Speciation: On the Scent of Mate Discrimination Genes

Jennifer M. Coughlan and Daniel R. Matute*

Biology Department, University of North Carolina, Chapel Hill, North Carolina, 250 Bell Tower Drive, Genome Sciences Building, Chapel Hill, NC 27514, USA
*Correspondence: dmatute@email.unc.edu
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Animals use smell to recognize individuals from their own species and find suitable mates. A study of female chemical cues in two species of fruit flies uses a creative genetic strategy to identify an allele that is involved in species recognition and may play an important role in keeping these species apart in nature.

The ability of species to distinguish conspecifics from close relatives is essential in the speciation process. The emergence of new species stems from the formation of reproductive barriers, which prevent hybridization between close relatives. These barriers can manifest prior to the formation of hybrids, as in the case of mate choice, or after a hybrid zygote is formed (pre- and post-zygotic reproductive isolation, respectively). While both are important, pre-zygotic barriers tend to evolve more quickly and thus may play a crucial role for young species pairs (reviewed in [1]). Determining the genetic basis of reproductive barriers is key to understanding speciation, and *Drosophila* has been a useful model system for such genetic analyses [2]. Still, relatively little is known about the genetic basis of pre-zygotic barriers, particularly behavioral barriers. Mate choice occurs when one sex simultaneously recognizes conspecifics and discriminates against heterospecifics of the opposite sex. This reproductive barrier involves the evolution of both a novel trait by which species can differentiate conspecifics and heterospecifics, as well as the evolution of a preference for that trait. Speciation geneticists are interested in both facets of isolation. Genetic loci underlying differences in mating cues between related species have been mapped to the gene level [3]. In some cases, the precise neurological circuits and genes involved in the ability to differentiate females from different species also have been identified (e.g. [4]). We now need to identify genes that underlie traits and behaviors involved in isolation between closely related species. In this issue of *Current Biology*, Acuña-Combs *et al.* [5] determine the role of *cis*-regulatory divergence of a single

gene in male mate choice in two closely related species of the *Drosophila melanogaster* species subgroup.

The *melanogaster* species complex includes four species: two widespread human commensals (*D. melanogaster* and *D. simulans*), and two island endemics (*D. sechellia* and *D. mauritiana*). In particular, *D. simulans* and *D. sechellia* have served as a test case to understand the evolution of reproductive isolation. These species diverged relatively recently (within the last 100,000 years [6]) and can produce viable hybrids. As only the female hybrids are fertile, much research has focused on hybrid male sterility (e.g. [7]). In addition, *D. sechellia* has evolved to breed on *Morinda* fruit, a substrate that is toxic to *D. simulans* and may serve as an ecological reproductive barrier [8]. Lastly, *D. sechellia* and *D. simulans* co-occur and hybridize in nature, and have a recent history of introgression [6,9]. Thus, *D. sechellia* and *D. simulans* present an interesting system to study reproductive barriers.

Many *Drosophila* species also show strong mate discrimination [10]. *D. simulans* males are able to discriminate against both *D. sechellia* and *D. melanogaster* females. This discrimination is largely based on female cuticular hydrocarbon (CHC) profiles [11], a species-specific scent produced by specialized cells called oenocytes (insect cells responsible for lipid processing and the only cells to produce CHCs [12,13]). While previous studies have used genetic mapping to identify the alleles involved in the CHC profile differences between *D. simulans* and *D. sechellia* [14], no candidate genomic region has been resolved to the gene level. Acuña-Combs *et al.* [5] now use a novel mapping approach to pinpoint the *cis*-regulatory

divergence of a single gene, *eloF*, which contributes to CHC profiles and mate discrimination between *D. simulans* and *D. sechellia.*

Acuña-Combs *et al.* [5] performed RNA sequencing on oenocytes and adjacent fat body tissue in both male and female F1 hybrids, and they searched for genes that show allele-specific expression and female- and oenocyte-biased expression (Figure 1). By looking for genes with expression patterns that fit these criteria, the authors were able to find genes where the expression differences both arise from *cis*-regulatory divergence and also specifically correlate with a particular female CHC profile. This two-step approach bypasses the need to generate hundreds of advanced intercrosses, which are usually required for genetic mapping.

Using the first criterion — identifying genes that have patterns of allele-specific expression in female oenocytes — the authors found 239 genes that show *cis*-regulatory divergence between *D. simulans* and *D. sechellia* in female oenocytes. Aided by the second criterion — filtering for genes with tissue- and sex-biased expression — the authors reduced this list to merely six genes, two of which had previously been implicated in the production of CHCs [14]. To confirm the role of these candidate genes in the CHC profile, the authors used RNAi knockdowns in *D. melanogaster* to manipulate gene expression. The reduced expression of a single gene — *eloF* — that is expressed in female *D. sechellia* oenocytes, but not in female *D. simulans* oenocytes, results in CHC profiles that begin to mimic female *D. simulans*. With this candidate in hand, the authors used CRISPR–Cas9 gene editing to ablate the expression of *eloF* in
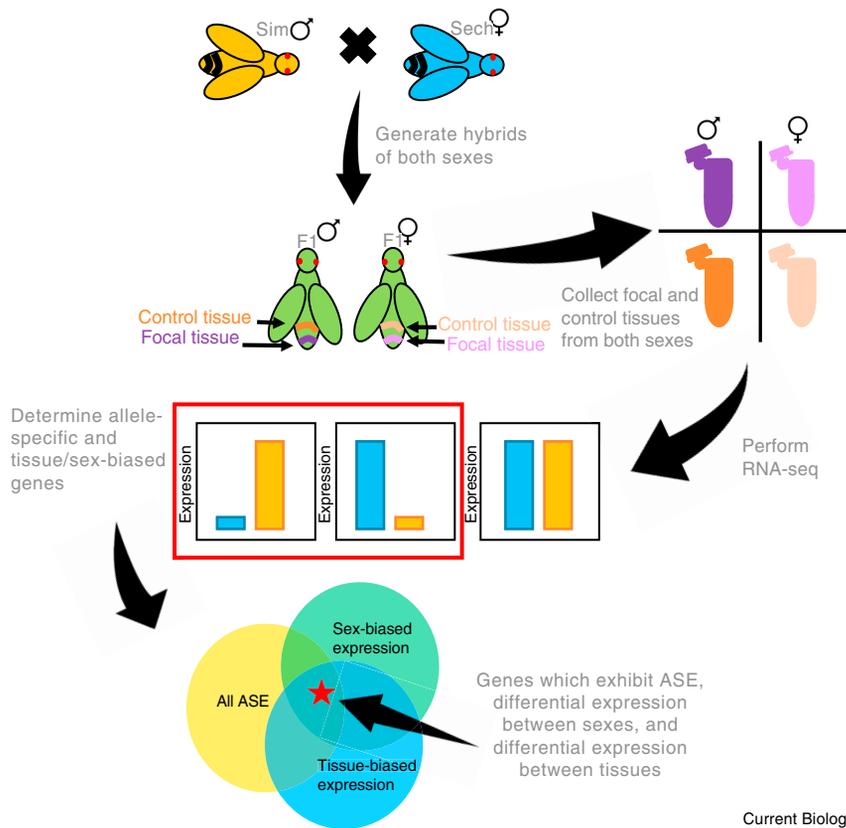
Figure 1. Experimental design to determine sex-, tissue-, and species-specific expression in regions of *cis*-regulatory divergence.

F1 hybrids, of both sexes, from a cross between *D. simulans* (Sim) and *D. sechellia* (Sech) are created, then the tissue of interest (focal tissue) is sampled alongside an appropriate control tissue, expression is measured (for example, using RNA sequencing (RNA-seq)), then genes are parsed based on patterns of allele-specific and sex- and/or tissue-biased expression. Genes that show allele-specific expression (ASE) unique to the sex and tissue of interest are good candidates for genes that have *cis*-regulatory divergence and regulate the trait of interest.

female *D. sechellia*, and again found that these *eloF⁻* females have a much more *D. simulans*-like CHC profile.

Finally, Acuña-Combs *et al.* [5] performed mate discrimination trials to test whether *D. simulans* males are able to recognize and discriminate against these *eloF⁻ D. melanogaster* and *eloF⁻ D. sechellia* females. *D. simulans* males court both *D. melanogaster* and *D. sechellia eloF⁻* mutant females as frequently as they court their own conspecific females. This suggests that *eloF* expression differences between *D. simulans* and *D. sechellia* females underlie the CHC profile differences, and these expression differences ultimately act as a trait by which *D. simulans* males can differentiate conspecific and heterospecific females. This study represents a tour-de-force examination of the genetic basis of pre-mating

reproductive isolation and behavior, and interrogates these parental genomes using a novel approach.

This alternative mapping approach, combined with a well-designed experimental setup as highlighted by Acuña-Combs *et al.*, should be useful to uncover the genetic basis of other traits. Allele-specific expression approaches can be powerful in interrogating trait differences caused by *cis*-regulatory divergence, but will miss trait differences that are due to *trans*-regulatory divergence or divergence in coding sequence. Additionally, knowledge of the relevant tissues (as well as controls) to analyze requires a deep *a priori* understanding of the developmental and/ or physiological basis of the traits. Lastly, trait differences that are caused by more minimal *cis*-regulatory expression divergence will be more challenging to

detect. In the case of this study by Acuña-Combs *et al.* [5], a carefully designed RNA-sequencing experiment was sufficient to obtain a clear set of candidate genes and then pursue an allele with a major effect, *eloF*. Allele-specific expression RNA-sequencing experiments and quantitative trait loci mapping have the potential to be integrated to jointly aid in the dissection of the genetic basis of specific traits.

The contribution of Acuña-Combs *et al.* [5] to speciation genetics is important and lays the groundwork for answering broader evolutionary questions. First, understanding the mechanisms responsible for mate preference evolution is crucial. One possible mechanism is reinforcement — the evolution of increased pre-mating isolation to decrease the costs of hybridization [15]. While reinforcement presents a potential explanation for the evolution of mate discrimination and is important in other *Drosophila* species where CHC profile differences contribute to mate choice (e.g. [16]), CHC divergence may have evolved in response to ecological conditions (e.g. [17]), sexual selection (e.g. [18]), or a combination of these factors. Second, it is also important to determine whether alleles that confer reproductive isolation in the lab are good barriers to introgression in nature; this can be accomplished by determining rates and spatial resolution of introgression of *eloF* in nature. Lastly, theory predicts that mate preference and the preferred trait will become genetically correlated [19]. In systems where mate preference is asymmetric, and the genetic basis of mate choice and preference traits are genetically complex (that is, both trait and preference are polygenic [14], and these candidate regions do not overlap [20]), it will be important to understand if and how linkage between trait and preference loci are maintained in the face of gene flow.

Understanding the genetic basis of reproductive barriers is one of the main goals of speciation research. The study by Acuña-Combs *et al.* [5] is one of the first to identify a gene controlling a trait involved in mate discrimination between naturally hybridizing sister species. As more mate preference genes are identified, we will be able to draw general patterns of how mating preferences evolve and, ultimately, how species arise.

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Active Sensing: Constancy Requires Change

Volker Hofmann and Maurice J. Chacron[*]

Department of Physiology, McGill University, Montreal, Quebec, H3G 1Y6, Canada
*Correspondence: maurice.chacron@mcgill.ca
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We constantly generate movements in order to enhance our ability to perceive the external environment. New research on electric fish has used augmented reality to demonstrate that animals dynamically regulate their movements to maintain variability in their sensory input.

During day-to-day life, our senses continually inform us about the external environment — a process that is very much influenced by our own movements. In some cases, movements act in a compensatory manner to stabilize the sensory input: for example, when fixating a given target by making compensatory eye movements while moving our head. In other cases, movements act to enhance sensory information: this is known as active sensing (Figure 1A): for example, when looking for keys and coins in our pocket, we make complex exploratory hand and finger movements to gather information about the characteristics of these objects, such as their shape, texture, weight and so on. Active sensing is found ubiquitously across sensory modalities and contexts [1,2]. Nevertheless, how such movements are controlled and adapted depending on context — for example when manipulating a dull *versus* a sharp object — is not known. A new study by Biswas *et al.* [3], reported in this issue of *Current Biology*, brings important insights into the control of active sensing movements. By studying weakly electric fish in an augmented reality setup, these authors were able to show how active sensing movements are dynamically regulated in relation to the sensory feedback that they generate.

Gymnotiform weakly electric fish such as *Eigenmannia virescens* (Figure 1B) rely on their active electric sense to explore their environment — they generate a three-dimensional electric field around their body