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Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Ninety Years of *Drosophila melanogaster* Hybrids

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ABSTRACT

Within 10 years of the beginning of experimental genetic research on *Drosophila melanogaster*, in 1919, A. H. Sturtevant discovered its sibling species, *D. simulans*. He hybridized the two species and made fundamental discoveries about the genetic basis of hybrid incompatibility. The complete sterility of surviving F_1 hybrids frustrated Sturtevant and his vision of comprehensively exploring the genetics of interspecific differences. But over the next 90 years, a combination of clever genetic tricks and close observation of natural variation has led to a wealth of discovery using these and other hybrids of *D. melanogaster* and *D. simulans*, resulting in an advanced understanding of speciation and the evolution of morphology, gene regulation, and behavior.

"MANY attempts have been made to hybridize different species of Drosophila, but hitherto all but the combination to be described here have been unsuccessful" (Sturtevant 1920, p. 488). So begins Sturtevant's article on hybrids between *Drosophila melanogaster* and *D. simulans*. What did Sturtevant hope to learn from studying species hybrids? His goals can be inferred from the disappointment that he expresses in the article's second sentence, where he describes the study as being only a "partial success" due to the parental species being extremely similar yet still producing only sterile or lethal F_1 hybrids. It is clear that, to study the genetic basis of species differences, Sturtevant was searching for species pairs with large phenotypic differences that were also amenable to backcrossing. Thus his disappointment. Sturtevant was too modest, because in his 1920 article he in fact had detailed important discoveries, particularly concerning the genetic basis of hybrid lethality. Here I review Sturtevant's findings and some of what has followed from studying these hybrids over the subsequent 90 years. Several themes recur:

Forced creativity: The limitation of being unable to get beyond the F_1 hybrid generation has motivated clever alternatives to backcrossing.

Exploiting natural variation: Without the many genetic reagents available in *D. melanogaster*, mutagenesis screens in *D. simulans* are much less feasible. The sterility of F_1 hybrids also precludes direct selection of mutants affecting F_1 hybrid phenotypes. These limitations have motivated the screening of natural populations for genetic variation in hybrid incompatibility traits as a way of identifying the causal genes and of overriding some of the incompatibilities.

Searching for better species: The lack of backcrossing led to the search for alternative species models, including the search for closer siblings species of *D. simulans*.

Premature pessimism: Sturtevant showed great insight in his early studies, but was overly pessimistic in implying that *D. melanogaster* and *D. simulans* have few interesting differences. Many behavioral, ecological, population genetics, and gene expression differences between these species have since been discovered, some of which were reviewed in a special issue of *Genetica* (Capy and Gibert 2004).

Two sibling species of *D. simulans*, *D. mauritiana* and *D. sechellia*, were discovered on islands in the Indian ocean (Tsacas and David 1974; Tsacas and Bächli 1981). Both species are able to hybridize with *D. melanogaster*. In terms of hybrid lethality and sterility, the outcome of these crosses is largely the same as with *D. simulans* (Lachaise *et al.* 1986). I will therefore discuss *D. melanogaster* hybrids with all three species. Nineteen years ago in this journal, Provine

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(1991) recounted the history of the discovery of *D. simulans* and of early experiments on *D. melanogaster/D. simulans* hybrids, as well as an appreciative assessment of Sturtevant's contributions to evolutionary biology.

Comparative genomics *ca.* 1920: Spontaneous visible mutations in *D. melanogaster* fueled the great series of fundamental discoveries in genetics made by the Columbia University fly group. By 1929 Sturtevant and colleagues had discovered 54 different visible mutants in *D. simulans* (Sturtevant 1929). Many of these mutations were similar to known *D. melanogaster* mutations and could be tested for allelism between species by asking whether they complemented in F_1 hybrids. Numerous examples of noncomplementation were found. As noted by Provine (1991, p. 2), Sturtevant (1921, p. 206) concluded that he had made the first discovery of "parallel mutations in distinct species" and thus had "definite proof that related species do have many genes in common, and that identical mutations may occur in different species." In modern terms, Sturtevant had demonstrated that the two species have orthologous genes and that the function of the genes in each species is the same. This discovery between species separated by only a few million years does not sound particularly surprising in light of contemporary knowledge, but it is difficult to imagine its reception during the 1920s. Little in the published record indicates whether Sturtevant thought his discovery was surprising.

Hybrid incompatibilities: Sturtevant (1920) found that only one sex of F_1 hybrids survives, and which sex survives depends on the direction of crossing. Crosses with *D. melanogaster* mothers produce only F_1 daughters, while those with *D. simulans* mothers produce mostly F_1 sons (F_1 female lethality having a variable penetrance). But digging deeper, he realized that F_1 lethality is not in fact sex-specific in either direction of crossing. Taking advantage of bridges' discovery (1916) that XXY females have an elevated rate of sex-chromosome nondisjunction, he crossed *D. melanogaster* XXY females to *D. simulans* males and obtained many exceptional F_1 male hybrids carrying the *D. simulans* X (henceforth referred to as X_{sim}), in contrast to the complete lethality of regular F_1 males carrying the *D. melanogaster* X (X_{mel}). He also noted the absence of any viable exceptional F_1 females carrying two copies of X_{mel}.

In the reciprocal cross, Sturtevant obtained the reciprocal effect. While X_{mel}/X_{sim} daughters of *D. simulans* are normally lethal, XXY *D. simulans* females crossed to *D. melanogaster* males produced viable X_{sim}/X_{sim} exceptional daughters but no corresponding exceptional X_{mel}/O males. Sturtevant had discovered that F_1 lethality in both directions of crossing is not sex-specific, but rather X chromosome-specific. I believe that this represents the first genetic mapping of a hybrid incompatibility effect. Furthermore, because the *D. melanogaster* X is associated with lethality and the *D. simulans* X with viability, Sturtevant demonstrated an asymmetric chromosome-specific effect. This asymmetry is an important prediction in subsequent models developed to explain the evolution of hybrid incompatibilities (Dobzhansky 1937; Muller 1940). As Sturtevant (1920, p. 206) concluded: "it appears that hybrids develop only if they carry a *simulans* X, but that in the presence of *simulans* cytoplasm a *melanogaster* X usually inhibits development even though a *simulans* X is also present." This hypothesis implies (1) that X_{sim} contains genes necessary for hybrid survival in both directions of crossing and (2) that X_{mel} contains genes deleterious for hybrid survival but only when they interact with maternally inherited factors of *D. simulans*. [Later experiments refuted an alternative hypothesis raised by Sturtevant (1929) that the *D. simulans* Y might be causing lethality (Yamamoto 1992)].

In an important way, however, his conclusion was only half right. In the cross of *D. simulans* mothers to *D. melanogaster* fathers, Sturtevant's interpretation turned out be entirely correct, at least concerning the X_{mel} inhibitory effect: X_{mel} does directly prevent development in a *D. simulans* cytoplasm. Sawamura *et al.* (1993c) discovered a pericentric heterochromatic region of X_{mel} called *Zygotic hybrid rescue* that is responsible for this lethal effect because deleting it suppresses hybrid female lethality. Subsequent research has demonstrated that lethality is caused by missegregation of this heterochromatic region of X_{mel} during early embryonic mitoses (Ferree and Barbash 2009). Thus X_{mel} indeed directly "inhibits development" in the presence of *D. simulans* maternal cytoplasm.

But in the reciprocal cross of *D. melanogaster* mothers to *D. simulans* fathers, it is not true that hybrids develop only if they carry a copy of X_{sim}. Rather, they are fully viable only if they do not carry a deleterious allele on X_{mel}. Sturtevant's hypothesis was that viability requires X_{sim} but is not inhibited by X_{mel} in this direction of crossing. This reasoning was based in part on the fact that X_{sim}/X_{mel} hybrid daughters are usually viable in this cross. But as Sturtevant (1929) himself noted, regular X_{mel}/X_{sim} females are poorly viable at high temperatures. This temperature-dependent female lethality as well as the fully penetrant lethality of X_{mel}/Y_{sim} hybrid sons are caused by the same X-linked *D. melanogaster* gene (*Hmr*; Barbash *et al.* 2000). When the *Hmr* gene is removed by mutation, then X_{mel}/Y_{sim} hybrid sons survive quite well, demonstrating that X_{sim} is not required for viability. The essential distinction is that lethality is caused by the presence of the wild-type activity of this *D. melanogaster* gene, not by the absence of an essential *D. simulans* gene product (as implied in Sturtevant's interpretation). X_{mel} therefore inhibits development in both directions of crossing.

In all crosses, the surviving F_1 hybrids remained sterile, precluding study of these hybrid lethality effects by conventional backcrossing approaches. Muller and

Pontecorvo, however, invented an ingenious solution that allowed further mapping (Muller and Pontecorvo 1940; Pontecorvo 1943). Triploids of *D. melanogaster* were widely used by the Morgan school to investigate a range of questions. Triploids segregate one or two copies of each chromosome during meiosis. When mated to diploids, progeny will be aneuploid if the triploid parent transmits different numbers of different chromosomes. Muller and Pontecorvo realized that they could create "partial hybrids" by mating triploid *D. melanogaster* females to heavily irradiated *D. simulans* males such that the *D. simulans* fathers transmitted zero or one intact copy of each chromosome, depending on the extent of radiation damage. These diploid partial hybrids, if viable, would contain different combinations of chromosomes, including genotypes normally obtainable only from a backcross of F_1 hybrids to *D. melanogaster* (albeit without the possibility of having recombinant chromosomes). They obtained several genotypes of female and male viable hybrids homozygous or hemizygous for X_{mel}. These combinations included $2_{mel}/2_{mel}$; $3_{mel}/3_{sim}$ and $2_{mel}/2_{sim}$; $3_{mel}/3_{mel}$, but not $2_{mel}/2_{sim}$; $3_{mel}/3_{sim}$. Pontecorvo (1943) concluded that hybrid male lethality in normal diploid crosses of *D. melanogaster* females and *D. simulans* males is caused by a three-way lethal interaction among X_{mel}, 2_{sim}, and 3_{sim}. The *D. melanogaster Hmr* and *D. simulans Lhr* genes are at least partially responsible for the X_{mel} and 2_{sim} effects, respectively (Brideau *et al.* 2006); the cause of the chromosome *3* effect remains unidentified.

In their initial experiment, Muller and Pontecorvo (1940) recovered a single fertile partial hybrid that turned out to be heterozygous for the *D. simulans Y* and *4th* chromosomes in an otherwise *D. melanogaster* background. Mating of this animal to *D. melanogaster* allowed them to introgress 4_{sim} into *D. melanogaster*. They found that 4_{sim} causes male but not female sterility when homozygous (Muller and Pontecorvo 1941). Masly *et al.* (2006) recreated an introgression strain of 4_{sim} into *D. melanogaster* and discovered that an essential male fertility gene, *Jyalpha*, has transposed from chromosome *4* in *D. melanogaster* and outgroup species to chromosome *3* in the ancestor of *D. simulans*. *D. melanogaster* flies homozygous for 4_{sim} thus lack any copy of *JYalpha* and are sterile. Although F_1 *D. melanogaster/D. simulans* hybrid males are sterile due to other causes, this process of gene transposition might cause incompatibility in other hybrids and also represents a mechanism distinct from divergence in gene function that can cause hybrid incompatibility.

After Pontecorvo's analysis of partial hybrids research, the genetics of *D. melanogaster/D. simulans* hybrid incompatibilities remained largely dormant until Watanabe's (1979) stunning discovery of the *Lethal hybrid rescue* (*Lhr*) mutation in *D. simulans*. This mutation, when crossed from *D. simulans* males to *D. melanogaster* females, dominantly suppresses the F_1 lethality of the hybrid sons. Preliminary experiments suggested that the *Lhr* mutation maps to a single locus on the second chromosome, leading to the conclusion that "the isolation of *melanogaster* and *simulans* may depend on fewer genes than previously thought" (Watanabe 1979). There was hope that isolating such a suppressor mutation would lead to an understanding of the reason why hybrid males normally die, but that hope was tempered by the formidable obstacle of identifying such a suppressor from *D. simulans* during this pre-genomic era. This challenge motivated Hutter and Ashburner (1987) to search for an analogous suppressor in *D. melanogaster*. They found one, but it clearly was a different gene because it mapped to the X chromosome rather than to the 2^{nd}. This suppressor, *Hybrid male rescue* (*Hmr*), was similar to *Lhr* in again mapping to a single region, confirming that F_1 hybrid male lethality can be overridden by single-gene mutations. Molecular analysis of *Hmr* and *Lhr* later showed that both suppressor alleles are loss-of-function mutations, demonstrating that it is indeed the wild-type activity of each gene that kills hybrids (Barbash *et al.* 2003; Brideau *et al.* 2006).

What of the female F_1 lethality found in the reciprocal cross of *D. simulans* females to *D. melanogaster* males? Crosses of *D. simulans Lhr* females to wild-type *D. melanogaster* males produced modest viability of F_1 daughters (Watanabe 1979). Likewise, crosses of wild-type *D. simulans* females to *D. melanogaster* males carrying the rescue chromosome *In(1)AB, Hmr*^{2} also produced viable F_1 daughters (Hutter *et al.* 1990). These observations led to a model in which the wild-type alleles of *Hmr* and *Lhr* cause lethality of both F_1 hybrid sons of *D. melanogaster* mothers and F_1 hybrid daughters of *D. simulans* mothers (Hutter *et al.* 1990). One complication, however, is Sturtevant's (1929) observation that F_1 hybrid female lethality is variably penetrant, depending in part on variation in *D. simulans* (Sawamura *et al.* 1993a; Orr 1996). Another complication is that Sawamura *et al.* (1993c) found that the previously mentioned *Zhr* gene suppresses the F_1 female lethality but not the F_1 male lethality. They further found that some *In(1)AB, Hmr*^{2} chromosomes carry *Zhr* mutations. These complicating facts underscore the need to demonstrate by mapping experiments that phenotypes associated with different hybrid rescue strains are actually caused by the specific hybrid rescue mutation and by not other variants in the genetic background. A data reassessment led to a convincing argument that the hybrid lethalities in the reciprocal *D. melanogaster/D. simulans* crosses have distinct genetic causes (Sawamura *et al.* 1993b).

Considering the many insights gained from study of these hybrid rescuing mutations and the ease of performing mutagenesis screens in *D. melanogaster*, it is surprising that no one has systematically screened for more such suppressors of lethality. Two screens have been performed, however, using large collections of *D. melanogaster* deletion strains that searched for the

opposite effect: genomic regions that when deleted cause rather than suppress hybrid lethality. Coyne *et al.* (1998) crossed females from >100 *D. melanogaster* deletion strains to *D. simulans* males in a search for chromosomal regions that when deleted cause F_1 female lethality and found only a handful of regions causing partially penetrant lethality. Presgraves (2003) repeated such a screen but used *Lhr* mutant *D. simulans* males to look for chromosomal regions that when deleted suppress *Lhr*-dependent male rescue. This screen was clearly more sensitive, identifying 20 lethal regions, and has since led to the discovery of incompatibilities caused by components of the nuclear pore (Tang and Presgraves 2009). These incompatibilities appear not to normally affect F_1 male viability, suggesting that if Sturtevant had achieved his goal of obtaining fertile F_1 hybrids, additional incompatibilities would become apparent among the backcross progeny.

The realization of Sturtevant's dream? In 1996, such backcross studies suddenly appeared possible when Davis *et al.* (1996) reported the discovery of fertile F_1 female hybrids. They found that, with certain strain combinations, F_1 hybrid daughters from *D. simulans* mothers and *D. melanogaster* fathers were partially fertile and could produce some backcross progeny. The genetic basis of sterility rescue was unclear, but this report suggested the exciting possibility of performing high-resolution genetic mapping between *D. melanogaster* and *D. simulans* through backcrossing—Sturtevant's dream realized. A subsequent study discovered fertility rescue in F_1 female progeny of the reciprocal cross of *D. melanogaster* females to either *D. simulans* or *D. mauritiana* males (Barbash and Ashburner 2003). Rescue was also shown to depend in part on mutations in *Hmr*, demonstrating that Hmr^+ is a dominant female sterility gene. Backcross progeny were again obtained, but with a low yield of less than one viable progeny per F_1 hybrid parent.

This low yield has tempered some of the hope that genetic mapping could be applied across the genome between *D. melanogaster* and *D. simulans*. Nevertheless, at least four successful interspecific introgressions have been obtained and used to address different questions. Two separate regions of chromosome *2L* were simultaneously introgressed from *D. simulans* into *D. melanogaster* and, when made homozygous, were inferred to contain several genes causing male sterility and at least one gene causing female sterility (Sawamura *et al.* 2000, 2004). These studies confirm that backcross hybrids do indeed have more incompatibilities than F_1 hybrids. The bw^D region on chromosome *2R* of *D. melanogaster* was introgressed into *D. simulans* to examine its nuclear organization in a foreign-species background (Sage and Csink 2003). In a third case, fertility rescue in F_1 hybrids was used to introgress mitochondria from *D. simulans* into *D. melanogaster*, allowing a detailed study of fitness effects in strains that are hybrids between their nuclear and mitochondrial genomes (Rand *et al.* 2006). Finally, in the fourth case, the recreation of the IV_{sim} introgression into *D. melanogaster* mentioned previously was created using fertility rescue strains (Masly *et al.* 2006).

Morphological differences and hidden regulatory divergence: *D. melanogaster* and *D. simulans* are morphologically very similar, with the key diagnostic differences being the size and shape of two cuticular structures that surround the male genitalia. Although first reported by Sturtevant (1919), he later gave credit for this discovery to Bridges (Sturtevant 1920). Closer examination discovered a difference between the species in the size of a region on the second leg devoid of tiny hairs (trichomes). By examining phenotypes in F_1 hybrids of *D. melanogaster* and *D. simulans Ultrabithorax* (*Ubx*) mutations, Stern (1998) showed that this phenotypic difference is caused in part by divergence in the *Ubx* gene.

Similar studies identified the genetic basis of a naked cuticle phenotype in larvae that is distinct to *D. sechellia*. F_1 *D. melanogaster/D. sechellia* hybrids were found to have the *D. melanogaster* phenotype, while F_1 hybrids mutant for the *D. melanogaster ovo/shavenbaby* gene display the *D. sechellia* phenotype. Additional experiments showed that this morphological difference is caused by divergence in regulatory regions of *ovo/svb* between *D. melanogaster* and *D. sechellia* (Sucena and Stern 2000).

These studies demonstrate the existence of distinct morphological differences between *D. melanogaster* and its siblings, which are in some cases attributable to divergence in single genes. The simple genetic basis of these phenotypes made them solvable using F_1 hybrids. F_1 hybrids have also revealed that gene regulatory pathways can have functionally detectable divergence, even for seemingly stable phenotypes. Sturtevant (1920) noted that F_1 hybrids are often missing large thoracic bristles (macrochaete), yet the number and location of these bristles are identical in both *D. melanogaster* and *D. simulans*. This observation of hybrid breakdown in the conserved bristle pattern was a key example in the concept of developmental systems drift (True and Haag 2001). This theory suggests that genetic regulatory mechanisms can evolve and diverge even as the phenotypes that they control remain under stabilizing selection.

The idea that apparently identical traits between species can arise from divergent genes was apparent to Muller and Pontecorvo (1941) when they reported that *D. melanogaster* flies homozygous for 4_{sim} manifest a range of phenotypes in addition to male sterility, including "slight, variable" morphological defects and "semi-*simulans* male genitalia," while heterozygotes show unusual complementation of two different 4_{mel} mutations. Summarizing all the effects attributable to divergence of the tiny chromosome *4* between *D. melanogaster* and *D. simulans* (as well as other effects of Y chromosome divergence), Muller and Pontecorvo

(1941, p. 157) concluded that "the fact that even these minor chromosomes exhibit so many gene differences indicates that the reaction systems producing the similar phenotypes of apparently closely related species may be highly divergent. Hybrid sterility is but one expression of this cryptic divergence, which need not in itself have had a selective value." This inference of extensive genetic variation was certainly unanticipated by Sturtevant in his initial comparison of the species, and other important implications of Muller and Pontecorvo's conclusion have been discussed by Provine (1991).

Weisbrot (1963) also observed a wide range of morphological defects in four female partial hybrids that he generated using the method of Muller and Pontecorvo. Like them, he also concluded that closely related and apparently similar species such as *D. melanogaster* and *D. simulans* are likely to have different alleles of many of their genes, and thus speciation would result from changes at many loci. Interestingly, Weisbrot (1963) cites Sturtevant (1948) as the clearest proponent of the alternative view that only a small number of loci directly involved in speciation would be different, even between well-separated species.

Further analyses have strongly suggested that the hybrid bristle-loss phenotype is due to divergence in genes that regulate the bristle pattern, rather than to an indirect consequence of unrelated hybrid incompatibilities. Biddle (1932) performed an extensive study of variation in bristle loss involving a total of 52,784 hybrids. Among Biddle's conclusions was that the degree of bristle loss did not correlate with other morphological defects and that hybrid females generally showed less bristle loss than X_{sim} hybrid males despite being poorly viable at higher temperatures. Biddle also found that the X chromosome was primarily responsible for variation in hybrid bristle loss among different *D. simulans* strains. Takano later showed that X_{sim} F_1 hybrid males have more severe bristle loss than X_{mel} F_1 males and identified a QTL on the *D. simulans* X responsible for variation among *D. simulans* lines (Takano 1998; Takano-Shimizu 2000). The identity of these gene(s) remains unknown. The pro-neural genes *achaete-scute* or their regulators are good candidates because genetic studies suggest that *achaete-scute* misregulation is a primary cause of the hybrid bristle-loss phenotype (Skaer and Simpson 2000).

Another conserved regulatory pathway, X chromosome dosage compensation, also shows evidence of disruption in hybrids. The X has reduced transcription levels in lethal hybrids, and several components of the dosage compensation complex fail to localize to the X (Pal Bhadra *et al.* 2006; Chatterjee *et al.* 2007).

Genome-scale regulatory divergence: The bristle-loss phenotype identified one example of a hybrid defect that is likely to reflect a failure in gene expression in hybrids. Gene misexpression now appears to be common in hybrids. Of 19 *D. melanogaster lacZ* enhancer trap lines, 13 showed aberrant expression in *D. melanogaster/D. simulans* or *D. melanogaster/D. mauritiana* hybrids compared with their expression in *D. melanogaster* (Hammerle and Ferrus 2003). A large-scale analysis of >4000 transcripts also found many changes in expression level in F_1 hybrid females that were not simply additive between the *D. melanogaster* and *D. simulans* parents (Ranz *et al.* 2004).

What is the underlying cause of such misexpression, and what is it revealing about the divergence of gene regulation between species? Wittkopp *et al.* (2004) developed a clever method of distinguishing whether gene expression changes between *D. melanogaster* and *D. simulans* are caused by divergence in *cis*-regulatory sequences of individual genes *vs.* divergence in *trans*-acting regulators. They quantified gene expression levels in both species, as well as the species-specific expression of each allele in reciprocal F_1 hybrids. Substantial *trans*-acting effects were found, but more strikingly, almost all genes demonstrating divergence in expression between the species showed evidence of divergence in their *cis* regulatory sequences. A recent larger-scale study has corroborated these conclusions (Graze *et al.* 2009).

Behavioral differences: Studies of interspecific differences in behavior provide a powerful complement to mutagenesis screens for identifying the genetic basis of behavior. Interspecific behavioral differences are also an important contributor to reproductive isolation and speciation. Hybrids helped to identify one of the first genes involved in such a behavioral difference. *D. melanogaster* and *D. simulans* males differ substantially in the courtship songs that they generate by wing vibration. These differences include the length of the interval between pulses (the interpulse interval) and the fluctuations in the interpulse interval that change rhythmically over tens of seconds (the rhythm). By using attached X chromosomes or the *Lhr* mutation, Kyriacou and Hall (1986) generated F_1 hybrid males from *D. melanogaster* mothers that carried either X_{mel} or X_{sim}. Their study found that the species difference for rhythm maps to the X, while the interpulse interval difference is autosomal. Subsequent transformation experiments demonstrated that the difference in song rhythm between the species is controlled by divergence in a small region of the X-linked *period* gene (Wheeler *et al.* 1991).

D. melanogaster and *D. simulans* females differ in their cuticular hydrocarbons, and these differences contribute to mating isolation between the species (Coyne and Oyama 1995). To map the causal genes, Coyne (1996) used a method of creating partial hybrids. This method was originally developed by Grell (1976) for mapping an interspecific difference in an isozyme. Mapping is done using *D. melanogaster* stocks that contain a compound chromosome for one autosomal arm along with

two free copies of the other arm. The *D. simulans* stocks used carry a translocation of the Y to one autosome arm, along with a freely segregating copy of the other arm and a wild-type version of the same autosome. When, for example, *C(3L) D. melanogaster* females are mated with *T(Y;3) D. simulans* males, F_1 female hybrids that are homozygous for either $3L_{mel}$ or $3L_{sim}$ and heterozygous for the remainder of the genome can potentially be produced. In crosses with all four possible *D. melanogaster* compound stocks, not all possible hybrid genotypes survive (Grell 1976). Nevertheless, the interspecific difference in female hydrocarbons was shown to map largely to chromosome *3*, with a particularly large contribution from recessive genes on $3L_{sim}$ (Coyne 1996).

Closer in: *D. melanogaster* and *D. simulans* show complete reproductive isolation, and it is thus difficult to distinguish which among the many hybrid incompatibilities may have been most relevant during early stages of speciation. The divergence of *D. simulans* from its sibling species *D. sechellia* and *D. mauritiana* occurred more recently (Kliman *et al.* 2000). One consequence is that crosses between pairs of these species produce fertile F_1 female hybrids (Lachaise *et al.* 1986), allowing for regions to be introgressed between them. Their similarity to *D. melanogaster* facilitated the use of genetic (Coyne 1983) and molecular (Perez *et al.* 1993) markers so that backcross progeny can be easily tracked and introgression lengths determined. Two of the most important results from these studies showed that hybrid male sterility genes are much more common among these species than either hybrid female sterility or hybrid inviability genes and that more hybrid male sterility genes have accumulated on the X compared to the autosomes (Hollocher and Wu 1996; True *et al.* 1996; Tao *et al.* 2003; Masly and Presgraves 2007). Fine-scale mapping of one introgression led to the identification of *Odysseus*, the first known hybrid sterility gene (Ting *et al.* 2000).

And farther out: The archives of unpublished results undoubtedly contain records of failures of *D. melanogaster* to mate to species more distant than *D. simulans*. The goal of making such hybrids has obvious appeal for studying morphological and behavioral traits that have diverged among Drosophila species. Several researchers have attempted to circumvent mating isolation by introducing the pole cells (germline precursor cells) from foreign species into *D. melanogaster* to create chimeras with a *D. melanogaster* soma and a foreign species germline. Such chimeras can then be mated to *D. melanogaster* to create hybrids essentially equivalent to the product of a mating between foreign species females and *D. melanogaster* males. Attempts using species from two non-*melanogaster* subgroups of the *melanogaster* group produced hybrids, but they died as embryos or larvae (Lawrence *et al.* 1993). The species used were too distant from *D. melanogaster*, which inspired Sánchez and Santamaria (1997) to try again using more closely related species from within the *melanogaster* subgroup. Remarkably, viable hybrids were obtained using pole cells transplanted from either *D. yakuba* or *D. teissieri*, demonstrating that no dominant hybrid lethality genes have evolved between these species and *D. melanogaster*. Even more striking was that at least some of these hybrids were male, and the *D. melanogaster*/*D. yakuba* hybrid males were almost as viable as hybrid females. Thus X-linked recessive hybrid lethality genes such as *Hmr* have also not evolved between these species, at least not on the *D. yakuba* X.

Matute *et al.* (2009) have recently discovered that *D. melanogaster* females will mate to males of *D. santomea*, a sibling species of *D. yakuba*, and produce viable F_1 female hybrids but no males. This cross is essentially the reciprocal of the above hybridization using *D. melanogaster* chimeras and suggests that there are X-linked recessive gene(s) on the *D. melanogaster* X that cause hybrid lethality with *D. santomea*. An intriguing question is whether *Hmr* is one of these genes.

D. santomea lacks abdominal pigmentation, a phenotype unique among *melanogaster* subgroup species. Divergence of the pigmentation locus *tan* was shown to contribute to this phenotypic difference between *D. santomea* and *D. yakuba* (Jeong *et al.* 2008). Matute *et al.* (2009) have used *D. melanogaster*/*D. santomea* hybrids to argue against this claim for the role of *tan* in pigmentation divergence, but Rebeiz *et al.* (2009) have persuasively countered that using hybrids between more distant species can lead to mis-inference about evolutionary divergence that has occurred between more closely related hybrids. For example, the *D. melanogaster*/*D. santomea* hybrids may have aberrations in gene expression that interact in unpredictable ways to affect expression of *tan* and other pigmentation genes. If so, then the goal of using *D. melanogaster* hybrids with distant species to infer the genetic basis of interspecific differences may be prone to artifactual results.

The past 20 years, and the next 90: Since Provine's (1991) review of *D. melanogaster*/*D. simulans* hybrids almost 20 years ago, major progress has been achieved in identifying some of the genes that cause the hybrid incompatibility phenotypes discovered by Sturtevant (1920) and by Muller and Pontecorvo (1940). Phenotypic differences in behavioral and morphological traits have been discovered, and in some cases the causal genes have been mapped and identified using hybrids. The discovery of partial fertility rescue has perhaps not been the dramatic breakthrough that was hoped for, but several studies have achieved interspecific introgressions and used them to address important questions.

Undoubtedly the major advance over the past 20 years of research into these two species has been the publication of their genome sequences (Adams *et al.* 2000; Begun *et al.* 2007). Looking ahead, interspecific differences and hybrid incompatibilities between *D. melanogaster* and *D. simulans* can now be mapped much more

rapidly. These genome sequences will also accelerate studies to address whether phenotypic differences and incompatibilities are direct consequences of adaptive evolution.

Hybrids will also offer many opportunities to dissect the genetic causes of behavioral differences beyond what has been discovered for the *period* locus. *D. melanogaster* and *D. simulans* differ markedly in mating behavior (Manning 1959), and these species-specific differences are an under-studied contributor to reproductive isolation in Drosophila.

Large-scale genome evolution is another research area for which hybrids may be profitably utilized. *D. melanogaster* and *D. simulans* are significantly diverged in heterochromatic satellite DNAs and transposable elements (Lohe and Roberts 1988; Vieira and Biémont 2004). It remains unclear, however, how much of such divergence contributes to differences in fitness and phenotype. One hybrid incompatibility has already been shown to be a direct consequence of heterochromatin divergence (Ferree and Barbash 2009). Because heterochromatic sequences cannot be easily manipulated for genetic analysis within species, examination of chromatin states and nuclear structure in hybrids can provide insight into the functional consequences of large-scale differences in DNA content.

Hybrids fascinate the biologist because they are the product of two separately evolved genomes combined in a single individual. Hybrid plants are of tremendous importance to agriculture, and hybrid animals have long served humans. Sturtevant's main contribution (1920) was to recognize the power in applying Drosophila genetics to a hybrid system. The greatest testimony to the impact of Sturtevant's 1920 article is that one can assert with confidence that it will continue to motivate discoveries beyond the imagination of its author.

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