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## Sexual selection and genetic colour polymorphisms in animals

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### Abstract

Genetic colour polymorphisms are widespread across animals and often subjected to complex selection regimes. Traditionally, colour morphs were used as simple visual markers to measure allele frequency changes in nature, selection, population divergence and speciation. With advances in sequencing technology and analysis

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methods, several model systems are emerging where the molecular targets of selection are being described. Here we discuss recent studies on the genetics of sexually selected colour polymorphisms, aiming at (1) reviewing the evidence of sexual selection on colour polymorphisms, (2) highlighting the genetic architecture, molecular and developmental basis underlying phenotypic colour diversification and (3) discuss how the maintenance of such polymorphisms might be facilitated or constrained by these. Studies of the genetic architecture of colour polymorphism point towards the importance of tight clustering of colour loci with other trait loci, such as in the case of inversions and supergene structures. Other interesting findings include linkage between colour loci and mate preferences or sex determination, and the role of introgression and regulatory variation in fuelling polymorphisms. We highlight that more studies are needed that explicitly integrate fitness consequences of sexual selection on colour with the underlying molecular targets of colour to gain insights into the evolutionary consequences of sexual selection on polymorphism maintenance.

## Introduction

Genetic colour polymorphisms are ubiquitous in nature (Figure 1) and constitute ideal model systems to study evolutionary processes for several reasons. First, many colour morphs have a simple genetic basis and show high heritability. Second, discrete colour morphs can be scored unambiguously in a large number of individuals and thus provide easy visual markers for examining selection in the wild. The suitability of genetic colour polymorphisms as marker phenotypes did not go unnoticed, and they were popular study systems in the pre-molecular ecological genetics era (Ford 1945). By following morph frequencies in natural populations over multiple generations, scientists could easily observe genotype frequencies and thus infer allele frequency changes over time and space (e.g. Ford 1945; Kettlewell 1961). In this sense, studies of heritable colour polymorphisms are the field equivalent of classical laboratory *Drosophila* studies, in which visual eye marker phenotypes were used to study evolution. Several studies have employed this approach since and have shown that colour morphs can be exposed to strong natural and sexual selection over short time scales (Gosden & Svensson 2009; Hughes *et al.* 2013; Iserbyt *et al.* 2013), that colour morphs are often genetically correlated with other traits (reviewed in McKinnon & Pierotti 2010) and that the processes generating and maintaining polymorphisms can affect speciation rates and/or extinction rates, either positively or negatively (Hugall & Stuart-Fox 2012).

A general finding from these studies is that colour morphs are often affected by natural selection in terms of e.g. crypsis, camouflage, mimicry and aposematism, whereby the common morph is preyed upon more frequently than the rare morph. This causes negative frequency dependent selection (NFDS) where the rare morphs have a selective advantage (Ford 1945; Kettlewell 1961). Yet another finding is that the colour of morphs commonly affect sexual mating interactions through NFDS and correlational selection, thereby altering morph specific fitness and maintaining genetic diversity (Hughes *et al.* 2013; Iserbyt *et al.* 2013; Roulin & Ducrest 2013;

Takahashi & Watanabe 2010). These insights have significantly increased our understanding of how multivariate selection acts on the covariance between phenotypes and fitness. However, lack of knowledge about the molecular genetic basis of colour in most polymorphic systems has hampered our ability to develop deeper insights into the evolution of these traits. This situation is changing rapidly as emerging genomic technologies offer an unprecedented opportunity to delve into the genetic basis of colouration. These advancements are particularly relevant for the many non-model organisms, where research has been impeded by the absence of available genomic resources. Due to these advances, the genomic location of colour has recently been described in several polymorphic species and in some cases the underlying genes and mutations have been identified (e.g. Kunte *et al.* 2014; Reed *et al.* 2011). With this new knowledge and much improved methods for elucidating the molecular basis of colour traits, we can improve the study of the evolutionary responses of colour genes to selection in general, and sexual selection in particular. For example, the evolutionary response to selection on coloration will be influenced according to whether the genes involved in pigmentation show multiple pleiotropic effects, whether they are tightly linked to other selected genes in the chromosomal vicinity, their dominance patterns and/or whether they form central nodes in integrated gene networks.

Here we review recent studies on genetic colour polymorphisms where sexual selection has been suggested to play a potential role in their maintenance and the colour loci have been mapped to genomic regions, or in some exceptional cases, even to mutations in genes and the specific nucleotide substitutions. We focus on heritable colour polymorphisms which we define as the co-occurrence of at least two discrete adult morphs in at least one sex coexisting within an interbreeding population (i.e. polychromatism), with the rarest morph being too common to be the product of recurrent mutations (Huxley 1955). In some instances, we also cover studies on polymorphic species for which colour variation is not truly discrete, but instead varies more along a continuous axis (e.g. the guppy). The key focus of this review is to conceptually integrate the emerging genomic data to the sexual selection theory and to identify knowledge gaps and priorities for future research. We also highlight emerging systems that promise to shed light on the genetics of sexual diversity in colour patterns.

### **Evidence of colour assortative mating and sexual selection on genetic colour polymorphisms**

Traditionally, natural selection was assumed to operate on colour polymorphisms. For instance, most classic colour polymorphism studies on the peppered moth *Biston betularia* and the grove snail *Cepaea nemoralis* were conducted in the British ecological genetics tradition in terms of predation risk and in relation to various abiotic factors (e.g. Kettlewell 1961, Figure 2). This bias towards natural selection might partly reflect the fact that many colour polymorphisms are not restricted to one sex in their expression, and researchers then assumed (for correct or incorrect reasons) that the absence of sex limitation indicates a lack of sexual selection.

Below and in Table 1, we list some representative examples where aspects of sexual selection on colour have been quantified.

A clear pattern that emerges from the literature is that colour assortative mating is manifold across diverse taxa, while disassortative mating is generally rare. Examples of assortative mating include the head colour morphs of the Gouldian finch *Erythrura gouldiae*. Both red and black morphs mate colour assortatively and colour preferences are maintained despite some gene flow through the coupled inheritance of mating preferences, colour expression and low fitness of intermorph hybrids on the Z chromosomes (Pryke 2010). This genetic association prevents recombination from breaking down these associations when the morphs interbreed and helps to maintain assortative mating. Assortative mating by colour has also been shown in several other birds, such as the polymorphic arctic skua *Stercorarius parasiticus*, lesser snow goose *Anser c. caerulescens* and common buzzard *Buteo buteo* (Krüger *et al.* 2001; Mundy *et al.* 2004, Table 1). In the latter case, the plumage polymorphism is maintained by heterozygote advantage, coupled with non-genetic mate choice based on sexual imprinting (Krüger *et al.* 2001). In fish, colour assortative mating has been demonstrated in cichlids displaying the barred/gold and blotch/plain polymorphisms. The barred/gold polymorphism is found in the Midas cichlid complex in Nicaragua and assortative mating creates significant genetic differentiation between morphs (Barluenga & Meyer 2004). The probably best studied species in the Midas complex is *Amphilophus citrinellus*, where a golden morph co-segregates with a more widespread white and black barred morph (Barlow 1973) and in this species around 95% of breeding pairs share the same body colouration. Although the golden morph makes up only a small percentage of the population (around 10%), they are present in most populations of the species, suggesting a balanced polymorphism (Barlow 1983). The cichlid *Neochromis omnicaeruleus* in Lake Victoria occurs either as a plain brown or as one of two blotched morphs (Figure 1D), though the blotched morphs are predominantly female, and interestingly, an identical polymorphism is also found in several other African Lake cichlids. Evidence for colour assortative mating in this species comes from morph frequencies in the wild (Seehausen *et al.* 1999b) and from laboratory mate choice experiments (Pierotti *et al.* 2009; Seehausen *et al.* 1999b). However, the scarcity of blotched males in natural populations in nearly all species for which the polymorphism occurs (Lande *et al.* 2001) limits the opportunity for assortative mating. The predominance of blotched females in some cichlid species is caused by a sexual conflict because blotches in females provide crypsis against the mottled habitat background, while blotches in males reduce fitness by disrupting species-specific male colour patterns used for mate recognition (Roberts *et al.* 2009). Work on the blotch/plain polymorphism in Lake Malawi has shown that the sexual conflict can be resolved by tight linkage of the blotch pattern to a dominant female sex determining locus (Roberts *et al.* 2009). Interestingly, females of the three colour morphs (plain, orange or white blotched morphs) have significant own-morph aggression biases, which could contribute to the evolution of a female-preference polymorphism by creating NFDS on female preference similar to what has been described in another polymorphic cichlid species (Dijkstra *et al.* 2010). The role of aggression in NFDS is a relatively unexplored aspect of colour maintenance so far, despite the existence of several species where conspicuous coloration is combined

with aggressive behaviour, such as in the Gouldian finch (Pryke *et al.* 2007) and the white-throated sparrow *Zonotrochia albicollis* (Figure 1C) (Tuttle 2003, see below).

The aforementioned examples describing colour assortative mating demonstrate that colour in several polymorphic species is used in the pursuit of reproduction, however, assortative mating alone does not help to maintain polymorphism (Box 1). For this, it needs to be linked with heterozygote advantage such as in the common buzzard, or co-occur with some form of NFDS or correlational selection (Figure 2). There is a lack of rigorous quantitative studies on colour polymorphisms that have clearly separated sexual selection on colour morphs and assortative mating between morphs (Box 1). One case is the throat colour morphs of the side-blotched lizard *Uta stansburiana* (Figure 1A). Here females show a preference for colour matching in the laboratory (Bleay & Sinervo 2007) but this preference is overridden in the wild by competitive exclusion of other males by the dominant male in their social neighbourhood. The male mating strategy is under NFDS: Orange males are territorial usurpers, blue are monogamous mate guards and yellow males adopt a sneaker strategy (Sinervo & Lively 1996). Unlike assortative mating, disassortative mating alone can prevent the loss of rare phenotypes (Box 1), but examples from polymorphic systems are rare. The probably best example of disassortative mating comes from the white and tan crown stripe morphs of the white-throated sparrow (Figure 1C). Here disassortative mating ensures that white-striped morphs almost always mate with tan-striped morphs. A chromosomal inversion separates white and tan morphs in both sexes (Table 1), and since white morphs are heterozygous for the inversion (Huynh *et al.* 2011), while tan-striped birds are homozygous, disassortative mating maintains the polymorphism at roughly 50/50% in the population (Tuttle 2003).

In species with sex-limited colour variation, both female- and male-limited colour polymorphisms are common. Sexual conflict over optimal mating rates has been implicated in the generation and maintenance of female-limited colour polymorphisms (Svensson *et al.* 2009), while when colour polymorphism is exclusively found in males, it mostly plays a role in male–male competition for access to receptive females (Plaistow & Tsubaki 2000). In birds, 23 species show female-limited polymorphism (Galeotti *et al.* 2003), and female-limited colour morphs are also common in many butterflies (e.g. *Papilio polytes*) and odonates. In fact, in odonates, trans-species colour polymorphism has been described for more than 100 species (Fincke *et al.* 2005). An emerging odonate model system for sexual selection and colour polymorphisms maintenance is the blue-tailed damselfly *Ischnura elegans*, which has three female morphs (Figure 1B). Studies on this damselfly have demonstrated that mating rates of morphs vary temporally and spatially (Gosden & Svensson 2009; Gosden & Svensson 2008) that they are frequency-dependent, whereby the common female morphs face fitness disadvantages (Svensson *et al.* 2005). The prevalence of female colour polymorphism in damselflies in general, and in *I. elegans* in particular, is thought to be the result of sexual conflict over optimal mating rates, where females benefit from lower mating rates than males, and where pre-copulatory male mating harassment is common (Gosden & Svensson 2009; Sánchez-Guillén *et al.* 2013b). By contrast,

male-limited colour polymorphisms are more rare (Van Gossum *et al.* 2008) and often include a territorial fighter male and a 'sneaky' male that resembles conspecific females in behaviour and phenotype and succeeds in intercepting females for mating. This is the case in the male-limited colour polymorphism of the Japanese damselfly *Mnais costalis* where morphs include territorial orange-winged 'fighter' males or non-territorial clear-winged 'sneaker' males. Orange-winged males are larger than clear-winged males and have a higher daily mating rate (Plaistow & Tsubaki 2000) but their reproductive life span and longevity is less. Across the lifetime of male morphs, however, the estimated lifetime reproductive success is comparable (Plaistow & Tsubaki 2000). In the male-limited polymorphism of the guppy *Poecilia reticulata*, replicated experimental manipulations of natural populations were able to show that males with rare colour patterns acquire more mates and also sire more offspring, thus demonstrating NFDS mediated by sexual selection (Hughes *et al.* 2013). Another famous example for male-limited polymorphism is the ruff *Philomachus pugnax* where alternative mating strategies are associated with plumage polychromatism in males (Lank *et al.* 1995).

**Box 1: Disentangling sexual selection from assortative mating**

*Sexual selection* and *assortative mating* are two important processes in colour polymorphic systems. Below, we clarify how they differ and how they can affect each other, both in general, and in the study of colour morphs in particular. We find it most convenient to define these two different processes in terms of their population genetic consequences, particularly how they alter allele or genotype frequencies.

Sexual selection alters allele frequencies by either fixing advantageous alleles or purging disadvantageous alleles, except when sexual selection is negatively frequency-dependent, in which case there will be no net directional long-term change in allele frequencies over generations and genetic variation is instead being maintained. Sexual selection may be directional or stabilizing, in which case selection may change the allele frequencies of the local gene pool. These forms classical forms of sexual selection do not maintain genetic diversity, but rather erode genetic variation, by favouring certain alleles. In contrast, NFDS rescues rare alleles from being lost from the population by genetic drift, as the bearers of rare alleles, i.e. rare morphs, have higher fitness.

Assortative mating alters *genotype frequencies*. Assortative mating can result from mate choice, but it does not need to, and it can arise simply due to spatial (Snowberg & Bolnick 2012) or temporal population structure (Hendry & Troy 2005) where non-random mating arises due to demographic fragmentation among subpopulations. Assortative mating between similar morphs (sometimes called "positive assortative mating") increases the frequency of homozygotes in local populations, at the expense of heterozygotes. Assortative mating does not, in itself, favour one allele over the other, and also does not maintain genetic polymorphisms. In contrast, disassortative mating (sometimes called "negative assortative mating") can maintain genetic diversity, if rare morphs (genotypes) obtain a frequency-dependent mating advantage. Assortative mating can also interfere with and influence sexual selection, through directional or stabilizing selection that disfavors rare morphs (rare homozygotes). This sexual selection effect of assortative mating

affects the search costs of mates, as the rarer morphs will experience a frequency dependent disadvantage in finding mates of their own phenotype (Kirkpatrick & Nuismer 2004).

Future empirical studies on sexual selection and assortative mating in colour polymorphic systems should try to quantify the relative importance of these different processes and investigate how they might interact with each other. For instance, a demonstration of sexual selection on colour morphs requires that some morphs have higher mating success, which neither requires morph assortative mating nor protects the polymorphism from being lost. Conversely, demonstrating that morphs mate assortatively within a colour polymorphic species does not demonstrate sexual selection in itself or provides any hard evidence that these morphs are being maintained. Finally, although disassortative mating might maintain colour polymorphisms in some systems (e.g. white-throated sparrows), most polymorphisms are probably maintained by some form of balancing selection, such as NFDS.

## **Emerging knowledge about genomics and genetic architecture of colour**

### **The genetic architecture of colour**

#### **Supergenes, inversions and tight linkage**

The recent advent of genome wide sequencing methods has shown that many colour loci cluster with functionally unrelated genes to form supergenes. Such supergenes link multiple genes into one segregating unit, thereby hardcoding for separate yet complementary phenotypes and preventing allelic combinations that create non-optimal intermediates. The degree of linkage in these supergene clusters depends on the physical distance between loci, but of particular importance is the effective recombination rate in the chromosomal region where they reside, which can be enhanced by structural variations such as chromosomal inversions, deletions, insertions, duplications, and translocations (Schwander *et al.* 2014). Below we will describe some representative cases where supergenes control the production of alternative colour phenotypes.

One of the best documented cases of a supergene architecture underlying colour morphs comes from the diverse clade of brightly coloured and chemically defended butterflies of the Neotropical genus *Heliconius*. Some of the species in this group have sympatrically co-occurring colour morphs (e.g. *H. numata* and *H. cydno alithea*, Table1), though most species in this genus vary along geographic gradients (e.g. *H. melpomene*, *H. cydno* and *H. erato*). Genetic mapping has revealed that only a few genomic regions code for hundreds of distinct wing patterns in this genus, demonstrating that some genes are unusually prone to facilitate rapid evolutionary change. Molecular work on wing colour patterns in *H. numata* (Figure 1E) has

identified a single supergene switch locus, which appears to have been assembled by increasing linkage between previously loosely linked ancestral loci (Joron *et al.* 2006). The supergene consists of two nested inversions on linkage group 15 that create three possible chromosomal rearrangements for the supergene locus *Pushmipullyu*, or simply *P*, each one of them corresponding to a specific wing colour pattern (Joron *et al.* 2011; Joron *et al.* 2006). The genomic location of *P* is orthologous with the major mimicry loci (*Yb*, *Sb*, *M*) that control the presence of yellow wing pattern in *H. melpomene*, *H. cydno* and *H. erato*. In other *Heliconius* species, however, loci on several different linkage groups are also implicated in colour control (Naisbit *et al.* 2003). For instance, *B* and *D* on linkage group 18 in *H. melpomene* control red wing colour, while *K* on linkage group 01 controls yellow versus white forewing bands in *H. cydno* (Naisbit *et al.* 2003). The diversity of colour loci in *Heliconius* demonstrates that phenotypic variation can be achieved in different ways, even in closely related species.

In the *Papilio* butterfly group, geographic colour phenotypes in *P. memnon* and *P. dardanus* underlie a supergene that controls different colour elements of the forewing and hindwing, as well as body colour and the presence of hindwing tails in *P. memnon* (Jones *et al.* 2011). Extensive genomic work on a similar colour supergene locus in *P. polytes*, which controls wing colour pattern and the presence of hindwing tails, was able to narrow down the supergene location to a 300 kb region with five genes (Kunte *et al.* 2014). One gene is the sex determining *doublesex* (*dsx*) gene, a transcription factor that regulates somatic sexual differentiation by producing alternatively spliced mRNAs encoding related sex-specific polypeptides, and subsequent detailed work showed that *dsx* alone acts like a switch between different wing pattern of this butterfly (Kunte *et al.* 2014). This finding is in stark contrast to the long-held view that supergenes are controlled by a tightly linked cluster of several loci, and suggests that the multiple linked loci scenario proposed by Clarke and Sheppard (1972) may indeed be composed of multiple, tightly linked mutations within a single gene. The region identified by Kunte *et al.* (2014) was further shown to lack recombination and analyses of whole genome re-sequencing data revealed signatures consistent with an inversion polymorphism harbouring the mimicry alleles and with the breakpoints flanking the *dsx* gene. Inversions prevent recombination in inversion heterozygotes, thereby allowing alleles to remain distinct from each other and to accumulate differing mutations, which has likely led to structural differences in the doublesex protein between mimetic and non-mimetic butterflies. That *dsx* can act as a supergene controller, despite being a fundamental gene controlling developmental processes, was presumably possible through the preservation of key coding parts, such as the DNA-binding motif or dimerization domains, which are essential components for the ancestral function (Kunte *et al.* 2014).

Supergenes have also been implicated in several colour polymorphic fish species, such as the blotch supergene polymorphism of cichlids occupying Lake Malawi and Victoria (Table 1). Species with blotched morphs (orange-, white-blotched or both) usually also possess a plain morph in both sexes, of which the latter is considered the ancestral condition, although in some species the blotched morph has become fixed in females (Seehausen *et al.* 1999b). The orange-blotch and plain



polymorphism was investigated using an interspecies cross between *Labeotropheus fuelleborni* and *Metriaclima zebra*, and could be mapped to a linkage group spanning approximately 30 cM (Streelman *et al.* 2003). The orange-blotch was shown to be tightly associated with the ski proto-onco gene *c-ski1* and a cis-regulatory mutation of the *pax7* gene (Roberts *et al.* 2009; Streelman *et al.* 2003). These genes lie in close proximity to a quantitative trait locus for tooth shape (Albertson *et al.* 2005) and a tandem array of three cone opsin genes (Carleton & Kocher 2001). Given that cichlid speciation probably involved the combined forces of natural selection on ecological traits (e.g. feeding morphology) and sexual selection on colour patterns (e.g. nuptial colour), the close genetic proximity of colour, morphology and putative mate choice characters has likely been a key component in the build-up of reproductive isolation.

In birds, a striking example of a colour supergene is the plumage polymorphism of the white-throated sparrow (Figure 1C, Table 1). This supergene is regulated by two paracentric inversions (ZAL2 and ZAL2<sup>m</sup>) that span almost 90% of chromosome two, which is the largest chromosome in passerine birds; thus includes hundreds of genes (Huynh *et al.* 2011). Alternative phenotypes in this species differ in several behavioural traits and mate preferences, although it is yet unclear whether (but certainly possible) the genes coding for these behavioural traits reside within the supergene cluster. Maintenance of alternative supergenes in the white-throated sparrow is ensured through disassortative mate choice preferences of morphs through NFDS (Tuttle 2003) (see section above).

### Gene duplications and introgression of colour genes

Genomic studies on colour genes are starting to show that the same genes are frequently causative of colour polymorphism in closely related taxa, and the most straightforward explanation for this is common ancestry. Such ancestral polymorphisms may be associated with historical gene duplication events which can cause expression differences between individuals having few or many gene copies. Moreover, having more than one gene copy may release one of the copies from pleiotropic constraints, and this can in turn allow evolution of novel functions of the newly derived paralogue. Copy number variation (CNV) is widespread across taxa and one extreme example includes the whole-genome duplication in teleost fish, which is a case where CNV has been shown to be important for the evolution of pigmentation gene families (reviewed in Braasch *et al.* 2007). An explicit role of gene duplications in the evolution of genetic colour polymorphisms is more limited, but some evidence exists. For example, the yellow/light and dark coat colours in many mammalian species, some being intraspecific polymorphisms, are in some cases caused by loss of function mutations in the melanocortin 1 receptor (*mc1r*), sometimes caused by increased expression of *Asip*, and thus production of ASIP, due to either gene duplication or mutations within the promoter region of *Asip* (Hofreiter & Schöneberg 2010; Linnen *et al.* 2009).

In addition to ancestral polymorphism, colour genes can also spread across species boundaries through introgression. Candidate groups for adaptive introgression of

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colour genes are adaptive radiations, since they often contain closely related and hybridizing species. Recent studies on the gene regulating red wing patterns in *Heliconius* butterflies provide evidence for a direct role of introgression in fuelling the mimetic wing pattern radiation. By sequencing regions linked and unlinked to the red colour locus, Pardo-Diaz *et al.* (2012) found a location with an almost perfect genotype-phenotype association in *H. melpomene*, *H. cydno*, *H. timareta*, and *H. heurippa*. The location is found 70 kb downstream of the red colour *optix* gene, and coalescent analysis indicates repeated introgression of alleles from *H. melpomene* into the *H. cydno* species clade. Another study on the orange-blotch/plain polymorphism in cichlids also supports the view that introgression can have a crucial role in generating adaptive colour diversity by transferring colour genes into new genomic backgrounds. Roberts *et al.* (2009) showed that the orange-blotch colouration originated once as a novel allele to up-regulate *pax7* expression, but then was subsequently incorporated into multiple species across four genera inhabiting Lake Malawi. Like in the *Heliconius* example, this spread has most probably occurred through multiple hybridization events, although sorting of an ancestral polymorphism cannot be excluded.

## Molecular genetics and developmental basis of colour morphs

### Regulatory vs protein coding evolution

Mutations in cis- and trans-regulatory mechanisms provide relatively simple ways to produce coordinated phenotypic changes, but it is yet unknown how frequent such regulatory changes are in relation to protein coding mutations. By looking into parallel colour evolution within closely related species it is possible to pinpoint if evolution has occurred through parallel molecular pathways, and also if independent mutations have taken place in the same genes or transcription factors. The comparatively rich literature in cichlid colouration suggests that colour diversity is related to comparatively little genetic variation in protein coding sequences, but rather to changes in gene regulatory factors (Maan & Seftc 2013). Of these, cis- and trans-regulatory mutations and a high diversity in untranslated mRNA regions have been documented, both potentially leading to colour gene expression differences (e.g. Colombo *et al.* 2013; Santos & Salzburger 2012). For example, the Lake Malawi orange-blotch/plain polymorphism is associated with a single QTL near the *c-ski1* gene, and is caused by a cis-regulatory mutation of the *pax7* gene (Roberts *et al.* 2009; Streelman *et al.* 2003). This cis-regulatory mutation affects a transcription factor that coordinates the development of melanocytes from neural crest precursors and leads to *pax7* upregulation. Studies on zebrafish *Danio rerio* showed that this upregulation leads to fewer and larger melanophores and exactly mimics the pattern of blotched female morphs (Roberts *et al.* 2009). To investigate if the same mutation also causes the Lake Victoria blotch morph, an orange-blotch Lake Victoria individual was examined for mutations in the *pax7* gene (Roberts *et al.* 2009). The haplotype was identical to the ancestral brown-banded female haplotype, indicating that blotches in the two lakes are caused by different mechanisms and suggests an independent origin of the blotch phenotypes in the two lakes (Roberts *et al.* 2009).

The barred/gold polymorphisms of Midas cichlids in the *Amphilophus* species group provide another example of a regulatory mechanism underlying colour differences. Unlike the blotch/plain polymorphism above, *Amphilophus* dark/gold colour morphs are not sex linked and very rare, with <10% of the population consisting of gold morphs. Large inter-individual variation in the timing of golden colour emergence exists, with some individuals displaying the golden colour when they are a few months old, but in others, it can start at an age of several years (Mattersdorfer *et al.* 2012). The onset of ontogenetic colour change is marked when the melanophores of dark juveniles degrade thereby allowing underlying xanthophores to become visible, and often this colour change does not progress uniformly. The gold polymorphism is determined by a Mendelian two-allele single locus model, with gold being dominant, and there is no *mc1r* sequence polymorphism (Henning *et al.* 2010). This is unlike the melanic colour polymorphism in the guppy (Tezuka *et al.* 2011) and many tetrapod vertebrates (Hoekstra 2006; Hofreiter & Schöneberg 2010), which are often associated with mutations in the coding sequence of the *mc1r* gene. Comparative analyses revealed high coding synteny between *Amphilophus* when compared to other teleosts, and yet, contradictory with its function in melanin synthesis, it was found that *mc1r* was upregulated in the gold morph (Henning *et al.* 2010).

In *Heliconius* butterflies, regulatory changes are causative of colour patterns in some geographic and sympatric colour variants. In *H. melpomene*, for example, between race red colour differences have been narrowed down to an 150 kb region (Nadeau *et al.* 2012) and analyses indicate that the homeobox transcription factor *optix* is the causal factor (Reed *et al.* 2011). Gene expression analyses and the highly conserved amino acid sequence variation in *optix* both suggest that variable red patterns are driven by cis-regulatory variation that generate differences in the spatial expression of *optix* (Reed *et al.* 2011). Phenotypic recombinants between red pattern elements have sometimes been observed (Mallet 1989), implicating the involvement of multiple, tightly linked, cis-regulatory variants. Likewise, black pattern divergence in *Heliconius* evolves through cis-regulatory variation of the morphogen *WntA* by inducing spatial changes in cell-signalling gradients across the wing (Martin *et al.* 2012).

### Alternative splicing

Alternative splicing is a fundamental mechanism for both gene regulation and the generation of proteomic diversity, and works by producing different mRNAs from the same gene, e.g. by exon skipping or intron retention. With this, alternative splicing increases the total coding capacity of the genome and enhances overall phenotypic diversity. For these reasons, alternative splicing has great potential in the context of colour polymorphisms, but with the most commonly used DNA-sequencing techniques, differently spliced variants will go undetected. This highlights the importance in colour polymorphic species to study expression differences through RNA-seq analyses of mRNA in the target species and target tissues. In cichlids, species-specific splicing patterns have been detected, but splicing differences have not yet been related to differences in colour pattern (Fan *et al.* 2012; Kocher 2004). Yet another example is the phylogenetically widespread sex-limited mimicry in the

genus *Papilio* described above (Kunte *et al.* 2014). The gene *dsx* controls the supergene mimicry in *P. polytes* and there is evidence that noncoding, regulatory DNA controls when and where *dsx* is expressed. Indeed, Kunte *et al.* (2014) showed that isoform expression differences (specific spliced iso-forms) contributed to the functional differences between *dsx* alleles, and that protein sequence evolution may also have a role. More studies are needed to understand the role of alternative splicing in the evolution of genetic colour polymorphisms.

### **Box 2: Polymorphism maintenance**

Maintaining colour polymorphisms within populations is usually thought to require some form of balancing selection, either in the form of overdominance (heterozygote advantage) or NFDS (Svensson *et al.* 2009, Figure 2). If no balancing selection operates locally, populations will lose one or several morphs over time by genetic drift and/or local selection and hence monomorphism is the default outcome. Although the presence of multiple morphs within local populations is consistent with balancing selection, it would be erroneous to argue that this pattern is proof of its existence. Rather, one needs to show that the rank ordering of morph specific fitness varies in response to changing environmental conditions, which is a population genetic prerequisite for the maintenance of genetic polymorphisms (Levene 1953). In the case of NFDS, it needs to be demonstrated (using fitness data from multiple subpopulations and/or local demes that differ in morph frequencies) that the relative fitnesses and the rank ordering of colour morphs varies predictably as morph frequencies change, either spatially, temporally or both. To date, few empirical studies have achieved this challenging and difficult goal. Notable exceptions are field studies on colour morphs of side-blotched lizard (Sinervo & Lively 1996), damselflies (Svensson *et al.* 2005; Takahashi & Watanabe 2010) and a recent study on rare-male mating advantages in the guppy (Hughes *et al.* 2013). In contrast to these studies which demonstrated balancing selection through NFDS, a recent study on an island population of Soay sheep *Ovis aries* instead provided evidence of balancing selection through overdominance both on coat colour polymorphism (a non-sexually selected character) (Gratten *et al.* 2008) and horn polymorphism (a non-colour but sexually selected character) (Johnston *et al.* 2013). Whether NFDS is more important than overdominant selection in maintaining sexually selected polymorphisms in colour and other traits is an open question that will require studies of a wider range of taxa. Note that in the case of NFDS, it is not enough to demonstrate differences between colour morphs in one or several phenotypic traits or fitness components, such as fecundity, parasite loads or behaviours. One needs to explicitly show how morphs fitness changes in relation to frequency if one wants to make a strong inference about NFDS.

Some of the studies above exemplify how NFDS might arise for purely intrinsic and social reasons, due to either intrasexual selection and male-male competition (e.g. side-blotched lizards) or frequency-dependent male mating harassment and sexual conflict (e.g. damselflies). However, it is possible, and even likely, that intrinsic factors caused by mating systems and mating interactions can interact with abiotic environmental factors, such as temperature or visual environments. For instance, if the lightning environment is variable, some male colour morphs might be more

visible than others, resulting in higher mating success of such locally visible morphs (e.g. Gray *et al.* 2008). However, theory suggests that such a varying abiotic environment is, in itself and alone, unlikely to maintain colour polymorphisms by frequency-independent natural selection, but the variable environments needs to operate in concert with negative frequency dependent sexual selection.

## Linking genetic architecture and sexual selection

The genetic architecture includes the number of loci affecting traits, the effect size of such loci, as well as the magnitudes of genetic variances and covariances between traits and the sign of covariances. Relatively few studies to date have explored how the genetic architecture evolves and how it may constrain phenotypic adaptation. Cheverud (1984) suggested that genetic correlations between traits would be expected to become aligned with the adaptive surface in the direction of maximum fitness, but it was not until recently that the generality of this hypothesis was confirmed (Roff & Fairbairn 2012). Using a meta-analysis, Roff & Fairbairn (2012) showed that when correlational selection (selection for combinations of characters, usually estimated as pair-wise fitness interactions) and genetic correlations are estimated in the same system, correlational selection gradients are concordant with the genetic correlations in terms of magnitude and sign. One explanation for this is that chronic correlational selection builds up genetic correlations between disparate traits, even if they are governed by separate sets of loci, through the formation of linkage disequilibrium (Sinervo & Svensson 2002). Alternatively, chronic correlational selection can favour the spread of pleiotropic mutations which impact both target traits of correlational selection (Roff & Fairbairn 2012). In either scenario and under both mechanisms, the result is the formation of adaptive genetic correlations, suggesting that the genetic architecture can be shaped by selection, as has been suggested theoretically by Wagner and Altenberg (1996). Empirical examples supporting this come mainly from field studies of reptiles, such as garter snakes (Brodie III 1989; Brodie III 1992) and side-blotched lizards (Sinervo & Svensson 2002; Svensson *et al.* 2009), and a few laboratory studies, such as guppies (Blows *et al.* 2003).

An interesting and empirically unexplored question is if the number of loci and their effect sizes in colour polymorphic systems have evolved from an initially polygenic architecture. A population genetic model on the effects of frequency-dependent selection on genetic architecture suggested that major effect loci (i.e. QTLs or supergenes) can evolve under chronic NFDS (Kopp & Hermisson 2006). Starting from a polygenic architecture of multiple loci, each one of small effect, Kopp and Hermisson (2006) demonstrated that the genetic architecture can become more asymmetric when the trait was targeted by NFDS and that the trait evolved into a single, large effect QTL over time. If this scenario is general and if it is applicable to colour polymorphic systems subjected to NFDS, then we would have a new avenue of research in future genomic studies on the evolutionary history of these polymorphisms. More specifically, researchers could reconstruct the evolutionary

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history of the genetic architecture of colour polymorphisms, starting with ancestral character states in the form of polygenic traits and ending up with oligogenic genetic architectures of the morphs that are governed by one or a few QTLs. Sexual selection may also promote the evolution of allelic dominance by making heterozygotes phenotypically more similar to homozygotes. This masking of recessive alleles would ameliorate heterozygote disadvantage and a stable polymorphism may emerge (Rueffler *et al.* 2006). Similar arguments could apply to the evolution of sex-limited colour gene expression, which is common in insects and fish (Table 1), such as in the polymorphism of the damselfly *I. elegans*, which is characterized by both females-limited colour morphs and an allelic dominance hierarchy (Sánchez-Guillén *et al.* 2005).

Studies on many colour polymorphic systems show that evolution is governed by the synergistic effects of natural and sexual selection (e.g. in guppies). Indeed, evidence is accumulating that speciation is favoured by genomic architectures that either eliminate or reduce recombination between traits involved in reproductive isolation through synergistic pleiotropy, and these traits have been termed 'magic' traits (Gavrilets 2004; Magalhaes *et al.* 2010; Servedio *et al.* 2011). In short, magic trait-models postulate a pleiotropic link between an ecological trait conferring adaptation and reproductive isolation, so that selection on the ecological trait results in reproductive isolation as a correlated response and by-product. These models can be extended to sexually selected traits, including colour polymorphisms, particularly if multiple other fitness-related traits are correlated with colour, which has been demonstrated in several cases (reviewed in McKinnon & Pierotti 2010). Hence, a consistent fitness advantage and selection on one morph would not only alter morph frequencies in the local population, but could also change other phenotypic traits, such as behaviours, physiology and developmental rates as correlated responses. Of particular interest here are colour polymorphisms where assortative mating has been demonstrated, such as in Gouldian finches, lesser snow goose or the barred/gold morphs in Midas cichlids (Table 1). In a wide range of taxa, colour also performs as a signal for individual quality, and may indicate varied traits ranging from ecological performance, immuno-competence, deleterious mutation load, to previous reproductive effort (e.g. Roulin 2004). The metabolic origin of colour may be important in determining the signal information content. For example in birds, melanin-based plumage colours are thought to be less likely indicators of individual quality than those containing carotenoid pigments, since carotenoid deposition in ornamentation may trade off with their use as immuno-stimulants and antioxidants or with other physiological functions (Rosenthal *et al.* 2012). Closely connected to the discovery of magic traits is a growing awareness that sexual and natural selection might not always be in opposition to each other, as has often been shown (e.g. Gray *et al.* 2008), but could work in the same direction and favour similar trait values (Maan & Seehausen 2011). In the case of colour polymorphisms, for instance, different colour morphs could be favoured by both natural and sexual selection. One example of this might be provided by *Heliconius* butterflies and *Dendrobates* poison dart frogs, which are brightly coloured, and warning colouration is thought to be an aposematic warning signal of toxicity or unpalatability (Joron *et al.* 2011; Maan & Cummings 2012). Interestingly, in at least some species, mating is assortative with respect to colour morph, demonstrating an association between non-random mating and an ecologically important colour trait (Summers *et al.* 1999). Another example is

provided by guppies, where rare male colour morphs are apparently favoured by NFDS in terms of less predation (Olendorf *et al.* 2006), but also in terms of NFDS through sexual selection and through rare-male mating advantage (Hughes *et al.* 2013). In cases where natural and sexual selection are working concordantly, the evolutionary pressure on the genetic architecture is expected to be strong and likely affect not only the reproductive life stages.

## Conclusions and future directions

Over the last years, significant progress has been made to describe the genomic underpinnings of genetic colour polymorphisms. These studies offer the opportunity to integrate sexual selection studies on colour polymorphisms with knowledge about the molecular targets of selection. Several prominent examples of colour genes point towards a tight clustering of colour loci with other traits, such as in the case of inversions and supergene structures that have been shown to control several adaptive colour phenotypes (Table 1). With the continuing development of genomic techniques, the numbers of cases where supergenes, large QTLs and inversions are found to be bearers of colour genes are likely to rise. Good study system candidates to study these relationships will be multivariate colour phenotypes that are genetically correlated with other phenotypic and fitness-related traits (McKinnon & Pierotti 2010), but that segregate in a fashion similar to a Mendelian locus. One of these systems could, for example, be the *Ischnura* damselfly system, where colour is found to segregate in a simple Mendelian fashion (Sánchez-Guillén *et al.* 2005), but affects many other fitness related traits (e.g. development time and fecundity) (Abbott & Svensson 2005). Interestingly, intense and ongoing hybridisation has been reported between several colour polymorphic *Ischnura* species, making this also a system to study adaptive introgression of colour genes (Sánchez-Guillén *et al.* 2013a; Sánchez-Guillén *et al.* 2011b; Sánchez-Guillén *et al.* 2012). A challenge for future studies in developmental genetics will be to determine which of the genes residing within these clusters are causative for the generation of novel colour phenotypes, since the tight linkage within clusters makes it difficult to map genes to function. Moreover, deciphering the dominance patterns of genes in such clusters, including the colour genes (which are often found to be dominant, see Table 1), would be valuable for understanding the consequence of selection acting upon them. With improved sequencing technologies and mapping, these hurdles can hopefully be overcome. Further, we foresee that knowledge about the temporal order of changes within gene clusters and supergenes will further clarify the sequence of evolutionary events. Suitable groups to explore such traits and events will be closely related species that carry, or likely carry, tightly linked genes in genomic regions that also harbour colour loci, such as butterflies, cichlids and damselflies (Table 1), and where a historical reconstruction is possible using phylogenetic comparative approaches. Deciphering the functional genetic properties of colour polymorphisms and the suite of correlated factors (e.g. mutational history, linkage disequilibrium, type of regulation and complexity of gene expression networks) can help elucidate how and when colour phenotypes may evolve in relation to natural and sexual selection. An interesting aspect that has so far received little attention concerns the future fate of species with colour supergene clusters or inversions. While both supergenes and inversions lead to reduced recombination between colour and other

linked loci, and are in the short term likely adaptive by preventing intermediate phenotypes, if selection regimes change, then such clusters can lead to restricted evolutionary potential. Because inversions and supergenes both act as one tight cluster, selection acting on only selected loci within these clusters cannot efficiently cause evolutionary change since recombination is minimal. In this sense, selection for the coupling of traits through pleiotropy or tight linkage could be disadvantageous for long term species survival and may constrain evolutionary divergence and speciation. Genetic hitchhiking around selected clusters will not only facilitate this process, but will also increase the genomic area affected, and together this can eventually lead to evolutionary dead-ends.

We would like to caution though, that the finding that many colour traits are caused by large effect loci and/or gene clusters is probably partly a methodological bias. The potential to detect certain types of genetic mechanisms over others with the commonly applied Next Generation Sequencing (NGS) approaches will e.g. skew the findings towards detecting large genomic areas due to the increased likelihood of having markers in such areas. Moreover, candidate gene approaches will bias findings towards certain protein sequences (e.g. in *mc1r*). In addition, with most current genomic approaches there will be a positive bias towards detecting cis-regulatory rather than trans-regulatory changes. This is because a causal cis-regulatory change must be located close to the gene with the divergent function, while evidence for a trans-regulatory change potentially implicates the entire genome. Moreover, while large inversions can be visualised with karyotypic and cytogenetic approaches, they are generally more difficult to detect with NGS approaches, although progress in this field is presently being improved (Lucas Lledó & Cáceres 2013).

Another interesting new avenue for research is that several studies show a linkage between colour loci and mate preferences or sex linkage of colour loci. In *Heliconius* butterflies, mate preference genes are genetically associated with those controlling colour pattern, such that a yellow/white colour pattern switch and the preference for yellow versus white is controlled by the same genomic region (Kronforst *et al.* 2006). Likewise, the loci for plumage colour, mating preferences and intrinsic postzygotic incompatibilities are coupled on the Z chromosome in *Ficedula* flycatchers (Qvarnstrom & Bailey 2008) and Gouldian finches (Pryke 2010). Sexually antagonistic selection appears to have also favoured linkage between colour loci and loci involved in sex determination in the orange-blotch allele of *pax7* in Malawi cichlids. Here, tight linkage of the orange-blotch with sex determination loci has occurred and this has subsequently been incorporated into multiple species across four genera of the Malawi cichlid flock, either by sorting of an ancestral polymorphism and/or hybridization (Roberts *et al.* 2009)

We think that colour polymorphism studies are entering an exciting new phase where the molecular targets of selection can be unravelled and connected to the ecological consequences acting on individual morphs, populations and ultimately, species (Forsman 2013; Forsman *et al.* 2008). We predict that this field will flourish over the



next years, but would like to emphasize that progress in our understanding of sexual selection on colour polymorphisms will only come from studies that explicitly combine ecology with genetics. Such combined approaches should ideally integrate rigorous studies evaluating colour effects on fitness and the ecological and behavioural causes of selection on morphs with studies on the molecular underpinnings of colour. Future molecular studies should also aim to provide increased insights into the genes in the vicinity of colour genes, which may be exposed to different selection pressures, and reveal possible genetic constraints on the effectiveness of sexual selection. Such studies, in addition with detailed knowledge about the molecular and functional genetics, may then facilitate the design of phenotype manipulations (e.g. through hormone treatments, crossing schemes or even genetic engineering), which in turn will allow for controlled experiments, the decoupling of correlated traits and enhanced understanding of how selection operates on colour morphs and other traits. Such an integrative research approach will help unify molecular and ecological studies, and would allow for inferences to be made about the genetic consequences of sexual selection on heritable colour polymorphisms.

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**Table 1: Representative examples of species with genetic colour polymorphism where both sexual selection on colour and the genetic basis of colour has been investigated to some extent. This list of polymorphic species is non exhaustive but highlights some systems where the molecular targets of sexual selection on colour seem traceable. The species column shows the scientific and common name, sex denotes the polymorphic sex in that species (F=female and M=male) and the column colour polymorphism described the colour variation, and number of morphs in brackets. Sexual selection summarises the evidence for sexual selection on colour in this species while genetics list the knowledge about the underlying genetics of colour in this system.**

Species	Sex	Colour polymorphism	Sexual selection on colour	Genetics of colour	References
<b>Arthropod</b>					
<i>Heliconius numata</i> , <i>Numata longwing</i>	F, M	Yellow, brown/orange, and black wing morphs (10).	Unknown	Single supergene switch locus consisting of two nested inversions that create three possible rearrangements for the supergene locus P, each one corresponding to a specific wing pattern.	(Joron <i>et al.</i> 2011; Joron <i>et al.</i> 2006)
<i>Heliconius cydno alithea</i> , <i>Cydno Longwing</i>	F	White and yellow wing morphs (2).	Yellow males prefer yellow females, but white males show no preference.	Autosomal supergene wing colour linked to male colour preference at same the location of the developmental gene wg. Linkage likely caused by an inversion (5.5-cM) including colour/preference/wg.	(Naisbit <i>et al.</i> 2001)
<i>Ischnura elegans</i> , <i>Blue-tailed damselfly</i>	F	Green, blue and pink body colour and patterning (3).	Negative frequency-dependent morph mating rates and fitness costs that vary temporally and spatially.	Autosomal Mendelian locus with three alleles in a dominance hierarchy. Linked with development time and fecundity. Similar morph inheritance patterns exist in related species.	(Abbott & Svensson 2005; Gosden & Svensson 2009; Gosden & Svensson 2008; Sánchez-Guillén <i>et al.</i> 2011a; Sánchez-Guillén <i>et al.</i> 2005)
<i>Papilio memnon</i> , <i>Great mormon</i>	F	Large diversity in wing pattern and colour of the basal forewing	Unknown	At least five loci in the mimicry supergene: T, W, F, E, and B.	(Jones <i>et al.</i> 2011)

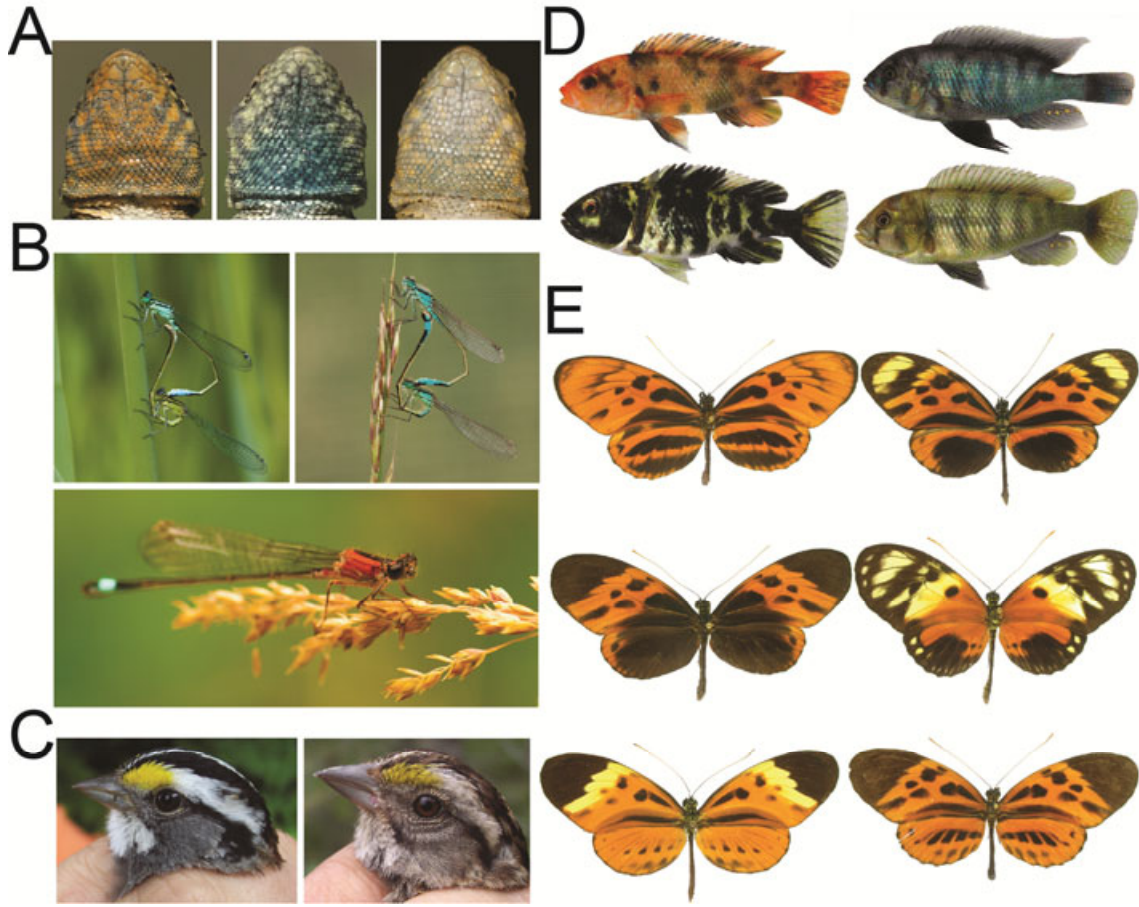
		triangle and abdomen (>20).			
<i>Papilio polytes</i> , <i>Common mormon</i>	F	One male mimic, and three morphs that mimic colours and patterns of toxic <i>Pachliopta</i> swallowtails (4).	Unknown	The gene <i>doublesex</i> controls supergene mimicry. Isoform expression differences contribute to the functional differences between <i>dsx</i> mimicry alleles.	(Kunte <i>et al.</i> 2014)
<b>Amphibians</b>					
<i>Dendrobates pumilio</i> , <i>Strawberry poison frog</i>	M, F	Many body colour and patterns, some with melanised spots (>15).	Females generally prefer males of their own colour. On an inter-population level, females prefer native male colours over foreign ones, and on an intra-population level, exhibit directional sexual selection for bright males.	Dark dorsal patterning is controlled by a single locus, with complete dominance of red over yellow.	(Maan & Cummings 2009)
<b>Reptiles</b>					
<i>Uta stansburiana</i> , <i>Common side-blotched lizards</i>	M, F	Orange, yellow and blue throat colours (blue mainly in males) (3).	Strong male-male competition that overrides female preferences for same coloured males.	Throat colour is heritable and segregates like a Mendelian factor with three alleles. In males, the o allele is the dominant allele, while the b allele is recessive to the y allele. Orange-throated males: oo, ob, or oy, yellow-throated males: yy or yb, and blue-throated males: bb. In females, all with the dominant o allele are orange-throated, while those lacking an o allele develop yellow throats.	(Bleay & Sinervo 2007; Sinervo & Lively 1996)
<b>Fish</b>					
<i>Amphilophus citrinellus</i> , <i>Midas cichlids</i>	M, F	Barred dark morph (~90%) and a rare gold morph in both sexes (2).	Gold and normal fish mate assortatively and genetic divergence of neutral markers	Mendelian inheritance pattern determined by a two-allele single locus model, with gold being dominant.	(Barlow 1973; Henning <i>et al.</i> 2010; Mattersdorfer <i>et al.</i> 2012)



			occurs between the two morphs.		
<i>Neochromis omnicaeruleus</i> , <i>Lake Victoria cichlid</i>	M, F	Females are plain or occur as orange or white blotched morphs. Intermediates rare (3).	Evidence for colour assortative mating from morph frequencies in the wild and laboratory mate choice experiments. Scarcity of blotched morphs in nature likely prevents phenotype matching in many pairs.	Blotch phenotype mapped to a linkage group of 30 cM, and is tightly associated to ski proto-oncogene <i>c-ski1</i> and a cis-regulatory mutation of the <i>pax7</i> gene. These genes lie in close proximity to a quantitative trait locus for tooth shape and a tandem array of three cone opsin genes.	(Maan & Sefc 2013; Pierotti <i>et al.</i> 2009; Seehausen <i>et al.</i> 1999a)
<i>Poecilia reticulata</i> , <i>Millionfish guppy</i>	M	Males display a diversity of spots of various colours, including black, white, red-orange, yellow and green (>10).	NFDS caused by rare-male mating advantage. Extent of orange male spots is also a basis for female choice.	Linkage mapping and QTL analyses showed that most male colour traits are controlled by multiple genes, including genes on autosomes.	(Hughes <i>et al.</i> 2013; Tezuka <i>et al.</i> 2011)
<b>Birds</b>					
<i>Anser c. caerulescens</i> , <i>Lesser snow goose</i>	M, F	White, intermediate, blue/grey (3 or more)	Assortative mating, imprinting on parental colour.	Melanism associated with variation in <i>mc1r</i> gene.	(Roulin & Ducrest 2013)
<i>Buteo buteo</i> , <i>Common Buzzard</i>	M, F	Pale, dark and white (3)	Assortative mating, possibly maladaptive due to morph heterozygote advantage.	Amino acid substitutions of <i>mc1r</i> (O. Kruger, pers. comm.)	(Krüger <i>et al.</i> 2001; Mundy <i>et al.</i> 2004)
<i>Erythrura gouldiae</i> , <i>Gouldian finch</i>	M, F	Red, yellow and black head colour patterns in males and females (3).	Both black and red morphs strongly prefer mates of their own morph. Z chromosome likely harbours genes for mating preferences, colour expression and the genes causing low fitness of intermorph-	Z-linked locus with a dominant red (ZR) and recessive black (Zr) allele. In females, the heterogametic sex in birds, phenotype matches genotype (Zr black, ZR red). Males can be homozygous ZrZr (black), ZRZR (red), or heterozygous ZRZr (red), with heterozygous and homozygous red males phenotypically indistinguishable.	(Pryke 2010; Pryke & Griffith 2006)

			hybrids.		
<i>Philomachus pugnax</i> , Ruff	M	Black, brown, white morphs (3)	Morph assortative mating strategies.	Autosomal, one-locus, two alleles.	(Lank <i>et al.</i> 1995)
<i>Stercorarius parasiticus</i> , Arctic skua	M, F	Pale, intermediate, dark (3)	Female preference for dark males and/or assortative mating.	Melanism associated with variation in <i>mc1r</i> gene.	(Mundy <i>et al.</i> 2004)
<i>Zonotrichia albicollis</i> , White throated sparrow	M, F	Tan and white striped morphs in males and females (2).	Strong disassortative mating pattern in which >96% of all breeding pairs comprise opposite morphs.	Supergene regulated by two paracentric inversions (ZAL2 and ZAL2m) that span almost 90% of chromosome two. Affects behaviour and mating preferences.	(Huynh <i>et al.</i> 2011, Tuttle 2003)

**Figure 1: Colour plate of some representative examples of colour polymorphic species. (A) Throat colours of orange (left), blue (middle) and yellow (right) homozygous males from a trimorphic population of *Uta stansburiana*. (B) The three female colour morphs of *Ischnura elegans*: infuscans (top left), male-mimicking androchrome (top right) and infuscans-obsoleta morph (bottom) in its juvenile phase (rufescens). (C) White (left) and tan (right) striped morph of *Zonotrichia albicollis*. (D) Blotched and plain morphs of *Neochromis omnicaeruleus*. Female orange-blotched (OB) morph (top left), white-blotched (WB) (bottom left), plain male (top right) and plain female (bottom right). (E) Colour morphs of *Heliconius numata* with the numata arcuella morph on the top left, aurora on the top right, bicoloratus (middle left), silvana (middle right), tarapotensis (bottom left) and timaeus (bottom right).**



**Figure 2: Schematic summary of main processes affecting phenotypic and genotypic evolution of colour polymorphisms.**

