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Magic Traits in Magic Fish: Understanding Color Patterns using Reef Fish

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Abstract

Color patterns provide an 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 perspective and we illustrate that such an approach can bring new insights on the evolution of complex phenotypes.

Why studying reef fish and their color patterns?

Questions regarding the diversity, evolution, and ecological significance of color patterns have caught scientist's attention for centuries [1]. Pigmentation has been studied using a wide variety of animal models from hexapods to vertebrates [1,2]. Fruit fly and mouse are still important models to study pigmentation genes [3] but, over the last years, teleost fish 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 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, 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, 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 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 pattern have not been studied in the model organisms cited above leading to a black box concerning how do 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 other chromatophores than the melanophores, xanthophores, and iridophores present in zebrafish (Box 2) and they are thus 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 inter-specific communication, and their color patterns may vary according to developmental stage, sex, social status and ecology (including colour polymorphism) [10–12]. Finally, extensive phylogenetic studies now provide a good comparative framework (*e.g.* damselfishes [13], snappers [14], etc.).

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. 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* (Fig. 1A) whereas others show complex patterns as seen in the clown triggerfish *Balistoides conspicillum* (Fig. 1B). 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 (Fig. 1).

The functionality of these patterns can be diverse however. It has often been assumed to be related to camouflage and/or communication [10] and the “prey-predator” relationship 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) (Fig. 1C) [15] or serving as cues for intra-school orientation [16]. On the other hand, a comparative study in butterflyfishes provided evidence that the number of diagonal body stripes are 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 ornaments in reef fish, **eye stripes**, have been attributed as serving to camouflage 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 was largely assumed that the large eyespot of the comet fish *Calloplesiops altivelis* has such an antipredatory function (Fig. 1D). However, the roles of eyespots might also be plural. 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 section.

The taxonomic diversity of reef fish [22] facilitates the identification of cases of parallel evolution (See examples on Fig. 1) and this might help to identify ecological and molecular mechanisms underlying convergence in color patterns. Methods for the quantification of color pattern become available [23] but, 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 sub-patterns defined by their nature (*e.g.* lines, spots, 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 provided a 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 for delineating repeated modes of trait evolution (Fig. 2).

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 very 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 (Fig. 3A). At the onset of metamorphosis, the adult pattern starts

developing. It is composed of longitudinal dark stripes of melanophores and iridophores contrasting with light inter-stripe regions containing xanthophores and iridophores (Fig. 3A). The generation of the adult color pattern is complex due to the variation in adult pigment cell origin. Experimental genetic analyses 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 persisting embryonic melanophores [30]. These results demonstrated that the underlying genetic architectures of the larval and adult patterns are only partially overlapping.

An important feature of this two steps-process that corresponds to a metamorphosis, is the role of thyroid hormones (TH). As in other teleosts, these hormones trigger and coordinate this elaborate transformation [31]. Interestingly, 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 chromatophores types have been deleted (*e.g. shady:pfeffer* having neither iridophores nor xanthophores) reveal that the single remaining chromatophore type (melanophores) 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 was shown in an elegant study that macrophages participate, via long distance cytoplasmic projections reaching xanthophores, to the network of cell interactions that govern the stripe patterning .

These dynamics of cell interactions are predicted by Turing models (also known as reaction-diffusion models), which is a standard for the modelling of complex pattern formation (Box 3). The Turing model effectively explains the formation of color pattern observed in zebrafish. Interestingly, artificial disturbance of the striped pattern by using laser irradiation (which ablates chromatophores) induces changes that can effectively be predicted by the model (Fig. 3B) [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 xanthophores stripe is ablated, only xanthophores will arise in the cleared area (Fig. 3C - upper panel). Conversely, when the two adjacent black stripes are also ablated in addition to the same part of the xanthophore stripe (Fig 3C-middle panel), melanophores will emerge in the former xanthophores domain suggesting that melanophores in the neighboring stripes had a repressive effect on the development of melanophores at a distant place [34]. Together, this puts forward that long-range interactions (*e.g.* xanthophores promoting melanophores emergence and melanophores inhibiting other melanophores) as well as short-range interactions are important in setting up final width of the stripes. Altogether, this reveals that this network of interactions possesses the properties necessary to form the Turing pattern (Fig. 3D) [34]. Another fundamental characteristic of 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 were observed in *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 zebrafish body trunk needs initial information and this is provided by the horizontal myoseptum in which iridophores precursors migrate to form the first horizontal stripe. The melanophores and xanthophores that will subsequently develop are then influenced by the position of iridophores. The crucial role of the horizontal myoseptum in providing directionality information was 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 showing different pigmentation patterns allowed deciphering some evolutionary mechanisms controlling the evolution of color patterns. For example, an interesting case is provided by *Danio albolineatus*, a non-striped *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 to *D. rerio.* Consequently, *D. albolineatus* individuals do not form stripes [38]. It was 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 the reduced iridophore proliferation and fewer stripes observed in another species, *D. 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.

Integrating ecology with Evo/Devo to understand the color patterns of reef fish

Integrating ecology with evolution and development allows addressing 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, their 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 the evolution of color patterns. Moreover, amongst those advantages, most of color patterns observed in reef fish are not reiterated patterns but rather results from the combination of simpler elements that cannot be explained by the Turing mechanisms. Thus, whereas Turing model have been successfully applied to angelfishes (*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. Pomacentrus amboiensis*) whereas the brown one is associated with brown damselfish species (*e.g. Pomacentrus chrysurus*) on coral rubbles. Experiments 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 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 then when the large-enough juvenile starts its mimicry strategy and select either yellow or brown victims [11]. This study therefore addresses how developmental plasticity can promote ecological adaptation.

The two other cases incorporate this time, evolution together with development and ecology. 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 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 preys ([44] but see [45]). Genetic analysis allowed to identify divergent loci among color

morphs [46–48]. Among them, an analysis using SNPs identify the HoxC cluster as being associated with color variation [47]. Hox genes have never been associated with pigmentation defect in teleosts but they have been linked to body pigmentation and eyespot formation in insects [49]. Developmental studies are much needed to better understand the role, if any, that these genes could play in the divergence of color patterns.

Clownfish offers a third case in which the mechanisms controlling pattern formation can be deciphered. These fishes are forming a tribe composed of 30 species within the damselfishes and displays a relatively simple color pattern made of zero to three white stripes containing iridophores well visible on a darker body background [50,51]. Vertical white stripes likely play a role in species recognition [50] but it was also suggested that this varied striped pattern might serve for camouflage or use as an aposematic signal [52]. Recently, we mapped the occurrence of these stripes on the clownfish phylogeny to reconstruct the ancestral state in terms of white stripes presence/absence [50]. Through this analysis, we provide evidence that the diversification of clownfish color pattern results from successive caudal to rostral losses of stripes during evolution. Interestingly, the juveniles of some species have supplementary stripes that disappear caudo-rostrally later on. The reduction of stripes number over ontogeny totally matches the sequences of stripe losses across evolution, demonstrating that the diversification in color pattern among clownfish lineages results from changes in developmental processes. This analysis illustrates that the clownfish model is very different from the zebrafish since the number of stripes is independent to body size [50]. Thus, Turing like mechanism cannot explain the disappearance of stripes during clownfish ontogeny and other mechanisms are obviously involved in white stripes formation. Genetic analyses are now required to understand the molecular mechanism at 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 works 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 reef "magic" 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. The first is the study of the numerous color polymorphisms existing in these fish (*e.g.* dottybacks, melanic clownfish, etc.) as well as the link between behaviour 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 these questions, it will be critical to bring together proximate and ultimate causations to understand the "Magic traits".

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Glossary box

- **Color pattern**: distribution of color across the body
- **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.
- **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].
- **Eco/Evo/Devo**: the interactions between an organism's environment, genes and development, and their consequences on evolution.
- **Melanosome**: in melanophores, organelles that effectively contain the dark pigment, melanin. This pigment is synthesized by enzymes through a process called melanogenesis.
- **Color pattern polymorphism**: consequence of developmental plasticity, in which the trajectories of developing organisms diverge under the influence of ultimate cues
- **Magic trait**: a trait subject to divergent selection and a trait contributing to non-random mating that are pleiotropic expressions of the same gene(s). Often these two traits will be one and the same. Thus, pleiotropy in the context of magic trait refers to the phenotypic effects on both selection and mating, rather than necessarily to two distinguishable phenotypic traits. See [53].

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Figure Legends

Figure 1. Illustrations of some pigmentation patterns in reef fishes. (A) The blue-green damselfish, *Chromis viridis*; (B) the clown triggerfish, *Balistoides conspicillum*; (C) the snapper, *Lutjanus kasmira*; (D) the comet fish, *Calloplesiops altivelis*; (E-H) Illustration of cases of convergence. The vertical black stripped pattern is observed in (E) the surgeonfish, *Acanthurus triostegus* and three damselfishes (F) *Abudefduf sexfasciatus*; (G) *Dascyllus aruanus*; (H) *Chrysiptera annulata*. The horizontal white bars 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).

Figure 2. Evolution of some markings in two groups of reef fish. Example from the clownfish *Amphiprion* (A) illustrating the caudal to rostral losses of white stripes during evolution [50]. The example from the snappers *Lutjanus* (B) shows the diversification of color patterns by disappearance of spots and longitudinal stripes. Phylogenetic hypothesis of snappers is from [14].

Figure 3. Understanding the ontogeny of pigmentation patterns using *Danio rerio* (A) Pigmentation pattern of larval (left) and adult *D. rerio* (right). (B) Regeneration of 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 [52]). (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 on a negative feedback loop between xanthophores and melanophores; II. an overall long-range

inhibition resulting on a long-range positive effect of xanthophores on melanophores and III. a long range auto-inhibition of melanophores. ((C) and (D) are adapted from [34]).

BOXES

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 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 (see Box 2) [8]. Studies in zebrafish and medaka have identified genes involved in specific teleost chromatophore differentiation [56]. To date, this makes a total of ca. 200 genes 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 involved in teleosts: for example, both *ltk* and *sox5* are known to be required for iridophores and xanthophores 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 needed in zebrafish [60].

During vertebrates' 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]. Interestingly, it was recently demonstrated that pigmentation genes have been globally more frequently retained as duplicates than other genes after teleosts-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.

Box 2: The diversity of pigment cells in reef fish

Reef fish contains many chromatophores in addition to the three types observed in zebrafish (*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 of leucophores responsible for the white coloration in medaka that have been recently 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 cyanophores [64]. The molecular nature of the pigment present in their specialized organelles has not been identified to date. 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 *S. 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 few seconds and this very fast color change is linked to a novel type of iridophores 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 in fish color [68].

Figure Box 2: Reef fish harbor a high diversity of pigment cells. (A) Chromatophores found in teleost. (B) Chromatophores only found in reef fish. Pictures of fishes are from: *O. latipes* [69]; *H. chlupatyi* [68]; *P. diadema* [66]; *E. pellucida* [70]. Pictures of chromatophores are from [14, 15, 41, 51, 73, 76–79]. Photo credit: Germain Boussarie (goby larva and *S. splendidus*).

Box 3: Turing Models

Originally introduced by the mathematician Alan Turing in 1952, the Turing or reaction-diffusion model (RD) explains the spontaneous formation of periodic biological patterns [73,74]. It involves two diffusing molecules that are interacting: 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 (see Box Figure, panel A). If the activator is sufficiently efficient and/or is in sufficient amount, 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 has been provided in the *Pomacanthus* marine angelfish [75]. Juveniles of *Pomacanthus semicirculatus* display three vertical white stripes on a dark background. During growth, new stripes insert between the preexisting ones, and this process is repeated several times to give rise to the final pattern. The RD 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 remain constant (See

Box Figure, panel B). By incorporating cell growth and movement in the models, it is possible to explain in a detailed manner the dynamic of stripes formation [42]. Recently, the arrangement the zebrafish stripes was also shown to be consistent with a RD [36,76].

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

Figure Box 3: Stripes formation is predicted by Turing model in fishes

(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* (up panel) and its computer simulation (down 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 move horizontally (to the anterior) like a zip resulting in its fusion and thus in the addition of a new line [75].

Highlights

Organisms live in continuously changing environments. Eco/Evo/Devo aims to uncover the rules that underlie the interactions between an organism's environment, genes and development, and by doing so aims to expand our current view of how evolution works to reach an integration of proximate and ultimate mechanisms.

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

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 and ultimate causations.

Outstanding questions box

- What are the molecular, 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 sih species suggests that they are formed by shared developmental modules. What are the genes and pathways controlling the formation of these typical domains?
- How 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 answer *i.e.* changes in colors or associated patterns?

Figure 1

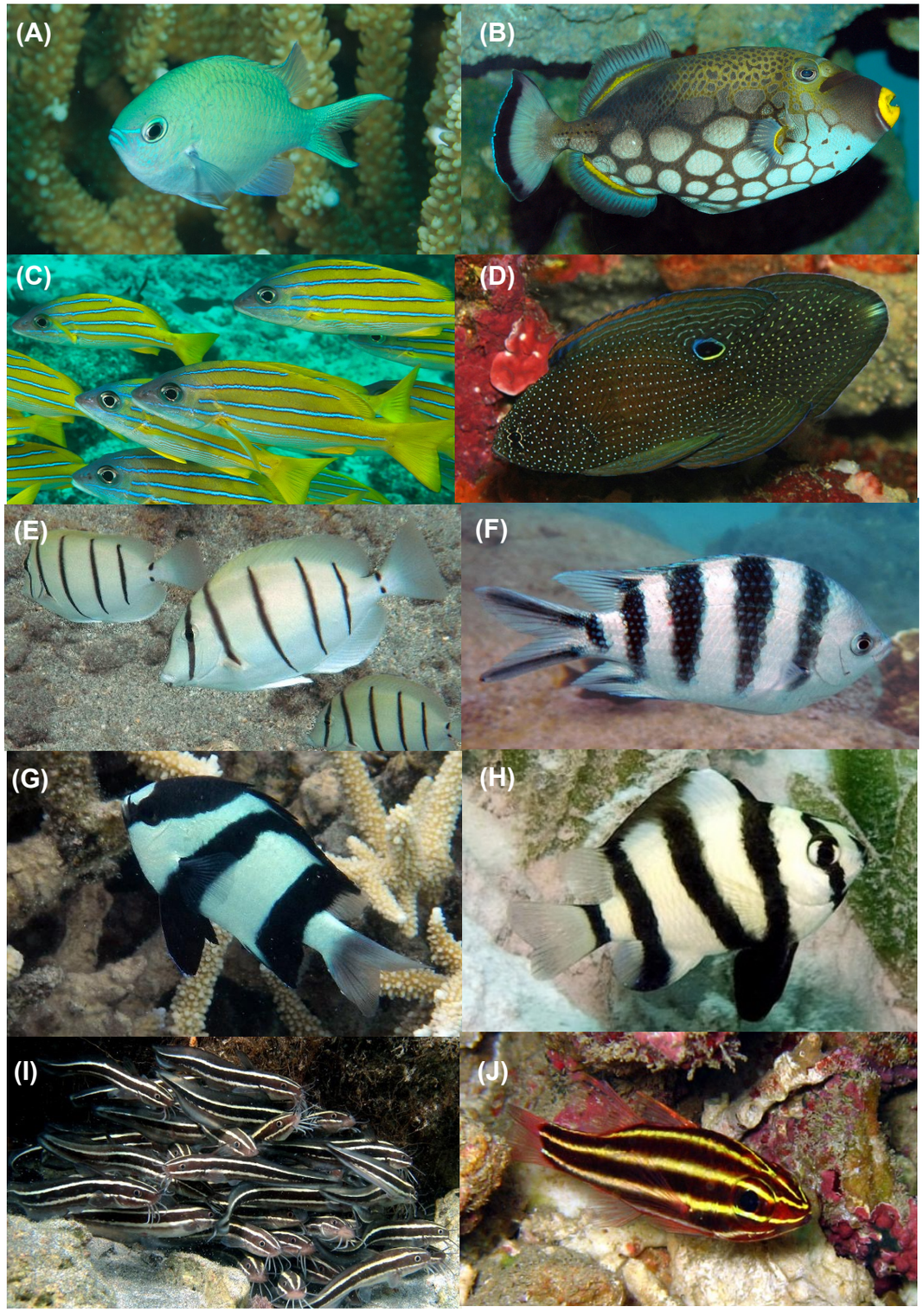


Figure2

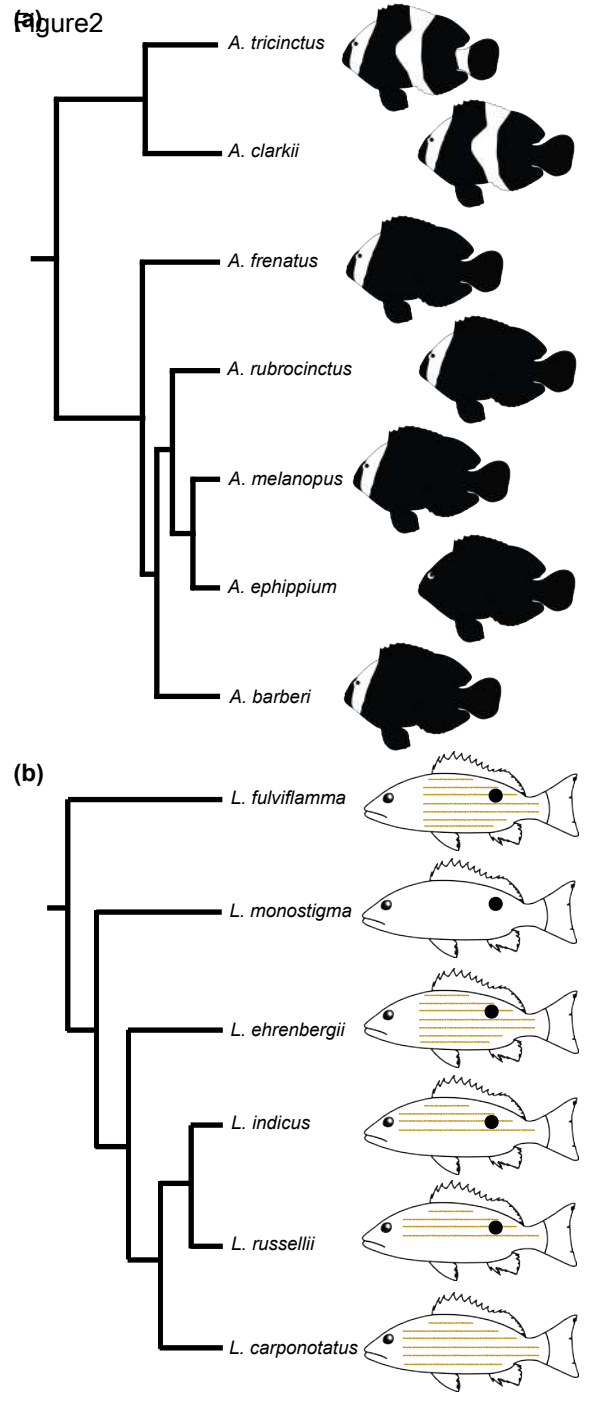
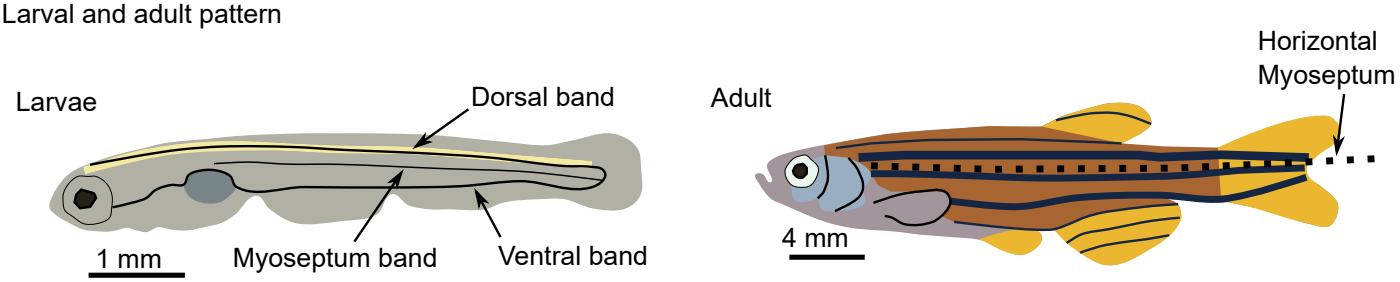
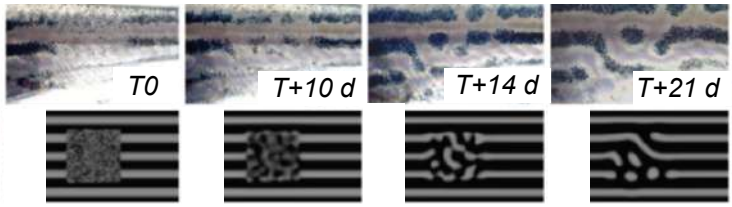

(a)
A. tricinctus
A. clarkii
A. frenatus
A. rubrocinctus
A. melanopus
A. ephippium
A. barberi
(b)
L. fulviflamma
L. monostigma
L. ehrenbergii
L. indicus
L. russellii
L. carponotatus

Figure3

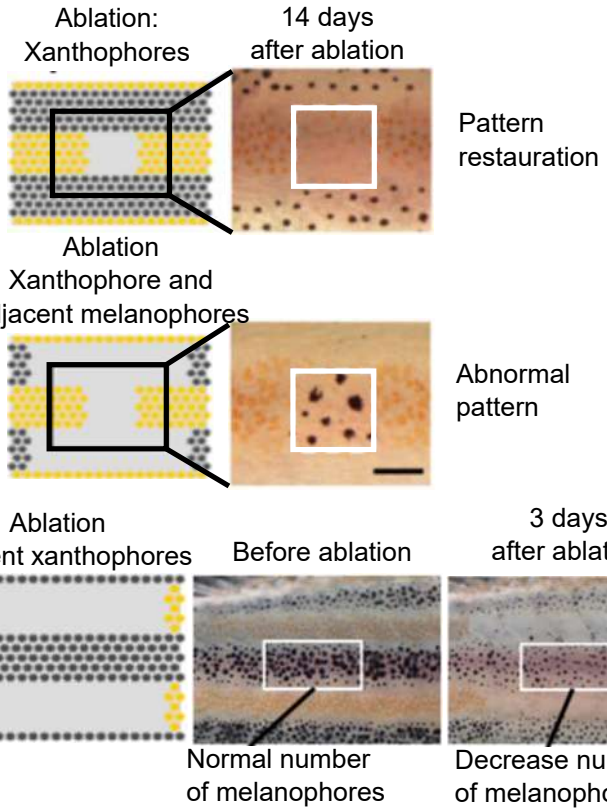
(A) Larval and adult pattern



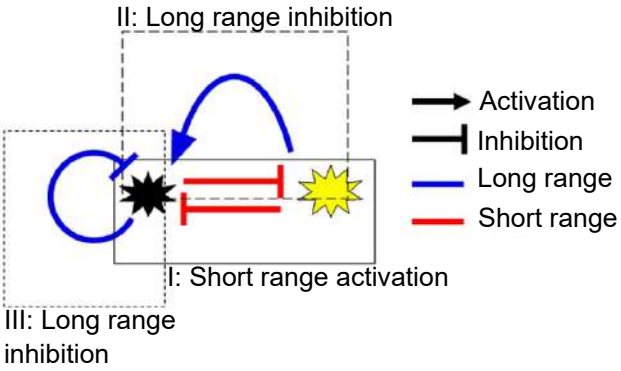
(B) Turing in *Danio rerio*



(C) Interaction between chromatophores



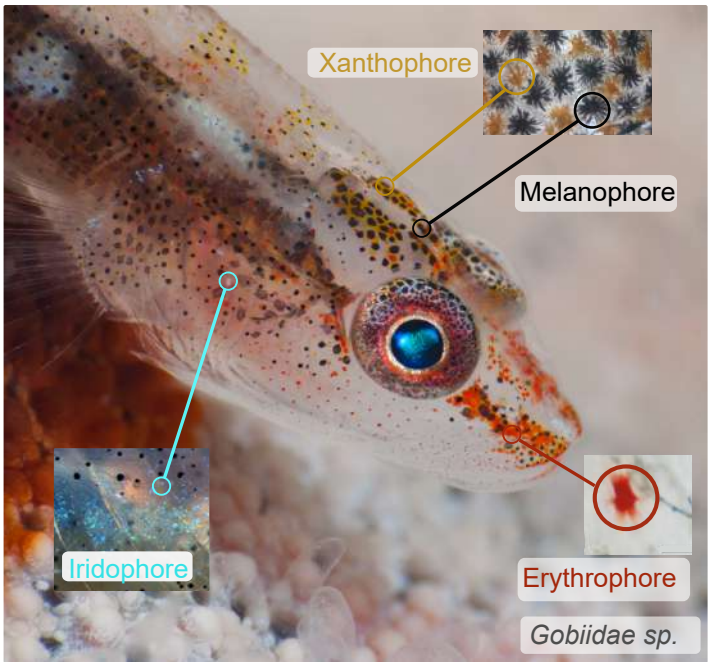
(D) Summary of interactions between chromatophores



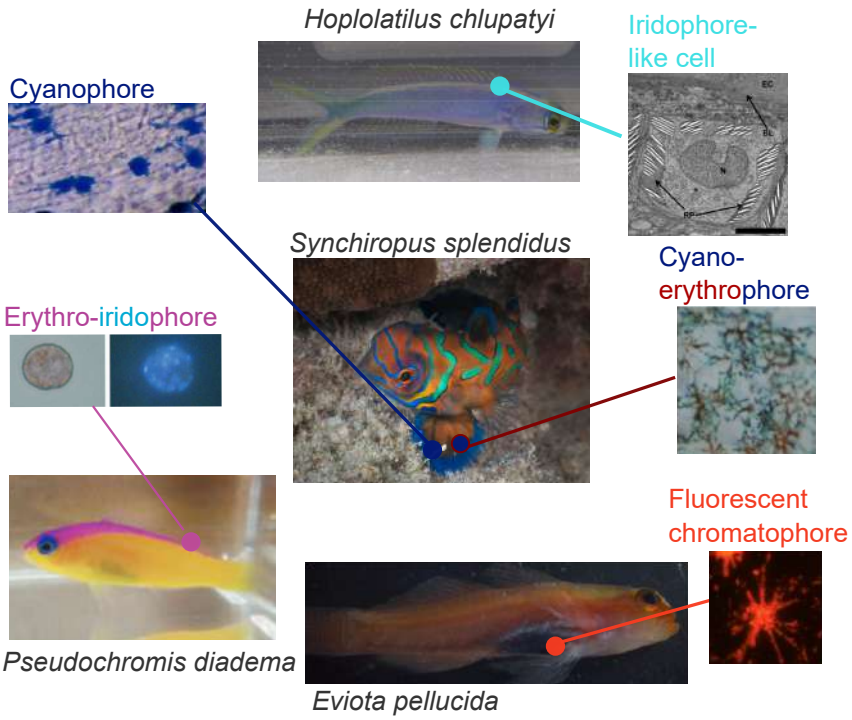
P.Salis et al., Figure 2

BOX2 Figure

(A) Chromatophores found in teleosts

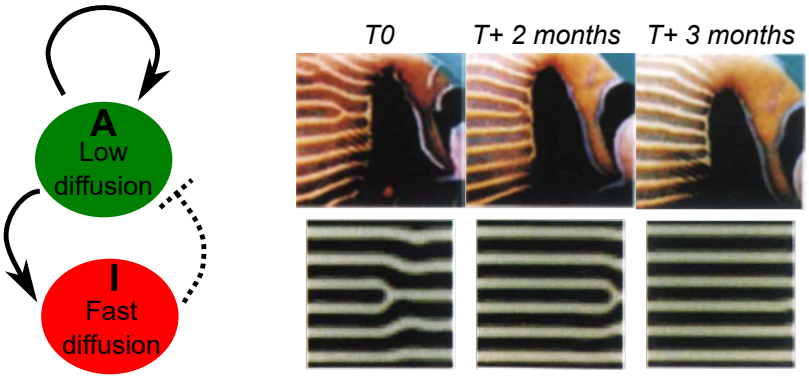


(B) Chromatophores found in coral reef fishes



P. Salis et al., Figure 3

(A) The Turing model (B) Turing in adult *Pomacanthus imperator*



Cover Letter

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Trends in Genetics

Banyuls/Mer, December 17th 2018

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Dear Caryn,

Please find enclosed our manuscript entitled "*Magic Traits in Magic Fish: Understanding Color Patterns using Reef Fish*" by Pauline Salis, et al., that we would like to submit to *Trends in Genetics*

In addition to the main text, the manuscript contains 3 short boxes of text and 5 figures including 2 in the boxes.

As discussed during our exchanges of e-mails this review presents an eco/evo/devo interdisciplinary perspective to understand the origin of pigmentation pattern diversification using reef fish as an example. We made all possible efforts to link the field discussed to broader, underlying questions of developmental biology and evolution. Our aim is effectively to attract a broad readership and to convince them that they should consider these fishes as interesting models. The first part focuses on the diversity and functions of color patterns in reef fishes. Then we reviewed the molecular, cellular and developmental basis for color pattern formation studied mainly in zebrafish. Finally, the last section explains how integrating ecology together with evo/devo allows uncovering the mechanisms promoting pigmentation diversity in these fishes.

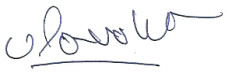
Observatoire Océanologique de Banyuls/Mer · Laboratoire ARAGO · 1882 · Sorbonne Université · CNRS

cnrs

As this review brings in information from a number of scientific disciplines in the service of a question of basic science we hope you will find it appropriate for the wide audience of *Trends in Genetics*.
Hoping that our manuscript will be regarded favourable, we look forward to hearing from you.

We would like to thank you in advance for taking care of our manuscript.

Yours sincerely,



Vincent Laudet

[MENTIONS LÉGALES À VENIR – Double cliquez ici pour modifier le pied de page – À faire également sur la première page]