Linking color polymorphism maintenance and speciation

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Here, we review the recently burgeoning literature on color polymorphisms, seeking to integrate studies of the maintenance of genetic variation and the evolution of reproductive isolation. Our survey reveals that several mechanisms, some operating between populations and others within them, can contribute to both color polymorphism persistence and speciation. As expected, divergent selection clearly can couple with gene flow to maintain color polymorphism and mediate speciation. More surprisingly, recent evidence suggests that diverse forms of within-population sexual selection can generate negative frequency dependence and initiate reproductive isolation. These findings deserve additional study, particularly concerning the roles of heterogeneous visual environments and correlational selection. Finally, comparative studies and more comprehensive approaches are required to elucidate when color polymorphism evolves, persists, or leads to speciation.

Introduction

Ever since Huxley [1], color polymorphism (CP) has been defined as the presence of two or more distinct, genetically determined color morphs within a single interbreeding population, the rarest of which is too frequent to be solely the result of recurrent mutation. A recent resurgence in CP research has focused on two areas fundamental to evolutionary biology: the study of the processes maintaining genetic variation in nature and the study of speciation. Here we review the literatures of these two fields, seeking in particular to identify insights arising from the joint consideration of these topics and to highlight areas ripe for further research. We begin by considering processes operating between populations, including differences in the visual environment that could influence both natural and sexual selection. We then consider processes that might act entirely within populations to maintain CP; these are of particular interest because they could contribute to sympatric speciation by sexual selection, a controversial process [2,3]. We focus our attention on those systems that have been studied the most thoroughly, and especially those in which both CP persistence and the possible contribution of CP to the evolution of reproductive isolation have been investigated (e.g. Figure 1, Table 1). Although plants also provide many interesting examples of CP, we limit our survey to animals.

Between-population processes

Regardless of the evolutionary forces acting within a population (e.g. random genetic drift or directional selection), we expect that gene flow (see Glossary) between divergent populations will have a nearly ubiquitous role in determining the degree to which populations diverge. Here we address two issues: (i) the contribution of non-adaptive processes, genetic drift and gene flow, to CP maintenance; and (ii) the selective pressures involved in divergence in color morph frequencies between populations, particularly the scale and consistency of the local visual environment and how these factors interact with gene flow to mediate CP persistence and speciation.

Glossary

Anti-apostatic selection: positive frequency-dependent selection by predators such that rare prey suffer higher predation; often found in aposematic mimicry systems.

Aposematism: warning coloration that advertises unpalatability of prey to predators.

Apostatic selection: negative frequency-dependent selection by predators such that rare prey suffer less predation than common prey.

Correlational selection: non-linear selection in which multiple traits have an interactive effect on fitness; specific suites of traits are favored over others.

Density-dependent selection: the case where the fitness of an allele (or trait) in the population depends upon its density.

Disruptive selection: selection where extremes of a phenotypic distribution are favored over intermediate phenotypes within a population.

Divergent selection: the case where different phenotypes are favored over intermediates in different environments and populations.

Frequency-dependent selection: selection where the fitness of an allele depends upon its frequency in the population; selection favoring the rare allele is negative frequency-dependent, selection favoring the common allele is positive frequency-dependent.

Gene flow: the movement of alleles between populations via migration and subsequent survival and successful reproduction between immigrants and non-immigrants.

Greenbeard: A gene or linkage group that can produce a signal, detect the signal in others and direct benefit to others possessing that signal.

Heterosis: the case where individuals heterozygous for an allele have a fitness advantage compared with homozygotes (also known as heterozygote advantage or over-dominance); it can be associated with disassortative mating between homozygotes.

Intra-locus sexual conflict: antagonism between genes at the same locus in the two sexes.

Inter-locus sexual conflict: antagonism between male and female phenotypes encoded by alleles at different loci in each sex.

Mimicry ring: a group of sympatric species with a common mimetic pattern.

Müllerian mimicry: two or more unpalatable species that converge on the same warning color pattern.

Rare-male effect: negative frequency-dependent sexual selection favoring rare male morphs over common ones (often via female mate choice).

Sexual conflict: the situation where characteristics that enhance the reproductive success of one sex reduce the fitness of the other.

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Genetic drift and gene flow

In a few well-studied cases, there appears to be a role for random genetic drift in maintaining CP [4], despite the fact that random genetic drift within populations should lead to the fixation of one color morph. We expect drift to maintain CP only in conjunction with other evolutionary forces (e.g. frequency-dependent selection, temporal variation in selection, or gene flow) acting on the population; consequently, drift might be difficult to detect (e.g. [5]). The 'locus comparison approach' [4] compares genetic variation between genes involved in CP and putatively neutral genetic markers, and has suggested that drift, rather than selection, is maintaining CP in the case of the northern leopard frog *Rana pipiens* [4]. Perhaps most interesting, however, is the finding that genetic drift can intermittently influence morph frequencies if the strength of selection varies temporally [4,6,7]. Oxford [7] proposes that when the frequency of one morph (*redimita*) of the candy-stripe spider *Enoplognatha ovata* is low (between ~ 0.05 and 0.3), weak selection operates and drift appears to mediate morph frequencies across generally small populations. By contrast, strong selection and possibly gene flow are thought to protect CP when a perturbation changes morph frequencies, although the agent of selection is not known. More long-term studies that test for a genetic signature of selection, both between and within populations, are needed to help determine the relative contribution of drift to the maintenance of CP in a larger sample of CP systems [4,6].

Divergent selection and gene flow

The terms divergent and disruptive selection are often used interchangeably in the CP literature; however, we distinguish them to clarify how each might contribute to CP evolution. Divergent selection occurs between two environments, each experienced by different populations [8], whereas disruptive selection in the generic sense describes selection for extreme phenotypes over intermediate forms within a single population [9]. The scale of environmental variation is important, in that we expect divergent selection when individuals generally experience only one environment (broad-scale variation), whereas we expect disruptive selection if individuals experience multiple environments or 'microhabitats' (fine-scale variation). Unfortunately, the associated line in nature is seldom so easy to draw, especially along environmental gradients.

Divergent selection in different visual environments could favor one color type while gene flow might simultaneously enable alternative color types to persist within the same populations [10–12]. Environment-contingent natural and sexual selection, the processes whereby morphs experience differential fitness dependent upon the environment (Figure 2) [13,14], should favor alternative morphs in alternative light environments. Distinct visual environments should have a role in promoting CP, depending on the consistency (or predictability) of environmental variation [14–17]. In general, consistent variation and selection are more likely to lead to divergence.

One of the best emerging examples of CP maintained by divergent natural selection and gene flow between alternative habitats is the convergent evolution of blanched versus dark dorsal coloration in the lizards of the White Sands Ecotone [18]. Distinct light and dark habitats select for crypsis via predation. Three lizard species have each evolved two genetic color morphs (i.e. polymorphism in the gene encoding melanocortin-1 receptor (*Mc1r*) [19]). In a set of elegant tests, Rosenblum [18] showed that the degree of CP (i.e. the amount of phenotypic divergence) is directly related to the level of gene flow between the habitats based on species-specific population structure: *Holbrookia maculata* is distributed patchily and shows the greatest amount of phenotypic and genetic divergence relative to *Sceloporus undulatus* and *Aspidoscelis inomata*, which range continuously throughout the habitats. Striking similarities in light and dark CPs (e.g. in the beach mouse *Peromyscus polionotus* [20] as well as in other mammals and birds [21,22]), coupled with variations at the gene encoding *Mc1r* [23], suggest that this pattern is common and that a balance between divergent selection and gene flow might act frequently to maintain CP.

In a less intuitive situation, multiple Müllerian mimic morphs can be maintained if multiple co-models are segregated spatially as separate mimicry rings, creating a mosaic of alternative selective environments [10,24–26]. *Heliconius* butterflies that face positive frequency-dependent selection in a given locality, but that can migrate between mimicry rings, provide the best example of this form of between-population morph persistence [10,26]. Where mimicry rings are separated on a larger geographic scale (e.g. in the sister species *Heliconius cydno galanthas* and *Heliconius pachinus*, which are separated by a mountain range), there is evidence for reproductive isolation in the form of male mating preference for wing color [25]. This finding supports the idea that the scale of environmental heterogeneity is a key factor determining when CP is maintained by divergent selection and gene flow, and when it can lead to speciation between populations.

The effect of gene flow on the evolution of reproductive isolation remains controversial, but theoretical analyses increasingly support the plausibility of reinforcement, in which matings between incipient species promote the evolution of reproductive isolation [27]. These issues have been studied in exceptional detail in the walking stick insect *Timema cristinae* (Figure 1g,h) [28,29]. Divergent selection on different host plants against less cryptic migrants, hybrids and migrant-native mating pairs makes a major contribution to reproductive isolation in this species [29,30]. Assortative mating is strongest at intermediate frequencies of migrants, when encounter rates are sufficient to promote reinforcement but not so great as to swamp divergence. Although color pattern is the most conspicuous phenotypic difference between populations of this species, it is not the basis of pre-mating isolation, where the best candidates at present are pheromones [30]. This finding suggests an important caution: even when color differences between incipient species are obvious, their role in reproductive isolation cannot be assumed automatically.

Sensory bias

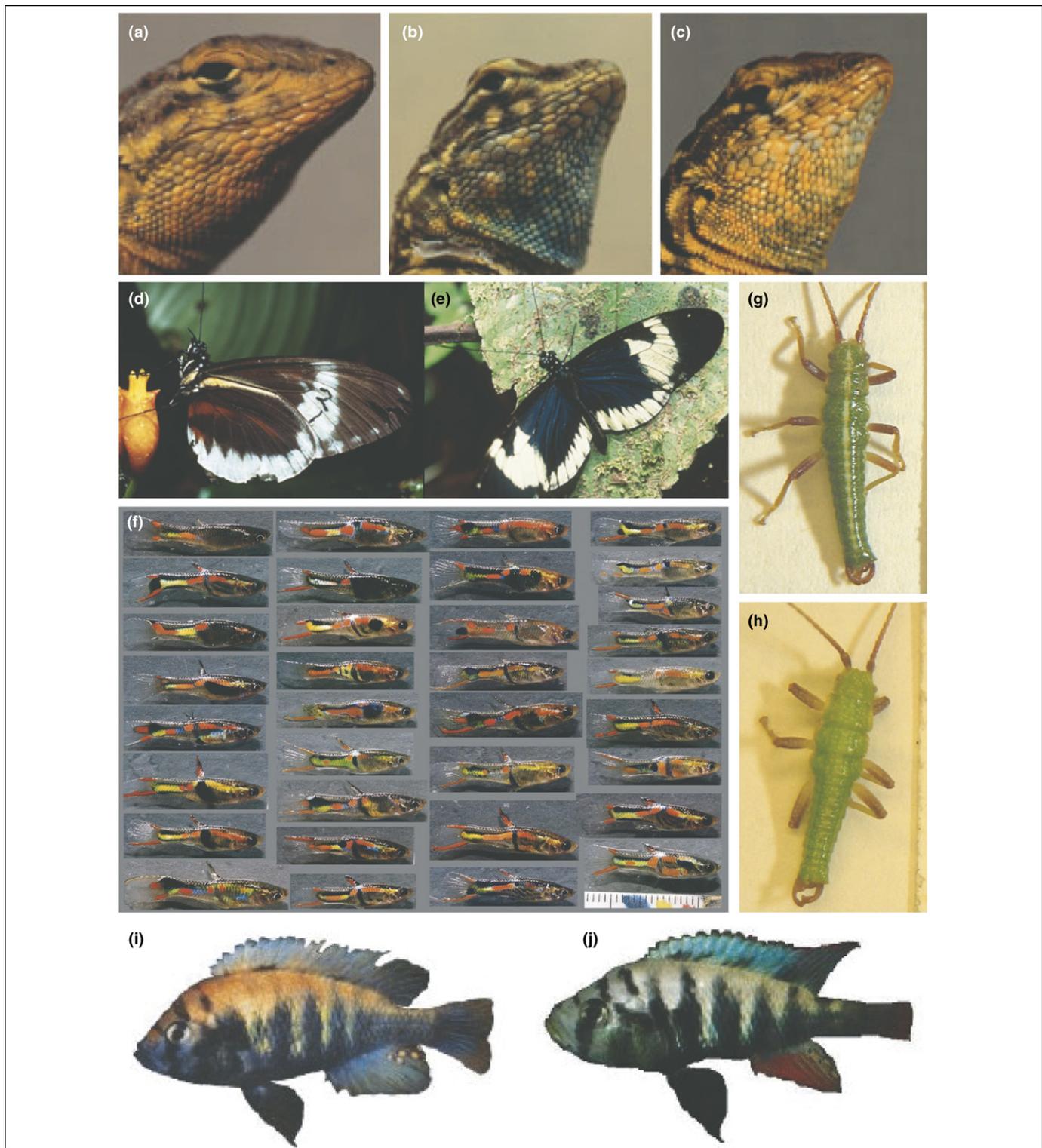Heterogeneous environments provide alternative visual habitats in which the visual systems of color-signal

Figure 1. Examples of model CP species. Three male color morphs of the side-blotched lizard *Uta stansburiana* play a cyclical 'rock–paper–scissors' game with correlated mating strategies: **(a)** aggressive orange males usurp the smaller territories of blue mate-guarders; **(b)** blue morphs defeat yellow sneakers by guarding mates; and **(c)** yellow sneakers defeat orange territory holders by cuckolding fertilizations [59]. This frequency-dependent male game is linked with the two-year cycle of two alternative female color morphs (and life-history strategies) whose frequency fluctuates with population density [73]. In Müllerian mimic butterflies, *Heliconius cydno*, **(d)** white and **(e)** yellow morphs are found in sympatry, probably as the result of multiple, sympatric mimicry rings acting as a heterogeneous environment in which anti-apostatic selection favors different morphs [10]. **(f)** The Cumaná guppy (geographical variant of *Poecilia reticulata* commonly known as Endler's livebearer) displays extreme variation in individual male coloration [76]. In walking stick insects *Timema cristinae*, **(g)** striped and **(h)** unstriped morphs are cryptic on alternative host plants and have a suite of reproductive isolating mechanisms, although some gene flow still occurs [28]. The sympatric sibling species of African cichlid *Pundamillia nyererei*, **(i)** red male, and *Pundamillia pundamillia*, **(j)** blue male are one of the best examples of sensory bias promoting color-assortative mating, which, when coupled with frequency-dependent male–male interactions, possibly leads to speciation [34]. Reproduced with permission from B. Sinervo (a–c); D. Kapan (d,e); H. Alexander/F. Breden, Ref. [76] (f); P. Nosil (g,h); and O. Seehausen (i,j).

Table 1. Representative examples[a] of model color-polymorphic systems and the processes promoting color polymorphism

Study system	Polymorphic sex(es)	Color polymorphism	Environmental variation[b]	Correlated trait(s)	Process(es) contributing reportedly to color polymorphism maintenance	CP or IS[c]	Refs
Invertebrates							
Candy-stripe spider *Enoplognatha ovata*	Both	Yellow, yellow with red dorsal stripes, and yellow with red dorsal surface	None	Unknown[d]	Intermittent natural selection and genetic drift	CP	[7]
Butterflies *Heliconius cydno galanthas* and *Heliconius pachinus*	Both	White, red, yellow and black patches on wings	Co-models in distinct geographic areas[e] (B)	Male mate preference (different color-pattern elements)	Divergent positive frequency-dependent selection, and pleiotropy between wing color and male preference for wing color (assortative mating)	IS	[25]
Blue-tailed damselfly *Ischnura elegans*	Female	Androchromes (blue male-mimic), olive green, and brownish	None	Female fecundity, thermoregulation and dispersal ability	Negative frequency-dependent sexual selection (male mate choice and sexual conflict)	CP	[5,66]
Reptiles							
Common lesser earless lizard *Holbrookia maculata*	Both	Blanched, and dark dorsal coloration	Gypsum dunes (white sand)/ Dark (B)	Unknown	Divergent natural selection (for crypsis) and gene flow	CP	[18]
Side-blotched lizard *Uta stansburiana*	Both	Orange, yellow and blue throat color (blue mainly in males)	None	Males: mate-guarding, territory size and sneaking; females: clutch size, egg size and immune function	Disruptive, frequency-dependent (cycling) correlational selection (for optimal character combinations)	CP	[59,73]
Fish							
Bluefin killifish *Lucania goodei*	Male	Yellow versus red/blue tail and anal fins	Tea-stained swamps and clear springs (B)	Visual sensitivity (?)	Sensory bias; environment-contingent sexual selection (?)	CP	[31,32]
Lake Victoria cichlid *Neochromis omnicaeruleus*	Both	'Plain' blue, white-blotched and orange-blotched	None (sympatric)	Color-linked sex-determination	Assortative mating (asymmetric); sexual conflict	CP/IS	[67,68]
Guppy *Poecilia reticulata*	Male	Orange, black and iridescent spots (individual variation)	Multiple (substrate, forest cover and predators, among others) (B/W)	Tail area and other color-pattern elements	Negative frequency-dependent predation and female mate choice; correlational, disruptive sexual selection (W); divergent selection and gene flow (B)	CP	[12,49,53, 70,75]
Birds							
White-throated sparrow *Zonotrichia albicollis*	Both	Tan and white	Unknown	Males: aggression, mate-guarding, parental care and extra-pair copulations; females: aggression and copulation-solicitation	Heterosis, disassortative mating and reproductive tradeoffs	CP	[45]
Mammals							
Rock pocket mouse *Chaetodipus intermedius*	Both	Light and dark	Light-colored rocks and lava flows (B)	Unknown	Divergent natural selection (predation favors crypsis) and asymmetrical gene flow between habitats	CP	[22]

[a]The examples here do not represent an exhaustive list of all systems in which CP has been studied, but rather a representative sample of relatively well studied cases.
[b]Environmental variation (that is known to be correlated with morph frequency, fitness or both) can be experienced between (B) and/or within (W) populations, although the distinction is difficult to make in some cases (e.g. across environmental gradients).
[c]CP, intraspecific color polymorphism; IS, incipient species.
[d]We use 'Unknown' to denote where no, or little, data exist.
[e]The co-models contributing to a mimicry ring create a geographic mosaic, and so whereas positive frequency-dependent predation acts within sympatric mimicry rings, movement within the mosaic helps maintain CP. Question marks (?) are used to denote cases in which evidence is ambiguous.
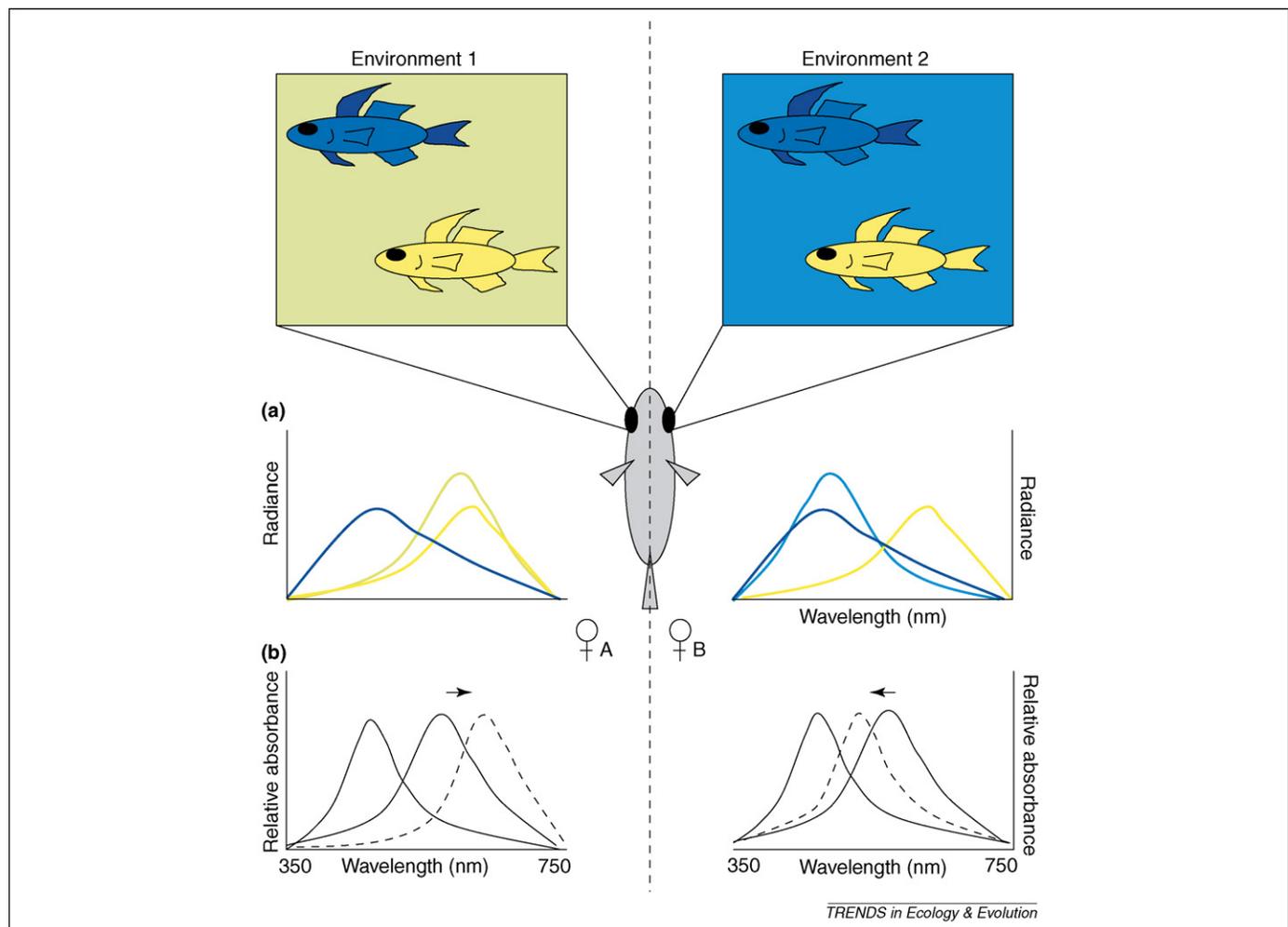
Figure 2. Visual ecology and a heterogeneous environment. Many organisms live in visually heterogeneous environments in which the visual background can change spatially and temporally. A color morph can therefore shift from being conspicuous to relatively cryptic depending upon the environment. Environments 1 [E1; **(a)**: green line] and 2 [E2; **(a)**: light-blue line] differ in visual properties (either spatially or temporally distinct habitats). The blue (B) and yellow (Y) morphs contrast differently with the background in each environment such that B [(a): dark-blue line] is more conspicuous in E1 and Y [(a): yellow line] in E2. The measurement of radiance (a) reveals a greater departure of the most conspicuous morphs from the background in each environment, whereas the measured spectrum for the more cryptic morph more closely matches the background spectrum. If the receiver (here, a female choosing a mate, although it could be a predator choosing prey) experiences both E1 and E2, her choice of mate might be environment-contingent. If the receiver experiences one environment only (e.g. there are two female populations or females are polymorphic within a population), the visual sensitivities of receivers might differ between environments because of divergent natural selection on visual systems. Even a small shift (e.g. a few nm, exaggerated here for illustrative purposes) in the long wavelength-sensitive opsins **(b)** can result in differences in the perception of a receiver that could affect her mating preferences substantially (i.e. sensory bias [14]). The arrows show the direction of the predicted shift in peak sensitivity in each environment, assuming selection favors sensitivity to the predominant background spectrum, with dotted spectra representing the derived state.

receivers are expected to be under natural selection [13,16,17,31–34] (Figure 2). We expect natural selection to often favor visual systems that match the local environment by shifting visual sensitivity to increase contrast (e.g. by shifting toward the predominant color of the environment; Figure 2b) [15]. The evolution of visual systems in this way can influence a female's perception of male color traits and hence her preference for those traits in a given environment, a process known as sensory bias [14]. For example, female visual sensitivity in threespine sticklebacks *Gasterosteus aculeatus* varies depending upon the degree of red-shift in the local light environment, with a corresponding shift in male nuptial coloration [13]. Thus, divergent sexual selection favoring different morphs in alternative light environments could be driven by sensory bias.

Theory predicts that the scale and consistency of environmental variation will influence the evolution of female visual systems (and consequently of mate preferences) and whether speciation or a stable CP should ultimately result. Consistent variation is more likely to lead to consistent selection and divergence, whereas unpredictable changes in the visual environment could favor flexibility in mate choice and the maintenance of CP [35]. The attenuation of light with depth in underwater environments provides an example of stable environmental variation for which some evidence shows divergent sensory biases and mate preferences in the incipient cichlid species *Pundamilia pundamilia* and *Pundamilia nyererei* [34]. Variation in cone opsin expression in bluefin killifish *Lucania goodei* (and perhaps many cichlids [36]) is determined both genetically and environmentally, suggesting that female preferences could vary as plastic responses to spatial and temporal fluctuations in the visual environment but also be subject to selective pressures [31].

Within-population processes

The non-overlapping phenotypic distributions that define CPs can result from genetic or developmental constraints, but are expected typically to be shaped by disruptive selection, which will often be accompanied by the negative frequency-dependent selection important to CP persistence within populations [9]. Both forms of selection can result from the same mechanisms if, for example, polymorphic female preferences lead simultaneously to poor mating success for intermediate males and greater success for the rarer morph. We consider these two forms of selection in turn. Genetic drift provides a non-adaptive alternative to these processes, but available evidence (see above) suggests that it typically has a secondary role to selection and it is not considered further here.

Disruptive selection

Evidence for the role of disruptive selection in CP evolution has been mainly indirect. In a comparative study of birds, for example, CP was associated with the use of both open and closed habitats and with daily rhythms extending across day and night [37]. Disruptive selection was thought to arise because different color patterns are cryptic in different lighting conditions. Of the few examples providing direct evidence of disruptive selection on color pattern within a species, one of the best is a study of sexual selection on yearling male lazuli buntings *Passerina amoena* [38]. Very dull and very conspicuous male buntings achieve greater mating success than intermediates, but color pattern variation is not discretely distributed. The paucity of direct evidence for disruptive selection on CPs might arise from a lack of appropriate studies or from weakened selection owing to previous evolutionary responses [9]. Another plausible explanation, however, is that non-linear selection frequently acts not on individual traits but on suites of characters through 'correlational selection' [39], with potentially important implications for speciation (Box 1).

Speciation might sometimes be driven by disruptive sexual selection on coloration, with cichlids providing possible examples (Table 1). Here, females of different incipient species often prefer males with different color patterns and assortative mating is reduced under narrowed ambient light spectra [40,41]. Recently, females have been shown to possess directional color preferences within a morph, thereby selecting for at least one extreme [34,42]; disruptive selection, however, has not yet been demonstrated directly. Studies of the passion-vine butterflies *Heliconius cydno* and *Heliconius melpomene* [43] and of sticklebacks [44] provide evidence of disruptive sexual selection against hybrid males, although not necessarily because of their intermediate color patterns.

Heterosis represents the opposite of disruptive selection because (genetically) intermediate forms are expected to be favored. It was considered historically to be a major process maintaining allelic diversity, including CP [17,31]; however, little recent evidence supports this possibility for CP systems (but see Ref. [45]).

Box 1. Disruptive correlational selection and CP evolution

A recent analysis suggests that disruptive selection (and non-linear selection generally) might often be correlational, involving suites of traits that do not appear to be under disruptive selection individually [39]. Documentation of disruptive correlational selection involving CPs raises the possibility that such selection might also be important in CP maintenance [46], particularly because CPs are often correlated with other characters (e.g. behavior, immune function, and other color traits; Table 1 in main text). In guppies, for example, three favored trait combinations occupy adaptive peaks and experience disruptive selection [70], whereas such selection was not detected in analyses of the individual traits [39].

The genetic mechanisms underlying correlations between CPs and other traits are now being elucidated, for example in *Heliconius* sp. Müllerian mimics. Here, linkage of some major color-pattern genes [71,72] leads to a genetic architecture minimizing production of individuals with mixed trait sets that mimic co-models poorly. In a more surprising result, *Heliconius* forewing color and male mate-preference genes both map to the regulatory gene '*wingless*' [25]. Consequently, natural selection for different color patterns in the young sister species *Heliconius cydno* and *Heliconius pachinus* would also cause evolution of corresponding mate preferences, thereby facilitating speciation. Consistent with this hypothesis, male preferences in a polymorphic population of *H. cydno* (Figure 1d,e in main text) (whose morphs experience divergent natural selection for Müllerian mimicry on different models [10]) are also assortative, at least for one morph; the second morph mates at random, possibly because most individuals are heterozygous at the mimicry locus [25]. The use of a single pigment in both color patterns and the eye is a potential mechanism for pattern-preference pleiotropy [25].

Correlations between CPs and other traits could increase the potential for CP to contribute to speciation generally, even when selection results mainly from social interactions as in the side-blotched lizard *Uta stansburiana* [60]. As frequency-dependent, disruptive correlational selection builds up co-adapted trait complexes in alternative sympatric color morphs, assortative mating should be favored to reduce the production of 'hybrids' with poorly adapted combinations of characters [73]. Moreover, as additional characters are added to a multi-trait CP, the probability that one will initiate positive assortative mating pleiotropically might increase (although other preferences might also appear).

Frequency-dependent selection

Frequency-dependent natural selection, whether relative or absolute (i.e. density [46]), has long been hypothesized to lead to CP maintenance within populations, particularly through predation [47,48]. A recent study of guppies provides the best experimental evidence yet obtained for a natural system in support of this hypothesis. Olendorf *et al.* [49] manipulated male morph frequencies in the field and found that rare morphs experienced elevated survival rates. The mechanism responsible for this apostatic selection is not known definitively, and recent work suggests that complexities of predator behavior might have important implications for CP persistence [48,50]. Nevertheless, laboratory studies using artificial prey provide considerable support for the straightforward hypothesis that predators form a search image for the most common prey types, making them less likely to eat rare morphs [48,51]. Such apostatic selection might not contribute directly to speciation, but it might contribute indirectly by favoring the persistence of multiple color morphs. This could, in turn, create conditions under which disruptive frequency-dependent selection on females, provided that males are in some respect limited, leads to a preference polymorphism and assortative mating [2].

Negative frequency-dependence can also arise through sexual selection. For males, several mechanisms that favor lower frequency morphs can lead to a 'rare-male effect'. In probably the best-known scenario, females shift their preference consistently toward whichever morph is uncommon or novel (e.g. as in guppies [52,53]), although this effect has been demonstrated only infrequently. Alternatively, female preferences can be polymorphic, with some females preferring one male morph and other individuals preferring a different morph. For example, individual females of the swordtail fish *Xiphophorus cortezi* strongly prefer males either with or without bar patterns [54]. Although heritable preference polymorphisms have rarely been confirmed directly in species with CP, extensive documentation of CP-based assortative mating in birds raises the possibility that such polymorphisms are widespread [17].

These two intersexual mechanisms of negative frequency-dependence have very different implications for speciation. Genetically-based female preference polymorphisms are key elements of sympatric speciation models and should facilitate the evolution of reproductive isolation [2]. By contrast, homogenous but shifting preferences for rare morphs would be expected to facilitate gene flow and prevent divergence, thereby slowing speciation. Phenotypically plastic preferences, possibly evolving as an adaptation to unpredictable environments (e.g. [55]), could have a similar effect.

Studies of divergent selection suggest additional possible mechanisms of frequency-dependent sexual selection. Microhabitat (fine-scale) or temporal variation in visual backgrounds could result in distinct visual 'niches' experienced by all morphs, but with each morph favored, for example as a result of high contrast, in one microhabitat (Figure 2). Overall, it appears that disruptive sexual selection and negative frequency-dependence could arise as a result, but a formal model is needed. Sensory bias could similarly have a within-population analogue, namely the evolution of polymorphic female visual sensitivity leading to polymorphic mate preference. Few studies have shown within-population polymorphic vision (e.g. as in spider monkeys *Ateles geoffroyi* [56] and guppies [57]), but advances in studying opsin expression and visual sensitivity should facilitate such research [33,58]. In future work, it will be important to look at causes of variation in sexual selection within populations and at the scale of variation in the light regime [16].

Recent studies have highlighted the role of intrasexual selection in maintaining male CPs. In the side-blotched lizard *Uta stansburiana*, a cyclical, 'rock-paper-scissors' game is played between the three male color morphs, each with their correlated mating strategy (Figure 1a–c) [59]. Remarkably, the different morphs also exhibit different patterns of settling and altruism. Some blue males with high genetic similarity across diverse elements of the genome establish adjacent territories disproportionately often and behave mutualistically and/or altruistically toward each other. Thus, color pattern might be one aspect of a 'greenbeard' genotype in this species [46,60]. One model suggests that such mechanisms could contribute to the evolution of reproductive isolation [61].

A different intrasexual mechanism might promote male CP in members of Lake Victoria's *Pundamilia* species complex of cichlids, in which males range from blue to red both within and between species (Figure 1i,j). Patterns of territory distribution and community assemblage suggest that, between distinct species with either blue- or red-colored males, higher aggression between males that share the same color pattern might mediate species coexistence [62]. Seehausen and Schluter [62] build on these and other results to develop a sympatric speciation model in which the territorial advantage enjoyed by a rare morph causes an increase in its frequency and a correlated increase in the frequency of rare females preferring such males, thereby initiating reproductive isolation. Male–male social interactions, which take place initially within species, ultimately become interspecific and, as long as such interactions persist, it should remain difficult for the alternative morph to reinvade either incipient species. Further support for this scenario comes from evidence of higher aggression by blue males, from relatively 'pure' populations and from reproductively isolated blue and red sister species, toward males of the same color. However, for partially isolated incipient species with different color patterns, stronger aggression toward same color males is not observed [63]. Further study of color and patterns of male–male interactions is clearly warranted in cichlids as well as other taxa, particularly those that are unequivocally polymorphic within a population.

Sexual conflict, in which the evolutionary interests of males and females differ (see Ref. [64]), has received much attention recently as a generator of both within-species diversity and speciation [65]. In the damselfly *Ischnura elegans* [5,66], rare female color morphs suffer less male harassment and have higher fecundity relative to common morphs, thereby contributing to CP maintenance. A comparison of inter-locus sexual conflict models has shown that CP maintenance is more probable than sympatric speciation in such cases because speciation would also require the male population to divide [3]. In cichlids, intra-locus conflict over offspring sex ratios, which are determined by a color-pattern-linked locus, might have contributed to male mating preferences and assortative mating [67]. The details of this scenario are complex, however, and empirical evidence for some elements of it is limited [68,69].

Conclusions

Our review reveals that several different mechanisms can contribute to CP maintenance and influence the evolution of reproductive isolation. In the most straightforward scenario, considerable evidence suggests that selection and, to a lesser extent, drift can cause divergence between populations, with the balance between selection and gene flow influencing the likelihood of speciation versus CP persistence. Environment-contingent sexual selection and sensory bias appear increasingly to be important in driving divergence.

More (and better) evidence is also accumulating in support of a diversity of within-population mechanisms of CP maintenance; however, we continue to have only a very limited understanding of the relative importance of

Box 2. Outstanding questions

What is the relative importance of between- versus within-population processes for CP maintenance?

When morph frequencies differ between adjacent populations, CP can be maintained primarily by gene flow or by within-population processes. Rarely, however, do we know which is more important.

Do CPs evolve mainly as a result of selection on the CPs themselves, on traits correlated with the CPs, or on suites of traits that include CPs?

Because the few studies that impinge on this question (e.g. Ref. [70]) have suggested that combinations of traits are sometimes the target of selection, it is essential that we increase our understanding of the correlation(s) between coloration and other traits. Genomic analyses will be central to elucidating the underlying genetics of color and associated phenotypic traits. Analyses of correlational selection and innovative experimental manipulations will be crucial to understanding the evolutionary significance of the genomic results. Such work could have important implications for understanding speciation.

What is the role of (micro) heterogeneous visual environments in maintaining CP and initiating speciation?

Differential color-pattern conspicuousness and different directions/patterns of selection in alternative visual environments have been demonstrated now, but we have limited evidence on how important such mechanisms are within populations. We also know little about whether sensory polymorphisms might be maintained by within-population processes and thereby contribute to CP persistence or initiate speciation. Both theoretical and experimental work are called for.

Among populations and species, what are the correlates of CP persistence, absence of CP, and CP as a transient stage in speciation?

Ultimately, comparative studies will be needed to test hypotheses of CP maintenance and speciation in the broader context of Ford's [47] questions about how genetic diversity is maintained in nature. Several analyses do this to some extent by examining relationships between CP, ecology and behavior in birds [17] and mating system in fish [74]. Future work on CP should strive to match and broaden this approach, at least in part through use of the comparative method.

each mechanism. In guppies for example, apostatic selection by predators, disruptive correlational selection, preferences for rare male morphs and sensory biases have all been implicated in CP maintenance, as well as divergent selection coupled with gene flow. However, we know little about which mechanism is the most important or how they might interact. Several of these mechanisms, together with intrasexual frequency-dependence, have also been implicated in the evolution of reproductive isolation and could contribute to sympatric speciation, thereby raising the plausibility of that controversial process.

A variety of studies are called for to better elucidate the processes that maintain CP and when these processes should contribute to speciation versus CP persistence (Box 2). Although considerable progress has been made since Huxley coined his definition, our understanding of CP maintenance and the role of CP in speciation remains fragmented. Even so, some of these fragments hold tremendous promise and rapid progress might be possible with a more integrated approach.

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