Speciation by postzygotic isolation: forces, genes and molecules

H. Allen Orr* and Daven C. Presgraves

Summary
New species arise as reproductive isolation evolves between diverging populations. Here we review recent work in the genetics of postzygotic reproductive isolation—the sterility and inviability of species hybrids. Over the last few years, research has taken two new directions. First, we have begun to learn a good deal about the population genetic forces driving the evolution of postzygotic isolation. It has, for instance, become increasingly clear that conflict-driven processes, like sexual selection and meiotic drive, may contribute to the evolution of hybrid sterility. Second, we have begun to learn something about the identity and molecular characteristics of the actual genes causing hybrid problems. Although molecular genetic data are limited, early findings suggest that ''speciation genes'' correspond to loci having normal functions within species and that these loci sometimes diverge as a consequence of evolution in gene regulation. ***BioEssays*** **22:1085–1094, 2000.**

Introduction

Speciation—the splitting of one species into two—occupies a unique place in the theory of evolution. Although a microevolutionary process, speciation ultimately gives rise to the macroevolutionary relationships we see reflected in phylogeny. Despite this special position, the history of speciation research has been curiously episodic: periods of intense work have been separated by many years of neglect.

In the first period of sustained work, Darwin, Wallace, Jordan, Wagner and others came to realize that species are mutable and wrestled with the roles of adaptation and geographic isolation in their origin. In the second period, the founders of the Modern Synthesis profoundly transformed our understanding of just what species are and, in turn, of what it means for species to split. Dobzhansky and Mayr, in particular, championed the Biological Species Concept, the view that species are characterized by their reproductive isolation from each other, not by differences in morphology. This reproductive isolation, they argued, takes two forms: prezygotic and postzygotic. In the former, barriers such as courtship differences prevent the actual formation of hybrid zygotes while, in the latter, barriers such as hybrid sterility or inviability bar the flow of genes between species through hybrids. In both cases, reproductive isolation ensures that species remain genetically distinct and, consequently, that they can undergo independent evolutionary fates.

In the third period—which began in the early 1980's and which followed forty years of relative neglect—attention shifted to the genetics of speciation. Although much has been learned during this period, progress has centered on a fairly narrow class of problems. We now know a good deal, for instance, about the number of genes that cause reproductive isolation as well as their locations in the genome. We also understand, at least roughly, the causes of several patterns that characterize speciation. The best known of these is Haldane's rule, the preferential sterility and inviability of hybrids of the heterogametic [XY] sex.[1–3] And last, we now possess a reasonably rich population genetic theory of speciation, a theory that did not exist fifteen years ago and that has yielded a number of novel predictions about the evolution of reproductive isolation.[4–6] While these developments represent real accomplishments, they share a certain focus. They all concern what might be called the classical or ''black box'' genetics of speciation. Only now are we beginning to open this box, getting our first glimpse of the detailed forces, genes and molecules that underlie reproductive isolation. We believe that these new studies may represent a nascent fourth period in the study of speciation.

Our goal here is to summarize some of these recent developments. Given, however, that the speciation literature has been heavily reviewed, it might be best if we first make clear what we will not do. We will not be concerned here with the geography or ecology of speciation. Nor will we consider prezygotic isolation in any detail. Instead, we focus on intrinsic postzygotic isolation, i.e., on hybrid sterility and inviability, particularly on the genetics and molecular bases of hybrid problems, rather than on comparative patterns like Haldane's rule. We make these restrictions for several reasons. For one, we know much more about the genetics of hybrid sterility and inviability than about any other form of isolation and there is good reason to believe that there will be major advances in this field soon. For another, while broad patterns like Haldane's rule have received a great deal of attention, progress on the

Department of Biology, University of Rochester, Rochester, New York.
Funding agencies: NIH; Grant Number: GM51932; David and Lucile Packard Foundation.
*Correspondence to: H. Allen Orr, Department of Biology, University of Rochester, Rochester, NY 14627.

detailed genetics of reproductive isolation has gone relatively unnoticed. Last and perhaps most important, the genetics of postzygotic isolation provides the most obvious meeting ground between evolutionary geneticists and molecular and developmental biologists. For while evolutionary developmental biology has focused primarily on macroevolutionary patterns, speciation genetics provides an obvious window onto the microevolutionary divergence of development. In any case, it is worth noting that postzygotic isolation likely plays an important role in nature, as evinced by the common observation of hybrid zones (i.e., hybrid fitness is routinely tested in such zones), and the fact that, in the sole study contrasting the rate of evolution of prezygotic and postzygotic isolation, the two forms of isolation accumulate at about the same rate among geographically separate taxa.[7,8]

Recent glimpses into the "black box" of the genetics of speciation have come in two forms. First, we have begun to get a clearer picture of the particular forces that drive speciation. In the past, evolutionary biologists could claim that reproductive isolation was, say, caused by natural selection but could go no further. Now we can begin to describe the forces involved in some detail. Second, we have begun to identify the genes that cause reproductive isolation. (It may come as a surprise to the non-specialist to learn that, when asked for the name of a gene causing reproductive isolation, we could not, until recently, answer.)

But before considering these developments in detail, it is important to consider a more general picture of how postzygotic isolation evolves. This picture, which forms the background for all that follows, is provided by the so-called "Dobzhansky-Muller model".

The Dobzhansky-Muller model

Speciation poses a problem that haunted Darwin and his contemporaries: how could something as patently maladaptive as the sterility or inviability of hybrids evolve by natural selection? How, to use the imagery of an adaptive landscape, could two lineages become separated by an adaptive valley unless one of the lineages passed through it, which would not be allowed by selection? Dobzhansky and Muller saw that this problem could be solved if postzygotic isolation results from an interaction between two or more genes. To see this, consider an ancestral species of genotype *aabb*. In one population, an *A* mutation appears and goes to fixation, yielding *AAbb*, which is fertile and viable (Fig. 1). In another (geographically separate) population, a *B* mutation appears and goes to fixation, yielding *aaBB*, which is also fertile and viable. The critical point is that, while *A* and *B* both function properly on their "normal" genetic backgrounds, we have no guarantee that they will function correctly when brought together in a common genome. They have, after all, never been tested together by natural selection. *AaBb* hybrids may well be sterile or inviable, either partially or fully. The evolutionarily important point is clear: hybrid sterility and inviability can evolve *without* either lineage having passed through an adaptive valley. There is now strong evidence for the Dobzhansky-Muller model. Indeed it appears that hybrid sterility and inviability in animals usually evolve as described by this model .[1]

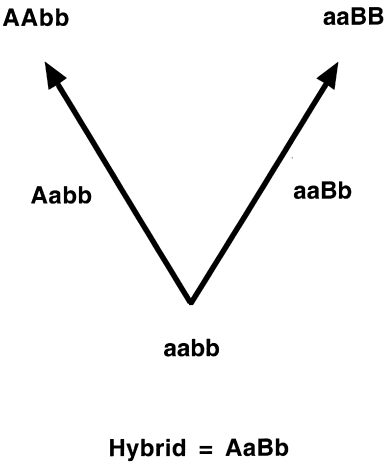

Figure 1. The Dobzhansky-Muller model of speciation. Time runs upwards and the common ancestor of the two species is shown at the bottom.

The Dobzhansky-Muller model highlights the role of epistasis in speciation. The two alleles, though singly fit, are unfit in combination. But recent work shows that the genes causing postzygotic isolation are also characterized by special dominance relations. In particular, "speciation genes"—genes that lower fitness when moved into another species—often act as partial recessives.[1] In other words, these genes lower hybrid fitness far more when homozygous or hemizygous than when heterozygous. (Nothing is implied about dominance of these genes within species.) In the case of hybrid inviability, the evidence for this recessivity is strong. *Drosophila* hybrid females that carry one X chromosome from each species are often viable, while those that are forced to carry two X chromosomes from the same species are often inviable.[9] Similarly, recent work shows that many more incompatibilities afflict *D. melanogaster–D. simulans* hybrids when pairs of loci are both made homozygous (one from each species) than when one of the loci remains heterozgygous (D.C.P., unpublished data). Last, comparative work shows that taxa having degenerate Y chromosomes—and thus hemizygous X chromosomes in males—suffer frequent hybrid male inviability, while taxa lacking degenerate Y's—and thus lacking hemizygous X's—do not (Fig. 2).[10] In the case of hybrid sterility, the evidence for hybrid recessivity is less voluminous, though still strong.[1,11,12] Indeed Sawamura et al.'s recent work shows that hybrid male steriles often have little or no

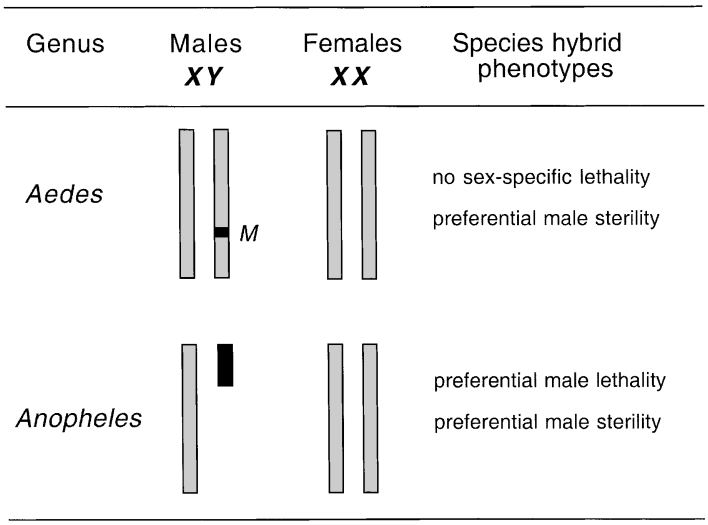

Figure 2. Speciation in taxa lacking hemizygous sex chromosomes. Mosquitoes of the genus *Aedes* lack differentiated sex chromosomes (M = male-determining lows) but still show preferential male sterility in species crosses. This finding is consistent with the idea that hybrid male steriles evolve faster than female, as expected if sexual selection drives postzygotic isolation. *Aedes* mosquitoes, however, fail to show sex-specific hybrid lethality. This finding is consistent with the recessivity of hybrid incompatibilities.

effect in heterozygous state, but are fully sterile when exposed by deficiency.[13]

The obvious question is: why do the genes causing hybrid problems act as partial recessives? Orr[14] originally speculated that hybrid lethals and steriles act recessively for the same reason that lethals and steriles within species do: such genes mimic loss-of-function mutations when placed on a foreign genetic background. If so, their recessivity might be explained by metabolic control theories of dominance.[15] This now seems unlikely. For one thing, metabolic calculations show that the resulting recessivity, while real, would probably be too weak to explain Haldane's rule (H.A.O., unpublished data). For another, it now seems unlikely that speciation genes typically encode enzymes (see below). Thus of the two properties known to characterize Dobzhansky-Muller incompatibilities—epistasis and recessivity—the reasons for the first are clear (postzygotic isolation cannot evolve under selection unless there are such interactions), while the reasons for the second are not.

Forces driving postzygotic isolation

Although the Dobzhansky-Muller model tells us that the evolution of reproductive isolation need not be opposed by natural selection, it does not tell us what, in particular, drives isolation. There are two possibilities. The first is that the alleles ultimately causing hybrid problems have little or no effect on fitness on their normal species background and randomly drift to fixation. Indeed there is a large literature suggesting that genetic drift plays a key role in speciation. Although we cannot rule out this possibility, there are several reasons for doubting that it applies generally. First, genetic drift-based theories of speciation were largely devised to explain how species could cross adaptive valleys—a problem that disappears under the Dobzhansky-Muller model. Second, phenotypic[16] and molecular[17] data strongly suggest that, in at least some cases, the genes causing hybrid problems did not diverge by genetic drift (see below). The alternative possibility is that the genes ultimately causing speciation were driven to fixation by some form of selection. Indeed recent work points to two varieties of selection, sexual selection and genetic conflict, that may play important roles in the origin of postzygotic isolation. We consider these in turn.

Faster male evolution

Sexual selection—the struggle for mates, not survival—doubtlessly drives the evolution of sex traits. In insects, for instance, it is overwhelmingly clear that male genitalia, sperm and female reproductive tract morphology evolve rapidly, and that these changes reflect sexual selection.[18–22] Sexual selection also surely plays a role in the evolution of prezygotic isolation. Divergence of male-limited characters and of female preferences for them in geographically isolated populations could easily give rise to prezygotic isolation upon secondary geographic contact: If female birds from population 1 prefer males with long tails, while those from population 2 prefer short tails, assortative mating will restrict gene flow if these populations come into contact. But it now appears that sexual selection may also drive the evolution of postzygotic isolation, in particular hybrid sterility.[23,24] Several lines of evidence support this surprising conclusion. Though each is indirect, they are, collectively, persuasive.

The first comes from introgression experiments. In these experiments, pieces of chromosomes from one species are moved into the genetic background of another by repeated backcrossing. Visible or molecular markers are then used to define the boundaries of the chromosome region introgressed.

The best of these experiments was performed by True et al.,[25] who separately introgressed 87 P-element-marked regions from *D. mauritiana* into a *D. simulans* background. When made homozygous in a largely *D. simulans* background, many more of these regions caused hybrid male than female sterility (36% versus 7%, respectively). Similar results were obtained by Hollocher and Wu[12] in a separate experiment. Although there are reasons for believing that these experiments may overestimate the magnitude of the sex difference,[5] there can be little doubt that hybrid male steriles evolve faster than hybrid female steriles, at least in *Drosophila*. The second line of evidence comes from taxa in which males and females have identical sex chromosomes except at a sex-determining locus or small chromosome region. Because males are not hemizygous for the X chromosome in such organisms, recessive hybrid steriles will not affect males more than females and the two sexes might be expected to show equal fitness in hybrids. Instead Presgraves and Orr[10] showed that crosses between mosquito species with homomorphic sex chromosomes produce sterile hybrid males far more often than sterile females (Fig. 2). Hybrid male steriles thus appear to accumulate faster than female. This pattern may also hold in a second group. Frogs of the genus *Xenopus* also have homomorphic sex chromosomes, although females are heterozygotes at the sex-determining locus, unlike in mosquitoes.[26] Once again however it appears that hybrid males are sterile more often than females[27,28] (detailed data have not, however, been published).

This picture of the rapid accumulation of hybrid male steriles is also supported by two indirect lines of evidence. First, many male reproductive proteins, including accessory gland proteins known to influence sperm competition and fertilization success, evolve rapidly, paralleling the rapid evolution of sexual ornaments and genitalia. Coulthart and Singh[29] showed, for example, that proteins from the male reproductive tract diverge between *Drosophila* species faster than proteins from most other tissues. Second, hybrid male sterility appears to evolve faster than hybrid inviability or hybrid female sterility in taxa in which males are heterogametic, e.g., *Drosophila* and mammals.[23]

The simplest explanation of these patterns is that sexual selection causes rapid evolution not only of male morphology but of male reproductive genes generally. Such genes, when brought together in hybrids, are therefore more likely to cause Dobzhansky-Muller incompatibilities than the presumably slower evolving female ones, a hypothesis championed by Wu and colleagues.[23,24] This view leads to several predictions that have not been previously noted. One is that the pattern of male-limited hybrid effects might extend to phenotypes other than hybrid sterility. In particular, one might expect (e.g. monoecious plant hybrids) hermaphrodite hybrids to show breakdown of male reproductive structures (e.g., flower parts) more often than female structures, regardless of whether these taxa possess differentiated sex chromosomes. This prediction has not been systematically tested. But if it and other predictions are confirmed, we will have arrived at a rather surprising conclusion: one of the chief forces driving the evolution of hybrid sterility may be sexual, not natural, selection.

Meiotic drive

There has been a good deal of speculation about the possible role of genetic conflicts in speciation. Frank[30] and Hurst and Pomiankowski,[31] in particular, have suggested that one form of conflict—meiotic drive—might play an important role in hybrid sterility. The reason is simple. Meiotic drive factors distort Mendelian ratios to their own advantage, typically by killing sperm that carry the homologous chromosome. The result is that the driving factors are vastly over-represented among viable gametes. While good for the drive factors, such drive imposes a fertility cost on its bearers (as well as on all other genes in the genome). As a result they should often be suppressed. One can therefore imagine that two geographically isolated populations might evolve different meiotic drive factors, each of which gets suppressed within species. Upon hybridization, however, normally-masked meiotic drive might become unmasked if suppressors are less than fully dominant. Thus X-linked drive factors might inactivate Y-bearing sperm in hybrids while Y-linked factors might inactivate X-bearing sperm, rendering hybrid males sterile. (Analogous autosomal drive systems are also possible.)

Although this idea is attractive-in part because meiotic drive is common among diverse organisms and in part because it preferentially afflicts males (perhaps helping to explain Haldane's rule in male heterogametic taxa)-it fell out of favor in the early 1990s. The reason is that tests found no meiotic drive among partially sterile males in several species hybridizations.[32,33] More recent findings, however, suggest that meiotic drive may indeed play a role in species differentiation and possibly in hybrid sterility. In particular, normally-masked meiotic drive systems have now been found in three different *Drosophila* hybridizations. In *D. simulans*, flies derived from within populations show little or no meiotic drive, while those produced by crossing individuals from the Seychelles or New Caledonia with those from Tunisia show meiotic drive.[34–36] Similarly, although meiotic drive is rare within *D. simulans* and *D. sechellia*, 10% of inbred hybrid introgression lines between them suffer strong segregation distortion (these lines are homozygous for some regions from *D. simulans* and homozygous for other regions from *D. sechellia*).[37] While these examples show that normally-masked meiotic drive systems can become unmasked in hybrids, neither implicates drive as a cause of hybrid sterility. The next example, however, does. We have recently found that, although individuals from the Bogota and USA subspecies of *D. pseudoobscura* rarely show meiotic drive, hybrid males between them invariably do (H.A.O. and S.Irving,

unpublished data). This meiotic drive was not previously detected as it occurs among hybrids that are normally completely sterile. Preliminary data, obtained via males who carry a newly recovered hybrid fertility rescue mutation (see below), suggest that the genes causing hybrid meiotic drive map to the same chromosome intervals as those causing hybrid sterility.

It thus appears that meiotic drive systems may often get fixed between closely related *Drosophila* populations or species. And it seems possible (though far from certain), that these normally-masked meiotic drive systems may sometimes contribute to postzygotic isolation. Two different interpretations of these findings are, however, possible. The first is that segregation distorters arise within populations, increase in frequency, but quickly become suppressed as in the Frank[30] and Hurst and Pomiankowski[31] scenario. The second, however, is that meiotic drive never occurred within species. Instead meiotic drive may represent a hybrid pathology unconnected to the original within-species effects of the alleles involved.[37] In this case, hybrid meiotic drive is simply a special case of a Dobzhansky-Muller incompatibility and neither lineage passed through the adaptive valley represented by the driving genotype. Thus while present data show that normally-masked meiotic drive is fairly common in hybrids, we do not know if meiotic drive within species caused the relevant evolutionary substitutions nor if meiotic drive plays a causal role in hybrid sterility. The hypothesis that segregation distortion within species may fuel the evolution of postzygotic isolation appears, however, far more plausible than a decade ago.

Speciation genes

A deeper understanding of the genetics of speciation requires that we ultimately find and characterize the genes causing hybrid problems. We could then ask: What are the normal functions of these genes within species (if any)? Are certain kinds of genes more likely to cause hybrid problems than others? Did these genes diverge by genetic drift or natural selection? Do the relevant substitutions occur in coding or regulatory regions?

Despite the explosion of molecular evolutionary studies over the last 30 years, the study of the genetics of speciation has lagged behind. Indeed only two genes causing postzygotic isolation have been cloned and characterized. We review these cases here. We also briefly touch on several speciation genes where the completion of molecular characterization seems imminent. We emphasize that, by "speciation gene", we merely mean any gene that reduces hybrid fitness. We cannot, of course, say whether a particular gene actually caused the initial split of two species as many incompatibilities may accumulate after the attainment of complete reproductive isolation. Analysis of such genes can nevertheless tell us a great deal about the identity and properties of the factors involved in Dobzhansky-Muller incompatibilities.

Hybrid lethality in Xiphophorus

The hybrid lethal system of *Xiphophorus* fish was once the best-known example of a gene causing fitness problems in species hybrids.[38–40] Having been neglected for some time by evolutionary geneticists, the story has recently developed dramatically.

Some *Xiphophorus* species, like the platyfish, *X. maculatus*, are polymorphic for dorsolateral spots comprising black-pigmented cells called macromelanophores. Other *Xiphophorus* species, like the swordtail, *X. helleri*, lack macromelanophores (Fig. 3A). When spotted *X. maculatus* and *X. helleri* are crossed, all F_1 hybrids develop an increased number of exaggerated spots. When F_1 hybrids are backcrossed to *X. helleri*, half of the resulting progeny lack macromelanophores while the other half develop phenotypes ranging from F_1-like to extreme invasive malignant melanomas that are often lethal, consuming most of the fish (see Fig. 3B).

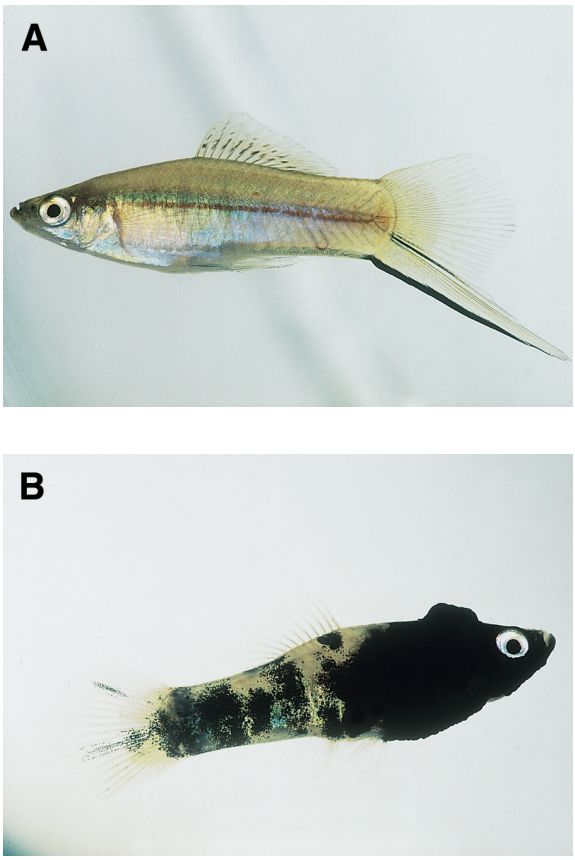

Figure 3. A: A melanoma-free swordtail parent, *X. helleri*; **B:** a *X. maculatus* platyfish–*X. helleri* swordtail backcross hybrid, showing the extreme melanoma phenotype. Many of these hybrids die. Photo published with permission of Steve Kazianis.

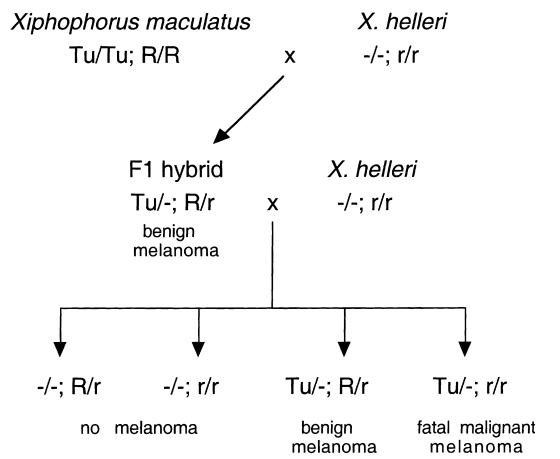

Figure 4. Species cross between *X. maculatus* and *X. helleri*. In the backcross generation, one-fourth of hybrids develop severe melanomas due to unregulated overexpression of the *Tu* locus. Hybrid inviability thus behaves as a simple two locus Dobzhansky-Muller incompatibility.

The genetic basis of this hybrid lethality has been studied intensively.[41–43] Classical work suggested a simple genetic basis and it is now clear that spotted *X. maculatus* fish carry a sex-linked complex, the *Tumor* (*Tu*) locus, that specifies macromelanophores and that is regulated by a major autosomal suppressor locus, *R* (*RR*). *X. helleri*, however, physically lacks the *Tu* locus, lacks macromelanophores, and lacks suppressor alleles at *R* (*rr*) (see Fig. 4). F_1 hybrids thus inherit *Tu* from *X. maculatus* and are heterozygous at the *R* locus (*Rr*). Consequently, they develop exaggerated macromelanophores due to overexpression of *Tu*. Half of the backcross hybrids (backcrossed to *X. helleri*) inherit *Tu* and half of these in turn lack *R* suppressors. As a result, they develop severe melanomas due to unregulated overexpression of *Tu*. Hybrid lethality in *Xiphophorus* hybrids thus behaves as a simple two-locus Dobzhansky-Muller incompatibility.

Schartl and colleagues have made remarkable progress on the evolutionary origin and molecular biology of the *Tu* locus. In 1989, they isolated a candidate gene by positional cloning[44] and showed that the *Tu* complex is composed of several tightly linked but separable loci[45,46]: the pigment-encoding *Macromelanophore determining locus (Mdl)* locus and two duplicate copies of a novel receptor tyrosine kinase (a transmembrane cell-signaling protein). These duplicate genes are called *Xmrk-1* and *Xmrk-2*, for *Xiphophorus melanoma receptor kinase*. *Xmrk-1* homologues reside on the X and Y chromosomes of all *Xiphophorus* species. *Xmrk-2*, however, apparently resulted from nonhomologous exchange between *Xmrk-1* and a second locus, *D* (for 'donor'), and is present only on the sex chromosomes of some species, including *X. maculatus*.[46] The two *Xmrks* are therefore nearly identical in structure and sequence except that *Xmrk-2* has come under the control of a *D* promotor.[47] The expression patterns of the two genes thus differ. *Xmrk-1* is ubiquitously expressed at low levels in all tissues while *Xmrk-2* is expressed at high levels *only* in hybrid melanomas.[44]

Three lines of evidence show that *Xmrk-2* causes tumorigenesis. The first is that transcription levels of *Xmrk-2* correlate with severity of melanomas across genotypes.[43] The second comes from two *Tu* mutant *X. maculatus* lines that fail to induce melanomas when hybridized to *X. helleri*. In one line *Xmrk-2* is disrupted by transposon insertion and, in the other, it is deleted.[48] Suppressor alleles at the *R* locus are thus thought to regulate *Xmrk-2* transcription: deregulation of the new promotor in *rr* backross hybrids causes six-fold overexpression of *Xmrk-2* in melanomas.[49] Finally, and most important, overexpression of *Xmrk-2* in transgenic fish causes tumor formation.[43]

Recent work has also begun to shed light on the *R* locus. *R* maps to linkage group V and a candidate gene, a member of the *cyclin dependent kinase 2* gene family (*CDKN2X*), has been identified.[49,50] While the critical tests have not yet been performed, *CDKN2X*'s candidacy seems plausible. For one thing, *CDKN2* genes act as tumor suppressors of human and rodent melanomas. For another, *CDKN2X* is expressed in the right place at the right time: expression assays reveal a seven-fold increase in *CDKN2X* RNA levels in melanoma tissues. Last, the *X. helleri* and *X. maculatus CDKN2X* alleles differ by two amino acid substitutions as well as major *cis*-regulatory region changes.[49] It is not yet clear, however, how *CDKN2X* is involved in transcriptional control of *Xmrk-2*. As *CDKN2X* is not a transcription factor, it cannot be a *direct* regulator of *Xmrk-2*. This finding, if correct, implies that Dobzhansky-Muller incompatibilities need not involve genes whose products directly interact molecularly.

Hybrid sterility in Drosophila

Hybrid male sterility is the most common form of postzygotic isolation in *Drosophila*.[51] and has been intensely studied in the *D. simulans–D. mauritiana* hybridization. As these species share a recent common ancestor[52] and are incompletely isolated (hybrid females remain fertile), they provide a window on a fairly early stage of speciation.

In the 1980s, Coyne showed, somewhat surprisingly, that a moderately large number of factors cause male sterility between these species.[53] This conclusion has since been confirmed by analyses that place the estimated number of male steriles near 100.[12,24] Hybrid male steriles thus accumulate rapidly. Using an introgression approach, Coyne and Charlesworth[54] mapped one of these hybrid male steriles to an approximately 2 cM segment of the *D. mauritiana* X chromosome. Using DNA markers, Wu and colleagues ultimately localized the hybrid male sterile to an 8.4 kb segment that includes three exons.[17,55,56] These exons, plus

an adjacent one, encode a 349 amino acid polypeptide, including a 60 amino acid sequence characteristic of homeobox transcription factors. The gene, called *OdysseusH* (*OdsH*, for *Odysseus*-site Homeobox gene), is expressed in testes, as expected for a gene causing sterility. Its function within the species remains unknown.

Sex-related genes often evolve rapidly[57] and *OdsH* is no exception. Despite the usual conservation of homeobox genes, *OdsH* has evolved at a spectacular rate. Indeed, since the split of *D. mauritiana* and *D. simulans* (~0.5 Mya), 15 replacement substitutions have occurred in the homeodomain alone. There is little doubt that these substitutions were driven by positive Darwinian selection as the ratio of replacement to silent site substitutions reaches a remarkable 10:1 in the lineage leading to D. mauritiana.[17]

One surprise to emerge from recent fine-scale introgression studies is evidence for complex conspecific epistasis.[58] Some hybrid sterility factors, including *OdsH*, cause complete sterility only when co-introgressed with other conspecific genes.[56] Indeed, when introgressed alone, the *D. mauritiana* allele of *OdsH* causes only a 40–50% reduction in fertility. This requirement for an unidentified but tightly linked conspecific factor has no doubt hampered attempts to perform the critical transformation experiments needed to prove that *OdsH* is in fact a hybrid sterility gene. Until these experiments are performed, *OdsH* must remain only a candidate speciation gene, albeit a strong one.

Hybrid rescue mutations

Until recently, speciation geneticists have been unable to routinely take advantage of the genetic tools available in *Drosophila melanogaster*. The reason is that *D. melanogaster* produces only dead or sterile hybrids when crossed to all other species.[59] This fact has posed the single greatest obstacle to the molecular genetic study of speciation. Fortunately, the discovery of hybrid rescue mutations—alleles that, when introduced into hybrids, restore fertility or viability[60]—has begun to change this.

Hybrid rescue mutations might act in either of two ways. They might represent rare compatible alleles at the genes that actually cause hybrid sterility or inviability. Alternatively, they might represent second-site suppressors of hybrid incompatibilities, i.e., they might involve a class of genes distinct from those that actually kill or sterilize hybrids. If the first hypothesis proves correct (and there is mounting evidence for it; see below), isolation of rescue mutations may provide a short cut to the molecular characterization of the genes underlying postzygotic isolation. We briefly review what is known about these candidate speciation genes.

Hybrid male rescue and Lethal hybrid rescue

When *D. melanogaster* females are crossed to *D. simulans* males, only hybrid females are produced. Males die at the larval–pupal transition due to an incompatibility between a recessive *X*-linked factor from *D. melanogaster* and an autosomal factor(s) from *D. simulans*.[9,61] Before death, hybrid male larvae develop slowly, often lack imaginal discs, and suffer mitotic defects in which chromosomes fail to condense properly.[62] Hybrid males can, however, be rescued with two mutations: *Hybrid male rescue* (*Hmr*) on the *D. melanogaster X*[61] and *Lethal hybrid rescue* (*Lhr*) on the *D. simulans* second.[63]

The rescue of hybrids via single mutations strongly suggests that F_1 male lethality has a simple genetic basis: it seems unlikely that a single mutation would simultaneously repair many independent developmental defects in hybrids. Recent evidence further suggests that this simple hybrid lethal interaction may involve wild-type alleles at the *Hmr* and *Lhr* loci themselves. Barbash, Roote and Ashburner[64] and Orr and Irving[65] manipulated the dose of the wild-type *D. melanogaster* allele, Hmr^{mel}, in species hybrids. Both studies found that increasing dosage of Hmr^{mel} kills hybrids, while decreasing dosage rescues them. Moreover, addition of another rescue mutation (*Lhr*) to hybrids carrying an extra copy of Hmr^{mel} largely reverses the lethal effect of increased Hmr^{mel} dosage. Though not conclusive, these results strongly suggest that Hmr^{mel} plays a causal role in hybrid lethality. Efforts are now underway to clone and characterize this candidate speciation gene.

Zygotic hybrid rescue and maternal hybrid rescue

When *D. simulans* females are crossed to *D. melanogaster* males, only hybrid males are produced. Females die as embryos due to an incompatibility between an *X*-linked factor(s) from *D. melanogaster* and a maternally acting factor(s) from *D. simulans*. Though little is known about the developmental basis of this lethality, hybrid females can be rescued via two mutations: *Zygotic hybrid rescue* (*Zhr*) on the *D. melanogaster* X chromosome and *maternal hybrid rescue* (*mhr*) on the *D. simulans* second chromosome.[60] Rescue of hybrids with single mutations again implies that lethality has a simple basis. Sawamura et al.[66] have shown that, like Hmr^{mel}, the wild-type allele Zhr^{mel} kills hybrids in a dose-dependent fashion. Once again, therefore, it appears that the wild-type allele of a rescue mutation may correspond to a speciation gene. *Zhr* maps to the centromeric region of the X chromosome which is rich in repetitive DNA. This led Sawamura et al. to speculate that Zhr^{mel} may be a repetitive sequence. This cannot be taken as conclusive, however, as *Zhr* resides in a region of β heterochromatin that includes "normal" coding genes.

Hybrid fertility rescue

In 1996, Davis, et al.[67] found a mutation that, remarkably, rescues the fertility of *D. simulans–D. melanogaster* hybrid females, albeit weakly. The recovery of fertile hybrid females

allows *D. simulans* material to be introgressed into a *D. melanogaster* background. Genetic and molecular technology from *D. melanogaster* can then be used to analyze the genes underlying species differences. The first practical use of this mutation, by Sawamura et al.,[13] appeared recently. In this work, two small regions of the *D. simulans* second chromosome were introgressed into *D. melanogaster*. Deficiency mapping showed that these regions, which together represent about 5% of the *D. simulans* genome, harbor six hybrid male steriles and no female steriles—further support for faster male evolution. (The fact that deficiencies "uncover" male steriles is also prima facie evidence that all six hybrid steriles are partially recessive—further support for the dominance theory.)

H.A.O. and S. Irving (unpublished data) have also recently recovered a mutation that weakly rescues the fertility of normally sterile male hybrids between the Bogota and USA subspecies of *D. pseudoobscura*. This factor resides in the USA subspecies, maps to the second chromosome and acts zygotically. Its recovery opens the door on a number of previously impossible experiments, including genetic analysis of the meiotic drive among these hybrids discussed earlier.

In general, *Drosophila* geneticists have recovered fewer hybrid fertility than viability rescue mutations and the former are far less effective than the latter. This greater difficulty in rescuing hybrid fertility likely represents a rarely appreciated line of evidence for the faster evolution of hybrid sterility than inviability, at least in *Drosophila*: at any point in time, it would seem harder to undo the tangle of incompatibilities underlying the sterility than lethality of species hybrids.

Conclusions

Although our understanding of the forces and genes causing speciation remains rudimentary, several facts now seem clear. (1) The genes causing postzygotic isolation typically act as partial recessives in hybrids, although the biochemical basis of this pattern remains unknown. (2) Sexual selection and, less clearly, meiotic drive may play roles in the evolution of hybrid sterility. (3) The two factors causing postzygotic isolation that have been molecularly characterized both correspond to genes having apparently normal functions within species. This fact should not be taken for granted. Indeed the history of speciation research has been marked by a great deal of speculation about the role of novel genetic elements in speciation (e.g., mass mobilization of transposable elements). It will be interesting to see if the factors causing meiotic drive in species hybrids also correspond to genes having normal functions within species. (4) Gene duplication may contribute to the origin of reproductive isolation, as several of the factors isolated thus far are products of duplication. (5) It appears that regulatory evolution might play an important role in the evolution of postzygotic isolation. The most conspicuously diverged aspects of both *Xmrk-2* and *CDKN2X* involve *cis*-regulatory regions, and substitutions in *OdsH*'s homeobox could correspond to change in its regulation of testis-specific target genes. This finding is perhaps not surprising given the growing evidence that morphological adaptation is often underlaid by regulatory changes, e.g., evolution at *tb1* between maize and teosinte[68] and at *ovo/svb* between *Drosophila sechellia* and its sister species.[69]

Despite considerable recent progress, it is important to stress that many, if not all, of the above inferences must remain tentative. The fourth phase in the study of speciation, the careful characterization of the forces, genes and molecules underlying speciation, has only begun. As evolutionists, we are of course most interested in broad patterns or rules that might characterize the genes underlying speciation. Are they typically transcription factors? Do they usually diverge at regulatory regions? Unfortunately these questions demand analysis of a large sample of genes. Not surprisingly, then, the single greatest obstacle to further progress in the genetics of speciation lies in the shortage of additional speciation genes for study. Our own laboratory has therefore recently turned to large systematic screens intended to identify many more speciation genes. In particular, we take advantage of the large arsenal of genetic tools available in *Drosophila melanogaster* to map factors from *D. simulans* that are lethal on a largely *D. melanogaster* genetic background. Preliminary results (D.C.P., unpublished data) show that this approach promises to reveal the location and identity of a large number of hybrid lethals, factors that can then be subjected to fine-scale molecular genetic analysis. Not until many such factors have been analyzed can we possibly hope to reach broad, firm conclusions about the nature of the genes underlying the origin of species.

References

1. Orr HA. Haldane's rule. Ann Rev Ecol System 1997;28:195–218.
2. Laurie CC. The weaker sex is heterogametic: 75 years of Haldane's rule. Genetics 1997;147:937–951.
3. Turelli M. The causes of Haldane's rule. Science 1998;282:889–891.
4. Orr HA. The population genetics of speciation: the evolution of hybrid incompatibilities. Genetics 1995;139:1805–1813.
5. Turelli M, Orr HA. Dominance, epistasis and the genetics of postzygotic isolation. Genetics 2000;154:1663–1679.
6. Gavrilets S. Evolution and speciation on holey adaptive landscapes. Trends Ecol Evol 1997;12:307–312.
7. Coyne JA, Orr HA. Patterns of speciation in *Drosophila*. Evolution 1989; 43:362–381.
8. Coyne JA, Orr HA. "Patterns of speciation" revisited. Evolution 1997;51: 295–303.
9. Orr HA. Haldane's rule has multiple genetic causes. Nature 1993;361: 532–533.
10. Presgraves DC, Orr HA. Haldane's rule in taxa lacking a hemizygous X. Science 1998;282:952–954.
11. Orr HA. Mapping and characterization of a "speciation gene" in *Drosophila*. Genet Res 1992;59:73–80.
12. Hollocher H, Wu C-I. The genetics of reproductive isolation in the *Drosophila simulans* clade: X vs. autosomal effects and male vs. female effects. Genetics 1996;143:1243–1255.
13. Sawamura K, Davis AW, Wu C-I. Genetic analysis of speciation by means of introgression into *Drosophila melanogaster*. Proc Natl Acad Sci USA 2000;97:2652.

14. Orr HA. A mathematical model of Haldane's rule. Evolution 1993;47: 1606–1611.
15. Kacser H, Burns JA. The molecular basis of dominance. Genetics 1981;97:639–666.
16. Christie P, Macnair MR. Complementary lethal factors in two North American populations of the yellow monkey flower. J Hered 1984;75: 510–511.
17. Ting CT, Tsaur SC, Wu ML, Wu CI. A rapidly evolving homeobox at the site of a hybrid sterility gene. Science 1998;282:1501–1504.
18. Eberhard WG. Sexual Selection and Animal Genitalia. Cambridge, Mass: Harvard University Press; 1985.
19. Eberhard WG. Female Control: Sexual Selection by Cryptic Female Choice. Princeton: Princeton University Press; 1996.
20. Andersson M. Sexual Selection. Princeton: Princeton University Press; 1994.
21. Arnqvist G. Comparative evidence for the evolution of genitalia by sexual selection. Nature 1998;393:784–786.
22. Rice WR, Holland B. The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. Behav Ecol Sociobiol 1997;41:1–10.
23. Wu C-I, Davis AW. Evolution of postmating reproductive isolation: the composite nature of Haldane's rule and its genetic bases. Amer Nat 1993;142:187–212.
24. Wu C-I, Johnson NA, Palopoli MF. Haldane's rule and its legacy: why are there so many sterile males? Trends Ecol Evol 1996;11:281–284.
25. True JR, Weir BS, Laurie CC. A genome-wide survey of hybrid incompatibility factors by the introgression of marked segments of *Drosophila mauritiana* chromosomes into *Drosophila simulans*. Genetics 1996;142:819–837.
26. Kelley DB. Sexual differentiation in *Xenopus laevis*. In: The Biology of *Xenopus*. Tinsley RC and Kobel HR, editor. Oxford: Clarendon Press; 1996. p. 143–176.
27. Kobel HR, Loumont C, Tinsley RC. The extant species. In: Tinsley RC and Kobel HR editors. The Biology of *Xenopus*. Clarendon Press: Oxford; 1996. p. 9–34.
28. Kobel HR. Reproductive capacity of experimental *Xenopus gilli* X X. l. laevis hybrids. In: Tinsley RC, Kobel HR, editors. The Biology of *Xenopus*. Clarendon Press: Oxford; 1996.
29. Coulthart MB, Singh RS. High level of divergence of male reproductive tract proteins between *Drosophila melanogaster* and its sibling species, *D. simulans*. Mol Biol Evol 1988;5:182–191.
30. Frank SH. Divergence of meiotic drive-suppressors as an explanation for sex-biased hybrid sterility and inviability. Evolution 1991;45: 262–267.
31. Hurst LD, Pomiankowski A. Causes of sex ratio bias may account for unisexual sterility in hybrids: a new explanation of Haldane's rule and related phenomena. Genetics 1991;128:841–858.
32. Johnson NA, Wu C-I. An empirical test of the meiotic drive models of hybrid sterility: sex ratio data from hybrids between *Drosophila simulans* and *Drosophila sechellia*. Genetics 1992;130:507–511.
33. Coyne JA, Orr HA. Further evidence against meiotic-drive models of hybrid sterility. Evolution 1993;47:685–687.
34. Mercot H. et al., Sex-ratio distortion in *Drosophila simulans*: co-occurrence of a meiotic drive and a suppressor of drive. J Evol Biol 1995;8:283–300.
35. Cazemajor M, Landre C, Montchamp-Moreau C. The *sex-ratio* trait in *Drosophila simulans*: genetic analysis of distortion and suppression. Genetics 1997;147:635–642.
36. Montchamp-Moreau C, Joly D. Abnormal spermiogenesis is associated with the X-linked sex-ratio trait in *Drosophila simulans*. Heredity 1997;79:24–30.
37. Dermitzakis ET, Masly JP, Waldrip HM, Clark AG. Non-Mendelian segregation of sex chromosomes in heterospecific *Drosophila* males. Genetics 2000;154:687–694.
38. Dobzhansky T. Genetics and the Origin of Species. New York: Columbia University Press; 1937. p. 364.
39. Stebbins GL.The inviability, weakness, and sterility of interspecific hybrids. Adv Genetics 1958;9:147–216.
40. Mayr E. Animal Species and Evolution. Cambridge, Mass.: The Belknap Press of Harvard University Press; 1963.
41. Anders F. Contributions of the Gordon-Kosswig melanoma system to the present concept of neoplasia. Pigment Cell Research 1991; 3:7–29.
42. Schartl M. Platyfish and swordtails: a genetic system for the analysis of molecular mechanisms in tumor formation. Trends Genet 1995;11: 185–189.
43. Malitschek B, Fornzler D, Schartl M. Melanoma formation in *Xiphophorus*: a model system for the role of receptor tyrosine kinases in tumorigenesis. Bioessays 1995;17:1017–1023.
44. Wittbrodt J, Adam D, Malitschek B, Maueler W, Raulf F, Telling A, Robertson SM, Schartl M. Novel putative receptor tyrosine kinase encoded by the melanoma-inducing *Tu* locus in *Xiphophorus*. Nature 1989;341:415–421.
45. Gutbrodt H, Schartl M. Intragenic sex-chromosomal crossovers of *Xmrk* oncogene alleles affect pigment pattern formation and the severity of melanoma in *Xiphophorus*. Genetics 1999;151:773–783.
46. Weis S, Schartl M. The macromelanophore locus and the melanoma oncogene *Xmrk* are separate genetic entities in the genome of *Xiphophorus*. Genetics 1998;149:1909–1920.
47. Adam D, Dimitijevic N, Schartl M. Tumor suppression in *Xiphophorus* by an accidentally acquired promoter. Science 1993;259:816–819.
48. Schartl M, Hornung U, Gutbrod H, Volff JN, Wittbrodt J. Melanoma loss-of-function mutants in *Xiphophorus* caused by *Xmrk*-oncogene deletion and gene disruption by a transposable element. Genetics 1999;153: 1385–1394.
49. Kazianis S, Morizot DC, Coletta LD, Johnston DA, Woolcock B, Vielkind JR, Nairn RS. Comparative sequence structure and characterization of a *CDKN2* gene in a *Xiphophorus* fish melanoma model. Oncogene 1999; 18:5088–0000.
50. Nairn RS, Kazianis S, McEntire BB, Della Coletta L, Walter RB, Morizot DC. A *CDKN2*-like polymorphism in *Xiphophorus* LG V is associated with UV-B-induced melanoma formation in platyfish-swordtail hybrids. Proc Natl Acad Sci USA 1996;93:13042–13047.
51. Coyne J. Genetics and speciation. Nature 1992;355:511–515.
52. Caccone A, Moriyama EN, Gleason JM, Nigro L, Powell JR. A molecular phylogeny for the *Drosophila melanogaster* subgroup and the problem of polymorphism data. Mol Biol Evol 1996;13:1224–1232.
53. Coyne JA. Genetic basis of male sterility in hybrids between two closely related species of *Drosophila*. Proc Natl Acad Sci USA 1984;81:4444–4447.
54. Coyne JA, Charlesworth B. Location of an X-linked factor causing male sterility in hybrids of *Drosophila simulans* and *D. mauritiana*. Heredity 1986;57:243–246.
55. Perez DE, Wu CI, Johnson NA, Wu ML. Genetics of reproductive isolation in the *Drosophila simulans* clade: DNA-marker assisted mapping and characterization of a hybrid-male sterility gene, *Odysseus* (*Ods*). Genetics 1993;134:261–275.
56. Perez DE, Wu C-I. Further characterization of the *Odysseus* locus of hybrid sterility in *Drosophila*: one gene is not enough. Genetics 1995; 140:201–206.
57. Civetta A, Singh RS. Sex-related genes, directional sexual selection, and speciation. Mol Biol Evol 1998;15:901–909..
58. Wu C-I, Palopoli MF. Genetics of postmating reproductive isolation in animals. Ann Rev Genetics 1994;27:283–308.
59. Sturtevant AH. Genetic studies on *Drosophila simulans*. I. Introduction. Hybrids with *Drosophila melanogaster*. Genetics 1920;5:488–500.
60. Sawamura K, Watanabe TK, Yamamoto MT. Hybrid lethal systems in the *Drosophila melanogaster* species complex. Genetica 1993;88: 175–185.
61. Hutter P, Roote J, Ashburner M. A genetic basis for the inviability of hybrids between sibling species of *Drosophila*. Genetics 1990;124: 909–920.
62. Orr HA, Madden LD, Coyne JA, Goodwin R, Hawley RS. The developmental genetics of hybrid inviability: a mitotic defect in *Drosophila* hybrids. Genetics 1997;145:1031–1040.
63. Watanabe TK. A gene that rescues the lethal hybrids between *Drosophila melanogaster* and *D. simulans*. Japn J Genet 1979;54: 325–331.

64. Barbash DA, Roote J, Ashburner M. The *Drosophila melanogaster Hybrid male rescue* gene causes inviability in male and female species hybrids. Genetics 2000;154:1747–1771.
65. Orr HA, Irving S. Genetic analysis of the *Hybrid male rescue* locus of *Drosophila*. Genetics 2000;155:225–231.
66. Sawamura K, Yamamoto MT. Characterization of a reproductive isolation gene, *zygotic hybrid rescue*, of *Drosophila melanogaster* by using minichromosomes. Heredity 1997;79:97–103.
67. Davis AW, Roote J, Morley T, Sawamura K, Herrmann S, Ashburner M. Rescue of hybrid sterility in crosses between *D. melanogaster* and *D. simulans*. Nature 1996;380:157–159.
68. Wang R-L, Stec A, Hey J, Lukens L, Doebley J. The limits of selection during maize domestication. Nature 1999;398:236–239.
69. Sucena E, Stern DL. Divergence of larval morphology between *Drosophila sechellia* and its sibling species caused by cis-regulatory evolution of *ovo/shaven-baby*. Proc Natl Acad Sci USA 2000;97:4530–4534.