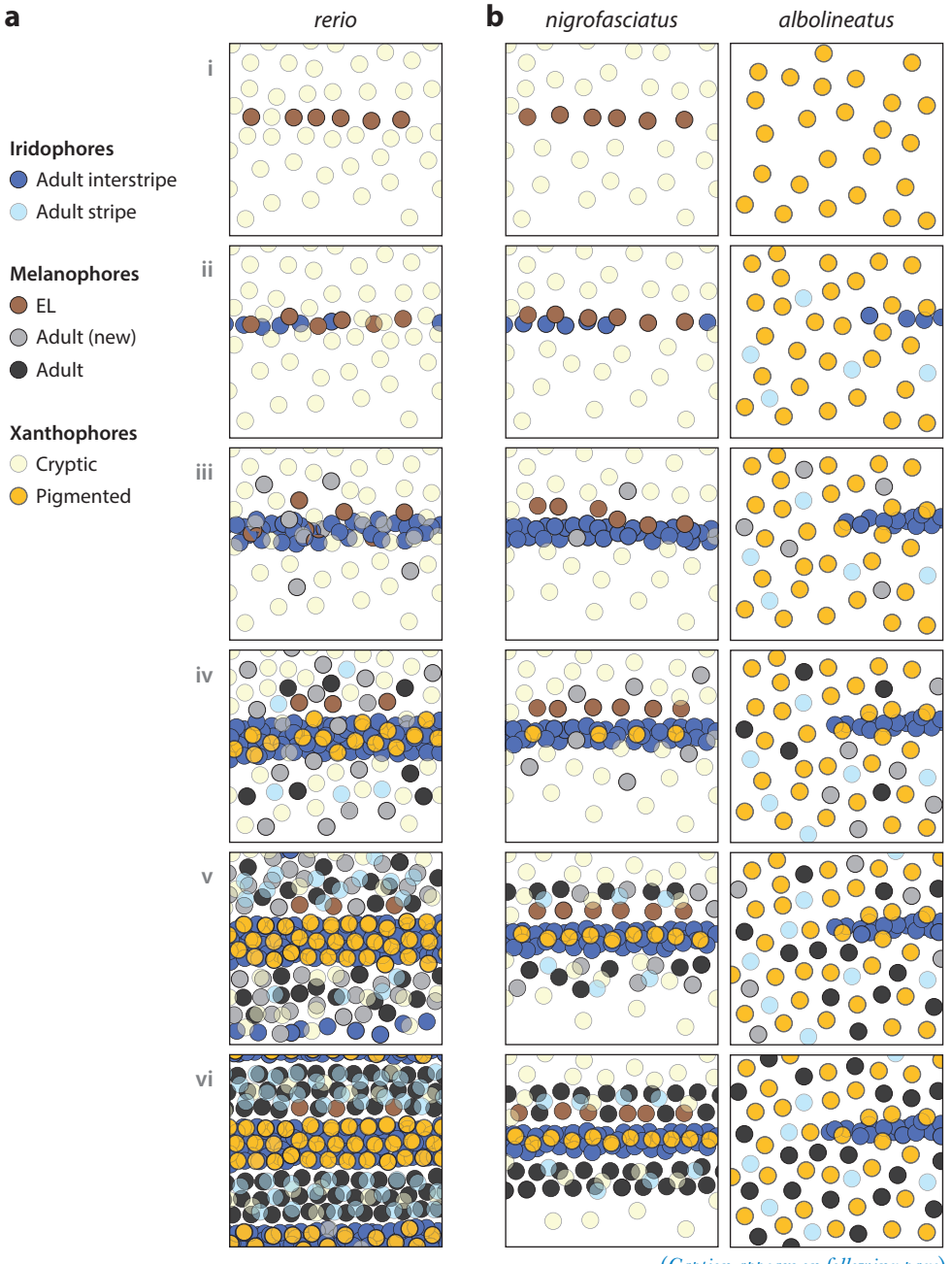


Figure 1

Pigment patterns of zebrafish. (*a*) The embryonic/early larval (EL) pattern changes during midlarval stages into a juvenile pattern of stripes and interstripes that persists into the adult stage. Extensive growth occurs during postembryonic development, as illustrated by insets showing EL and midlarval fish at the same scale as the adult. (*b*) Close-ups of boundaries between stripes and interstripes on adult fish (*boxed region* in panel *a*), showing an individual as seen typically (*left*) and another individual after treatment with epinephrine (*right*), which contracts pigment granules toward cell centers and thereby nicely reveals cellular arrangements. Inset shows iridophores typically found in stripes (I_s), iridophores of interstripes (I_i), and xanthophores (*circled*).

is reiterated with additional interstripes and then stripes forming ventrally and dorsally to make a juvenile pattern by approximately day 28 (**Figure 2*a*, subpanels *v,vi***). The seemingly choreographed sequence of events, and their reiteration, suggests that attainment of a stripe pattern requires precise regulation. Within pigment cell lineages, specification and differentiation must be coordinated with morphogenetic behaviors of proliferation, migration, and death. Between



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Developmental anatomy of pigment pattern formation. (*a*) In zebrafish, *Danio rerio*, cryptic xanthophores are scattered over the flank and early larval (EL) melanophores reside at the horizontal myoseptum (*i*). EL iridophores are not shown. Prospective adult interstripe iridophores appear in the middle of the flank (*ii*), and shortly thereafter new adult melanophores are scattered over the flank while EL melanophores move into prospective adult stripe regions, die, or disappear (*iii*). Iridophores begin to be seen within the developing stripe, and pigmented xanthophores become evident in the developing interstripe; melanophores continue to appear and become more distinctly melanized (*iv*). Finally, the pattern becomes increasingly well organized, with stripes of melanophores, sparsely arranged iridophores, and cryptic xanthophores bordering interstripes of densely arranged iridophores and pigmented xanthophores (*v*, *vi*). (*b*) Different events of pattern formation in *D. nigrofasciatus* and *D. albolineatus*, as described in Section 5. Figure adapted from Reference 113 with permission.

pigment cell lineages, and between pigment cells and nonpigment cells in their tissue environment, there must be transfer of information, in the form of cell–cell interactions, ensuring that cells differentiate appropriately and come to occupy the correct locations. We address these issues below.

3. ADULT PIGMENT CELL LINEAGES

New adult pigment cells differentiate throughout the larva-to-adult transition, but the three major adult pigment cell lineages differ dramatically in their time of first differentiation. The relative timing of pigment cell appearance can have substantial impacts on the pattern (113, 114). A major challenge, therefore, is to identify mechanisms that specify adult pigment cell lineages and promote their differentiation, within the broader context of overall somatic growth and a changing anatomical milieu. The recent finding that at least some neural crest–derived progenitors remain multipotent into postembryonic stages, capable of producing iridophores, melanophores, and xanthophores, adds additional complexity (55, 133). Here, we review some of what is known about adult pigment cell specification, differentiation, and morphogenesis and highlight gaps in our current understanding.

3.1. Postembryonic Progenitors Contribute Most Adult Melanophores, Iridophores, and Some Xanthophores

Most melanophores of the adult pattern develop from postembryonic neural crest–derived progenitors rather than directly from migrating neural crest cells (**Figure 3*a***). A distinction between EL and adult melanophore populations might be expected given the different times at which these populations first appear (57) (**Figure 2*a***). The first indication that EL and adult populations are genetically separable came from mutants of *sparse* (53), shown later to encode the Kita receptor tyrosine kinase, mammalian homologs of which are well known for their roles in melanocyte development (36, 109, 124). EL melanophores differentiate in *sparse* but mostly fail to migrate and begin to die within the first week. Subsequently, the fish lack melanophores until a new population of *kita*-independent melanophores differentiates a few weeks later, suggesting the presence of latent cells with melanogenic potential even at postembryonic stages.

Support for a distinct population of adult precursors also came from analyses of *puma* and *picasso* mutants with lesions in *tubulin, alpha 8 like 3* (*tuba8l3*) and the receptor tyrosine kinase gene *erb-b2 receptor tyrosine kinase 3b* (*erbb3b*), respectively (6, 65, 111, 112). Both mutants have normal complements of EL melanophores but develop very few additional melanophores during the larva-to-adult transformation. Both have defects in the peripheral nervous system as well. Pharmacological analyses demonstrated that requirements for Erbb3 signaling coincide with the embryonic

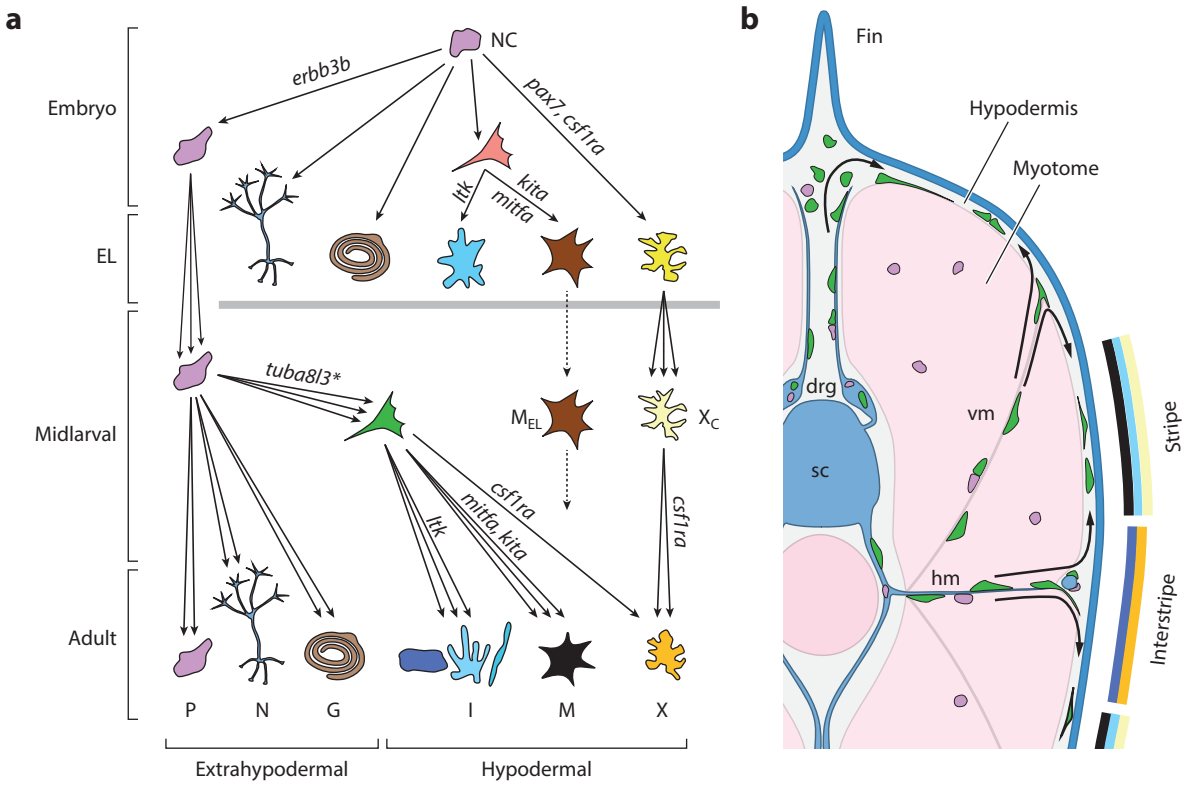


Figure 3

Models for lineages and migration routes of pigment cell precursors during postembryonic development. (*a*) In the embryo, neural crest cells (*mauve cell*, *top*) generate neurons (*dendritic blue cell*), glia (*taupe cell*), iridophores (*blue cell*), melanophores (*brown cell*), xanthophores (*yellow cell*), and other derivatives (not shown) of the embryonic/EL phenotype. Additional cells are set aside (*mauve cell*, *far left*) as postembryonic progenitors, some of which are multipotent. These may or may not be bona fide stem cells, with the capacity to self-renew while also generating differentiated progeny. As fish enter midlarval stages, progenitors associated with the peripheral nervous system (*mauve cells*) and potentially other niches expand in number (*multiple arrows*) and begin to differentiate as postembryonic derivatives (e.g., glia, *taupe cell*) within the fish, though events underlying development of nonpigmentary cells remain speculative. Other progenitor derivatives migrate to the hypodermis of the skin to generate pigment cells of the adult pattern (*green cell*). These amplifying pigment cell progenitors contribute to three classes of adult iridophores (*blue cells*), associated with interstripes, stripes, and deeper skin strata (*left to right*), each with its own morphology and physiological properties (40, 41, 103). These same progenitors contribute to adult melanophores (*black cell*) and at least some xanthophores (*orange cell*). Additionally, some embryonic/EL melanophores persist into the adult stage (*brown cell*), and EL xanthophores lose their pigment, entering a cryptic phase (*light yellow cell*), and then reacquire pigment at a late stage of adult pattern development (*orange cell*). Also shown are transitions promoted by several genes mentioned in the main text, elucidated largely through analyses of conditional alleles or pharmacological approaches (6, 7, 28, 54, 110, 112, 121). Additional activities and loci are omitted here for clarity. Asterisk marking *tuba8l3* indicates that allelic effects may be neomorphic (65). (*b*) Cross section of midlarval fish illustrating inferred locations of different progenitor classes (colors as in panel *a*) and migration routes, including along nerves, to reach the hypodermis (*arrows*). Bars at right indicate relative strata occupied by pigment cell classes in the adult hypodermis (deeper, spindly iridophores not shown). Abbreviations: EL, early larval; drg, dorsal root ganglia; G, glia; hm, horizontal myoseptum; I, iridophores; M, melanophores; M_{EL}, early larval melanophores; N, neurons; NC, neural crest cells; P, postembryonic progenitors; sc, spinal cord; vm, vertical myoseptum; X, xanthophores; X_C, xanthophores in cryptic phase. Figure adapted from Reference 7 with inferences from additional sources (7, 16, 52, 84, 133).

migration of neural crest cells contributing to dorsal root ganglia and other elements of the peripheral nervous system, weeks before adult melanophores differentiate. These findings raised the possibility that Erbb3-dependent progenitors of adult melanophores are established in association with the peripheral nervous system, an idea confirmed by molecular marker analyses, fate mapping, time-lapse imaging, and experimental tests of melanogenic competence (7, 17, 133, 135). Together, these lines of evidence showed that proliferative progenitors of adult melanophores occur in the peripheral nervous system, and potentially other locations, and then migrate to the hypodermis to differentiate as melanophores (**Figure 3*b***), in a manner somewhat reminiscent of nerve-associated melanocyte progenitors in avian embryos and perhaps mammalian embryos (1, 15, 94).

Adult iridophores and some adult xanthophores are also derived from postembryonic progenitors (7, 84, 135) (**Figure 3*a***). Adult iridophore development is disrupted in *erbb3b* mutants (6), and lineage tracing and other approaches suggest that cells initially in the vicinity of dorsal root ganglia and other locations generate not only melanophores but also iridophores and occasionally xanthophores, neurons, and glia (9, 17, 133, 135), implying that some cells have a multipotentiality similar to that of trunk neural crest. Consistent with this idea, transcriptomic analyses of postembryonic neural crest–derived cells at single-cell resolution have revealed differentiation trajectories linking presumptive progenitors through intermediate states to terminal cell types (130).

Insights into potential mechanisms of fate specification from bipotent or multipotent postembryonic precursors may come from analyses of similar processes during embryogenesis. Melanophore specification from neural crest cells depends on the basic helix-loop-helix transcription factor, microphthalmia-associated transcription factor (Mitf), which functions as the master regulator of melanocyte fate in mammals (69, 72, 101). The zebrafish homolog, melanocyte inducing transcription factor a (Mitfa), is not essential for the establishment of progenitors of adult melanophores but is required for specification and survival of both EL and adult melanophores, similar to roles in mammals (54, 72, 156). Requirements for signaling via WNT/β-catenin are shared with mammals as well (18, 37, 143). Iridophore fate specification requires repression of *mitfa* by the forkhead transcription factor, Foxd3 (13), and stable expression of the basic helix-loop-helix transcription factor gene *tfec* (transcription factor EC) (71, 116), while maintenance of *mitfa* expression and WNT signaling specify melanophores. The important signals that recruit adult melanophore or iridophore precursors from multipotent progenitors during postembryonic stages and the guidance cues that direct precursor migration to the hypodermis are not known.

Once they reach the hypodermis, adult melanophore precursors are highly motile and proliferative, and time-lapse imaging of *mitfa:GFP*$^{+}$ cells in wild-type and mutant backgrounds has revealed some of the factors influencing morphogenesis and differentiation (7, 25). For example, precursor proliferation is defective in a temperature-sensitive allele of *tuba8l3a* (that may be neomorphic); in this genetic background the cells are more likely to differentiate and less likely to divide. Migration of melanophore precursors and melanophores in the hypodermis also requires immunoglobulin superfamily member 11 (*igsf11*), a cell surface receptor containing two immunoglobulin-like domains expressed by these cells (25). Igsf11 mediates adhesive interactions in vitro, but the precise roles of these interactions and binding partners in vivo remain unclear. Analyses in a regenerative context further indicate that progenitors of melanophores persist in the adult (7, 98, 102) and that at least some of these cells reside in the hypodermis and can divide asymmetrically to self-renew while also producing differentiated progeny in a manner dependent on WNT/β-catenin (52).

Differentiation and survival of a subset of adult melanophore precursors are promoted by paracrine factor Kit ligand a (Kitlga) signaling through Kita. During the larva-to-adult transformation, *kitlga* is expressed in the hypodermis, and its ectopic expression induces melanophore differentiation in extrahypodermal locations (7). Melanophores and their precursors express Kita,

and both *kita* and *kitlga* null alleles develop far fewer adult melanophores than wild-type fish do (17, 109). The number of hypodermal *mitfa*$^+$ cells does not differ significantly between *kita* mutants and wild type, but cells in mutants are more likely to die and less likely to differentiate, revealing roles for Kita signaling in not only migration but also survival during maturation (7). Mitfa and Kita have complex interactions in other systems (43), but functional relationships between these gene products and their pathways have yet to be dissected extensively in zebrafish.

Not all adult melanophores require Kita. Early studies showed that most adult melanophores (and all EL melanophores) are absent in *kita* mutants. Nevertheless, some melanophores develop at late stages and form a regulative stripe pattern even in fish homozygous for a *kita* null allele (53, 88, 109). The relatively few melanophores that differentiate in *kita* mutants proliferate, whereas wild-type melanophores do not, suggesting that a small number of escaping precursors could account for late-appearing, regulative melanophores in the mutant (7). The molecular factors that substitute for Kit signaling and allow apparently regulative melanophore development are unknown. Interestingly, however, Kita-independent melanophores are lost when fish are doubly mutant for *kita* and either *endothelin receptor Ba* (*ednrba*) or *colony stimulating factor-1 receptor, a* (*csf1ra*) (53, 88, 107–109). The essential functions of both *ednrba* and *csf1ra* are nonautonomous to melanophores, and implied mechanisms through which they act are only vaguely understood (31, 110, 136) (see later in this section and Section 3.2). Single mutants of both *ednrba* and *csf1ra* have adult melanophore deficiencies (albeit less severe than *kita* mutants); the numbers of hypodermal *mitfa*$^+$ cells in *ednrba* and *csf1ra* mutants are similar to those in wild type, but these cells are far more likely to both differentiate and die (7).

Lineage tracing suggests that most adult iridophore precursors initially access the hypodermis through the horizontal myoseptum, where they differentiate and proliferate extensively to generate a primary interstripe with densely packed iridophores (135, 136) (**Figure 3*b***). Multiple aspects of adult iridophore development require the cell surface leukocyte receptor tyrosine kinase (Ltk) expressed in this lineage (28, 74). *ltk* mutants lack nearly all EL and adult iridophores, and many adult melanophores dependent on iridophores (31, 74). The secreted ligands, ALK and LTK ligand 2a (Alkal2a) and ALK and LTK ligand 2b (Alkal2b) (previously Augmentor-α1a and Augmentor-α1b), interact with Ltk to promote iridophore differentiation and survival (29, 93). Inhibiting Ltk signaling during postembryonic stages results in the loss of adult iridophores, whereas ectopic iridophores are induced by Aklal2a overexpression and develop in *moonstone*, an *ltk* gain-of-function mutant (28, 29, 145). Expression of *alkal2a* and *alkal2b* has not been examined postembryonically, so the cells expressing these ligands are not yet identified.

In mutants with defects in endothelin signaling, iridophores differentiate near the horizontal myoseptum but fail to populate other regions of the hypodermis (62, 107, 114, 136). In mice, *Ednrb* is required autonomously for melanocyte precursor migration and proliferation (68, 115). In zebrafish, *ednrba* is expressed by melanophore and iridophore lineages but is dispensable in the former (31, 107). Proliferation of iridophores is decreased dramatically in mutants for the *ednrba* ligand endothelin 3b (Edn3b), whereas Edn3b overexpression results in iridophore population expansion and, indirectly, more melanophores through signals yet to be identified (136).

3.2. Mixed Contributions from Neural Crest and Postembryonic Progenitors: Xanthophores and Melanophores

Not all pigment cells of the adult pattern come from postembryonic progenitors (**Figures 2*a*** and **3*a***). A few EL melanophores persist into the adult and are somewhat browner than newly differentiating melanophores, making them easy to spot (24, 120); the reason for the color difference is not known. By contrast, many of the adult xanthophores that develop by juvenile stages are

direct descendants of EL pigment cells. Throughout larval development EL xanthophores persist and proliferate in the hypodermis, so that at the onset of the larva-to-adult transition these cells cover most of the flank (78, 84). Proliferation may dilute pteridine pigments, as xanthophores are not visible during these intermediate larval stages and instead persist in an unpigmented, cryptic state (84). Additional xanthophores arise independently of EL xanthophores during normal development, and such cells can populate the flank when EL xanthophores are ablated (84, 110). As noted in the preceding section, at least some of these cells can be traced back to neural crest–derived progenitors in the peripheral nervous system able to produce adult melanophores and iridophores (133).

The relative contributions of EL and progenitor-derived xanthophores to the adult pattern and their specific genetic requirements are not yet understood. Specification of EL xanthophores from embryonic neural crest requires paired box homeodomain transcription factors encoded by *pax7a* (*paired box 7a*), *pax7b* (*paired box 7b*), and *pax3* (*paired box 3*), but how these promote xanthophore specification and differentiation is not understood (92, 100). Once specified, xanthophores and xanthoblasts express and require the receptor tyrosine kinase Csf1ra, an ancient homolog of Kit (108). The ligands of Csf1ra, Csf1a and Csf1b, are sufficient to drive repigmentation of cryptic xanthophores or de novo differentiation of progenitor-derived xanthophores (114). Mechanisms that promote EL xanthophore proliferation and other aspects of morphogenesis in the hypodermis are not known.

3.3. Paracrine Versus Endocrine Signaling

As is true for other neural crest derivatives, pigment cells receive essential information from other cells in their local environment and can be exposed to a wide variety of signals as they migrate through different tissues. Beyond some specific factors already mentioned (e.g., WNTs, Edn3) as well as a ventral-to-dorsal gradient of *agouti signaling protein 1* (*asip1*) expression, which represses melanization and *mitfa* expression ventrally (11), the nature of other paracrine signals and the cells that provide them has been mysterious.

Signals also can be more global. The α-melanocyte stimulating hormone (α-Msh) and melanin-concentrating hormone (Mch) regulate reversible color change that depends on the dispersion or contraction of pigment granules within pigment cells (8, 73). Long-term exposure to either factor can alter cell numbers as well (138). A role for insulin signaling has been revealed by analyses of mutants for *beta-secretase 2* (*bace2*, formerly *wanderlust*) in which melanophores are hyperdendritic and found ectopically in interstripes and other locations (157). *bace2* is expressed by melanophores and encodes a sheddase that cleaves insulin receptors from the plasma membrane, thereby curtailing insulin/PI3K/mTOR signaling cell autonomously. The salient insulin gene, *insulin b*, is expressed in the head at EL stages (157) and subsequently in muscle and other tissues (10), suggesting endocrine roles early and possibly endocrine and paracrine roles later.

Thyroid hormone—best known for coordinating disparate cellular processes during amphibian metamorphosis as well as its functions in mammalian metabolism and neural development—has profound effects on all three major classes of adult pigment cells (84). Zebrafish that lack thyroid hormone owing to transgenic ablation of the thyroid gland, or loss-of-function alleles of thyroid-stimulating hormone receptor, develop about twice as many adult melanophores as wild-type fish and lack visible xanthophores. In these hypothyroid fish, melanophores proliferate inappropriately, whereas xanthophores are present but fail to acquire pigmentation. Conversely, a hyperthyroid mutant develops too many pigmented xanthophores and too few melanophores. Despite having opposite effects on melanophore and xanthophore population sizes, thyroid hormone promotes the maturation of both cell types, as revealed by single-cell transcriptomics coupled with

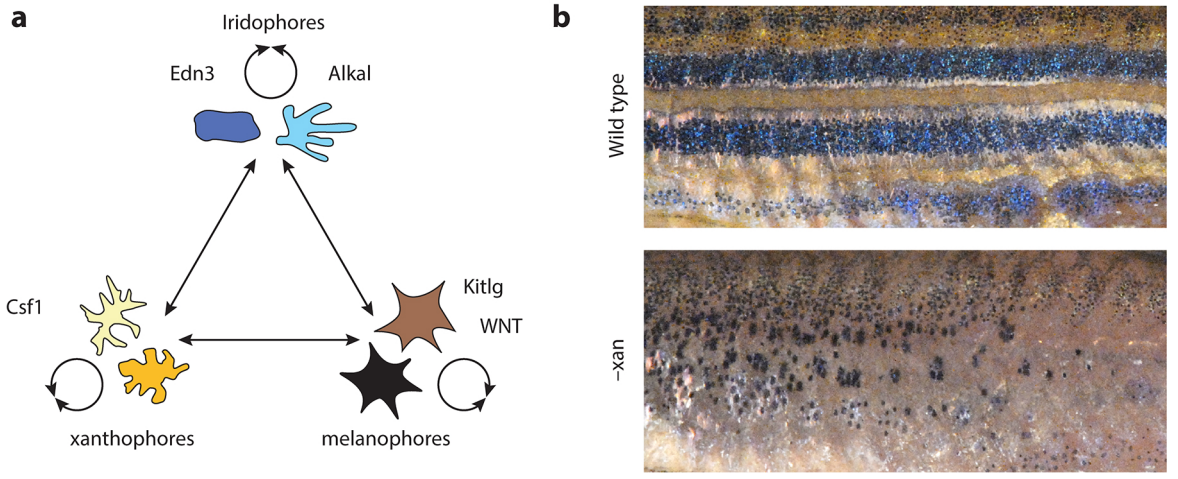


Figure 4

Interactions among pigment cell classes are required for adult pattern formation. (*a*) A variety of reciprocal positive and negative interactions—occurring over short and long ranges—are required to generate and maintain pattern. Interactions also can be specific to particular subclasses of pigment cells, the details of which are omitted for simplicity. Pigment cells also exhibit regulatory interactions within classes (*circular arrows*) and depend on signals in their environment that may be permissive or instructive (e.g., Edn3, Alkal, Csf1, Kitlg, WNT). (*b*) Mutants that lack even a single class of pigment cells can have profound pattern defects owing to the loss of essential interactions, here illustrated by the *csf1ra* mutant that lacks xanthophores (−xan) and develops only disorganized stripes anteriorly and an even more severe defect posteriorly.

mutational and other analyses (130). For melanophores, thyroid hormone promotes a terminally differentiated, binucleated state in which proliferation ceases. For xanthophores, it drives acquisition of carotenoid-based pigmentation, making previously cryptic cells visible. Without thyroid hormone, melanophores remain "young" and continue to divide, whereas xanthophores stay hidden.

It seems likely that other systemic factors affect zebrafish pigmentation, such as growth-hormone-related somatolactin, which promotes xanthophore differentiation while repressing leucophore proliferation in medaka (32–34). Determining how global signals are integrated with gene regulatory networks to produce cell-type-specific responses remains a major challenge for understanding the developmental basis of adult traits.

4. CELLULAR INTERACTIONS UNDERLYING PATTERN FORMATION

Once pigment cells and their precursors reach the hypodermis, interactions between all three pigment cell types and their tissue environment are required for adult stripe development (**Figure 4**). Evidence for such interactions comes from genetic, transgenic, or laser ablation of specific cell types, cell transplants, direct observations of cell behaviors, and analyses of gene loss-of-function in mutants and gain-of-function in transgenic lines (24, 26, 31, 38, 48, 51, 62, 75, 77, 78, 84, 98, 108, 110, 113, 114, 131, 134, 136, 141, 145–148, 154, 155). Collectively, these studies indicate that adult stripe development occurs in three principle stages: establishment, refinement, and reiteration. Establishment depends critically on the local tissue environment and interactions that might be permissive or instructive in nature. The other phases depend more extensively on interactions among pigment cells themselves, in the context of permissive signals provided by the local tissue environment as well as global endocrine factors: Once a starting point has been set, autonomous interactions between pigment cells provide an engine to complete and extend the stripe pattern.

4.1. Iridophores Establish the Location and Orientation of the Pattern

Iridophores are the first adult pigment cell type to differentiate during the larva-to-adult transformation, appearing near the horizontal myoseptum and proliferating extensively to establish the primary interstripe (**Figure 2*a***, **subpanels *ii,iii***). Localized proliferation and differentiation of adult iridophores are critical for establishing interstripe and stripe orientation and position. Mutants lacking the horizontal myoseptum, including *mesenchyme homeobox* (*meox*; previously *choker*) and *T-box 6* (*tbx6*; previously *fused somites*), have defects in interstripe development and subsequent stripe formation; differentiation of adult iridophores (but not melanophores) is delayed and iridophores appear in patches with haphazard locations and orientations (31, 99, 139, 152). Whether iridophore defects in the mutants result simply from changes in precursor migratory pathways and loss of an important exit point (**Figure 2*b***), the loss of specific localization and mitogenic signals at the surface, or both, remains unclear. Melanophores and xanthophores adopt their correct configurations with respect to the initially sparse and mislocalized patches of iridophores that do form in these mutants, suggesting that self-organizing, autonomous interactions between pigment cells can generate pattern once iridophores specify location and orientation.

Additional analyses hint at the nature of the pattern-forming interactions between iridophores and the other two pigment cell types (114). In iridophore-deficient mutants, cryptic xanthophores are distributed across the flank, as in wild type, but the development of visibly orange xanthophores is delayed. Consistent with iridophores promoting the maturation of cryptic xanthophores, iridophores express xanthogenic Csf1a. Xanthophores that develop in association with interstripe iridophores are densely packed and brightly pigmented, whereas cryptic xanthophores in the stripe regions remain dispersed and unpigmented. Iridophores also interact with melanophores. Following transgenic ablation of iridophores, numerous melanophores localize to what should be the interstripe, and individual melanophores migrate toward residual iridophore patches. These changes in melanophore behavior occur even in the *csf1ra* mutant that lacks xanthophores, suggesting though not proving that iridophore effects on melanophores are direct. Similarly, iridophore mutants *ltk* and *ednrba* develop melanophores in stripes and spots, but these are mispositioned relative to wild-type stripes (114), and both of these genes, and another in the endothelin pathway having a similar phenotype, act autonomously to iridophores rather than melanophores (31, 62). Together, these observations suggest that iridophores normally prevent melanophores from accessing the interstripe region and simultaneously support stripe formation dorsally and ventrally. The molecular bases of these interactions have not been identified. Despite their importance for establishing and orienting stripes on the body, iridophores are not required for stripe development in the fins. There are comparatively few iridophores in fins, and mutants lacking these cells retain fin stripes (64, 74, 107).

4.2. Refining and Maintaining the Striped Pattern: Roles for Melanophores and Xanthophores

Once rudimentary stripes and interstripes are established, the pattern becomes increasingly distinct as boundaries become more regular (**Figure 2*a***, **subpanels *iv–vi***). This process includes the consolidation of dispersed melanophores into stripes. Some new adult melanophores, as well as a few residual EL melanophores initially in the interstripe, exit this territory to join the stripes; other melanophores in this region die or are covered by iridophores and lost from view (108, 111, 131, 141). Numerous studies indicate that interactions between melanophores and xanthophores contribute to this stage of pattern refinement. Early evidence came from analysis of mutants for *csf1ra*, which is expressed by xanthophores but not melanophores (110). These mutants lack all xanthophores but also have fewer melanophores and develop highly disorganized stripes (**Figure 4*b***).

Melanophore numbers and pattern can be rescued, however, by transplanting wild-type xanthophore precursors into the mutant or by restoring Csf1ra activity with a temperature-sensitive allele. Restored patterns develop in the correct orientation on the body, consistent with the prior patterning of iridophores and the cascading effects of this cellular prepattern, whereas stripe orientation in fins, which mostly lack iridophores, is randomized, suggesting that whatever cue sets the orientation of pattern in this locale is either missing or no longer recognized at late stages. This observation and others nicely illustrate the competence of melanophores and xanthophores, on their own, to generate stripes, albeit disoriented ones, an issue that has itself generated some controversy (77, 84, 134, 148, 149). Interactions between these cells are also required to maintain pattern in both body and fins, as melanophore stripes degenerate when xanthophores are ablated in juvenile fish (110), a result confirmed by local changes in melanophore numbers and positions after laser ablation of xanthophore patches (98). Notch/Delta signaling was identified as a mediator of interactions between xanthophores and melanophores. Though classically a juxtracrine mechanism for patterning tissue, Notch/Delta signaling between zebrafish melanophores and xanthophores can occur over long distances through cellular projections (24, 38), one class of which requires macrophages for extension (26).

Additional insight into melanophore–xanthophore interactions comes from analysis of *leopard* mutants resulting from mutations in *gap junction protein, alpha 5b* (*gja5b*; previously *connexin 41.8*) (75, 146, 147). Both melanophores and xanthophores express *gja5b*, and *leopard* mutants develop melanophore spots instead of stripes. Mutants for another gap junction component encoded by *connexin 39.4* (*cx39.4*) have a similar but less severe phenotype with disrupted stripes and spots, and double mutants (*gja5b; cx39.4*) have very few melanophores that are broadly dispersed (50, 150). Gja5b forms heteromeric gap junctions with Connexin 39.4, and these appear to be required for cell–cell interactions between melanophores and xanthophores.

Short-range interactions are also somehow dependent upon *kcnj13* (*potassium inwardly-rectifying channel, subfamily J, member 13*), an inwardly rectifying potassium channel that is expressed by melanophores. Mutants for *kcnj13* (*jaguar* and *obelix*) develop broad melanophore stripes that include intermingled, pigmented xanthophores (51, 75). Strikingly, melanophore membranes depolarize in response to contact with xanthophores and this depolarization triggers melanophore migration away from xanthophores (48). Depolarization does not occur in melanophores of *kcnj13* mutants, suggesting that mixing of melanophores and visibly pigmented xanthophores in stripes may result from a failure of short-range interactions. Mutants for *tetraspanin 36* (*tspan36*; previously *dali*) have a similar phenotype and exhibit defects in melanophore–xanthophore interactions in vitro (49). The inward rectification of potassium channels as well as gap junctional communication requires the polyamine spermidine, and genetic or transgenic alterations in spermidine availability generate stripe defects, consistent with an essential role for this factor in cell–cell communication (30, 151).

4.3. Stripe Reiteration and Maintenance

Iridophores play a central role not only in establishing but also in reiterating the striped pattern as the fish grows, a process that begins even as the first stripes are still being refined (**Figure 2*a***, **subpanels *v,vi***). When iridophore proliferation is curtailed in mutants such as *ednrba* and *edn3b*, melanophore stripes or spots develop in association with the residual primary interstripe, but secondary stripes do not develop further ventrally or dorsally (136). During adult stripe formation, iridophores appear sparsely distributed among melanophores of the dorsal and ventral primary stripes and subsequently develop at high density farther ventrally where they establish a secondary interstripe (113, 135). Development of this secondary interstripe results in the expulsion or death

of melanophores in this region; when these iridophores are ablated, melanophores persist and stripes extend to the ventral margin of the flank (113). This suggests that ventral iridophores (forming the secondary ventral interstripe) promote termination of the primary melanophore stripe and simultaneously specify the position of the next, secondary melanophore stripe. In mutants of *tight junction protein 1a* (*tjp1a*), iridophores do not develop in the stripes. Instead, densely packed iridophores spread dorsally and ventrally into stripe regions, generating melanophores spots (27). Stripe reiteration is also disrupted in the *kcnj13* and *tspan36* mutants. Differentiated xanthophores can suppress development of secondary interstripe iridophores, allowing stripe expansion (113), but it remains unclear whether the pigmented xanthophores that develop ectopically in stripes of *kcnj13* and *tspan36* mutants themselves explain the failure of pattern reiteration.

4.4. Non-Pigment-Cell Autonomous Factors in Stripe Formation

The local tissue environment plays crucial but understudied roles in stripe formation and maintenance. Some insight into the role of the extracellular environment in this process has been provided by analyses of mutants for *basonuclin 2* (*bnc2*), which encodes a highly conserved zinc finger protein (64, 114). Fewer iridophores develop in these mutants, and many of the iridophores—as well as melanophores and xanthophores—that do differentiate do not survive. Cell transplantation associates Bnc2 requirements with dermis, where the gene is expressed. Bnc2 activity promotes *kitlga*, *csf1a*, and *csf1b* expression, and restoration of these factors in *bnc2* mutants rescues melanophore and xanthophore survival but not stripe development (114). One could imagine that Bnc2 effects on iridophores are mediated through secreted Edn3 and Alkal (29, 93, 136) but this has yet to be tested. What other factors extrinsic to the pigment cells themselves permit them to organize and whether specific instructive signals designate interstripe locations are important issues also awaiting investigation.

5. DEVELOPMENT AND EVOLUTION OF *DANIO* PIGMENT PATTERNS

Teleosts exhibit the most diverse pigment patterns of all vertebrates. Zebrafish lineage and stripe-forming mechanisms (**Figures 2*a*** and **3*a***) provide a conceptual framework for making predictions about cellular and genetic mechanisms that underlie this diversification. Broadly speaking, pattern variation could be produced by (*a*) changes in pigment cell lineage development, including alterations in the timing or location of differentiation that might have cascading effects on whether pigment cells can participate in specific interactions, or (*b*) changes in the nature of interactions between pigment cells or between pigment cells and their environment.

An excellent system for investigating these issues is the pigment patterns of other *Danio*, given the wide variety of forms that they take, including horizontal stripes, spots, vertical bars, uniform patterns, and even variation within these general themes (24, 82, 88, 106, 113, 119, 120, 136) (**Figure 5**). *Danio* patterns are likely to be ecologically relevant, as they are conspicuous, zebrafish imprint on pigment patterns, and variation in these patterns influences choice of shoalmates in the laboratory (21–23, 70, 76, 81, 123). Nevertheless, specific roles for *Danio* pigment patterns in the wild—and whether species differences reflect different functions or simply different solutions to the same ecological problems—remain unknown. Whatever their roles, the phylogenetic proximity of these other danios to zebrafish means that tools and techniques developed initially for developmental genetic analyses are readily available for dissecting evolutionarily relevant pattern variations and transformations in pattern state. These species are also of sufficiently close relationship to generate viable and in some cases fertile hybrids (**Figure 6**), allowing for evolutionary genetic analyses. A few species of *Danio* even resemble zebrafish mutants (see below), suggesting

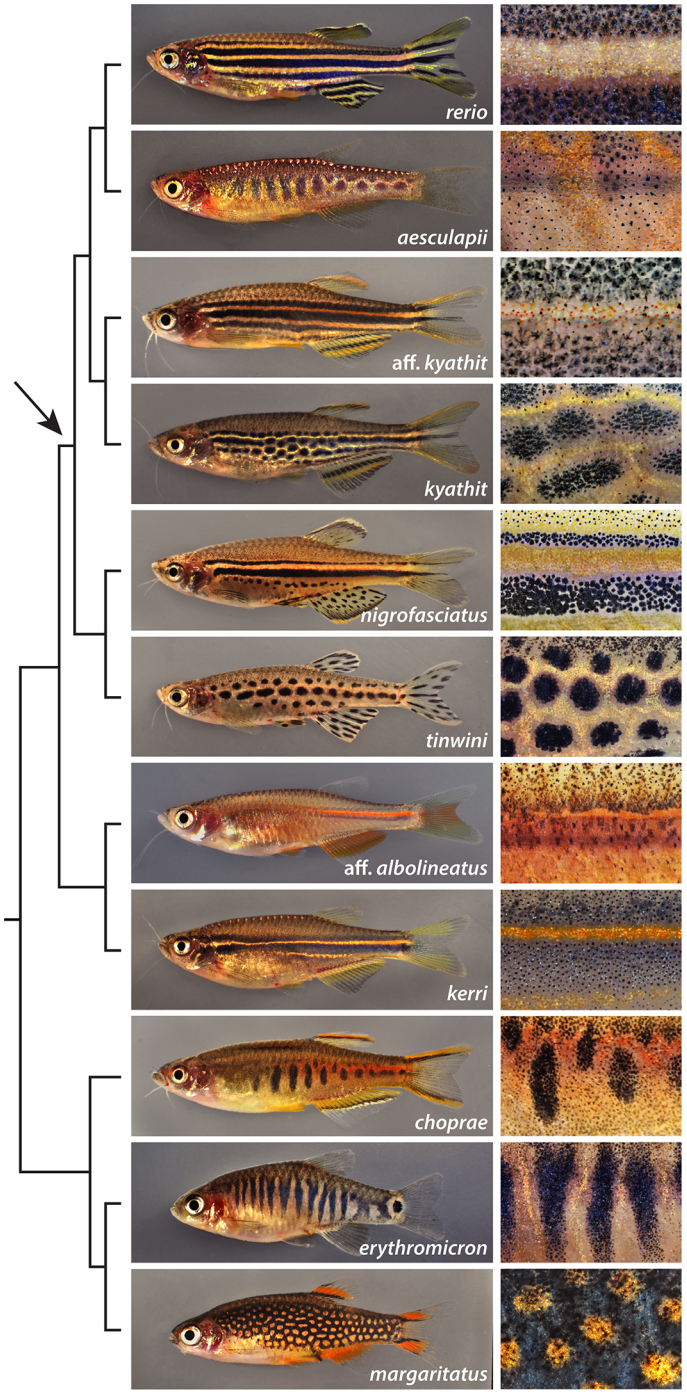


Figure 5

Stunningly diverse pigment patterns of *Danio* spp. Shown are whole fish and close-ups of pattern elements for zebrafish and 10 additional *Danio* taxa. Phylogenetic relationships are one of several possible topologies (83), with the arrow indicating a subset of the *Danio rerio* species group, within which branch order is particularly ambiguous owing to rapid divergence times and horizontal gene flow. To date, ≥25 *Danio* species are recognized (**https://www.fishbase.in/search.php**); additional taxa (e.g., *D.* aff. *kyathit*) have affinities with described species though their taxonomic status is unclear. Many species develop stripes that may be lasting or transient [*D. choprae* and *D. dangila* (119), not shown], suggesting a rudimentary stripe pattern may be ancestral for the group, though incomplete taxonomic and stage-specific sampling and extensive evolutionarily lability have so far precluded assigning polarities to character state transformations.

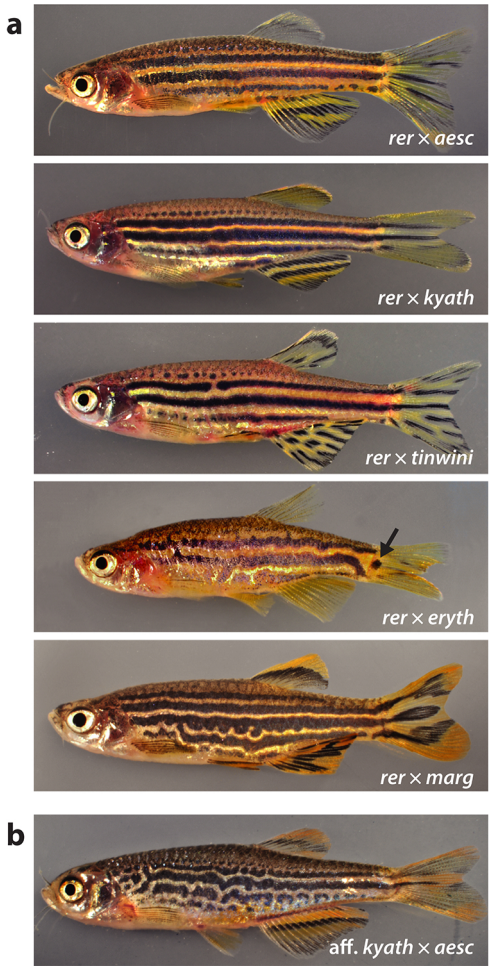


Figure 6

Pigment patterns of *Danio* hybrids reveal stripe dominance. (*a*) Crosses with *D. rerio* have patterns more similar to those of *D. rerio* than of the other species (for additional species, see References 106, 119, 136). A posterior eyespot (*arrow*) of *D. erythromicron* is penetrant even in the hybrid. A similar spot occurs in other *Danio* as well (63). (*b*) Stripe dominance is not limited to crosses with *D. rerio*, as hybrids between *D. kyathit* and *D. aesculapii* also develop irregular stripes rather than vertical bars. Abbreviations: *rer*, *D. rerio*; *aesc*, *D. aesculapii*; *kyath*, *D. kyathit*; *eryth*, *D. erythromicron*; *marg*, *D. margaritatus*; aff. *kyath*, *D.* aff. *kyathit*.

that fixed species differences may result from changes at relatively few loci. Whether this is the case—and how genetic effects are translated through cell behaviors of differentiation and morphogenesis into specific pattern outcomes—is just beginning to be explored.

5.1. Fewer Stripes: *Danio nigrofasciatus*

The critical roles played by iridophores in establishing and reiterating pattern suggest that evolutionary changes in iridophore development could alter stripe number, width, or orientation. This seems to have happened in *D. nigrofasciatus* (**Figure 5**), in which the earliest events of adult pattern formation resemble those of zebrafish (**Figure 2*b***, **subpanels *i–iii***). Subsequently,

however, fewer adult melanophores develop, more EL melanophores persist and integrate into stripes, and iridophores fail to populate other areas of the flank (**Figure 2*b***, **subpanels *iv–vi***), owing in part to reduced rates of proliferation compared with *D. rerio* (136). Altogether these events result in a pattern of stripes and spots (**Figure 5**). Differences in melanophore behavior between *D. nigrofasciatus* and *D. rerio* are nonautonomous to these cells as revealed by interspecific cell transplantation (120). This finding and a phenotype reminiscent of *ednrba* mutant *D. rerio* (53, 107, 114) raised the possibility that endothelin signaling might be involved. Indeed, mutants for *edn3b* strongly resemble *D. nigrofasciatus*, interspecific complementation tests revealed that *D. nigrofasciatus* is fixed for a hypomorphic allele at this locus, and *edn3b* is expressed at lower levels in the skin of *D. nigrofasciatus* owing to an unidentified *cis*-regulatory difference between species. Further confirming a role for endothelin—and iridophores—as a contributor to this difference in phenotype, restoration of Edn3b expression allowed iridophores to form a secondary interstripe and resulted in a corresponding reorganization of ventral melanophores into stripes (136). While not excluding roles for other factors in the species difference, these findings illustrate how an evolutionary change at a single locus can curtail pattern reiteration to generate novelty. How changes in iridophore behavior contribute to other species patterns—such as vertical bars in *D. aesculapii* and *D. erythromicron*—is an exciting question just beginning to be addressed.

5.2. Stripes or Not? *Danio albolineatus*

A different pattern is evident for *D. albolineatus* (**Figure 5**), which nicely illustrates how heterochronic and heterotopic changes in pigment cell differentiation can impact pattern outcome. This species, hybrids of which are often sold in pet stores as the pearl danio, exhibits all three major classes of pigment cells, as well as late-developing red erythrophores. In contrast to *D. rerio* and *D. nigrofasciatus*, the first adult pigment cells to differentiate in *D. albolineatus* are xanthophores, which occur not only in the hypodermis (**Figure 2*b***, **subpanel *i***) but also deep within the fish and independently of thyroid hormone (84, 113, 119). Subsequently, and in comparison to *D. rerio*, few interstripe iridophores develop, few melanophores differentiate, more melanophores die, and few of the remaining cells migrate (88, 113). These events culminate in a nearly uniform pattern in which the different pigment cell classes are intermingled and only a vestigial interstripe is evident posteriorly.

At least some of the core pigment cell interactions of *D. rerio* (**Figure 4*a***) appear to be intact in *D. albolineatus*, as mutants for *kita* develop melanophores in seemingly atavistic primary stripes requiring little migration, similar to *D. rerio kita* mutants (88). A plausible explanation for the lack of stripes in wild-type *D. albolineatus* comes from a failure of initially dispersed melanophores to consolidate, owing to the precocious differentiation and exuberant population of xanthophores. These fully differentiated xanthophores can repel melanophores and suppress iridophores in *D. rerio* (110, 113), and their presence all across the flank of *D. albolineatus* likely abolishes the directionality of cues that would otherwise be provided to melanophores specifically by the iridophores and xanthophores of the interstripe. Genetic analyses of *D. albolineatus* suggested that extra and early xanthophores result from changes in the Csf1r pathway, relative to *D. rerio* (106, 119), and molecular analyses identified a *cis*-regulatory difference at *csf1a* that drives earlier, stronger, and broader expression of this xanthogenic factor in *D. albolineatus*. That such a change in the timing and location of xanthophore differentiation is sufficient to inhibit stripe formation is apparent when Csf1a is expressed similarly in *D. rerio*: These fish develop only two stripes extending to the margins of the flank, similar to *D. albolineatus* (113). Additional factors clearly contribute to the pattern of *D. albolineatus*, especially its paucity of iridophores, which cannot solely be explained by precocious xanthophore development (L.B. Patterson & D.M. Parichy, unpublished data). Yet

the phenotype of Csf1a-overexpressing *D. rerio* is striking. It shows how changes in timing and abundance of pigment cell types can dramatically alter pattern even when the underlying network of cellular interactions remains largely intact.

That earliest-developing pigment cell classes and patterns can exert a priority effect on subsequent pattern development seems likely not only from these studies of *D. albolineatus* but also from phenotypes of hybrids. A rudimentary primary interstripe is probably the ancestral state for these fishes but this pattern can be elaborated on, or masked, in different ways, and other pattern elements, such as vertical bars, develop long after horizontal stripes are established and refined in *D. rerio* (88, 119). It is tempting to speculate that in hybrids with other danios, the robust stripe-forming mechanisms of zebrafish are sufficient to exert a priority effect over other patterns that might be specified by the heterospecific *Danio* genome, an idea that is testable using modern methods of zebrafish developmental genetics.

5.3. Gain and Loss of Fin Ornamentation? New Cell Types in *Danio rerio*

The preceding discussion has focused largely on body patterns. But fishes can also have striking pattern elements on or near their fins and *Danio* is no exception (e.g., *D. erythromicron* and *D. margaritatus* in **Figure 5**). One feature recently examined is the white edging on the dorsal fin and the distal tips of the caudal fin of *D. rerio*. Analyses of these and other fins unexpectedly revealed two previously undescribed classes of white-containing pigment cells, leucophores (70). The cells contributing to white edging of dorsal and caudal fins develop by transdifferentiation of melanophores, and so have been termed melanoleucophores. By contrast, other cells in the anal fin, xantholeucophores, contain white and orange pigmentation and develop from a xanthophore-like cell. Transcriptomic, chemical, and lineage analyses of these cells suggest they have arisen convergently relative to one another and leucophores of more distant species, such as medaka. Of particular interest in the present context, melanoleucophores contribute to shoaling preference in the laboratory, and different *Danio* species have independently gained or lost these cells in different fins, suggesting an interesting system for understanding both the evolution of cell fate plasticity and intraspecific signals in particular ecological contexts.

6. PIGMENT PATTERN DEVELOPMENT AND EVOLUTION IN DIVERSE TELEOSTS

Here, we highlight a few emerging systems that offer new opportunities to investigate the cellular and molecular bases of pattern formation and the evolution of adult form and provide interesting parallels to *Danio*.

Clownfishes, *Amphiprion*, have variable numbers of white vertical bars on backgrounds ranging from yellow to red and brown to black (126). *A. ocellaris* has a relatively simple adult pigment pattern of three white bars, outlined in black, on an orange background (**Figure 7*a***). Similar to the pattern in zebrafish, this pattern includes xanthophores and melanophores, and ultrastructural and transcriptomic analyses indicate that the white bars themselves constitute iridophores having a specific cytological organization (125, 126). Also similar to pattern formation in zebrafish, inhibition of Ltk signaling ablates the iridophores, bars, and the organization of adjacent melanophores. Despite potentially similar roles for iridophores in horizontal stripe and vertical bar development, some pattern-forming mechanisms are likely to differ significantly between species. It will be interesting to compare the cellular mechanism of vertical bar development between *Amphiprion* and *Danio* and whether they have other commonalities.

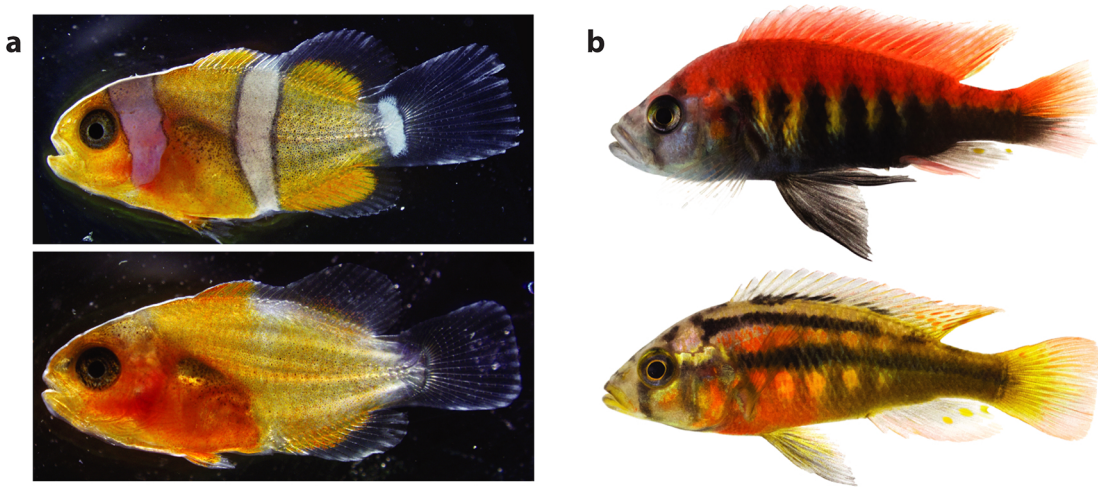


Figure 7

Pigment patterns of diverse teleosts. (*a*) Wild-type *Amphiprion ocellaris* develops a pattern of white bars, outlined in black, on an orange background (*top*) that is lost following inhibition of Ltk signaling melanophores (*bottom*). (*b*) Pigment patterns of Lake Victoria cichlids *Pundamilia* (*Haplochromis*) *nyererei* (*top*) showing vertical bars and no horizontal stripe, and *Haplochromis sauvagei* (*bottom*) with a horizontal stripe but no vertical bars. Photographs courtesy of Pauline Salis and Vincent Laudet (*a*) and Claudius Kratochwil and Axel Meyer (*b*).

In the East African Lakes Victoria, Tanganyika, and Malawi, repeated adaptive radiations have generated a diversity of pigment patterns in more than 1,000 species of cichlid fishes (58, 127). The independent evolution of similar patterns offers an outstanding opportunity to test whether alleles at the same genes are responsible for the acquisition of common pattern states across phylogenetic lineages. A recent study (61) focused on vertically barred and horizontally striped forms. Genetic mapping using a vertically barred species that lacks horizontal stripes and a striped species that lacks bars (**Figure 7*b***) identified a causal role for the inhibitor of melanophore differentiation, a*gouti-related peptide2* (*agrp2*), encoding an ortholog of the inhibitor of melanophore differentiation in zebrafish, Asip1 (agouti signaling protein 1). In the cichlids, naturally occurring *cis*-regulatory variation at this locus generates melanophore environments that are either repressive (high Agrp2) or permissive (low Agrp2) for melanophore differentiation, and in the latter state, a horizontal stripe can develop. This inference was confirmed by mosaic knockout of *agrp2* in the barred species, which resulted in stripe formation. Additional pairs of species with and without stripes have exploited the same allelic variation.

Finally, *cis*-regulatory evolution also appears to underlie the development of anal fin egg-spots in male cichlids that provide a visual signal relevant to mating behavior (58, 128, 129). Insertion of a transposable element into the regulatory region of the transcriptional activator gene, *four and a half LIM domain 2b* (*fhl2b*), results in extra iridophores in the anal fins, preceding xanthophore development in egg-spots. By analogy (or homology) with zebrafish, a role for iridophore-derived Csf1 in promoting xanthophore differentiation (114) at egg-spots seems likely but has yet to be tested.

7. CONCLUSIONS

Tremendous progress has been made in identifying genetic and cellular bases of adult stripe formation in zebrafish, but many aspects of this process remain only poorly understood. Identifying

the signals that promote specification of the three pigment cell lineages from multipotent progenitors, which guide cells to the hypodermis and localize them in specific places, and the molecular mechanisms that run an engine generating pigment-cell autonomous patterns are all areas that will benefit from additional analysis in zebrafish. Beyond zebrafish, we are only just beginning to understand how cell lineage maturation and cellular interactions have been altered to produce diverse patterns in *Danio* and other teleosts. The future is bright, and emerging genetic, genomic, and imaging technologies mean that almost any species can be the next model species. The only question now is, Will the model be wearing stripes or spots?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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