Minireview

Assessing the origin of species in the genomic era

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Abstract

Advances in genomics have rapidly accelerated research into the genetics of species differences, reproductive isolating barriers, and hybrid incompatibility. Recent genomic analyses in *Drosophila* species suggest that modified olfactory cues are involved in discrimination that is reinforced by natural selection.

Ever since Darwin laid out overwhelming evidence for the mutability of species, biologists have sought to explain the forces driving the genesis of new species and the genetic changes involved in speciation. Frequently, this goal has been translated into the study of the genetic basis of species differences, especially the genetic causes of inviability or sterility in hybrids between species. Despite creative early approaches to these problems [1], however, classical genetic studies provided insufficient resolution for identifying the specific genomic regions and genes responsible for these traits. It is unsurprising, then, that the field of speciation genetics is being revolutionized by the rapidly expanding availability of genomic tools, techniques, and data, especially in the model speciation systems such as *Drosophila*. The resulting contemporary studies of the genetics of speciation most frequently involve detailed linkage-mapping analyses of the quantitative trait loci (QTLs) underlying the isolating barriers and hybrid incompatibility between closely related species.

The genomics of species divergence and hybrid incompatibility

By far the most likely, and most explicable, form of speciation occurs when populations diverge from each other while separated by an external barrier to gene flow, such as simple physical distance. Genetic changes can accumulate in these isolated populations, either in response to different environmental pressures or purely through random sampling processes (genetic drift). As a consequence, when diverged populations are brought back into contact, exchange of genes between them is restricted; for example, diverged mating signals may prevent hybridization, or hybrids may be unfit either because of inappropriate genic interactions or because they are phenotypically intermediate and thus ill-suited to either parental environment. Most of the recent QTL mapping approaches have examined species differences and hybrid incompatibility in the context of this model of 'allopatric' speciation (speciation during physical isolation in the absence of gene flow). The most detailed studies have focused on identifying the number, genomic location, and distribution of individual effects of QTLs underlying hybrid male and female sterility among *Drosophila* species [2,3]. So far, the results indicate a number of general patterns in the genetics of *Drosophila* hybrid incompatibility; for example, incompatibility is frequently highly polygenic and epistatically complex, and within any specific cross many more loci confer hybrid male sterility than confer female sterility or hybrid inviability [2-6].

Hot on the heels of these landmark *Drosophila* studies are a suite of related analyses of speciation in a newer but increasingly developed wave of model systems, including sunflower [7], monkeyflower (*Mimulus*) [8,9], mosquito [10], and tomato [11]. With the inclusion of these new systems, evolutionary geneticists are beginning to piece together a general understanding of the genetic architecture of speciation, as well as the biological factors that might contribute to the differences observed between phylogenetically divergent

groups [11]. In addition to expanding the phylogenetic scope of speciation genetics, the increasing availability of genomic tools is also enabling the dissection of more complex modes of speciation. One recent study is that of Ortiz-Barrientos and colleagues published in *PLoS Biology* [12]; they examine the genetics of speciation by reinforcement - one of the most attractive but controversial models of speciation.

The genetics of speciation by reinforcement

Speciation by reinforcement has long held biologists' attention because it unites two classical evolutionary processes: speciation and natural selection. During allopatric speciation, natural selection can play only an indirect role in the evolution of reproductive barriers, by bringing about trait changes that inadvertently prevent gene flow between diverging populations. Under reinforcement, however, natural selection directly favors the evolution of barriers to mating between incipient species. The most straightforward conceptual model of this process imagines two species (or highly diverged populations) that have accumulated some degree of genetic incompatibility (in isolation or allopatry), such that hybrids between them have reduced fitness. Nonetheless, because genetic differentiation between the groups is incomplete, when they co-occur in the same geographical location (that is, they are in 'sympatry'), less fit hybrids can be formed. In this case, any individuals that preferentially mate with only their own kind will have a selective advantage because they do not waste any reproductive effort on producing sterile hybrid offspring. In regions of geographical overlap, natural selection will thus act directly to 'reinforce' the partial isolation between two groups by favoring traits that reduce inter-type matings. Although the frequency of speciation by reinforcement (especially in comparison with simple allopatric speciation) continues to be debated, it now seems clear that there is solid theoretical support for this mode of speciation, as well as empirical support in a few well described cases [13].

Ortiz-Barrientos and colleagues [12] have examined the genetics of mate discrimination in one such probable case of reinforcement between two very closely related fruit-fly species. *Drosophila pseudoobscura* and *D. persimilis* co-occur in coastal northwestern USA, but *D. pseudoobscura* is also found alone throughout a large proportion of its natural range. In artificial mating trials between the two species, *D. pseudoobscura* females from allopatric populations show weak mating discrimination against *D. persimilis* males (described as 'basal' mate discrimination), whereas females from sympatric populations show enhanced mating discrimination (described as 'reinforced' mate discrimination) [14]. This pattern is consistent with the operation of reinforcement, as selection is expected directly to favor strong mating discrimination in sympatry only: no hybrids can be produced in allopatry, so there is no direct selective pressure for increased mate discrimination in allopatric populations.

Ortiz-Barrientos and colleagues confirmed these mate-discrimination patterns and went on, in a series of backcrosses, hybrid mating trials, and QTL mapping analyses, to identify the genomic locations of traits that are responsible for the reinforced mate discrimination of *D. pseudoobscura* against *D. persimilis*. Their analysis is particularly novel in that it capitalizes on within-species variation in mating propensity in order to understand the genetic basis of trait changes involved in reinforcement between species. To do so, the general strategy was to cross allopatric to sympatric populations within *D. pseudoobscura* and then to mate the resulting hybrid females with *D. persimilis* to assess their level of mating discrimination (Figure 1). This allowed Ortiz-Barrientos *et al.* [12] to map the QTLs associated with reinforced mate discrimination against *D. persimilis*: using two separate sympatric-allopatric population pairs of *D. pseudoobscura* derived from four different locations, they first analyzed whole-chromosome effects on mating discrimination by backcrossing to F1 males (there is no meiotic recombination in *Drosophila* males so chromosomes are inherited as unrecombined blocks in this case). They showed that whole-chromosome effects differ between different allopatric-sympatric population pairs, suggesting that there is a different genetic basis for reinforced mate discrimination against *D. persimilis* in the two different sympatric *D. pseudoobscura* locations examined. Second, using a recombinant backcross population (BC1) derived from a single *D. pseudoobscura* allopatric-sympatric combination, they localized two strongly supported and two probable QTLs to regions on chromosome 4 and the

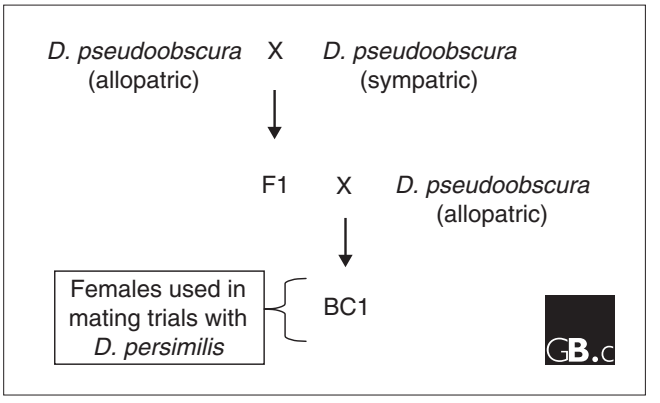

Figure 1
A generalized scheme of the crossing procedure used by Ortiz-Barrientos *et al.* [12] to analyze mating discrimination against *D. persimilis* by *D. pseudoobscura* females in sympatric populations. Sympatric and allopatric populations of *D. pseudoobscura*, differing in their levels of mating discrimination against *D. persimilis*, were crossed to produce F1 hybrids, which were then backcrossed to the same allopatric population to produce BC1 flies with segregating variation in mating-discrimination traits. BC1 females were tested in mating trials with *D. persimilis* to assess the degree of mating discrimination. Females were then genotyped at 70 markers distributed throughout the genome in order to map sympatric mating-discrimination loci.

X chromosome, respectively, using standard QTL mapping. Finally, by consulting the recently sequenced *D. pseudoobscura* genome, as well as functional genomic information from *D. melanogaster*, the researchers identified likely candidate loci that lie within the mapped chromosomal regions. These include two loci (*bru-3* and *CG13982*) whose mutation with P-elements leads to smell impairment, as well as several UTP-glycosyltransferases that encode enzymes involved in detoxification and olfaction. Although these candidate genes are necessarily tentative until the requisite functional assays are performed, the results implicate olfactory changes as important factors in female mating discrimination under reinforcement.

In the light of prior studies, several substantive conclusions follow from this novel combination of analyses. First, the QTLs underlying reinforced mate discrimination clearly differ from those previously identified as underlying the basal isolation that separates all *D. pseudoobscura* and *D. persimilis* populations. This basal isolation, expressed as weaker female mate discrimination, was previously mapped to two inverted regions on chromosome 2 and the X chromosome [15]. Along with other studies [16], this finding ignited the recent interest in models of speciation involving regions of substantially reduced recombination such as chromosomal inversions. In the new study, however, there is no evidence for the role of inversions in reinforced mate discrimination, suggesting that very different genetic mechanisms underlie this second layer of isolation between species. The evidence that the genetic basis of mate discrimination differs even among different sympatric populations within *D. pseudoobscura* also emphasizes the fact that different genetic systems may be recruited during the evolution of reproductive barriers. The second major conclusion is that different mate-signaling modalities appear to be involved in reinforced versus basal layers of reproductive isolation. Basal isolation is thought to be due to changes in auditory cues during mating [15], whereas reinforced mate discrimination probably involves modified olfactory signals between sympatric *D. pseudoobscura* and *D. persimilis* [12].

Many genetic paths to speciation

In combination, these two substantive conclusions support the intuition of many biologists that overall reproductive isolation between species is likely to be due to the combined effect of numerous different trait changes. Nonetheless, whether the particular genetic mechanisms or kinds of traits involved in speciation differ systematically between phylogenetic groups, or between different stages of reproductive isolation, remains to be clarified in future studies on other complementary systems. It is reasonable to expect, for example, that pre-mating barriers to interspecific gene flow will frequently involve trait changes that are directly connected with mating or reproductive interactions. In the case of *D. pseudoobscura* and *D. persimilis*, these traits are both the olfactory and the auditory factors that presumably affect perception of potential mating partners. In comparison, mating isolation between adjacent monkeyflower species involves changes in floral traits that influence the attractiveness of flowers to pollinators, and thus reduce interspecific pollinations [8]; much of this variation in pollinator visitation is associated with loci that control flower coloration [9]. In both cases, although the specific trait changes are quite different, they have straightforward biological links to their corresponding species barriers. In contrast, it seems less certain that the genetic underpinnings of hybrid inviability and sterility will be biologically unified or predictable. Indeed, in the handful of cases in which researchers have identified individual genes that confer hybrid inviability or sterility [17-19], there is little indication that particular classes or kinds of genes are routinely involved in hybrid incompatibility, although all such loci do appear to be rapidly evolving. Other evidence similarly suggests that the genetic complexity of speciation traits might also differ systematically between different stages of reproductive isolation [15] or among different biological systems [11].

Finally, beyond enhancing our understanding of the details of reinforcement, the work of Ortiz-Barrientos and colleagues [12] also clearly illustrates how genetic studies of speciation can be facilitated by additional (seemingly unrelated) paths of genomic research. In particular, the authors use prior functional analyses (specifically in mutant lines) of *D. melanogaster* to generate hypotheses about the functional role of genes falling within the identified QTL regions. Functional genomic parallels can also be drawn constructively across more distant phylogenetic connections. In a recent analysis, An *et al.* [20] used circadian pathways described in *D. melanogaster* to generate and test hypotheses about the role of altered gene expression in mating isolation among two sympatric tephritid fruit flies. Using a combination of gene-expression assays and artificial mating experiments, they found evidence that changes in the circadian cycling of the *cryptochrome* gene - a light-sensitive component of the circadian clock - was associated with shifts in the timing of diurnal mating between species. Changed gene expression was specifically localized to the antennal lobe within the brain [20], again implicating a role for altered olfactory processes in the development of mating isolation. It is through careful studies such as these - which include creative and judicious use of genomic information developed in other contexts - that evolutionary biologists can continue to make such great strides in understanding the genetic basis of, and evolutionary forces involved in, the generation of biodiversity through speciation.

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