

NEWS AND VIEWS**Perspective****Balancing selection maintains cryptic colour morphs**Maren Wellenreuther 

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Animals display incredibly diverse colour patterns, a testament to evolution's endless innovation in shaping life. In many species, the interplay between males and females in the pursuit of mates has driven the evolution of a myriad of colour forms, from the flashy peacock tail feathers to the tiniest colour markings in damselflies. In others, colour provides crypsis by allowing to blend into the background and to escape the eyes of predators. While the obvious benefits of this dazzling diversity for reproduction and survival seem straightforward, its maintenance is not. Theory predicts that genetic drift and various forms of selection reduce variation over time, making the persistence of colour variants over generations a puzzle. In this issue of *Molecular Ecology*, Lindtke et al. (2017) study the cryptic colour morphs of *Timema cristinae* walking sticks to shed light on the genetic architecture and mechanisms that allow colour polymorphism maintenance over long timescales. By combining genome-wide data with phenotyping information from natural populations, they were able to map the green and melanistic colour to one genomic region with highly reduced effective recombination rate between two main chromosomal variants, consistent with an inversion polymorphism. These two main chromosomal variants showed geographically widespread heterozygote excess, and genomic signatures consistent with long-term balancing selection. A younger chromosomal variant was detected for the third morph, the green-striped colour morphs, in the same genomic regions as the melanistic and the green-unstriped morphs. Together, these results suggest that the genetic architecture of cryptic *T. cristinae* morphs is caused by nonrecombining genomic blocks that have been maintained over extended time periods by balancing selection making this study one of the few available empirical examples documenting that balancing selection of various forms may play an important role in maintaining adaptive genetic variation in nature.

KEYWORDS

colour polymorphism, crypsis, heterosis, inversions

Colour variation is ubiquitous among animals and often subjected to complex selection regimes, yet little is known about the underlying genetic basis of these phenotypes. However, emerging results over the last years are shedding light on the genetic basis of colour and the processes by which phenotypic evolution occurs. Species with heritable polymorphisms have been

indispensable in this quest, as they allow to test the basic tenets of population genetics to provide insights into the mechanisms leading to colour variation maintenance over time. The basic question is simple: How are multiple alleles or discrete heritable morphs maintained generationally against the forces of genetic drift and recombination?

Timema cristinae walking sticks are ideal species to address these questions, because colour variation has a simple genetic basis, involving just one or a few loci of large effect. Furthermore, *T. cristinae* has distinct colour morphs that coexist as an integral property of all natural populations, often even on the same host plant (Comeault et al., 2015). Unlike many other insects that flee from predators, this species is wingless and relies entirely on colour crypsis as protection against avian predators while resting on their host plants *Adenostoma fasciculatum* and *Ceanothus spinosus* in the chaparral vegetation of California. Three colour morphs can be distinguished: a rare melanistic morph that is cryptic against plant stems, fruits and soil; a common green colour morph that resembles plant leaves; and a green morph with a white longitudinal dorsal stripe that is adapted to the narrow needle-like leaves of *A. fasciculatum* (Comeault et al., 2015; Figure 1).

Lindtke et al. (2017) study populations of *T. cristinae* to understand how colour morphs are determined and what processes allow coexistence of morph. In that quest, the authors combine genotyping-by-sequencing and phenotyping data from hundreds of samples across 21 populations, making this study a powerful “ecological genomics” example to study the genetic mechanisms underlying responses of organisms to their natural environments. A genome-wide PCA analysis found that the three colour morphs were associated with three highly divergent chromosomal variants, existing in three homo- and three heterokaryotypic combinations. The homokaryotypic clusters showed strong genetic differentiation that could be mapped to one region of around 13 Mb covering 29% of LG 8, consistent with a chromosomal inversion that suppresses the effective recombination rate and genetic exchange among chromosomal variants. Association of these variants was in agreement with known dominance and linkage patterns both within and among colour morphs, and candidate SNPs for colour and pattern genes could be mapped within the boundaries of the defining divergent variants, adding further support that these regions are linked to colour morph variation.

Owing to the relatively recent advent of genome-wide sequencing technologies, we now know that colour loci of several polymorphic species are co-expressed with functionally unrelated genes by either tight physical linkage in the genome, through epistatic

interactions, or inversions (Wellenreuther, Svensson, & Hansson, 2014). Of these, inversions have been increasingly linked to colour polymorphisms across the animal kingdom, from the bright colour polymorphism in the mimetic butterfly *Heliconius numata* (Joron et al., 2011) to the plumage colours of the white-throated sparrow *Zonotrichia albicollis* (Thomas et al., 2008; Figure 2). The association of inversions with complex colour phenotypes is no coincidence, but instead occurs because inversions effectively prevent allelic combinations in heterokaryotypes, and thus are powerful genetic architectures to prevent the creation of nonoptimal intermediates. The finding that *T. cristinae* colour morphs are likely hardcoded by an inversion suggests that evolution can occasionally take large leaps across phenotypic space and that the evolution of inversions to reduce recombination between co-adapted alleles may be a common phenomenon in colour polymorphic species.

Lindtke et al. (2017) then took a closer look at the genetic signatures of the genetic clusters and found that genome-wide F_{ST} ranged from 0 to 1 and that the highest differentiated regions were mostly falling within the putative colour morph region on LG8, indicating extended coexistence of clusters. Indeed, additional genome-wide statistics reflecting the accumulation of an independent set of mutations between the melanistic and either green variant, Dxy and Zg, were also elevated, indicating varying selection between variants, in line with an old inversion polymorphisms maintained by balancing selection. In contrast, divergence estimates between the two green variants were only slightly elevated and showed increased haplotype homozygosity, suggestive of a recent selective sweep of the green-striped variant (Guerrero, Rousset, & Kirkpatrick, 2012). The age of the split between the melanistic and green-unstriped chromosomal variant was estimated to be ancient (13.5–8 mya), whereas the split between the unstriped- and striped-green variants was considerably more recent (2.7–1.8 mya). Moreover, increased levels of linkage disequilibrium as well as elevated levels of Tajima's D, the latter being a more unbiased indicator of historical processes when inversions are involved, were detected along colour morph-associated genetic clusters stretching several hundreds of kb, further indicating that balancing selection has been acting on morphs in the distant past (Guerrero et al., 2012).



FIGURE 1 Three colour morphs of *Timema cristinae*. The left panel shows the rare melanistic morph that is most cryptic against plant stems, fruit or soil. The middle panel shows the common green (unstriped) morph that is best hidden against green leaves. The right panel shows the green-striped morph that gains crypsis by matching the needle-like appearance of the *Adenostoma fasciculatum* leaves (copy credit Aaron Comeault)

FIGURE 2 Colour plate of some representative examples of colour polymorphic species. The top panel shows the white (left) and tan (right)-striped morph of the white-throated sparrow *Zonotrichia albicollis* (photograph credit Elaina Tuttle). Colour in this species resides inside an inversion polymorphism that is maintained by negative-assortative mating. The middle panel shows the three female colour morphs of the blue-tailed damselfly *Ischnura elegans*: the male-mimicking androchrome, infuscans and infuscans-obsoleta morph (photograph credit Maren Wellenreuther). Colour polymorphism in this species is female limited to reduce excessive male mating harassment and is maintained via negative frequency-dependent sexual selection on female colour. The genetic architecture of colour in *I. elegans* is not known, but is currently being investigated. The bottom panel shows some of the colour morphs of the mimetic butterfly *Heliconius numata* including the silvana, bicoloratus and tarapotensis morphs (photograph credit Mathieu Joron). Colour in this butterfly has also been linked to an inversion polymorphism and is maintained via frequency-dependent sexual and natural selection on warning colour patterns

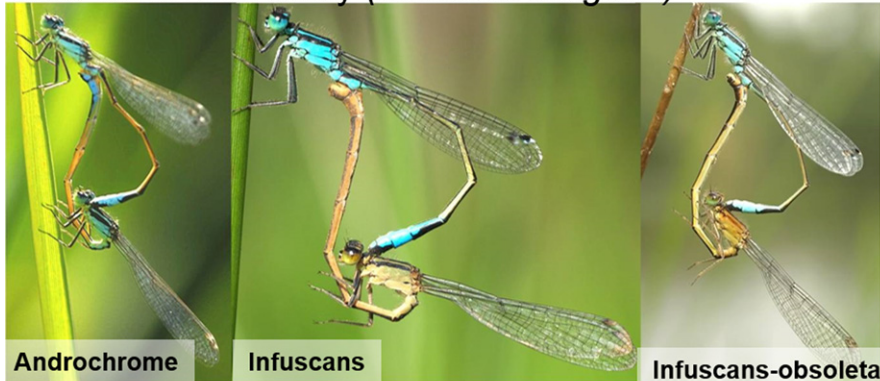
White-throated sparrow (*Zonotrichia albicollis*)



White-striped

Tan-striped

Blue-tailed damselfly (*Ischnura elegans*)

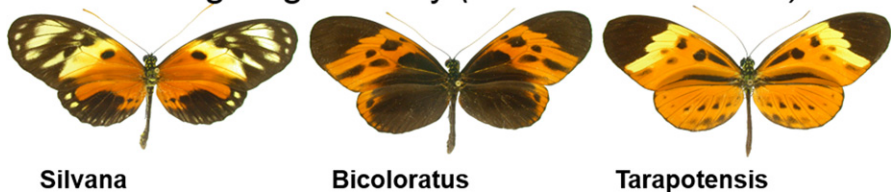


Androchrome

Infuscans

Infuscans-obsoleta

Numata longwing butterfly (*Heliconius numata*)



Silvana

Bicoloratus

Tarapotensis

Given this ancient split of the melanistic and green variants, the authors were intrigued to disentangle the mechanisms that allow inversion persistence over such long time frames. Analysing all populations for the presence of the melanistic and green morphs, they found that the corresponding chromosomal variants were abundant across the species range and present at similar frequencies, a pattern that is compatible with geographically widespread balancing selection. Balancing selection refers to a suite of selective processes that allow multiple alleles to be maintained at frequencies larger than expected from genetic drift alone and includes frequency-dependent selection, heterozygote advantage (overdominance), selection that varies in direction and intensity across space or time and antagonistic evolution within species, including sexual conflicts, as well as disassortative mating. The finding that balancing selection may allow *T. cristinae* colour morphs to be maintained over extended time frames is important for two main reasons. First, empirical examples for balancing selection are still rare, because the discovery of locally adaptive polymorphisms subject to balancing selection has long been hampered by a lack of suitable methods, resulting in many false positives, even when the selection coefficients are large relative to the migration rate (Beaumont & Balding, 2004). Moreover, recombination is expected to rapidly erase the effects of selection in close proximity to the selected sites, such that soft sweeps on new targets

of balancing selection often only leave transient genomic footprints, which will diminish the chances of detection. Second, evidence that balancing selection can operate over long timescales is debated. Recent theoretical studies indicate that balancing selection may only be a short-term process that cannot efficiently promote the long-term maintenance of adaptive polymorphisms because of the genetic load they create (Fijarczyk & Babik, 2015). In addition, environmental changes may cause allele frequency shifts that could cause the loss of formerly stable polymorphisms, something that is supported by an increasing number of studies that report the transient nature of balancing selection (Fijarczyk & Babik, 2015). Consequently, the commonly held view is that balancing selection mainly affects a few classes of genes (e.g., those related to immune responses) and that its overall role in maintaining variation is relatively minor (Hedrick, 2012).

To further explore the types of balancing selection on *T. cristinae* colour morphs, Lindtke et al. (2017) then calculated a number of population genetic parameters and found that heterokaryotypes were in excess relative to their expected Hardy–Weinberg frequencies. This excess was detected both in the majority of populations and in all populations with a sufficiently large sample size. Two main candidate mechanisms are then explored to explain heterozygote excess, namely negative-assortative mating and heterozygote

advantage selection, also known as overdominance. Negative-assortative mating is the main balancing selection mechanism operating in colour morphs of the white-throated sparrow (Figure 2), and in which almost all breeding pairs are between birds with different male plumage colours (Thomas et al., 2008). In *T. cristinae*, however, both the frequencies of recessive colour alleles and mating trials do not support a scenario where negative-assortative mating could cause heterozygote excess. Heterozygote advantage selection occurs when the combination of two different alleles has a positive synergistic effect on the phenotype and takes place when heterozygosity in itself produces a benefit. Empirical evidence that heterokaryotype advantage selection can maintain adaptive polymorphisms is rare, particularly for old polymorphisms. One of the few examples include the seaweed fly *Coelopa frigida*, where a large inversion polymorphism on chromosome I causes a threefold size difference in males, affecting mating success and development time, and where heterozygotes are nearly always found in excess of Hardy–Weinberg expectations in natural populations and show higher viability than either homozygote under laboratory conditions, especially at high larval densities (Butlin & Day, 1984).

In conclusion, the genomic underpinnings of crypsis in *T. cristinae* walking sticks show several parallels with colour polymorphic traits in other species, suggesting that a genetic architecture that prevents the formation of maladapted intermediates without reducing gene flow genome-wide might be a common evolutionary outcome. Finally, the finding that heterokaryotype advantage selection might be acting on the ancient colour polymorphism provides an important empirical example to better understand the role of balancing selection in the long-term maintenance of adaptive polymorphisms in phenotypic evolution.

AUTHOR CONTRIBUTIONS

M.W. wrote the manuscript.

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