Trends in Genetics

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Opinion

Magic Traits in Magic Fish: Understanding Color Pattern Evolution Using Reef Fish

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Color patterns provide easy access to phenotypic diversity and allow the questioning of the adaptive value of traits or the constraints acting on phenotypic evolution. Reef fish offer a unique opportunity to address such questions because they are ecologically and phylogenetically diverse and have the largest variety of pigment cell types known in vertebrates. In addition to recent development of their genetic resources, reef fish also constitute experimental models that allow the discrimination of ecological, developmental, and evolutionary processes at work. Here, we emphasize how the study of color patterns in reef fish can be integrated in an Eco/Evo/Devo (ecological evolutionary developmental) perspective and we illustrate that such an approach can bring new insights on the evolution of complex phenotypes.

Highlights

Organisms live in continuously changing environments. Eco/Evo/Devo aims to uncover the rules that underlie the interactions between the environment, genes, and development of an organism.

Color patterns have a clear ecological and behavioral significance, with a wide range of functions in animals and in teleosts in particular.

Study of model species such as zebrafish allows the understanding of the developmental mechanisms underlying phenotypic evolution.

Changes in expression of key molecular factors coupled with changes in cell–cell interactions can lead to color pattern diversification during evolution.

Recent studies about color patterns in reef fishes emphasize the need to address such questions in this group in an Eco/Evo/devo perspective, integrating **proximate causation** and **ultimate causation**.

Why Study Reef Fish and Their Color Patterns?

Questions regarding the diversity, evolution, and ecological significance of **color patterns** (see Glossary) have caught scientists' attention for centuries [1]. Pigmentation has been studied using a wide variety of animal models from hexapods to vertebrates [1,2]. Fruit flies and mice are still important models to study pigmentation genes [3] but, over the last few years, teleost fish have also became efficient systems for addressing questions related to color patterns. Zebrafish and medaka are helpful models for combining genetic manipulations with live imaging, and their study has provided new insights on the cellular and molecular mechanisms that drive the development of color patterns [4]. Other fish such as cichlids and guppies have also provided valuable insight into genes and molecular mechanisms underlying specific traits (egg spots and stripes) and various color ornaments [5–7].

While mammals only possess melanocytes, the teleost lineage harbors the highest number of pigment cell types – also called chromatophores (e.g., melanophores, xanthophores, and iridophores) [8]. This diversity can explain the diversity of color and their patterns and implies the involvement of many pigmentation genes. The list of identified genes has increased in recent years (Box 1) and the whole genome duplication that occurred at the basis of the teleost lineage has been identified as a major contributor to this diversity [9].

To be able to fully understand the evolution of traits such as those displayed in color patterns and the genetic mechanisms underlying the responses of organisms to their natural environment, it is important to perform **Eco/Evo/Devo** approaches. However, ecological and behavioral roles of color patterns have not been studied in the model organisms cited above, leading to a black box concerning how proximate factors shape color patterns and their diversity over evolution. Reef fish offer promising models to address such questions because they do express much of the amazing diversity of color patterns as well as associated behavioral and ecological variation. Their original color patterns include dark or conspicuous colors, and can be made of a diverse combination of spots, stripes, bands, and **eyespots**. Reef fish exhibit many

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Box 1. Pigmentation Genes

Pigmentation patterns are mainly controlled by genes deployed during the development of chromatophores [8]. In vertebrates, these cells are neural crest cell (NCC) derivatives and the acquisition of a functional, pigment NCC-derived cell is a multiple step process that requires a fine orchestration of the expression of a specific set of genes [8].

Pigmentation genes have been studied in mammals, in which melanocytes are the only chromatophore type. Genes involved in (i) melanocyte differentiation, (ii) **melanosome** biogenesis, (iii) melanogenesis regulation, and (iv) melanosome transport are often distinguished [55]. The situation is even more complex in other vertebrates, and in particular in teleosts, that have more chromatophore types (Box 2) [8]. Studies in zebrafish and medaka have identified genes involved in specific teleost chromatophore differentiation [56]. To date, a total of ~200 genes are known to be involved in pigmentation [9]. Some genes, such as *mitf* (important for melanocyte development) and *agouti* (controls dorso-ventral patterning), have conserved mechanisms of action throughout vertebrates [57]. Others are specifically associated with teleosts: for example, both *Itk* and *sox5* are known to be required for iridophore and xanthophore development [58,59]. Recent work has shown that the same gene can be involved in the development of the same pigment cell type but in different ways in various fish species. For instance, xanthophore differentiation requires the expression of *sox5* in medaka whereas the repression of this gene is required in zebrafish [60].

During vertebrate evolution, the pigmentation gene repertoire has been shaped by several whole-genome duplications (WGDs). After a WGD event, genes are either retained or lost. The retention pattern greatly varies with the function of the encoded protein, and genes that are retained in two copies often provide the raw material for the acquisition of new functions [61]. It was recently demonstrated that pigmentation genes have been globally more frequently retained as duplicates than other genes after teleost-specific WGDs [9,62]. This high pigmentation gene repertoire is thus expected to be linked to the highest pigment cell diversity and the great diversity of pigmentation patterns observed in teleosts.

Glossary

Color pattern: distribution of color across the body.
Color polymorphism: consequence of developmental plasticity, in which the trajectories of developing organisms diverge under the influence of ultimate cues.
Eco/Evo/Devo: the interactions between the environment, genes, and development of an organism, and their consequences in evolution.
Eyespots (or ocelli): concentric markings that contrast with the surrounding area.
Eye stripes: a dark bar that runs through the eye, matching the eye color and therefore hiding the eye.
Magic trait: a trait subject to divergent selection and a trait contributing to nonrandom mating that are pleiotropic expressions of the same genes. Often these two traits will be one and the same. Thus, pleiotropy in the context of the magic trait refers to the phenotypic effects on both selection and mating, rather than necessarily to two distinguishable phenotypic traits. See [53].
Melanosome: in melanophores, organelles that effectively contain the dark pigment, melanin. This pigment is synthesized by enzymes through a process called melanogenesis.
Proximal causation: explanation of a trait when considering direct mechanistical aspects (for instance, a change in the levels of a given hormone explain a particular color change). See [54].
Ultimate causation: explanation of a trait when considering long-term evolutionary forces (for instance, prey-predator interactions can lead to better background matching in preys). See [54].

chromatophores other than the melanophores, xanthophores, and iridophores present in zebrafish (Box 2), and thus, are of particular importance to fully grasp the range of possible pigmentation systems in vertebrates. In addition, these fish live in a complex environment with extremely rich intra- and interspecific communication, and their color patterns may vary according to developmental stage, sex, social status, and ecology (including **color polymorphism**) [10–12]. Finally, extensive phylogenetic studies now provide a good comparative framework (e.g., damselfishes [13] and snappers [14]).

Here, we aim to illustrate how the analyses of functions of color patterns in reef fish combined with developmental knowledge and phylogenetic information will provide new insights into processes generating complex phenotypes (Figure 1, Key Figure). For this, we focus on the diversity of color patterns in reef fish and relate this to what is known from the development of pigmentation in zebrafish. We then argue why reef fish constitute excellent models to understand the evolution of color patterns.

Diversity and Function of Color Patterns in Reef Fish

Reef fish harbor a myriad of colors and associated patterns. Some display uniform body color such as the blue–green damselfish *Chromis viridis* (Figure 2A), whereas others show complex patterns as seen

Box 2. Diversity of Pigment Cells in Reef Fish

Reef fish possess other chromatophores in addition to the three types observed in zebrafish (Figure I) (i.e., melanophores, xanthophores, and iridophores) [8]. Reef fish are therefore of particular importance to fully grasp the range of possible pigmentation systems in vertebrates.

Some of the extra pigment cells present in reef fish appear to be variants of the three main types. This may be the case for leucophores which are responsible for the white coloration in medaka and which have recently been described as similar to xanthophores [63]. White hue is also present in clownfish and has been shown to be based on iridophores [50,51].

However, new chromatophore types have also been recently described. For example, the blue color observed in the mandarin fish *Synchiropus splendidus* is linked to a specialized cell type, the cyanophore [64]. Another fascinating case is provided by the red fluorescent system observed in the pigmy reef goby *Eviota pellucida* [65]. Reef fish are also providing the only known case of dichromatic pigment cells. The erythro-iridophores, found in the diadem dottyback *Pseudochromis diadema*, contain both a

reddish carotenoid pigment and reflecting platelets similar to those found in iridophores [66]. The mandarin fish *Synchiropus splendidus* also possesses dichromatic cells, the cyano-erythrophores [67].

Lastly, the mechanism allowing color change of some species have started to be analyzed. The chameleon sand tilefish *Hoplolatilus chlupatyi* can exhibit color change from blue to red in a matter of a few seconds and this very fast color change is linked to a novel type of iridophore in which the reflecting platelets are concentrated in the periphery of the cell. Adrenergic stimulation leads to changes in the reflecting platelet organization and therefore changes in the color of the fish [68].

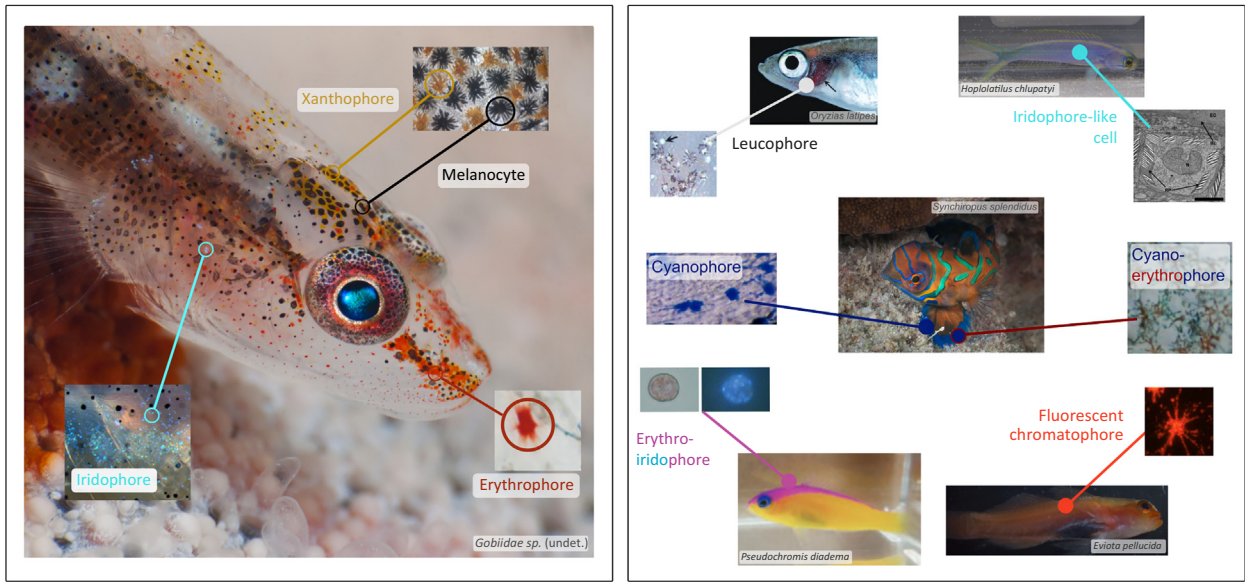

Figure I. Reef Fish Harbor a High Diversity of Pigment Cells. (A) Chromatophores found in teleost. (B) Chromatophores only found in reef fish. Fish pictures are from: *Oryzias latipes* [69]; *Hoplolatilus chlupatyi* [68]; *Pseudochromis diadema* [66]; *Eviota pellucida* [70]. Pictures of chromatophores are from [11,33,64,66–68,71,72]. Photo credit: Germain Boussarie (goby larva and *Synchiropus splendidus*).

in the clown triggerfish *Balistoides conspicillum* (Figure 2B). The latter combines a series of large ventral white spots, with a dorsal yellow shield punctuated with small brown spots. Strikingly, some reef fish species share ornamental similarities, whereas others have the exact same color pattern (Figure 2).

However, the functionality of these patterns can be diverse. It has often been assumed to be related to camouflage and/or communication [10] and the prey–predator relationship has probably led to a large variety of color patterns. Caudo-rostral stripes have been shown, for example, to have a role in inducing a confusion effect during shoaling behavior of snappers (*Lutjanus* spp.) (Figure 2C) [15] or serve as cues for intraschool orientation [16]. In contrast, a comparative study in butterflyfishes has provided evidence that the number of diagonal body stripes is associated with social behavior and dietary complexity: social species, living in groups, have fewer diagonal stripes, while species with greater dietary diversity have more of these markings [17]. Another frequently observed ornament in reef fish, **eye stripes**, have been attributed to camouflage of the eyes from predators, hence hiding a primary target [18]. Eyespots have also been linked to various antipredatory functions, such as deterring hunting predators to initiate an attack (intimidation hypothesis), or diverting their attacks toward less vital body parts (deflective hypothesis) [19]. For example, it is assumed that the large eyespot of the comet fish *Calloplesiops altivelis* has such an antipredatory function (Figure 2D). However,

Key Figure

An Eco/Evo/Devo Perspective Will Provide New Insights into Processes Generating Complex Color Patterns in Reef Fish, as Illustrated by Clownfishes (*Amphiprion*)

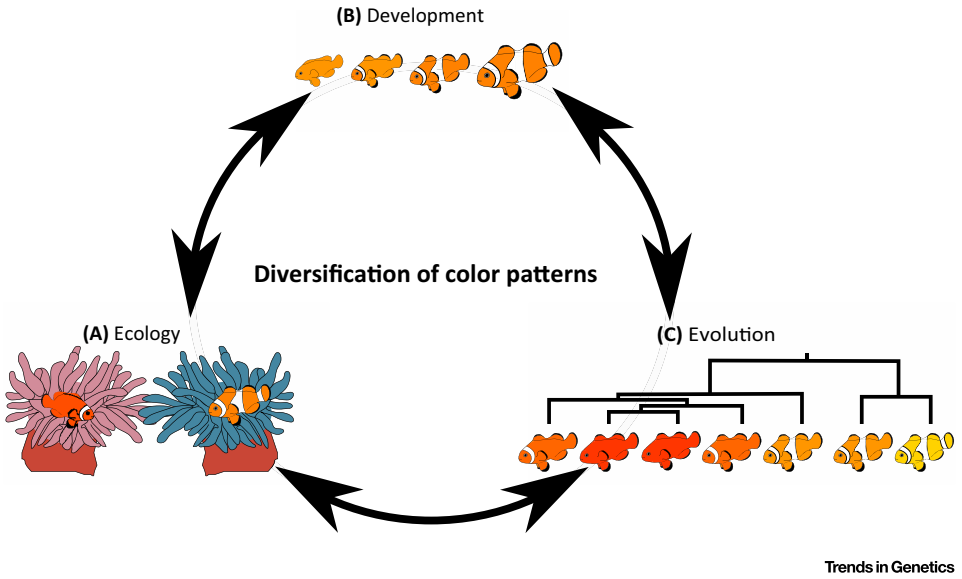

Figure 1. (A) White bars could be necessary for species recognition and could be adaptive for camouflage or even use as an aposematic signal. (B) The three white bars arise sequentially from anterior to posterior body parts during ontogenesis (*Amphiprion ocellaris*) whereas (C) during evolution, bars are lost in the opposite sequence of ontogenesis: from the posterior to anterior region. In the example of clownfishes, the loss of white bars during evolution is driven by the ecology of the species and constrained by the developmental processes of white bars.

the roles of eyespots might also be multifaceted. In the juveniles of the ambon damselfish *Pomacentrus amboinensis*, these markings serve as a signal of subordinance from juveniles to reduce aggression by mature males [20]. Moreover, the function of eyespots in *P. amboinensis* changes over ontogeny. Indeed, some mature males of *P. amboinensis* retain eyespots, when others do not (i.e., the mature dominant males), and adopt a deceptive appearance [21]. These studies from *P. amboinensis* reveal that markings may have multiple roles and beautifully illustrates the (sometimes conflicting) effects of natural and sexual selection.

The taxonomic diversity of reef fish [22] facilitates the identification of cases of parallel evolution (Figure 2) and this might help to identify ecological and molecular mechanisms underlying convergence in color patterns. Methods for the quantification of color pattern have become available [23] however, often, even the most complex patterns can be interpreted by the combination of several simpler elements/markings. Usually, we can reduce this complexity by fragmenting them into well-characterized modular subpatterns defined by their nature (e.g., lines, spots, and borders) and associated body regions. This property offers a unique opportunity to explore the evolution of color patterns through the biological concepts of integration and modularity [24]. The above-mentioned comparative study of butterflyfishes has provided a

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Figure 2. Illustrations of Some Pigmentation Patterns in Reef Fishes. (A) Blue–green damselfish, *Chromis viridis*; (B) clown triggerfish, *Balistoides conspicillum*; (C) snapper, *Lutjanus kasmira*; (D) comet fish, *Calloplesiops altivelis*. (E–H) Illustration of cases of convergence. The vertical black bars pattern is observed in (E) surgeonfish, *Acanthurus triostegus* and three damselfishes (F) *Abudefduf sexfasciatus*, (G) *Dascyllus aruanus*, and (H) *Chrysiptera annulata*. Horizontal white stripes evolved in (I) the eel catfish, *Plotosus lineatus* and (J) the cardinalfish, *Ostorhinchus nigrofasciatus*. Photo credits: Mark Rosenstein (A), Derek Ramsey (B), Alan Sutton (C), Guido & Philippe Poppe (D), Franck Merlier (E-G), Joe De Vroe (H), Philippe Bourgeon (I), Anders Poulsen (J).

first demonstration that some markings evolved differently: eyespots are evolutionary labile, whereas eye stripes are more phylogenetically conserved [17]. Correlated evolution of some specific markings, such as spots and eye stripes or eyespot and adjacent eye stripe in butterflyfishes [17], allows the suggestion of ultimate and proximate mechanisms driving the pigmentation patterns. Fragmenting complex patterns and isolating markings with extensive comparative studies across various reef fish families will help to delineate repeated modes of trait evolution (Figure 3).

Understanding the Ontogeny of Color Patterns Using Fish Models

Developmental studies are needed to provide additional information on proximate mechanisms, allowing the emergence of various color patterns during development and evolution. Up to now, cellular and molecular studies have mainly been carried out using zebrafish (*Danio rerio)*, a widely used model. Thanks to the genetic and live imaging tools developed in this species, it has been possible to investigate the mechanisms underlying color pattern formation and evolution.

The Cellular Context of Adult Pigmentation

In zebrafish, three distinct types of chromatophores are present: black melanophores, yellow xanthophores, and iridescent iridophores [25]. As in most teleosts, the zebrafish shows two different pigmentation patterns during ontogeny: a larval pattern and an adult one. The larval pattern consists of loose longitudinal stripes of melanophores, in the dorsal and ventral apex, as well as laterally at the level of the myoseptum on a subtle yellowish background caused by scattered xanthophores (Figure 4A). At the onset of metamorphosis, the adult pattern starts developing. It is composed of longitudinal dark stripes of melanophores and iridophores contrasting with light interstripe regions containing xanthophores and iridophores (Figure 4A).

The generation of the adult color pattern is complex due to the variation in adult pigment cell origin. Experimental genetic analyses have revealed that the largest number of melanophores and iridophores found in adults (often called metamorphic chromatophores) differentiate during metamorphosis and later [26–28], whereas almost all adult xanthophores differentiate earlier, during the larval stage [29]. Additionally, the melanophores found in adults have a dual origin: the largest number of melanophores differentiates at the adult stage, whereas a minority corresponds to persistent embryonic melanophores [30]. These results demonstrate that the underlying genetic architectures of larval and adult patterns only partially overlap.

An important feature of this two-step process that corresponds to metamorphosis is the role of thyroid hormones (THs). As in other teleosts, these hormones trigger and coordinate this elaborate transformation [31]. The different types of chromatophores are differentially sensitive to alterations of TH levels. For example, treatment with TH leads to a marked xanthophores excess and deficiency in melanophores in adults [32]. The role of TH is therefore central for controlling the differentiation and the ultimate presence of the three types of chromatophores, generating the observed adult pattern.

Cell–Cell Interactions Are Instrumental for Patterning

Genetic studies in zebrafish have revealed the major role of the interactions among the three types of chromatophores in the development of the color pattern. For example, in some xanthophore-deficient mutants (*pfeffer* mutants), the melanophore stripes are reorganized into spots [33]. Mutants in which two chromatophore types have been deleted (e.g., *shady:pfeffer* having neither iridophores nor xanthophores) reveal that the single remaining chromatophore type (melanophore) is not able to form the precise pattern [33]. Moreover, such interdependency is also

important for sustaining formation and/or survival of chromatophores. For example, it was shown that iridophores promote and sustain melanophore differentiation [26,33], whereas depletion of xanthophores leads to a reduction in melanophore number [34]. These interactions go beyond pigment cells, as it has been shown in an elegant study that macrophages are key players in long-range communication between xanthoblasts and melanophores and consequently participate in the network of cell interactions that govern stripe patterning [35].

These dynamics of cell interactions are predicted by the Turing model (also known as the reaction–diffusion (RD) model), which is a standard for the modeling of complex pattern formation (Box 3). The Turing model effectively explains the formation of the color pattern observed in zebrafish. Artificial disturbance of the striped pattern using laser irradiation (which ablates chromatophores) induces changes that can effectively be predicted by the model (Figure 4B) [36]. Moreover, ablation experiments of chromatophore types in different regions leads to the disruption of various short-range and long-range interactions that are essential in the Turing model. For example, when part of a xanthophore stripe is ablated, only xanthophores will arise in the cleared area (Figure 4C, upper panel). Conversely, when the two adjacent black stripes are ablated in addition to the same part of the xanthophore stripe (Figure 3C, middle panel), melanophores will emerge in the former xanthophore domain, suggesting that melanophores in the neighboring stripes have a repressive effect on the development of melanophores at a distant location [34]. Together, this suggests that long-range interactions (e.g., xanthophores promoting melanophore emergence and melanophores inhibiting other melanophores) as well as short-range interactions are important in establishing the final width of the stripes. Altogether, this reveals that this network of interactions possesses the properties necessary to follow the Turing model (Figure 4D) [34]. Another fundamental characteristic of the Turing model is that the number of repeated stripes or spots is intimately connected to body size, and therefore to the growth of the organism. Such characteristics are observed in the *long-fin* zebrafish mutant (for which the fins never stop growing), which continues to form perfectly new stripes as the fins grow [28].

If cell interdependency shapes the width of the stripes, the global directionality of the pattern has to be established. Some biological indicators must specify the direction of stripe formation. Accordingly, the pigmentation pattern of the zebrafish body trunk needs initial information and this is provided by the horizontal myoseptum in which iridophore precursors migrate to form the first horizontal stripe. The melanophores and xanthophores that subsequently develop are then influenced by the position of iridophores. The crucial role of the horizontal myoseptum in providing directionality information is illustrated by the *choker* mutants in which the myoseptum is lost. In adult mutants, the pigmentation develops into a labyrinth-like pattern because of the loss of the initial positional indicator [33,37].

Evolution of Color Patterns

The study of cellular and molecular mechanisms of color pattern generation in *D. rerio* and their closely related species that show different pigmentation patterns allowed some of the evolutionary mechanisms controlling the evolution of color patterns to be deciphered. For example, an interesting case is provided by *Danio albolineatus,* a nonstriped *Danio* species characterized by the presence of intermingled populations of the three pigment cells. In this species, differentiation of xanthophores occurs earlier than in *D. rerio* because of an increased expression of *csf1* (due to a change in its gene regulatory region); a growth factor supplied by iridophores and other cells in the skin [38]. This earlier differentiated population of xanthophores in *D. albolineatus* modifies the positioning information provided to melanophores compared with *D. rerio.* Consequently, *D. albolineatus* individuals do not form stripes [38]. It has been

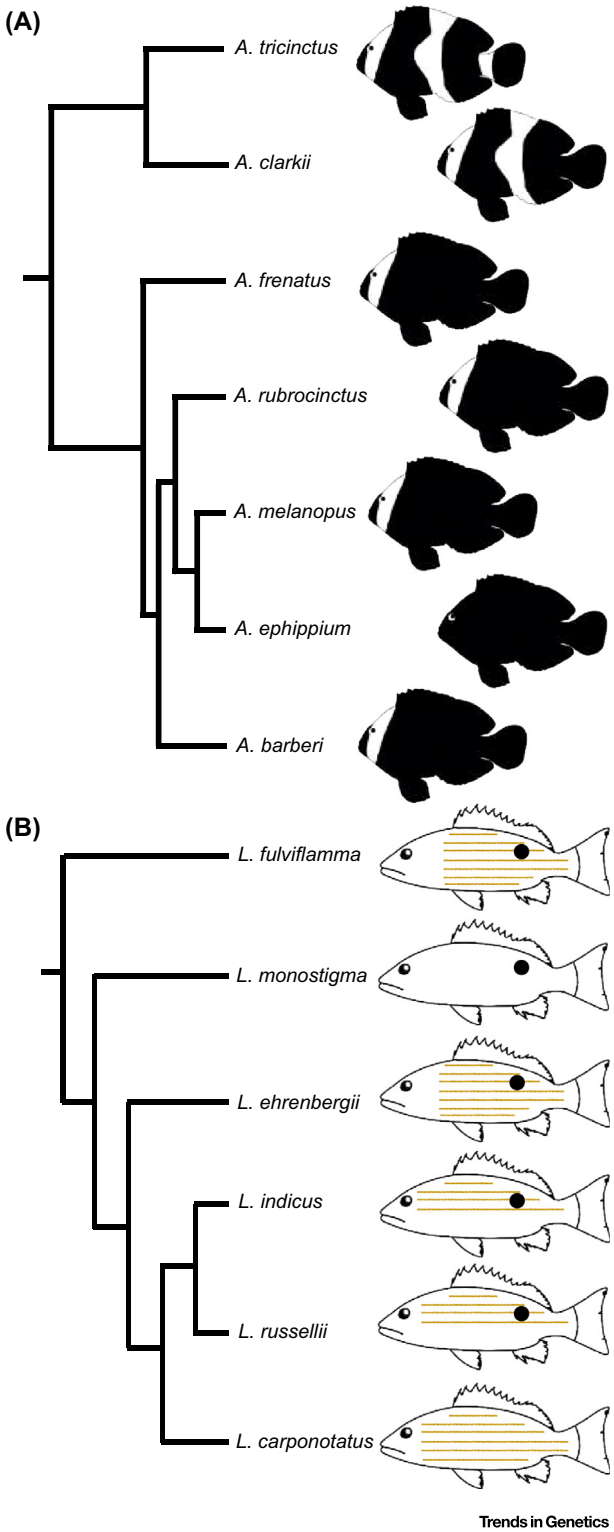

Figure 3. Evolution of Some Markings in Two Groups of Reef Fish. Example from the clownfish *Amphiprion* (A) illustrating the caudal to rostral losses of white bars during evolution [50]. The example from the snappers *Lutjanus* (B)

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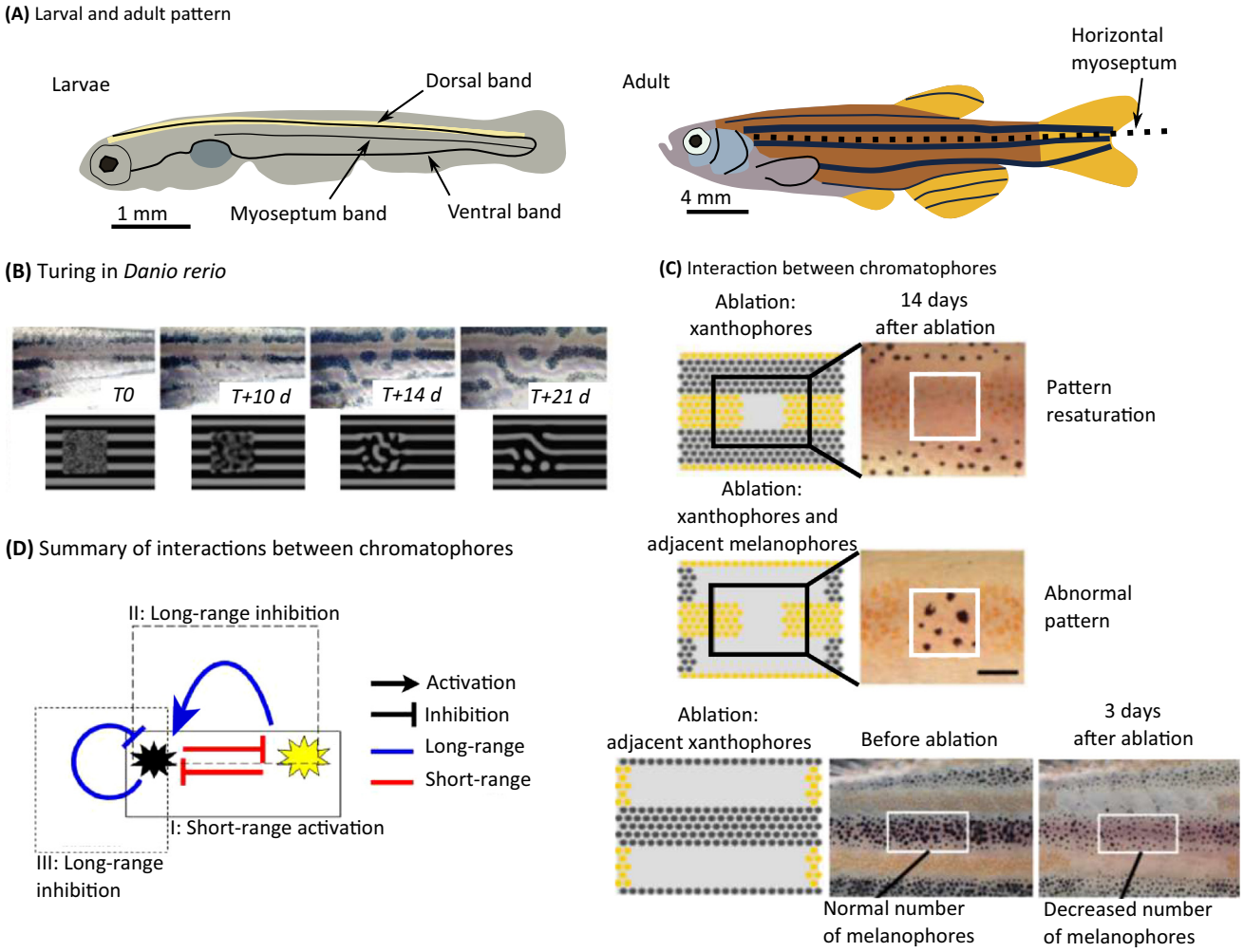

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Figure 4. Understanding the Ontogeny of Pigmentation Patterns Using *Danio rerio*. (A) Pigmentation pattern of larval (left) and adult *D. rerio* (right). (B) Regeneration of the labyrinthine pattern of adult zebrafish after laser ablation (ablation of pigment cells) (upper panel) and its computer simulation (lower panel): the stripes developed but the directionality is lost (picture from [36]). (C) Ablation experiments showing long- and short-range interactions between xanthophores and melanophores. (D) Cartoon summarizing interactions between xanthophores and melanophores consistent with Turing Model: I. a short-range activation resulting in a negative feedback loop between xanthophores and melanophores; II. long-range inhibition resulting in a long-range positive effect of xanthophores on melanophores; and III. a long-range autoinhibition of melanophores. [(C) and (D) are adapted from [34]].

shown experimentally that increased expression of *csf1* in zebrafish results in similar cascading effects giving rise ultimately to a similar intermingling of all three pigment cell types and stripe loss [38]. Recently, the secreted peptide endothelin-3, a known melanogenic factor, was shown to contribute to reduced iridophore proliferation and fewer stripes observed in another species, *Danio nigrofasciatus* [39]. These data illustrate how changes of expression of key molecular factors coupled with changes in cell–cell interactions can lead to the evolution of a new color pattern.

shows the diversification of color patterns by disappearance of spots and longitudinal stripes. Phylogenetic hypothesis of snappers is from [14].

Box 3. Turing Models

Originally introduced by the mathematician Alan Turing in 1952, the Turing or reaction-diffusion (RD) model explains the spontaneous formation of periodic biological patterns [73,74]. It involves two diffusing molecules that interact: a slowly diffusing activator and a rapidly diffusing inhibitor. As the inhibitor molecule diffuses more rapidly than the activator, it impairs activation at long range (Figure IA). If the activator is sufficiently efficient and/or is present in sufficient amounts, it can prevent its inhibition at short range. It is the balance between the reaction of the two molecules and their diffusion that explains how various periodic patterns can spontaneously emerge from an initially homogeneous pattern. The parameters that can vary in models (relative strengths of the activator and inhibitor and their diffusion abilities) explain the wide variety of patterns (stripes, spots, etc.).

An illustration of the RD model has been provided in the *Pomacanthus* marine angelfish [42]. Juveniles of *Pomacanthus semicirculatus* display three vertical white stripes on a dark background. During growth, new stripes insert between the pre-existing ones, and this process is repeated several times to give rise to the final pattern. The RD model can predict this dynamic change. The same authors also show how rearrangement of the parallel striped pattern of the adult *Pomacanthus imperator* can also be predicted. During growth, the number of horizontal stripes increases proportionally to body size and the space between them remains constant (Figure IB). By incorporating cell growth and movement in the models, it is possible to explain in a detailed manner the dynamic of stripe formation [75]. Recently, the arrangement of zebrafish stripes was also shown to be consistent with an RD model [36,76].

RD models have also been applied to a variety of other biological systems. As the model is particularly easy to implement in a simple 2D space it has been used to better understand the formation of several ectodermal appendages such as hair follicle spacing in mouse [77], or feather patterning in birds [2]. More complex systems, such as branching morphogenesis in the lung, or teeth patterning have also been explored [78]. By changing parameters and initial conditions of the systems, such as developmental landmarks as shown in birds [79], RD models can generate a virtually unlimited variety of periodic patterns [76]. We thus expect that a large proportion of pigmentation patterns observed in reef fish could be explained through RD models.

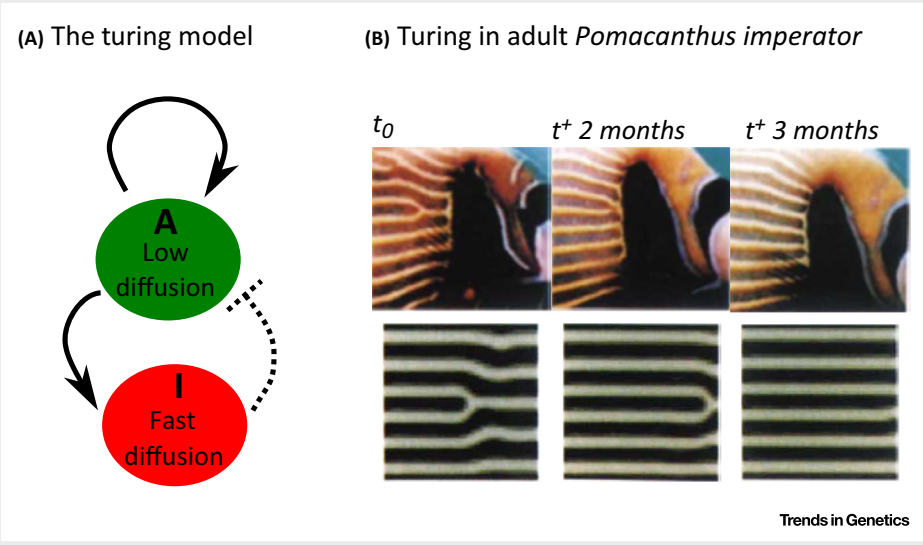

Figure I. The Turing Model and Application in Reef Fish. (A) The activator stimulates the production of both itself and its inhibitor (arrows). The inhibitor turns off the production of the activator (dashed line). As the inhibitor molecule diffuses more rapidly than the activator, it impairs activation at long range. (B) Rearrangement of the stripe of the same adult *Pomacanthus imperator* (upper panel) and its computer simulation (lower panel): as they grow, the number of lines increases proportionally to body size whereas the width remains constant. At t_0, *P. imperator* contains a branching point, during growth, the branching point moves horizontally (to the anterior) like a zip resulting in its fusion and thus in the addition of a new line [42].

Integrating Ecology with Evo/Devo to Understand the Color Patterns of Reef Fish

Integrating ecology with evolution and development allows us to address how developmental mechanisms modified during evolutionary changes are selected. If zebrafish with its unique

toolkit is an excellent model to understand the development of reiterated striped pattern, its ecological diversity is limited, and thus how the developmental mechanisms at the origin of variation in the pigmentation patterns have been selected remains unknown. This is why reef fish, with their diversity of pigment cell types (Box 2), combined with the vast knowledge gathered on their ecology and the new development of genomic resources [40,41], are becoming attractive models to reach a full understanding of the diversity and evolution of color patterns. Moreover, among those advantages, most color patterns observed in reef fish are not reiterated patterns but rather result from the combination of simpler elements that cannot be explained by the Turing model. Thus, although the Turing model has been successfully applied to angelfish (*Pomacanthus* spp.) [42], it is clear that it will only explain a subset of the patterns observed in reef fish and that other mechanisms must be at work.

To exemplify analyses that beautifully illustrate the potential of incorporating the ecological and developmental approaches in the evolution of complex color patterns, we have chosen three recent studies. The first concerns phenotypic plasticity, a major tenet of Eco/Evo/Devo. It is well exemplified by the dusky dottyback *Pseudochromis fuscus*, a small predatory fish [11,12]. This species can exhibit numerous uniform color morphs from orange to brown, yellow, pink, or gray. At the Great Barrier Reef, the yellow morph inhabits living coral heads with yellow damselfishes (e.g., *P. amboiensis*) whereas the brown morph is associated with brown damselfish species (*e.g. Pomacentrus chrysurus*) on coral rubbles. Experiments have revealed that yellow morphs can transform into brown morphs within 2 weeks if translocated from living corals to coral rubbles [43]. Strikingly, however, the dottyback does not change color because of the environment but because of the presence of colored damselfishes. The advantages of this strategy are double for *P. fuscus*. First, by mimicking adults of a damselfish species, it increases its predation success on their juveniles. Second, the color change helps the dottyback to escape its own predator by providing a habitat-associated crypsis. The study of associated cellular mechanisms has revealed that this change in color is explained by a change in the respective proportions of xanthophores and melanophores [12]. In a fascinating follow-up study, this color change has been placed upon an ontogenetic trajectory and it has been shown that, in fact, dottybacks change color twice during development: once during metamorphosis, when a pelagic translucid larvae is transformed into a grey juvenile, and again when the large-enough juvenile begins its mimicry strategy and selects either yellow or brown victims [11]. This study therefore addresses how developmental plasticity can promote ecological adaptation.

The two other examples incorporate this time, evolution together with development and ecology approaches. One concerns the radiation of the Caribbean hamlets (*Hypoplectrus* spp.) and shows how color polymorphisms allow the understanding of the ecological and developmental basis of phenotypic adaptation. Detailed analysis of their radiation has revealed that a single trait, color pattern, has driven incipient speciation in this fish [44]. It is often considered that, as a predatory fish, *Hypoplectrus* mimics harmless fish in order to increase their predation success on their prey [44,45]. Genetic analysis allows us to identify divergent loci among color morphs [46–48]. Among them, an analysis using SNPs has identified the *HoxC* cluster as being associated with color variation [47]. *Hox* genes have never been associated with a pigmentation defect in teleosts but they have been linked to body pigmentation and eyespot formation in insects [49]. Developmental studies are needed to better understand the role, if any, that these genes could play in the divergence of color patterns.

Clownfish offer a third case in which the mechanisms controlling pattern formation can be deciphered. These fishes form a tribe composed of 30 species within the damselfishes and display a simple color pattern made of 0–3 white bars containing iridophores visible on a darker

body background [50,51]. Vertical white bars likely play a role in species recognition [50] but it has also been suggested that this varied bar pattern serves for camouflage or use as an aposematic signal [52]. Recently, we have mapped the occurrence of these bars on the clownfish phylogeny to reconstruct the ancestral state in terms of white bars presence/absence [50]. Through this analysis, we have provided evidence that the diversification of the clownfish color pattern results from successive caudal to rostral losses of bars during evolution. The juveniles of some species have supplementary bars that disappear caudo-rostrally later. The reduction of bar number over ontogeny totally matches the sequence of bar loss across evolution, demonstrating that diversification in color pattern among clownfish lineages results from changes in developmental processes (Figure 1). This analysis illustrates that the clownfish model is different from the zebrafish since the number of bars is independent of body size [50]. Thus, a Turing-like model cannot explain the disappearance of bars during clownfish ontogeny and other mechanisms are obviously involved in white bar formation. Genetic analyses are now required to understand the molecular mechanism of the origin of such color pattern evolution within clownfish.

Concluding Remarks and Future Perspectives

Color patterns in reef fish, with their extreme divergence and plasticity, can indeed be considered as a '**magic trait**' that may easily lead to speciation [53]. Thanks to work on the zebrafish model, we have more knowledge about the developmental mechanisms generating color patterns. The combination of ecological analysis with genomic and/or developmental analysis using magic reef fish as model systems (in addition to other valuable models such as cichlids and guppies) will help to provide an integrated understanding of the evolution of such complex phenotypes. We have identified several concrete directions in which the study of reef fish could have specific advantages (see Outstanding Questions). The first is the study of the numerous color polymorphisms existing in these fish (e.g., dottybacks and melanic clownfish), as well as the link between behavior and color. In both cases, the vast ecological knowledge accumulated can be advantageously combined with the transcriptomic and functional approaches to understand how ecological and developmental constraints intermingle to generate novel phenotypes. Another promising aspect is to study the developmental and evolutionary rules governing the assembly of various patterns. For all of these questions, it will be critical to bring together proximate and ultimate causations to understand the magic traits.

Outstanding Questions

What are the molecular and cellular processes shaping color pattern during development in reef fish? What genes and developmental pathways contribute to the variation of their color patterns?

The frequent occurrence of some specific ornaments in different reef fish species suggests that they are formed by shared developmental modules. What are the genes and pathways controlling the formation of these typical domains?

How do changes in the molecular, cellular, and developmental processes result in beneficial trait differences that are favored by selection during the course of evolution?

How does the organism integrate the environment to give an appropriate response, for example, changes in colors or associated patterns?

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