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SYMPOSIUM ARTICLE

Molecular Plasticity in Animal Pigmentation: Emerging Processes Underlying Color Changes

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Synopsis Animal coloration has been rigorously studied and has provided morphological implications for fitness with influences over social behavior, predator–prey interactions, and sexual selection. In vertebrates, its study has developed our understanding across diverse fields ranging from behavior to molecular biology. In the search for underlying molecular mechanisms, many have taken advantage of pedigree-based and genome-wide association screens to reveal the genetic architecture responsible for pattern variation that occurs in early development. However, genetic differences do not provide a full picture of the dynamic changes in coloration that are most prevalent across vertebrates at the molecular level. Changes in coloration that occur in adulthood via phenotypic plasticity rely on various social, visual, and dietary cues independent of genetic variation. Here, I will review the contributions of pigment cell biology to animal color changes and recent studies describing their molecular underpinnings and function. In this regard, conserved epigenetic processes such as DNA methylation play a role in lending plasticity to gene regulation as it relates to chromatophore function. Lastly, I will present African cichlids as emerging models for the study of pigmentation and molecular plasticity for animal color changes. I posit that these processes, in a dialog with environmental stimuli, are important regulators of variation and the selective advantages that accompany a change in coloration for vertebrate animals.

Introduction

Animal colors and their variation play a critical role for adapting to a changing visual environment (Spaeth 1913; Bagnara et al. 1968; Price et al. 2008). Coloration can be as conspicuous as a peacock's tail feathers (Zi et al. 2003) or as cryptic as the graded coloration of field mice across environmental clines (Bedford and Hoekstra 2015). The study of animal pigmentation has been a cornerstone for several disciplines such as the study of genetics, cellular biology, and physiology. In genetics, pigmentation has facilitated the ease at which a phenotype can be screened such as the colors of fruit fly eyes (MORGAN 1911) or mosaic kernels of maize (McClintock 1950). However, our reliance on the mechanistic contributions of genes has also generated a blind spot regarding the importance of plasticity and their accompanying processes (Alvarado et al. 2014). While countless studies have elucidated the genetics of developmental pigmentation patterns or sexually determined pigmentation (Gazda et al. 2020), it often builds a narrative that such traits do not change over the lifetime of an animal since genes do not.

Undoubtedly, there is a genetic component in animal coloration and the biology of a developing embryo where genes can have the most pronounced effects on phenotype. In vertebrates, lineages of pigment cells migrate and proliferate from the neural crest where they generate progenitor pools of cells that migrate to dermal layers (Lapedriza et al. 2014). Pigmentation patterns in fish and mammals are subject to genetic mutations and have been essential for our current understanding of animal pigmentation and genetics (Johnson et al. 1995; Kelsh et al. 1996; Parichy et al. 2000; Frohnhofer et al. 2013;

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Cal et al. 2019). However, pigmentation patterns that rely on hereditary information do not take into account environmental stimuli that are also capable of shaping patterns of animal pigmentation. In nature, the examples of plasticity are various and provide fertile ground for improving our understanding of animal coloration and molecular plasticity. Several arctic animals such as the ptarmigan, ermine, and snowshoe hare demonstrate dramatic changes in coat color to become more cryptic in winter seasons (Ferreira et al. 2017, 2020; Zimova et al. 2018). In the snow shoe hare, *Lepus americanus*, such plasticity is built on cis-regulatory variation in the genome with phenotypic plasticity relying on environmentally coded information between seasons (Jones et al. 2018). Similarly, subtle changes in environment allow morphological color changes with background adaptation in various vertebrate fish (Masazumi 1993; Mizusawa et al. 2018). It simply stands to reason that if a given trait is reversible, its underlying molecular processes could also be reversible. While genes provide a blueprint for candidate regulators of various processes, they also require environmental interactions to shape their appropriate function in time and space.

Chromatophores: a cellular palette for coloration

While a variety of coloration patterns exist across the animal kingdom, in vertebrates, attention has focused primarily on pigment-containing stellate cells known as chromatophores. These cells function through transitions between dispersed and aggregated states. During dispersal, subcellular pigment granules are translocated throughout the cytoskeleton to distal processes or aggregated to the center of the chromatophore (Fingerman 1959). In each state, pigments are spread over tissues differentially leading to variation in coloration. The position of chromatophores across dermal layers and the overlapping of their stellate processes contribute to the coloration observed across a given tissue (Ligon and McCartney 2016). While neural and physiological changes can affect the range of color seen within a tissue, morphological changes shift the limits of its intensity or spread over a tissue. Pigment cells are further categorized based on their color, regardless of lineage or pigment biosynthesis. For example, red chromatophores are referred to as erythrophores. Similar considerations are given to orange/yellow xanthophores, black/brown melanophores, white leucophores, and reflective iridophores. Together these cells create a complex palette that forms the dermal chromatophore unit (DCU). Collectively the DCU considers several subtypes and their respective position between one another to describe tissue level coloration.

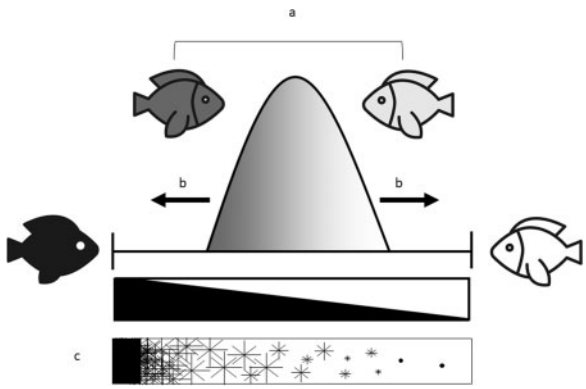

Fig. 1 Overview on the limits of morphological and physiological change. Coloration of an animal can be seen to occur rapidly over a narrow norm of reaction (**a**) or over a longer time (**b**) through changes in cellular substrates represented in (**c**).

Neural, morphological, and physiological roles in color change

In plastic traits, the reliance of static and dynamic molecular mechanisms depends largely on the time to change. For example, neural regulation (seconds to a minute) precede physiological (minutes to hours) and morphological (hours to days) changes to color, respectively. During rapid color changes, transient neural and physiological signals shape existing cellular infrastructure that leads to dramatic changes in color. Over time, signaling under these cues can cause robust changes to cellular function and composition driving more robust changes in color (Fig. 1). Ultimately, while some types of color change can be categorized as predominantly neural, physiological, or morphological they work integratively to generate a norm of reaction for phenotypic plasticity.

Neural control

Various visual and peripheral cues present an environment capable of shaping the function of chromatophore cells. With information primarily being processed through the visual axis, neural signaling between the retina, suprachiasmatic nuclei, thalamus, and peripheral nervous system mediate fast changes in color. The neural control and development of chromatophores is in part due to a shared lineage with neuronal cell substrates in the peripheral nervous system (Nüsslein-Volhard and Singh 2017). This neural control of coloration relies on the

innervation of chromatophore progenitor pools and their subsequent differentiation and cellular interaction and the direct modulation of dispersal/aggregation. For example, in murine models, overstimulation of the sympathetic nervous system can cause the migration of melanocytes to hair follicles thus leading to their depletion (Zhang et al. 2020). Once depleted, melanocytes are unable to deposit melanin in the hair follicle leading to a graying of fur. Interestingly, while yet to be connected to such neural processes in humans, the reversal of graying has also been naturally observed in humans along with a suite of proteomic changes, reinforcing its phenotypic plasticity at a molecular level (Rosenberg et al. 2020).

Similarly, some pigmentation patterns can also be under neural control in the social signals seen in African cichlids (Muske and Fernald 1987a). Specifically, innervation of the black eye bar is an important social signal displayed among dominant males that is constantly being suppressed by subordinate conspecifics. Lesions made to the maxillary nerve that innervates the eyebar leads to the immediate dispersion of melanophores that make up this pattern. In the golden mbuna, a dark and yellow morph also appears to be linked to the innervation of dermal layers. In a transcriptional screen between both morphs, several genes were related to synapse formation with scales in the dark morph being innervated 1.3–2 times more axonal fibers (Liang et al. 2020b).

Physiological control

In contrast, similar neural processes transition to endocrine control (i.e., via pituitary, pineal, and adrenal glands) and the systemic distribution of intermediate signals in the circulatory system, leading to color changes. For example, in the lightening or darkening of body color, various hormonal substrates can act on chromatophore receptor biology to mediate changes in coloration (Sköld et al. 2013) (Fig. 1a). One of the most studied neuroendocrine responses involves the melanocortin system which is involved in the regulation of most vertebrate melanophores. The secretion of melanocortins like melanin stimulating hormone (MSH), Agouti signaling peptide (ASIP), and adrenocorticotropic hormone (ACTH) work in concert to elicit effects of organismal lightening and darkening (Logan et al. 2006; Shiraki et al. 2010). Briefly, MCs/ACTH binds the melanocortin receptor (MC1R) on the surface of melanophores which in turn lead to increases of intracellular cAMP causing pigment granule translocation toward stellate processes causing dispersal and organismal darkening. In contrast, ASIP acts as an antagonist to MC1R reducing the effects of MCH/ACTH and contributing to aggregated states and organismal lightening (Dijkstra et al. 2017; Cal et al. 2019).

In red porgy, changes to background color during rearing can cause marked changes in plasma cortisol during crowding and variation in interrenal sensitivity to α-MSH and ACTH (Rotllant et al. 2003). In zebrafish, a loss of Agouti-signaling protein can lead to a loss of dorso-ventral gradients during development required for countershading (Cal et al. 2019). Other forms of physiological color change can also be linked to sexual behaviors in amphibians. For example, stony creek frogs can initiate the dispersal of xanthic chromatophores allowing a discrete change from brown to yellow in less than 5 min via epinephrine (Kindermann et al. 2014).

Morphological control

Following rapid changes, morphological control requires discrete changes in the density and function of cellular substrates which take significantly more time (Fig. 1b). As such it often requires the programming of molecular substrates via gene transcription. These changes can coincide with visual ecology, seasonality, or development. For example, zebrafish moved to a dark background will signal a physiological response that can cause an organismal darkening effect that is limited to its current suite of pigment cells (Logan et al. 2006; Shiraki et al. 2010) (Fig. 1c). Over time, morphological changes follow, causing an increase in melanophores which in turn can push the limits of how dark this individual can become. This often involves cellular reprogramming, differentiation, proliferation, or apoptosis suggesting regulation that must be reversible to accommodate change.

These changes have been extensively studied in the migration, proliferation, and interactions between chromatophores. For example, microphthalmia-associated transcription factor (MITF) and Wnt transcriptional regulation mediate several aspects of pigment synthesis and pigment cell fate from neural crest derived pigment progenitors (Widlund and Fisher 2003). Other interactions between different (Frohnhofer et al. 2013) and similar (Walderich et al. 2016) chromatophore cell types also determine the development of zebrafish stripes during development. In plastic coloration, many of these phenotypic changes are driven by robust changes in gene transcription within pigment cells and their processes (see Table 1) underlying various cellular

Table 1 Overview of recent transcriptome studies examining differences in pigmentation across vertebrate models and classification into tentative neural, physiological, and morphological contributions

			Differential transcription and putative role in			
Model	**Species**	**Study**	**Neural control**	**Physiological control**	**Morphological control**	**Reference**
Mammals	*Lepus americanus*	White-brown coat color transitions of snowshoe hare	GAL	ASIP	FGF10, RUNX1, TP63, RORB, NR1D1, KRT71, ID2, GFPT1, PPARGC1A, IGFBP5, PTGS2, PTCH2, HPSE, WNT10B, MYO7A	(Ferreira et al. 2017, 2020)
	Ovis aries	Bashibai (brown red), Yemule (white), and Tulufan (black) breeds of sheep			DCT, TYR, TYRP1, PMEL, SLC45A2, MLANA	(Yao et al. 2019)
	Neovison vison	Comparative differences between white and black morphs of American mink		EDN3	KITLG, LEF1, MITF, SOX10, ARCN1, PAX3, DCT, TYRP1, PMEL, LYST, OCA2, RAB38, MLPH, MYO5A, MYO7A, RAB27A, OSTM1, SOX18	(Song et al. 2017)
Avian	*Gallus gallus*	Comparative differences between white-black plumage in chicken		RGN, RYR2	HOXB9, CHRDL1, BKJ, CHAC1, GPX3, IRX5, NTN1, LAMA2, BMP5, STMN2	(Yu et al. 2018)
Fish	*Danio rerio*	Melanophore-iridophore transcriptional differences during larval development of zebrafish		EDNRB1	GCH2, MLPHB, KITA, PMELA, DCT, TYRP1B, ATIC, LTK, SOX10, FOXD3, SNAI2, PAX6A, NR2E1, MYO7AB	(Higdon et al. 2013)
	Amphilophus citrinellus	Yellow–brown body color transitions of the midas cichlid	IER2	STC1	TYR, TYRP1A/B, SLC24A5, PMELA, MREG, CXCL13/IL8, CX41.8, SLC6A15, RT1/2, RIMKA, FYNA, RASEF, PTGIS, ZBTB20, C-FOS, RTN3, JUN-B	(Henning et al. 2013)
	Astatotilapia burtoni *Cynotilapia pulpican* *Pseudocrenilabrus philander* *Callochromis macrops*	Comparative differences in anal fin spots in various cichlid species	NPYR1, TACR3	EDNRB, ADRB1, CALCRL, MC5R, ACKR3, GCGR	RAB38, PAX7, ALK, GPNMB, SOX9A, MITF, MATP, RGR, JUP, POSTN, EDIL3, CADM3	(Santos et al. 2016)
	Melanochromis auratus	Dark-yellow morphs of the Malawi golden cichlid	GPM6AB, SNAP25A, VSNL1A, NAPB, SNAP25B, SYT1A, NSFA, SNCB, SEZ6L2, NSG2		HSD3B1, TTC39B, PLIN6, MITFA, GCH2, FOXQ1A	(Liang et al. 2020)
	Lutjanus erythropterus	Black–red skin differences within individuals of red snapper		MC1R, ASIP, EDN3	TYRP1, DCT, MITFA, MLPH, FOXD3, PAX3A, RAB11, GK, WNT5A, MITFB	(Zhang et al. 2015)
	Pristella maxillaris	Comparative differences between black gray–silver-transparent morphs of tetra		ACT3, CASQ2, CKB, CKMT2, HRC, MYH2, TPM1-3	ACTA1, ACTN2, COL10A1, DHRS7CB, FHL1, HHATL, MYBPC3, MYH13, MYL4, TNNT3	(Yan et al. 2020)

(continued)

Table 1 Continued

Model	Species	Study	Differential transcription and putative role in: Neural control	Physiological control	Morphological control	Reference
	Salmo marmoratus *Salmo trutta*	Comparative differences between marble-brown morphs of trout	GJA9, GJB1, GJD, TJP1	MC1R, EDNRB	DCT, MITF, PMEL, SOX10, CDKN1A, WNT10A, TJP1, SLC7A2	(Djurdjevič et al. 2019)
	Oreochromis spp.	Comparative differences between pink-pink/red-white morphs of tilapia		EGFR	TYRP1B, SFRP3/5, TYR, MAPK14A, ERBB3, SOX10, SLC24A5, PMEL, RAB11B, SLC45A2, SLC7A11, FGFR1, SOX10, CBS, RYK, FER, MAPK8IP1, HSP70	(ZHu et al. 2016)
Amphibian	*Dendrobates auratus*	Comparative differences between green, blue, gold, white morphs of poison dart frogs		DIO2, EDNRB, EGFR,	ADAM17, ARFGAP1/3, AIRC, ATIC, ATOX1, ATP12A, BBS2/5, BMPR1B, BRCA1. CTR9, DERA, DTNBP1, FBXW4, GART, GAS1, GNE, HPS3, ITGB1, LEF1, LEO1, MITF, MLPH, MTHFD1, MREG, NOTCH1, PRTFDC1, QDPR, QNR-71, RAB3D/7A, RABGGTA, SCARB2, SHROOM2, SOX9, TBX15, TYRP1, XDH	(Stuckert et al. 2019)

Neural changes were categorized on involvement with synaptic or electrical junctions. Physiological changes were categorized based on receptor signaling via calcium, adrenergic, endothelin, melanocortin, and extracellular signaling. Morphological changes were categorized based off of transcriptional signaling, cellular trafficking, metabolism, and organellar function.

functions. Such morphological changes are the most pronounced in mammalian vertebrates (Table 1) where the deposition of melanin granules from melanocytes to keratinocytes allows the darkening of hair follicles.

Transcriptional regulation of chromatophores across neural, physiological, and morphological substrates

Across neural, physiological, and morphological layers, these substrates depend heavily on the transcriptomic suite of molecular changes that prepare an animal for a change in color. For example, the increased density of a given receptor on a chromatophore will also lead to sensitization to endogenous endocrine signals. Considering that chromatophore receptor biology is not restricted solely to the physiological control of the melanocortin system, endothelin (Murata and Fujii 2000; Parichy et al. 2000; Regazzetti et al. 2015) and adrenergic systems (Hadley and Goldman 1970; Taylor and Teague 1976; Morishita et al. 1985; Ryozo et al. 1985) may also contribute to chromatophore subtype aggregation and dispersal. As a result of this complex regulation, the systemic distribution of endocrine signals provides an effective means to generate phenotypic diversity across diverse chromatophore types. For example, two chromatophores may express varying suites of receptors that allow them to differentially aggregate and disperse to the same hormone. This can lead to phenotypic diversity and the formation of patterns throughout the organism dependent on centralized signaling (Mizusawa et al. 2018) and chromatophore-to-chromatophore interactions (Frohnhofer et al. 2013).

If we hone into the molecular function within chromatophore cells, we begin to reorient our focus on the molecular substrates underlying biological traits, genes. Genes and their transcribed products form our current basis for understanding trait variation and are often conflated as one molecular process. It is important to draw the distinction between genetic variation which is static over the lifetime of an animal and gene transcription which changes between cells and environmental niches. The latter has been more helpful in understanding plasticity whereas the former aids in understanding population level variation that is relevant over longer evolutionary timescales. In the study of plasticity, transcriptomic screens continue to reaffirm the relevance of

canonical regulators of animal pigmentation such as the melanocortin, adrenergic, and endothelin signaling systems and their accompanying ligands, receptors, and catabolizing enzymes. In addition to physiological signaling, other cellular pathways related to pigment biosynthesis, transcriptional regulation, and cytoskeletal function continue to play their respective roles (Table 1).

Transcriptomic screens done in red snapper (Zhang et al. 2015) and an African cichlid (Santos et al. 2016) have revealed the role of endothelin signaling for xanthic coloration consistent with previous physiological studies (Murata and Fujii 2000). The physiological control of chromatophore signaling, however, is different from roles in early development where pigment cell progenitors signal migration from the neural tube (Lee et al. 2003; Square et al. 2016). In chromatophores, endothelin ligands (ET1–3) produced within the pituitary/skin are released to bind their corresponding G-protein coupled receptor (EdnRA/B) where intracellular signaling leads to changes in intracellular phosphate leading to pigment aggregation. In fish, this leads to a masking of yellow/orange/red coloration in relevant chromatophores. For example, in the orange/yellow fin spots of *Astatotilapia burtoni*, the expression of EdnRB would mediate control over the intensity of this color (Santos et al. 2016) whereas dorsoventral xanthic coloration countershading appears to be in part mediated by marked differences in endothelin ligand expression in red snapper (Zhang et al. 2015). However, endothelin signaling is not solely restricted to control over xanthic cell types since genetic variation of endothelin receptors in fowl (Kinoshita et al. 2014), horses (Bellone 2010), and humans (Edery et al. 1996; Lapedriza et al. 2014) also have pronounced effects on melanophore patterns (see also Table 1).

In the polymorphic midas cichlid, the transcriptomes of brown, intermediate, and yellow male morphs highlight several pathways committed to regulating melanin production (Henning et al. 2013). In this study, coloration focused on an increase or decrease in melanophores underlying morphological variation collected from scales. Genes related to melanin and melanophore biosynthesis were shown to be overexpressed in brown morphs such as tyrosinase related protein 1a (TYRP1a), pre melanosomal protein a (PMEL), and melanoregulin (MREG). Together, TYRP1a is involved with the biosynthesis of melanin and PMEL/MREG facilitate the development and transport of melanosomes in melanophores. While this study sampled tissues laterally across a population that displayed variation in pigmentation, it may not necessarily be indicative of developmental plasticity since yellow morphs may have been the result of genetic differences within the population.

In mammals, the melanocortin system helps resolve our understanding of phenotypic plasticity initiated by seasonal change. In a study by Ferreira et al., the transcriptional landscape of the snowshoe hare was studied during white/brown coat transitions in the arctic tundra. Within their screen, genes such as ASIP were downregulated in brown animals supporting their role in antagonizing melanocortin receptors, stimulating melanin production, and deposition into hair follicles. While several other genes were shown to change, additional pathways related to circadian rhythms and metabolic shifts identified functions independent of processes directly related to animal pigmentation. Ultimately, this phenotypic plasticity is afforded by gene-by-environment interactions within an individual due to a complete loss of fur and transitional morphs between seasons. This suggests a phenotype that is not entirely genetically determined, but the result of seasonal variation that is shaping transcriptional programs.

Molecular plasticity in pigment-bearing cells

There is a necessary integration of various layers of biological function from genes, cellular function, and physiology that each play a role in how pigmentation changes in a dynamic environment. Additional challenges present themselves in the thoughtful interpretation of transcriptomic screens which often reaffirm canonical pathways involved in pigmentation (Table 1). This creates an overly conservative interpretation of the molecular underpinnings of plasticity and pigmentation. Even genetic variation over evolutionary time occurs at a higher frequency outside of the gene body, underlining the important intersection of cis-regulatory gene regulation and variation (Stone and Wray 2001). This begs for a more holistic and inclusive consideration of molecular processes that change independent of fixed genetic substrates (Laland et al. 2014).

Genes are subject to an intricate concert of transcription factors that regulate gene expression and function. Various environmental stimuli ranging from temperature, ambient light, and hormonal cues mediate diverse signaling pathways that converge on transcription (Hunter 2005). Most importantly, unlike genes themselves, these processes have reversible biochemistry. DNA methylation and histone post translational modifications are among the

most rigorously studied processes that lend plasticity to gene function. These processes have also been foundational to the study of epigenetics, a field that studies how a gene's function can change without changes to genetic sequence (Allis and Jenuwein 2016). Fundamentally, epigenetic regulation relies on the accessibility of regulatory machinery to loci where open and closed chromatin conformation shapes gene function. Genetic organization occurs on chromosomes following several degrees of compaction through protein–protein and protein–DNA interactions. These collective interactions act on promoters and enhancers to regulate transcriptional activity.

For example, the transcription factor MITF is a well established regulator of pigment cell fate that can also be subject to regulation via epigenetic mechanisms. In various melanocyte and keratinocyte lineages, genome-wide mapping of DNA methylation have revealed the cis-regulatory regulation of MITF transcription (Lauss et al. 2015). However, MITF is not only the subject of epigenetic regulation but is also capable of initiating genome wide regulation of histone posttranslational modifications. In zebrafish, MITF has been shown to activate carbonic anhydrase 14 which in turn increases intracellular pH and activation of the histone acetyltransferase p300/CBP. This leads to H3K27 acetylation of downstream MITF activated genes amplifying their expression (Raja et al. 2020). Additionally, integrative analysis of transcription and DNA methylation in the hair follicles of cashmere goats has shown a role for DNA methylation in the regulation of Wnt-signaled genes along with long non-coding RNAs (Wang et al. 2020). This regulation is not limited to function within the nucleus as the expression of microRNAs is also capable of mediating variation in color morphs. Specifically, mir-137 can exert repressive control over MITF and its downstream targets TYR and TYRP1/2 causing differences between black and brown morphs in murine models (Dong et al. 2012). MicroRNAs such as mir-141-3p and mir-200a-3p also target MITF and subsequent melanin production leading to its repression (Itoh et al. 2020).

One notable study by Waterland and Jirtle (2003) demonstrated that *in utero* administration of a methyl donor, folate, can result in darker coat colors in mice. Mechanistically, the supplementation of a methyl donor increased promoter methylation of the Agouti/ASIP gene which acts as an antagonist to MSH/ACTH binding. Hypermethylation of this gene reduces its transcription and subsequent antagonism of the MC1R allowing endogenous control of the melanocortin system and subsequent melanism deposition in hair follicles. While remaining one of the most compelling studies supporting the role of DNA methylation and pigmentation, this study became seminal for highlighting the importance of maternal diet and its effects on offspring.

Currently, the mapping of DNA methylation in relation to pigmentation is relatively novel. In zebrafish, candidate regulation of a cis-regulatory region of the ASIP peptide was under the control of DNA methylation following pharmacologically induced hypomethylation *in vivo* (Laura et al. 2014). Similarly, in a follow up to previous analysis of skin color pigmentation in carp (Yan et al. 2020), methylomes provided additional regulatory information with promoter hypermethylation/hypomethylation. This screen described hypermethylation of the gene tyrosinase, a promoter of melanin synthesis, to be repressed in red versus dark morphs of carp. This marked difference in transcription and protein abundance was inversely proportional to DNA methylation within a putative promoter of the gene (Zhang et al. 2017).

Other plastic mechanisms such as RNA editing (Rosenthal 2015) or RNA binding proteins (Mukherjee et al. 2011) are capable of modulating transcript stability, abundance, and recoding after it has left the nucleus. This requisites the study of morphological changes in pigmentation within the individual longitudinally or the adoption of models where robust changes in coloration occur in a cyclical and predictable manner.

Consideration for plastic molecular processes such as DNA methylation will complement existing paradigms in the study of complex traits and gene function. For example, animal pigmentation can be a continuous phenotype triggered by various environmental cues (San-Jose and Roulin 2017). Classically, such phenomena were best described by the additive contribution of genetic variation at quantitative trait loci across the genome (Matthysse et al. 1979). Simply put, the more graded a trait is, the more genetic variation must be present in trait-related biosynthetic pathways. However, this provides a complex mechanism where a simpler process can be considered if DNA methylation is involved. In animal sizing, I have been able to show that continuous DNA methylation of a single conserved locus is just as important in generating a continuum of a trait as various mutations at quantitative trait loci (Alvarado et al. 2015a). Provided that phenotypic plasticity of animal pigmentation is subject to similar processes, one can speculate that quantitative DNA methylation at static loci provides a parsimonious explanation for continuous variation in animal coloration in

addition to describing discrete differences in pigmentation.

While no single molecular mechanism (genetic or epigenetic) can represent the breadth of phenotypic plasticity seen in coloration, they can intersect to provide a population adaptive potential in changing environments. Ultimately, various epigenetic mechanisms rely on genetic substrates in cis-regulatory regions to provide heritable means for molecular plasticity (see Jones et al. 2018). This suggests that epigenetic processes present a probabilistic model via its dialog with environmental stimuli. I speculate that the interaction between environment–epigenetic–genetic factors itself can define a substrate that can be selected on and inherited via sustained interaction (or washed out by a lack thereof). For a deeper examination of inherited gene regulation see Adrian-Kalchhauser et al. (2020).

Considerations for the study of pigment-related molecular plasticity

As a result of the current approach to sequencing whole tissues, the use of transcriptional screens provides a bird's eye view of putative contributions to pigmentation. Many of these studies take whole tissues that are heterogeneous in chromatophores and other cell types. While necessary in phenotyping the DCU, it presents a challenge in the study of gene function and transcription which is unique to individual cell types. The DCU, as a heterogeneous population of cells, is subject to subtle molecular differences between chromatophores that define their discrete phenotypic differences. For example, a red erythrophore and yellow xanthophore with similar transcriptomes will have subtle differences in pigment synthesis and/or receptor biology but robust contributions to phenotypic coloring. This reduces tentative interpretation of transcriptomic findings and their relevant biology and cellular function thus requiring rigorous empirical testing and validation.

To circumvent some of these confounds, Higdon et al. (2013) have used cell sorting technology to separate iridescent and melanic chromatophores using innate chromatic properties in developing zebrafish larvae. This allows the collection of chromatophore cell populations of specific colors, but also their quantification and a robust assessment of morphological change. Furthermore, this approach allows the collection of chromatophores for comparative analyses that are unrestricted by immunological resources and tools. Additionally, whole genome coexpression network associations may also allow the *in silico* deconvolution of cell heterogeneity and the putative identification of cell type expression patterns (Monaco et al. 2018). Ultimately, the nascent field of single cell sequencing (Shapiro et al. 2013) and the promise of *in toto* workflows may 1 day solve many of these concerns but have several caveats to overcome before wide adoption (Lähnemann et al. 2020).

The additional emergence of novel sequencing platforms such as the PacBio Single Molecule Real Time (SMRT) and Oxford Nanopore Technologies (ONT) have also provided workflows for measuring DNA methylation and hydroxymethylation *in situ* during sequencing reactions (Gouil and Keniry 2019). This reduces biases often seen in other methylation mapping approaches such as bisulfite mapping (Olova et al. 2018) while allowing the measurement of additional modifications such as hydroxymethylcytosine, 6-methyladenosine, and possibly other unknown modifications in the genome. Lastly, advances in computer vision for visual ecologists have afforded a cheap comprehensive view of animal coloration with multispectral imaging, color quantification, and pattern extraction (Belleghem et al. 2018; van den Berg et al. 2020).

African cichlids as emerging models in the study of phenotypic plasticity and pigmentation

In order to best study the diverse effects of the environment on the developmental plasticity of pigmentation, it is important to adopt model systems (and families) with natural phenotypic diversity and adaptive plasticity. The adaptive speciation of African cichlids has afforded us a rich palette of colors that are particularly important for their evolution (Maan and Sefc 2013; Kratochwil et al. 2019). For example, study of cichlid lineages with and without horizontal stripe patterning has revealed cis regulatory regions in the control of Agouti related peptide 2 and convergent evolution of horizontal stripes (Kratochwil et al. 2018). Furthermore, candidate screens in the developing *Haplochromis latifasciatus* show developmental differences in gene expression that can be attributed to molecular plasticity during cell differentiation (Liang et al. 2020a). Similarly, in the Malawi cichlids, the orange blotch pattern is regulated by a cis-regulatory mutations to *Pax7* and plays a role in sexual conflict by promoting female crypsis while disrupting male nuptial coloration.

Interestingly, as a family, their phenotypic diversity is not entirely reflected in their genetic diversity

(Braw et al. 2015; Malinsky et al. 2018), suggesting an emerging role for epigenetic plasticity. Cichlid fish have a robust DNA methylation machinery (Hilliard et al. 2019) that is responsive to pharmacological manipulation (Lenkov et al. 2015) and bear various pigmentation patterns that change in response to social cues (Alvarado et al. 2015b). Specifically, the cichlid *A. burtoni* has genomic (Braw et al. 2015) and proteomic (Hu et al. 2016) resources along with reversible pigmentation marks that are controllable through social and visual cues (Fernald and Hirata 1979; Muske and Fernald 1987a, 1987b; Korzan and Fernald 2007; Korzan et al. 2008).

Cichlid coloration is most dramatic within the context of nuptial coloration among males and is believed to affect male–male competition and sexual selection from females (Maan and Sefc 2013). Various social behaviors among males involve physiological and morphological control over chromatophore intensity. In *A. burtoni*, yellow and blue morphs occur naturally in the lab and in the wild and provides a fighting advantage for yellow males competing for territory and mating opportunities over blue males (Korzan and Fernald 2007; Korzan et al. 2008; Border et al. 2019). Physiologically, yellow–blue coloration seems to be in part regulated by the melanocortin system since alpha melanocyte stimulating hormone was capable of stimulating yellow xanthophore dispersal in a dose dependent manner (Dijkstra et al. 2017). While a majority of literature examining body coloration focus primarily on social behavior, the visual ecology and algal blooms in lake Tanganyika itself (Horion et al. 2010) could possibly be playing into their social life history. Specifically, increases in algal phytoplankton could provide nutrient rich resources that would reduce the need for territorial behavior maintained by more aggressive yellow males while providing a cryptic advantage to blue males (Fig. 2b, c).

Social status of *A. burtoni* males also provides various other reversible changes to pigmentation that are evident in specific body markings (Fernald and Hirata 1979). For example, dominant males bear a robust black eye bar that is under neurophysiological control of the maxillary nerve (Muske and Fernald 1987a). Similarly, socially dominant males bear a red humeral blotch and melanism along the throat and pectoral fins that can change morphologically compared with subordinate males (Fig. 2). Provided that social status is a reversible state in these animals (Maruska and Fernald 2010; Maruska et al. 2013), the accompanying pigmentation patterns are also reversible and change over the course of 2–4 weeks.

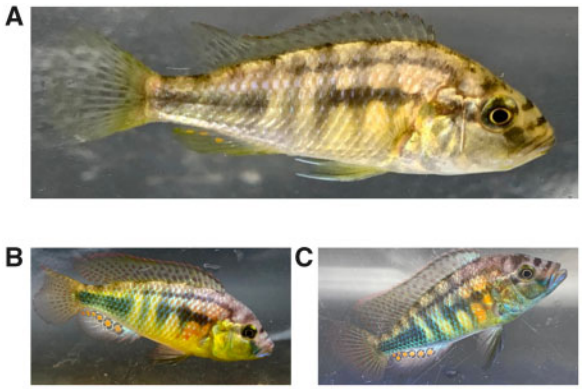

Fig. 2 Overview of the phenotypic plasticity seen in *Astatotilapia burtoni*. (**a**) A subordinate male with no visible nuptial coloration, (**b**) a yellow male, and (**c**) a blue male. Parts (b) and (c) show visible humeral blotch behind their operculum, darkened pectoral fins, and bright anal fin spots.

Furthermore, the presence of anal fin spots has been studied as an important contributions to fitness and male–female interactions (Theis et al. 2012; Santos et al. 2016). These egg spots are also subject to what could be developmental bias or plasticity based off of studies describing larger and more conspicuous egg spots in riverine tribes of *A. burtoni* (Theis et al. 2017). This suggests that water turbidity and visual ecology may drive conspicuous visibility of egg spots as social signals.

Cichlid fish are emerging as powerful model systems for the study of epigenetic processes due to their easy to manipulate phenotypes. As a model family several cichlids, specifically those that mouthbrood, are further tractable for several genetic manipulations. *Astatotilapia burtoni* and *H. latifasciatus* have both been successfully modified to carry transgenes or undergo genetic editing by CRISPR platforms (Juntti et al. 2013; Kratochwil et al. 2018). Transient knockdowns of gene expression are also achievable through the adoption of electroporation workflows that have been applied to fin tissues zebrafish (Hyde et al. 2012; Gosse et al. 2017). These advances in transcriptional regulation *in vivo* continue to revolutionize our approach to the study of complex traits and may eventually include epigenome editing platforms capable of modifying single gene cell specific epigenomic substrates *in vivo* (Liu et al. 2016).

Concluding remarks

The prolific characterization of gene transcription and pigmentation in the past several decades has laid the foundations of various candidate pathways that may be regulated by epigenetic processes. Here,

I presented evidence for DNA methylation, histone modifications, and microRNAs as a plastic regulators of transcription and briefly suggested roles for post-transcriptional regulation. Integration between higher order chromatin structures, histone posttranslational modification, and long non-coding RNAs can have additional profound effects on gene function and pigmentation. I anticipate that tracing changes in chromatophores will shed light on the plasticity underlying the control of pigmentation during color transitions. Additional consideration of neurophysiological regulation will complement our understanding of chromatophore biology in the periphery. For example, which centralized circuits within the visual axis descend into chromatophores in the periphery? How does this neural activity modulate exocrine and paracrine signals that initiate signal transduction in chromatophores? How long do these signals take to result in epigenetic changes in gene expression? Do such changes require sustained external cues or cascades of signaling between chromatophores in the periphery? I anticipate that answers to these questions will be broadly enlightening to our understanding of animal coloration, visual ecology, and molecular plasticity.

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Conflict of Interest

The author declares no conflicts of financial interest.

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