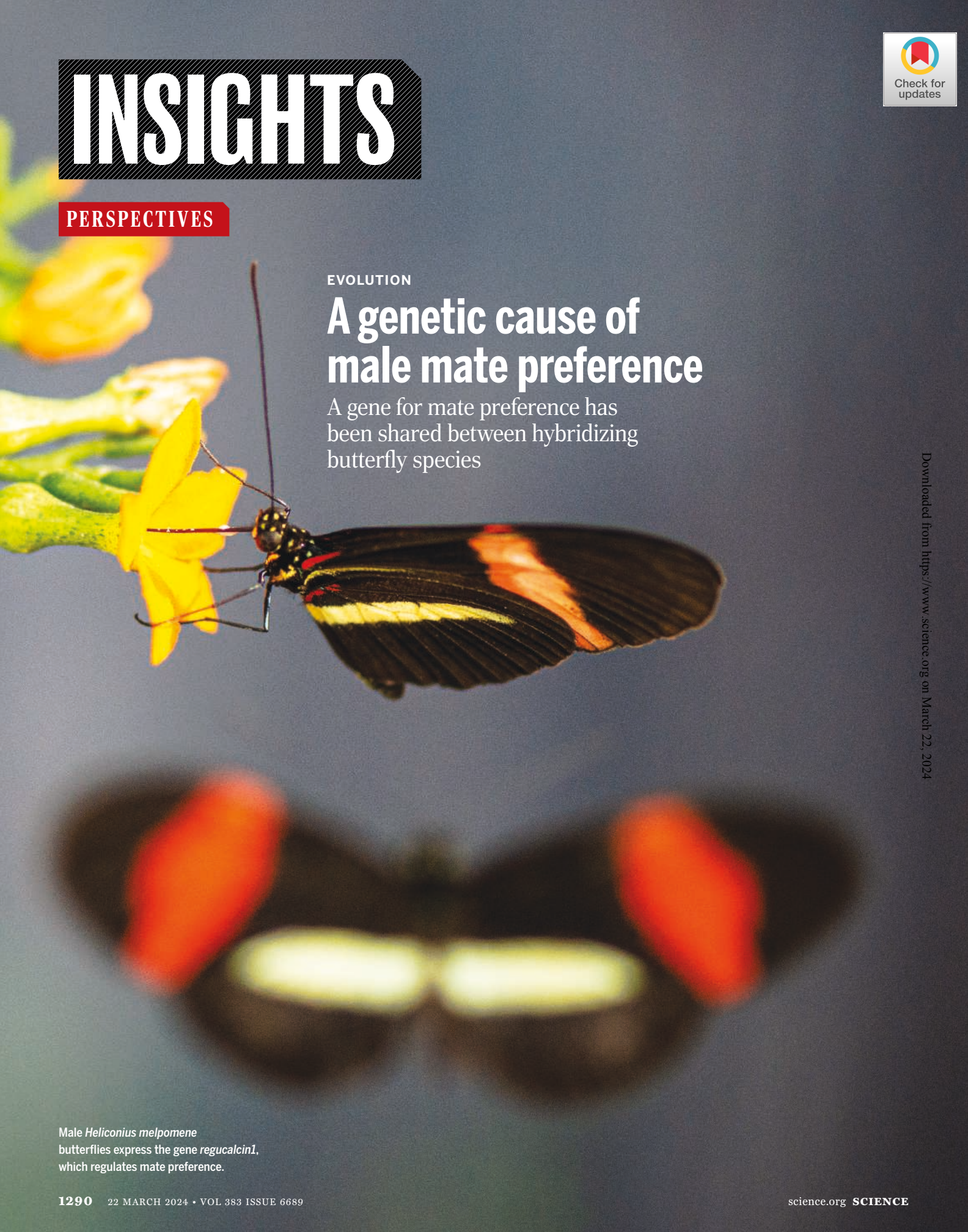
INSIGHTS

PERSPECTIVES

EVOLUTION

A genetic cause of male mate preference

A gene for mate preference has been shared between hybridizing butterfly species



Male *Heliconius melpomene* butterflies express the gene *regucalcin1*, which regulates mate preference.

By **Adriana D. Briscoe**

For millions of years, brightly colored and unpalatable *Heliconius* butterflies have flitted around vine-entangled forests of the Neotropics (*1*). Between geographical regions, a single species of *Heliconius* can have substantially different wing color patterns, while resembling local unrelated species. The speed with which these mimetic wing color patterns evolve can be accelerated if the butterflies' mate preference coincides with the locally adapted wing color pattern (cue). Several genes involved in wing color pattern have been identified (*2*), but genes that underlie behavioral preferences (*3*), and the extent to which color pattern is genetically linked to mate preference in *Heliconius,* and animals in general, remain to be uncovered. On page 1368 of this issue, Rossi *et al*. (*4*) report the genetic basis for behavioral wing color preference in *Heliconius* butterflies. Their findings reveal some of the mechanisms by which cues and preferences are most likely to evolve, while ruling out less likely hypotheses.

Although pheromones play a role in butterfly mate choice, the decision to approach or court is initially visual. Behavioral experiments revealed that *Heliconius* butterflies most often prefer to mate with the locally adapted wing color pattern, indicating that natural and sexual selection reinforce each other (*5*). Linkage disequilibrium—the nonrandom association of alleles between loci—is expected to evolve between preference and cue genes owing to assortative (nonrandom) mating. The relative prevalence of genetic mechanisms reinforcing linkage disequilibrium are less clear. More than 50 years ago, physical linkage between cue and preference loci was predicted to facilitate speciation (*6*). Taken to a logical extreme, some theorists proposed that both preference and cue loci might be encoded by the same gene with pleiotropic effects, a single-gene "magical trait" model (*7*). Magical traits such as wing color pattern in *Heliconius* evolve under divergent ecological selection and can result in differences in mate preference (*7*). Other examples include the fruit fly *Drosophila serrata*, in which methyl-branched cuticular hydrocarbons that are encoded by a single locus allow populations to adapt to different water-limited habitats, which generates reproductive isolation as a by-product. Inactivating the gene for methyl-branched hydrocarbons affects female mate preference, although the preference genes themselves are unknown (*3*).

To identify genes involved in both signal generation and male mate preference in *Heliconius*, polymorphic populations or closely related partially fertile species have been studied (*8, 9*). Mate preference for live *Heliconius* butterflies of species with distinct wing colors or for butterflies with color patterns that were altered by the authors were measured. Next, genetic crosses were created, and the mate preferences of F1 and F2 backcross hybrid males for females of the parental species were analyzed. This led to the mapping of *wingless*—a candidate gene associated with the K locus (which underlies white versus yellow forewing color)—to the same genomic region as that of male mate preference for wing color in *H. cydno* and *H. pachinus* (*8*). Because *wingless* was subsequently found to be expressed in butterfly larval wing margins (*10*) (but not in the parts of the wing giving rise to the K locus's color pattern element), it is unlikely to be directly involved in the generation of the color pattern cue, and the preference gene or genes in this genomic region have yet to be identified.

Subsequent work that mapped loci responsible for male mate preference between *H. cydno* and *H. melpomene* identified three loci affecting mate choice on three chromosomes (*11*). Two of the preference loci were unassociated with the K locus or any other known wing color pattern gene. By contrast, a preference locus on chromosome 18 was identified near the *optix* gene, which controls red wing color (*2*). Could *optix* and the mate preference gene be the same, that is, a single gene that encodes a magical trait and a behavioral preference for that trait? The apparent lack of recombination (or independent segregation of alleles) between the unknown preference gene and *optix* in hybrid males—and the absence of an inversion in this genomic region between species (which would lead to a reduction in recombination) (*12*)—suggested it might be a rare example of a single locus that determines the cue and preference for it.

Rossi *et al*. suggest several lines of evidence to rule out the single-gene magical trait hypothesis. They investigated the genetic history of white-winged *H. cydno* and red- and yellow-winged *H. timareta* and *H. melpomene,* identifying a region of admixture between *H. timareta* and *H. melpomene* around the genomic region associated with male mate preference for wing color (and *optix*), which suggested that these two species have shared DNA in this region (an example of adaptive introgression, or sharing of genetic material between species driven by selection). They classified genomic segments into regions in which either *H. timareta* and *H. melpomene* are most closely related or into regions where *H. cydno* and *H. timareta* are most closely related. One region of similarity between *H. timareta* and *H. melpomene* corresponds to the region that includes both the behavioral mate preference locus and *optix*. They then identified a selective sweep in *H. timareta* in part of the region containing the behavioral mate preference locus that excludes *optix*, which suggests that regions that control wing color (*optix*) and preference are different.

There are at least 200 genes that comprise the genomic interval encompassing the behavioral preference peak identified by Rossi *et al*. A single gene, *regucalcin1*, was more highly expressed in *H. cydno* compared with *H. timareta* and *H. melpomene* males. Rossi *et al*. showed that *H. melpomene* males in which *regucalcin1* was inactivated lost interest in courting females, whereas other traits such as foraging for nectar and wing color were not affected. Therefore, *regucalcin1* regulates mate preference in *H. melpomene* males, and likely also in *H. timareta* owing to their sharing of *regucalcin1* alleles (through hybridization between the two species). Notably, *regucalcin1* is one of many genes likely involved in mate preference, and although an impressive number (794) of wild-type and hybrid males were behaviorally tested (across 3637 trials) and genotyped, the number of butterflies involved may still be too few to resolve loci with smaller effects on behavior. In addition, the specific mechanism of action in determining male mate preference of *regucalcin1,* which is expressed in eyes and parts of the brain, is not clear. Because regucalcin appears to be a broad-acting protein involved in intracellular Ca^{2+} signaling (*13*), there will be many possible functional roles to investigate. Might *regucalcin1* be involved in modifying the expression of other mate preference genes? Examining the mechanisms by which *regucalcin1* acts to regulate mate preference at the neuronal level could be a promising direction for future research. ■

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