Speciation in *Drosophila*: From Phenotypes to Molecules

H. ALLEN ORR, J. P. MASLY, AND NITIN PHADNIS

From the Department of Biology, University of Rochester, Rochester, NY 14627.

Address correspondence to H. Allen Orr at the address above, or e-mail: aorr@mail.rochester.edu.

Abstract

Study of the genetics of speciation—and especially of the genetics of intrinsic postzygotic isolation—has enjoyed remarkable progress over the last 2 decades. Indeed progress has been so rapid that one might be tempted to ask if the genetics of postzygotic isolation is now wrapped up. Here we argue that the genetics of speciation is far from complete. In particular, we review 2 topics where recent work has revealed major surprises: 1) the role of meiotic drive in hybrid sterility and 2) the role of gene transposition in speciation. These surprises, and others like them, suggest that evolutionary biologists may understand less about the genetic basis of speciation than seemed likely a few years ago.

The 2006 Wilhelmine E. Key Lecturer
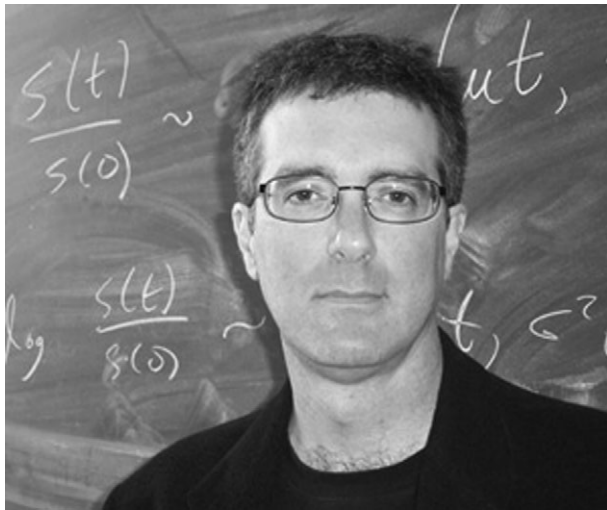
H. Allen Orr is the Shirley Cox Kearns Professor of Biology at the University of Rochester. He received his Ph.D. from The University of Chicago and performed postdoctoral research at the University of California, Davis. His research focuses on experimental and theoretical studies of speciation and the genetics of adaptation. He recently published the book *Speciation* (with Jerry A. Coyne), a scholarly review of research on the origin of species. In addition to his scientific work, Orr is a frequent contributor of book reviews and critical essays to such publications as *The New Yorker* and *The New York Review of Books*. He has been the recipient of a Guggenheim Fellowship, a David and Lucile Packard Fellowship in Science and Engineering, an Alfred P. Sloan Foundation Postdoctoral Fellowship, and a Rockefeller Foundation Scholar in Residence Fellowship (Bellagio Study Center). This Wilhelmine E. Key Lecture as delivered on July 24, 2006 at the "Genetics of Speciation" meeting in Vancouver, CA.

There has, over the last 20 years, been an explosion of interest and progress in speciation (Coyne and Orr 2004). Perhaps the easiest way of parsing this progress is by the organism studied. One of the more satisfying aspects of the recent work in speciation is that it has not focused on one model system or even one kingdom. Instead, important advances in our understanding have followed from work in plants—especially in systems like *Mimulus* (Bradshaw et al. 1995, 1998; Ramsey and Schemske 1998; Fishman and Willis 2001) and *Helianthus* (Rieseberg, Linder, and Seiler 1995, Rieseberg, Van Fossen, and Desrochers 1995; Rieseberg 1997; Ungerer et al. 1998)—and in animals—especially in systems like the stickleback (Schluter and McPhail 1992; Hatfield and Schluter 1999; Rundle 2002), mouse (Guenet et al. 1990; Pilder et al. 1991; Tucker et al. 1992; Said et al. 1993; Payseur and Nachman 2005), and *Drosophila* (Coyne 1985; Coyne and Orr 1989b; Zeng and Singh 1993; Civetta and Singh 1995; Davis et al. 1994; Palopoli and Wu 1994; Wu and Davis 1993; Wu and Ting 2004).

Another, and surely more informative, way of parsing recent progress is by the approach taken. In our view, 4 approaches have been particularly powerful. The first involves broad comparative surveys like those performed in *Drosophila*, birds, Lepidoptera, frogs, and mammals (Coyne and Orr 1989a, 1997; Sasa et al. 1998; Presgraves 2002; Price and Bouvier 2002; Lijtmaer et al. 2003; Fitzpatrick and Turelli 2006). These studies ask whether any patterns characterize the evolution of reproductive isolation in a large group of species. One of the clearest lessons to emerge from this approach is that biology matters: although postzygotic isolation, for instance, evolves at about the same rate as prezygotic between allopatric species of *Drosophila* (Coyne and Orr 1989a, 1997), the same is not true in birds, where prezygotic isolation

typically evolves long before postzygotic (Price and Bouvier 2002). The second approach is the opposite of the first and features detailed analysis of a pair of species. One of the best examples involves the monkeyflowers, *Mimulus lewisii* and *Mimulus cardinalis*, which occur sympatrically in nature. In a painstaking study, Ramsey et al. (2003) partitioned the contributions of various forms of reproductive isolation (e.g., pollinator isolation, hybrid sterility) to total reproductive isolation between these species. They found that pollinator and habitat isolation accounted for the vast majority of reproductive isolation between these taxa. The third approach is theoretical. It is worth remembering that, as late as 1974, Lewontin (1974) could claim that evolutionary biology possessed no quantitative theory of speciation. This is certainly no longer true. Indeed, we have seen rapid progress on several theoretical fronts, including fitness landscape theory (Gavrilets 2004), reinforcement (Liou and Price 1994; Kelly and Noor 1996; Kirkpatrick and Servedio 1999; Servedio 2000), and the evolution of postzygotic isolation (Orr 1995; Turelli and Orr 2000; Orr and Turelli 2001; Turelli et al. 2001). This last topic has featured a large body of work on the Dobzhansky–Muller model, that is, the accumulation of hybrid incompatibilities between pairs (or more) of loci, an accumulation that can occur unopposed by—or even driven by—natural selection within allopatric populations.

The final approach involves experimental genetic studies. There seems little doubt that this approach has yielded the greatest progress in our understanding of speciation. Indeed, to the extent that there has been any fundamental reformulation of our view of the origin of species, it has been here. Progress has been both rapid and broad and has involved advances in our understanding of both extrinsic postzygotic isolation (e.g., analysis of phenotypes involved in ecological speciation in sticklebacks) and intrinsic postzygotic isolation (e.g., analysis of hybrid sterility and inviability in *Drosophila*). For most of the last 20 years, these types of genetic studies were performed at the classical genetic level. A large set of backcross, F2, and introgression analyses allowed mapping and counting of chromosome regions causing reproductive isolation and shed light on a number of genetic problems, including the basis of Haldane's rule, the large X-effect, faster-male evolution, the snowball effect, reinforcement, and the role of inversions in speciation (reviewed in Coyne and Orr 2004). Recently, though, progress has pushed beyond the classical genetic level to the molecular level.

It is well known that the genetics of speciation has been in an awkward, if not embarrassing, position for several decades. In particular, the genetics of speciation has not resembled the genetics of anything else: speciation geneticists have not been able to point to the actual genes that cause reproductive isolation. Instead, we have largely continued to point to particular (and often large) regions of the genome that are, say, involved in Dobzhansky–Muller incompatibilities. The reason for this awkward situation is that genetic study of speciation requires genetic analysis where such a thing is nearly impossible—between reproductively isolated taxa. The key challenge facing the genetics of speciation has thus been clear: to bring the study of speciation together with the study of molecular evolution, a task that requires identifying the DNA sequences that cause reproductive isolation.

In what surely represents one of the most significant developments in speciation studies, this challenge has been met. This accomplishment has, to a considerable extent, taken advantage of whole genome sequences that were not available to earlier workers. Several genes that cause reproductive isolation have been identified and characterized. These genes are *Xmrk-2*, which causes inviability in backcross hybrids between the platyfish *Xiphophorus maculatus* and the swordtail *Xiphophorus helleri* (Wittbrodt et al. 1989); *OdsH*, which causes male sterility in backcross hybrids between the flies *Drosophila simulans* and *Drosophila mauritiana* (Ting et al. 1998; Wu and Ting 2004); *Hmr*, which causes inviability in F1 hybrids between the flies *Drosophila melanogaster* and *D. simulans* (Barbash et al. 2003, 2004); *Nup96*, which causes inviability in F2-like hybrids between *D. melanogaster* and *D. simulans* (Presgraves et al. 2003); and *Lhr*, which causes inviability in F1 hybrids between the flies *D. melanogaster* and *D. simulans* (Brideau et al. 2006). Although this list suffers several problems—most obviously, it is short and focuses entirely on intrinsic postzygotic isolation—analysis of the genes on this list has already revealed several striking patterns. First, the loci causing postzygotic isolation are ordinary genes; there is no evidence so far to support the early suggestion that novel genetic factors or processes, for example, the mass mobilization of transposable elements, play a part in speciation. Second, these genes have a variety of functions. Some are enzymes, some are transcription factors, whereas others are structural proteins; there is no support, then, for the idea that postzygotic isolation involves a special functional class of gene. Third, many of these genes are rapidly evolving. *OdsH*, for instance, has experienced 15 replacement substitutions in its homeodomain alone, a remarkable number for 2 species that separated only ~250 000 years ago. Similarly, *Hmr* is among the fastest evolving loci known in the genus *Drosophila*, and *Nup96* has experienced many replacement substitutions between *D. melanogaster* and *D. simulans*. Finally, and most important, molecular population genetic analysis shows that this rapid evolution is driven by positive natural selection (Coyne and Orr 2004). The genetics of speciation thus provides strong support for the traditional view that reproductive isolation evolves as an epiphenomenon of Darwinian adaptation, a result that has rightly received much attention.

The Awkward Question

This progress in the molecular genetics of speciation—and the remarkable strength of the above patterns—raises a possibility that has been largely ignored in the literature: Is the genetics of postzygotic isolation wrapped up? At one level, this question is absurd. We have, after all, isolated only a few genes causing hybrid sterility or inviability and many more remain to be identified (indeed several laboratories are hot on their trail). But there are always more genes to be found, whatever the phenotype of interest. The more serious question is whether the genetics of postzygotic isolation is wrapped up in the sense that all patterns of any intellectual

significance—patterns like those listed above—have been discovered. Put differently, have we reached a point in the genetics of postzygotic isolation where nothing fundamental is at stake and the identification of additional "speciation genes" represents little more than a filling in of the blanks of the Dobzhansky–Muller model? Research that proceeds by momentum alone is, of course, dismayingly common in the history of science (e.g., the extended practice of protein gel electrophoresis), and there seems no reason to think that speciation studies are exempt from the problem.

For the remainder of this paper, we review recent findings, some published and others not, that lead us to conclude that postzygotic isolation is not in fact wrapped up. To the contrary, a number of surprising findings suggest that evolutionary biologists may understand less about speciation by postzygotic isolation than believed a few short years ago. And there seems every reason to think that we are in for further surprises.

Recent unexpected findings in the genetics of speciation fall into 2 classes: 1) the role of meiotic drive in postzygotic isolation and 2) mechanisms other than Dobzhansky–Muller incompatibilities that cause postzygotic isolation. We discuss each in turn.

Does Meiotic Drive Cause Speciation?

Although never mainstream, the idea that genetic conflict might play a role in speciation has a long history (reviewed in Coyne and Orr 2004). In its modern form, the idea dates to the theoretical work of Frank (1991) and Hurst and Pomiankowski (1991) on the role of meiotic drive in hybrid sterility. These authors considered a scenario in which 2 allopatric populations of a species evolve independently. In one population, a mutation that causes meiotic drive appears (say, on the X chromosome). Because this allele enjoys a segregation advantage, it will increase in frequency. But because the mutation causes the destruction of Y-bearing gametes—and thus the distortion of normal 50:50 sex ratios in the direction of an excess of daughters—there will be strong selection to suppress it. Following the fixation of a suppressor mutation, segregation and sex ratios return to normal within the population. In the other, allopatric, population, another meiotic drive mutation appears (say, on the Y chromosome). This mutation also distorts segregation and sex ratios, now causing an excess of sons. Following the above logic, this mutation will also increase in frequency but will ultimately be suppressed, restoring normal segregation and sex ratios within the population. But if the 2 populations meet, hybrids might well lack proper suppression and thus suffer meiotic drive. Indeed, drive would be expected whenever suppressor mutations are less than fully dominant. One can even imagine a scenario in which X-bearing sperm destroy Y-bearing sperm in hybrids, while Y-bearing sperm destroy X-bearing, rendering hybrids sterile (Frank 1991; Hurst and Pomiankowski 1991). Although many variations on this idea are possible, all share the theme that an unmasking of normally suppressed meiotic drive in species hybrids might yield intrin-

sic postzygotic isolation. More recent forms of the meiotic drive theory emphasize the role of "centromeric drive," that is, competition among homologous chromosomes to enter the egg, thereby avoiding the evolutionary dead end of the polar body (Henikoff et al. 2001; Malik and Henikoff 2001; Henikoff and Malik 2002). Although these theories focus on segregation distortion in the female germ line, they are similar in spirit to those of Frank (1991) and Hurst and Pomiankowski (1991).

Although intuitively appealing, the idea that meiotic drive plays a part in reproductive isolation fell out of favor in the early 1990s. At that time, several experiments were performed that appeared to falsify, or at least to lessen the plausibility of, the idea (Johnson and Wu 1992; Coyne and Orr 1993). These experiments showed that species hybrids that were partially sterile—thus allowing recovery of some gametes—suffered no discernible segregation or sex-ratio distortion. (The theory is difficult, if not impossible, to test when hybrids are completely sterile. The theory maintains that meiotic drive causes sterility but drive cannot be observed because of sterility.) In retrospect, it appears that these early experiments were unlucky in their choice of taxa and/or hybrid genotype: many cases of normally masked meiotic drive have since been observed in species or population hybrids (see Coyne 2004). Although these cases are concentrated in *Drosophila*, this almost certainly reflects the intense scrutiny of hybridizations in this genus.

In one of the most interesting cases, Montchamp-Moreau and colleagues (Mercot et al. 1995; Cazemajor et al. 1997; Montchamp-Moreau and Joly 1997) have shown that segregation distortion occurs in hybrids between certain populations of the fly *D. simulans*. Whereas individuals from within populations show normal segregation ratios, "hybrids" that result from crossing flies from Tunisia with those from either the Seychelles or New Caledonia suffer segregation distortion. Recent work (Montchamp-Moreau et al. 2006) has revealed that this meiotic drive involves 2 regions of the X chromosome and that both regions are required for expression of drive. Interestingly, one of these regions includes a small duplication of 6 genes of known identity; the other region has also been fine mapped to a small number of loci (Montchamp-Moreau et al. 2006).

Although this work demonstrates that meiotic drive can be unmasked in population hybrids, the resulting segregation distortion is not associated with any known postzygotic isolation. However, Tao et al. (2001) showed that normally masked meiotic drive that arises in hybrids between *D. simulans* and *D. mauritiana is* associated with postzygotic isolation. In particular, they identified a small region of chromosome 3 from *D. mauritiana* that, when introgressed into a *D. simulans* genetic background, causes both hybrid male meiotic drive and hybrid male sterility. Given the fine resolution of their mapping (the putative gene involved, *too much yin* (*tmy*), has been located to less that 80 kb), it seems likely that the same locus causes both drive and sterility in hybrids, consistent with the theory described above.

Our laboratory has also characterized a case of meiotic drive associated with postzygotic isolation in *Drosophila* (Orr
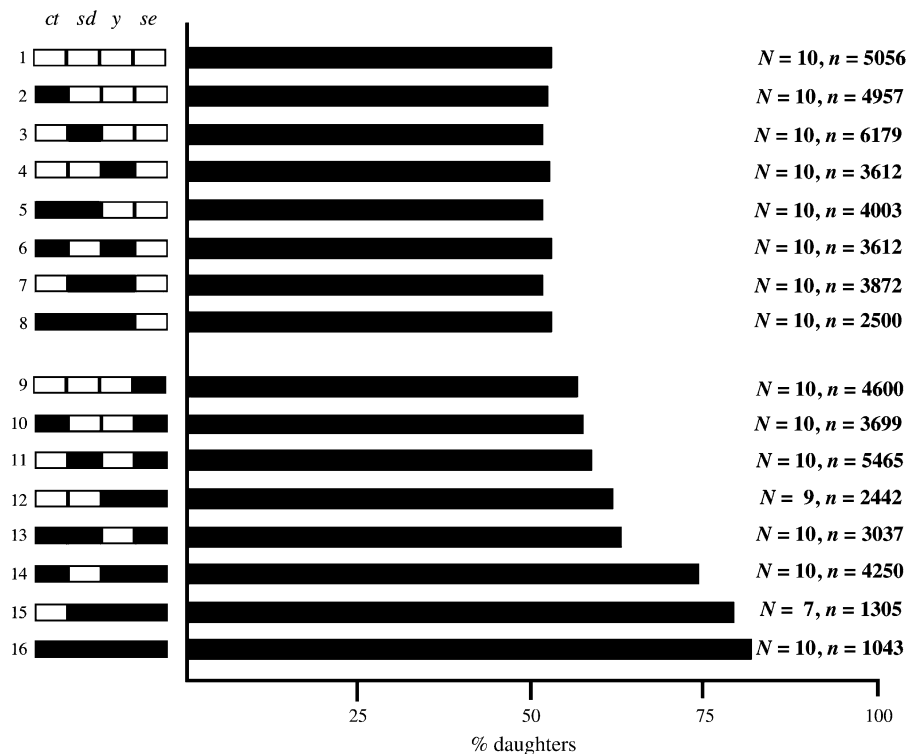
Figure 1. Mapping of chromosomal regions causing segregation distortion in hybrids between the Bogota and USA subspecies of *Drosophila pseudoobscura*. Recombinant X chromosomes among backcross males are shown on the *y* axis (region in black are from Bogota, whereas regions in white are from USA; *ct*, *sd*, *y*, and *se* correspond to mapped visible markers on the X chromosome); progeny sex ratio from these backcross males is shown on the *x* axis. Strong segregation distortion occurs only when hybrid males carry all relevant regions from the Bogota subspecies.

and Irving 2005). *Drosophila pseudoobscura* includes 2 subspecies which split only ~200 000 years ago (Machado et al. 2002; Machado and Hey 2003): the USA subspecies, found throughout western North America, and the Bogota subspecies, found in the highlands near Bogota, Colombia. Hybridization between these taxa results in postzygotic isolation. The cross of Bogota females to USA males yields sterile F1 males, whereas the reciprocal cross yields fertile F1 males; all F1 females are fertile (Orr and Irving 2001). No other form of reproductive isolation separates these subspecies. Although F1 hybrid males with Bogota mothers have invariably been described as sterile, we recently discovered that they are in fact weakly fertile. More surprisingly, these weakly fertile hybrids produce all or nearly all daughters (Orr and Irving 2005). This sex-ratio bias almost certainly reflects segregation distortion, not the inviability of sons. Because we know a great deal about the genetics of male sterility in Bogota–USA hybrids, we were able to ask if the genes that cause segregation distortion in hybrids map to the same chromosomal regions as the genes that cause sterility in hybrids. The answer is yes (Orr and Irving 2005). Factors causing hybrid male sterility and segregation distortion map to the same 3 regions of the Bogota X chromosome (Figure 1). Moreover, these chromosomal regions show the same pattern of epistasis for both phenotypes: F1-like levels of hybrid sterility and segregation distortion arise only when

hybrid males carry all relevant regions from Bogota. These results are consistent with the hypothesis that the same genes cause both hybrid sterility and distortion.

In one of the X-linked regions implicated above, the genes causing hybrid sterility and segregation distortion are fortuitously tightly linked to a visible marker, *sepia* (*se*). In recent work, our laboratory has performed an introgression experiment to determine if the genes causing these 2 hybrid phenotypes can be separated genetically. Although this work remains in progress, the answer thus far is no. After 28 generations of introgression—14 of them with recombination—we have mapped hybrid sterility and segregation distortion to a tiny chromosomal region near *se* that includes a small number of predicted genes. Again, these findings are consistent with the idea that the same genes cause both hybrid segregation distortion and hybrid sterility.

It is worth emphasizing that, in the Bogota–USA hybridization, we are considering a very young pair of subspecies as well as the only form of reproductive isolation known to separate them. Furthermore, we are considering a small chromosomal region that is required for the expression of hybrid sterility; we are not, in other words, considering genes that evolved after the attainment of complete reproductive isolation. Although we cannot yet draw firm conclusions, it is growing difficult to escape the view that the same genes that cause

meiotic drive can cause hybrid sterility and thus that meiotic drive may play a role in the earliest stages of speciation.

If further data support this surprising conclusion, these results, and others like them, may demand a considerable change in our traditional view of the role of natural selection in speciation. In particular, we may well be forced to conclude that genetic conflict, including meiotic drive, plays an important role in the evolution of postzygotic isolation, at least in its intrinsic form. (There is no reason, of course, to think that genetic conflict plays an important role in extrinsic postzygotic isolation.) Reproductive isolation may, then, sometimes reflect arms races within the genome, races that involve natural selection on selfish genetic elements—not Darwinian adaptation to the external environment. It is even conceivable that these 2 forms of natural selection (adaptation to the internal genomic environment vs. adaptation to the external ecological environment) might map neatly onto the 2 forms of postzygotic isolation (intrinsic vs. extrinsic isolation). In particular, we might conjecture that "internal" forms of natural selection often (though not always) drive the evolution of intrinsic postzygotic isolation, whereas "external" forms of natural selection often (though not always) drive the evolution of extrinsic postzygotic isolation. Although this idea remains wholly speculative, it seems to us both possible and plausible. In any case, this tentative view of postzygotic isolation obviously differs considerably from the one that has, until recently, guided speciation research.

The Universality of the Dobzhansky– Muller Mechanism

The Dobzhansky–Muller model provides a simple explanation of the evolution of intrinsic postzygotic isolation (Dobzhansky 1937; Muller 1942; Orr 1995). According to this model, hybrid sterility and inviability result from normal evolution within 2 allopatric populations. Each population evolves independently, accumulating genic substitutions under the action of natural selection and/or genetic drift. But if these populations come into contact and hybridize, we have no guarantee that mixtures of genes from them will function properly. Instead, some genes from one population might fail to function correctly with other genes from the other population. The result is (partial or complete) intrinsic sterility or inviability of hybrids.

The Dobzhansky–Muller model has played an important part in both theoretical and experimental studies of speciation. Indeed, it represents one of the most significant unifying ideas in the genetics of speciation. Although evolutionary geneticists have always appreciated that mechanisms other than Dobzhansky–Muller incompatibilities can cause postzygotic isolation—polyploidy in plants represents the most obvious alternative—a good consensus holds that hybrid sterility and inviability in animals almost always reflect between-locus incompatibilities in hybrids. To our surprise, recent work in our laboratory has shown that this consensus could be wrong.

Drosophila melanogaster and its sister species *D. simulans* split several million years ago. Not surprisingly, they are completely reproductively isolated; indeed all F1 hybrids are completely sterile or inviable (Sturtevant 1920). Although the low fitness of *D. melanogaster–D. simulans* hybrids has blocked most genetic analyses of this hybridization, Muller and Pontecorvo (1940, 1942; Pontecorvo 1943) were able to sidestep the problem, at least partly. By crossing triploid females from *D. melanogaster* to heavily irradiated males from *D. simulans*, they were able to recover hybrids having backcross-like genotypes. Only one of these "pseudo-backcross" hybrids was fertile: a female carrying the small fourth chromosome from *D. simulans* and having all remaining major chromosomes from *D. melanogaster*. (The fourth or "dot" chromosome represents only ∼1–2% of the genome and carries approximately 90 genes; under normal conditions the fourth does not recombine.) Muller and Pontecorvo showed that hybrid males that are homozygous for the "4-sim" chromosome in an otherwise *D. melanogaster* background are sterile—their sperm are immotile—whereas hybrid males that are heterozygous for one 4-sim chromosome and one *D. melanogaster* fourth chromosome are fertile. Deficiency mapping revealed that the genes causing this recessive hybrid male sterility reside within a small, minute deletion near the proximal end of the chromosome (Muller and Pontecorvo 1942).

Given concerns that use of X-irradiation by Muller and Pontecorvo may have induced a (artifactual) male sterile on the *D. simulans* fourth chromosome, we set out to obtain a new 4-sim chromosome without use of X-rays. Employing a combination of weak viability and fertility rescue mutations, we introduced a new fourth chromosome from *D. simulans* into an otherwise *D. melanogaster* genetic background (Masly et al. 2006). This new chromosome behaves exactly as did that of Muller and Pontecorvo. Phenotypically, the chromosome again causes male sterility when homozygous in *D. melanogaster*; cytologically, the spermatogenic lesion again involves sperm immotility (electron microscopy reveals that sperm flagella are normal ultrastructurally); genetically, hybrid sterility is again recessive and maps to the same small, minute deficiency as before. The 4-sim chromosome clearly causes true hybrid male sterility.

We performed additional deficiency mapping and complementation tests that ultimately revealed that 4-sim hybrid male sterility is caused by a single gene, *JYAlpha* (Masly et al. 2006). *JYAlpha* encodes the catalytic subunit of a Na-K-ATPase, a protein that, among other things, maintains proper pH across cellular membranes. *JYAlpha* from *Drosophila* is similar to 1 of 4 isoforms of Na-K-ATPases from mammals—the so-called alpha4 isoform. Importantly, alpha4 plays a critical role in sperm function in mammals: it is necessary for sperm motility. By remobilizing a *P* element insertion in *JYAlpha*, we were able to obtain a null allele of *JYAlpha* in *D. melanogaster*. Homozygotes for this null allele are completely sterile, showing that, as in mammals, Na-K-ATPase function is essential for male fertility within species.

Although we assumed initially that *JYAlpha* from *D. simulans* was incompatible with a locus or loci from

D. melanogaster—indeed we designed experiments to identify this partner gene—we soon discovered otherwise. In particular, we discovered that this gene is not involved in a traditional Dobzhansky–Muller incompatibility; instead, *JYAlpha* causes hybrid sterility by a different mechanism. A combination of genomic, genetic, and molecular analyses showed that *JYAlpha* has different chromosomal locations in *D. melanogaster* and *D. simulans*: whereas *JYAlpha* resides on the fourth chromosome of *D. melanogaster*, it resides on the third chromosome of *D. simulans*. *JYAlpha* appears to be single copy in both species (Masly et al. 2006). The cause of hybrid sterility thus appears surprisingly simple. Homozygous *4-sim* hybrid males lack a copy of *JYAlpha* from either species, and postzygotic isolation results from the absence of this essential fertility locus. Although we understand little about the mechanism of *JYAlpha*'s transposition (except that it did not involve an RNA intermediate), we do understand something about its evolutionary history. Genomic and molecular data show that *JYAlpha* resides on the third chromosome in the entire *D. simulans* clade (including *D. simulans*, *D. mauritiana*, and *D. sechellia*); *JYAlpha* resides on the fourth chromosome in *D. melanogaster* and in the outgroup *D. yakuba*. It appears, therefore, that *JYAlpha* resided ancestrally on chromosome 4 and transposed to chromosome 3 before the split of *D. simulans*, *D. mauritiana*, and *D. sechellia*. Interestingly, we have also found that *JYAlpha* resides on the left arm of the X chromosome in *D. pseudoobscura*; *JYAlpha* has thus transposed to a new chromosome at least twice during the evolutionary history of the subgenus Sophophora (Figure 2).

The significance of these findings is that they reveal that postzygotic isolation in animals sometimes involves mechanisms other than the traditional Dobzhansky–Muller one. Hybrid sterility here is due not to functional incompatibilities between two or more diverged loci but to gene movement between chromosomes. Because *JYAlpha* almost surely passed through a phase in the history of the *simulans* clade in which it was duplicated (present on both chromosomes 4 and 3) within the population, our results lend strong support to the prescient suggestion of Lynch and Force (2000) that gene duplication followed by "divergent resolution" can lead to postzygotic isolation. (Note that, formally, gene transposition can be interpreted within a 2-site Dobzhansky–Muller model; it differs biologically from this model, however, in that postzygotic isolation involves the physical movement of a gene, not a functional incompatibility between 2 diverged loci.)

The key question now is whether gene transposition is a common cause of intrinsic postzygotic isolation. The answer is that we do not know. All we can say with confidence is that of the 6 genes identified thus far that cause postzygotic isolation, one involves gene transposition. Transposition-based reproductive isolation could, however, be more common than it first appears, for one theoretical and one practical reason. The theoretical reason is that gene transposition, even if infrequent on the timescale of speciation, might still make an important contribution to reproductive isolation as F1, F2, or backcross hybrids need lack only one essential gene to be rendered sterile or inviable (this is what it means for a gene to be essential). The practical reason is that, if it were com-
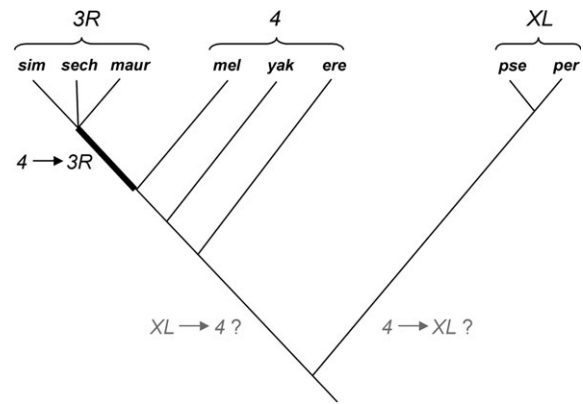
Figure 2. *JYAlpha* location in the subgenus *Sophophora* of the genus *Drosophila*. The branch on which *JYAlpha*'s transposition from chromosome 4 to chromosome 3 occurred is shown in bold. Another *JYAlpha* transposition event occurred between *D. pseudoobscura*–*D. persimilis* and the *melanogaster* group species, although its directionality is presently unclear. Chromosome arm XL of *D. pseudoobscura* and *D. persimilis* is not homologous to an autosomal arm of *D. melanogaster* or its sister species. Abbreviations: *sim* = *D. simulans*; *sech* = *D. sechellia*; *maur* = *D. mauritiana*; *mel* = *D. melanogaster*; *yak* = *D. yakuba*; *ere* = *D. erecta*; *pse* = *D. pseudoobscura*; *per* = *D. persimilis*.

mon, transposition-based reproductive isolation would not necessarily have been detected in genetic analyses performed to date. The reason is that gene transpositions that sterilize or kill certain F2 or backcross hybrids will behave formally like Dobzhansky–Muller incompatibilities in quantitative trait locus style experiments. Some combinations of chromosomal regions from the 2 species will be viable or fertile (as these combinations carry at least one copy of an essential gene), whereas other combinations of chromosomal regions from the 2 species will be inviable or sterile (as these combinations lack any copies of the essential gene). It is difficult, therefore, to distinguish Dobzhansky–Muller versus transposition mechanisms of postzygotic isolation when using genetic—not molecular or genomic—data alone.

Conclusions

Recent discoveries in the genetics of speciation suggest that the field—including the subfield of the genetics of postzygotic isolation—is far from complete. Five years ago, there was little reason to suspect that genetic conflict, including meiotic drive, played an important part in speciation. Nor was there substantial empirical reason to believe that mechanisms other than the traditional Dobzhansky–Muller one played a role in postzygotic isolation. Both scenarios now seem likely. Indeed, it now seems clear that intrinsic postzygotic isolation can have a variety of causes, ranging from Dobzhansky–Muller incompatibilities to gene transpositions to endosymbiont infections to polyploidy.

Although perhaps not comforting, these recent surprises are, nonetheless, exciting. The genetics of speciation is a young enterprise—surely one of the youngest areas of evolutionary biology—and young scientific disciplines are, of course, the ones prone to surprise. Although we may understand less about the genetics of speciation than we believed several years ago, it is at least encouraging to realize that future work in the genetics of speciation may demand considerable changes in our views, not minor and inconsequential modifications.

Acknowledgments

References

Barbash DA, Awadalla P, Tarone AM. 2004. Functional divergence caused by ancient positive selection of a *Drosophila* hybrid incompatibility locus. Public Libr Sci. 2:839–848.

Barbash DA, Siino DF, Tarone AM, Roote J. 2003. A rapidly evolving MYB-related protein causes species isolation in *Drosophila*. Proc Natl Acad Sci USA. 100:5302–5307.

Bradshaw HD, Otto KG, Frewen BE, McKay JK, Schemske DW. 1998. Quantitative trait loci affecting differences in floral morphology between two species of Monkeyflower (*Mimulus*). Genetics. 149:367–382.

Bradshaw HD, Wilbert SM, Ott KG, Schemske DW. 1995. Genetic mapping of floral traits associated with reproductive isolation in monkeyflowers (*Mimulus*). Nature. 376:762–765.

Brideau NJ, Flores HA, Wang J, Maheshwari S, Wang X, Barbash DA. 2006. Two Dobzhansky-Muller genes interact to cause hybrid lethality in *Drosophila*. Science. 314:1292–1295.

Cazemajor M, Landre C, Montchamp-Moreau C. 1997. The *sex-ratio* trait in *Drosophila simulans*: genetic analysis of distortion and suppression. Genetics. 147:635–642.

Civetta A, Singh RS. 1995. High divergence of reproductive tract proteins and their association with postzygotic reproductive isolation in *Drosophila melanogaster* and *Drosophila virilis* group species. J Mol Evol. 41:1085–1095.

Coyne JA. 1985. The genetic basis of Haldane's rule. Nature. 314:736–738.

Coyne JA, Orr HA. 1989a. Patterns of speciation in *Drosophila*. Evolution. 43:362–381.

Coyne JA, Orr HA. 1989b. Two rules of speciation. In: Otte D, Endler J, editors. Speciation and its consequences. Sunderland (MA): Sinauer Associates. p. 180–207.

Coyne JA, Orr HA. 1993. Further evidence against meiotic-drive models of hybrid sterility. Evolution. 47:685–687.

Coyne JA, Orr HA. 1997. "Patterns of speciation in *Drosophila*" revisited. Evolution. 51:295–303.

Coyne JA, Orr HA. 2004. Speciation. Sunderland (MA): Sinauer Associates.

Davis AW, Noonburg EG, Wu CI. 1994. Evidence for complex genic interactions between conspecific chromosomes underlying hybrid female sterility in the *Drosophila simulans* clade. Genetics. 137:191–199.

Dobzhansky T. 1937. Genetics and the origin of species. New York: Columbia University Press.

Fishman L, Willis JH. 2001. Evidence for Dobzhansky-Muller incompatibilities contributing to the sterility of hybrids between *Mimulus guttatus* and *M. nasutus*. Evolution. 55:1932–1942.

Fitzpatrick BM, Turelli M. 2006. The geography of mammalian speciation: mixed signals from phylogenies and range maps. Evolution. 60:601–615.

Frank SH. 1991. Divergence of meiotic drive-suppressors as an explanation for sex-biased hybrid sterility and inviability. Evolution. 45:262–267.

Gavrilets S. 2004. Fitness landscapes and the origin of species. Princeton (NJ): Princeton University Press.

Guenet JL, Nagamine C, Simon-Chazottes D, Montagutelli X, Bonhomme F. 1990. Hst-3: an X-linked hybrid sterility gene. Genet Res. 56:163–165.

Hatfield T, Schluter D. 1999. Ecological speciation in sticklebacks: environment-dependent hybrid fitness. Evolution. 53:866–873.

Henikoff S, Ahmad K, Malik HS. 2001. The centromere paradox: stable inheritance with rapidly evolving DNA. Science. 293:1098–1102.

Henikoff S, Malik HS. 2002. Selfish drivers. Science. 417:227.

Hurst LD, Pomiankowski A. 1991. Causes of sex ratio bias may account for unisexual sterility in hybrids: a new explanation of Haldane's rule and related phenomena. Genetics. 128:841–858.

Johnson NA, Wu CI. 1992. An empirical test of the meiotic drive models of hybrid sterility: sex-ratio data from hybrids between *Drosophila simulans* and *Drosophila sechellia*. Genetics. 130:507–511.

Kelly JK, Noor MAF. 1996. Speciation by reinforcement: a model derived from studies of Drosophila. Genetics. 143:1485–1497.

Kirkpatrick M, Servedio MR. 1999. The reinforcement of mating preferences on an island. Genetics. 151:865–884.

Lewontin RC. 1974. The genetic basis of evolutionary change. New York: Columbia University Press.

Lijtmaer DA, Mahler B, Tubaro PL. 2003. Hybridization and postzygotic isolation patterns in pigeons and doves. Evolution. 57:1411–1418.

Liou LW, Price TD. 1994. Speciation by reinforcement of premating isolation. Evolution. 48:1451–1459.

Lynch M, Force AG. 2000. The origin of interspecific genomic incompatibility via gene duplication. Am Nat. 156:590–605.

Machado CA, Hey J. 2003. The causes of phylogenetic conflict in a classic *Drosophila* species group. Proc R Soc Lond B Biol Sci. 270:1193–1202.

Machado CA, Kliman RM, Markert JA, Hey J. 2002. Inferring the history of speciation from multilocus DNA sequence data: the case of *Drosophila pseudoobscura* and close relatives. Mol Biol Evol. 19:472–488.

Malik HS, Henikoff S. 2001. Adaptive evolution of Cid, a centromere-specific histone in Drosophila. Genetics. 157:1293–1298.

Masly JP, Jones CD, Noor MAF, Orr HA. 2006. Gene transposition as a cause of hybrid sterility. Science. 313:1448–1450.

Mercot H, Atlan A, Jacques M, Montchamp-Moreau C. 1995. Sex-ratio distortion in *Drosophila simulans*: co-occurrence of a meiotic drive and a suppressor of drive. J Evol Biol. 8:283–300.

Montchamp-Moreau C, Joly D. 1997. Abnormal spermiogenesis is associated with the X-linked sex-ratio trait in *Drosophila simulans*. Heredity. 79:24–30.

Montchamp-Moreau C, Ogereau D, Chaminade N, Colard A, Aulard S. Forthcoming 2006. Organization of the *sex-ratio* meiotic drive region in *Drosophila simulans*. Genetics.

Muller HJ. 1942. Isolating mechanisms, evolution, and temperature. Biol Symp. 6:71–125.

Muller HJ, Pontecorvo G. 1940. Recombinants between Drosophila species, the F_1 hybrids of which are sterile. Nature. 146:199.

Muller HJ, Pontecorvo G. 1942. Recessive genes causing interspecific sterility and other disharmonies between *Drosophila melanogaster* and *simulans*. Genetics. 27:157.

Orr HA. 1995. The population genetics of speciation: the evolution of hybrid incompatibilities. Genetics. 139:1805–1813.

Orr HA, Irving S. 2001. Complex epistasis and the genetic basis of hybrid sterility in the *Drosophila pseudoobscura* Bogota-USA hybridization. Genetics. 158:1089–1100.

Orr HA, Irving S. 2005. Segregation distortion in hybrids between the Bogota and USA subspecies of *Drosophila pseudoobscura*. Genetics.

Orr HA, Turelli M. 2001. The evolution of postzygotic isolation: accumulating Dobzhansky-Muller incompatibilities. Evolution. 55:1085–1094.

Palopoli MF, Wu CI. 1994. Genetics of hybrid male sterility between Drosophila sibling species: a complex web of epistasis is revealed in interspecific studies. Genetics. 138:329–341.

Payseur BA, Nachman MW. 2005. The genomics of speciation: investigating the molecular correlates of X chromosome introgression across the hybrid zone between *Mus domesticus* and *Mus musculus*. Biol J Linn Soc. 84:523–534.

Pilder SH, Hammer FM, Silver LM. 1991. A novel mouse chromosome 17 hybrid sterility locus: implications for the origin of t-haplotypes. Genetics. 129:237–246.

Pontecorvo G. 1943. Hybrid sterility in artificially produced recombinants between *Drosophila melanogaster* and *D. simulans*. Proc R Soc Edinb Sect B (Biol Sci). 61:385–397.

Presgraves DC. 2002. Patterns of postzygotic isolation in Lepidoptera. Evolution. 56:1168–1183.

Presgraves DC, Balagopalan L, Abmayr SM, Orr HA. 2003. Adaptive evolution drives divergence of a hybrid inviability gene between two species of *Drosophila*. Nature. 423:715–719.

Price TD, Bouvier MM. 2002. The evolution of F1 postzygotic incompatibilities in birds. Evolution. 56:2083–2089.

Ramsey J, Bradsha HD, Schemske DW. 2003. Components of reproductive isolation between *Mimulus lewisii* and *M. cardinalis* (Scrophulariaceae). Evolution. 57:1520–1534.

Ramsey J, Schemske DW. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. Annu Rev Ecol Syst. 29:467–501.

Rieseberg LH. 1997. Hybrid origins of plant species. Ann Rev Ecol Syst. 28:359–389.

Rieseberg LH, Linder CR, Seiler GJ. 1995. Chromosomal and genic barriers to introgression in *Helianthus*. Genetics. 141:1163–1171.

Rieseberg LH, Van Fossen C, Desrochers AM. 1995. Hybrid speciation accompanied by genomic reorganization in wild sunflowers. Nature. 375:313–316.

Rundle HD. 2002. A test of ecologically dependent postmating isolation between sympatric sticklebacks. Evolution. 56:322–329.

Said K, Saad A, Auffray JC, Britton Davidian J. 1993. Fertility estimates in the Tunisian all-acrocentric and Robertsonian populations of the house mouse and their chromosomal hybrids. Heredity. 71:532–538.

Sasa MM, Chippindale PT, Johnson NA. 1998. Patterns of postzygotic isolation in frogs. Evolution. 52:1811–1820.

Schluter D, McPhail JD. 1992. Ecological character displacement and speciation in sticklebacks. Am Nat. 140:85–108.

Servedio MR. 2000. Reinforcement and the genetics of nonrandom mating. Evolution. 54:21–29.

Sturtevant AH. 1920. Genetic studies on *Drosophila simulans*. I. Introduction. Hybrids with *Drosophila melanogaster*. Genetics. 5:488–500.

Tao Y, Hartl DL, Laurie CC. 2001. Sex-ratio distortion associated with reproductive isolation in *Drosophila*. Proc Natl Acad Sci USA. 98:13183–13188.

Ting CT, Tsaur SC, W ML, Wu CI. 1998. A rapidly evolving homeobox at the site of a hybrid sterility gene. Science. 282:1501–1504.

Tucker PK, Sage RD, Warner J, Wils AC, Eicher EM. 1992. Abrupt cline for sex chromosomes in a hybrid zone between two species of mice. Evolution. 46:1146–1163.

Turelli M, Barton NH, Coyne JA. 2001. Theory and speciation. Trends Ecol Evol.

Turelli M, Orr HA. 2000. Dominance, epistasis and the genetics of postzygotic isolation. Genetics. 154:1663–1679.

Ungerer MC, Baird SJE, Pan J, Rieseberg LH. 1998. Rapid hybrid speciation in wild sunflowers. Proc Natl Acad Sci USA. 95:11757–11762.

Wittbrodt J, Adam D, Malitschek B, Maueler W, Raulf F, Telling A, Robertson SM, Schartl M. 1989. Novel putative receptor tyrosine kinase encoded by the melanoma-inducing *Tu* locus in *Xiphophorus*. Nature. 341:415–421.

Wu CI, Davis AW. 1993. Evolution of postmating reproductive isolation: the composite nature of Haldane's rule and its genetic bases. Am Nat. 142:187–212.

Wu CI, Ting CT. 2004. Genes and speciation. Nat Rev Genet. 5:114–122.

Zeng LW, Singh RS. 1993. The genetic basis of Haldane's rule and the nature of asymmetric hybrid male sterility among *Drosophila simulans*, *Drosophila mauritiana* and *Drosophila sechellia*. Genetics. 134:251–260.

Corresponding Editor: Loren Rieseberg