The Selfish *Segregation Distorter* Gene Complex of *Drosophila melanogaster*

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ABSTRACT *Segregation Distorter* (*SD*) is an autosomal meiotic drive gene complex found worldwide in natural populations of *Drosophila melanogaster*. During spermatogenesis, *SD* induces dysfunction of SD^+ spermatids so that SD/SD^+ males sire almost exclusively *SD*-bearing progeny rather than the expected 1:1 Mendelian ratio. *SD* is thus evolutionarily "selfish," enhancing its own transmission at the expense of its bearers. Here we review the molecular and evolutionary genetics of *SD*. Genetic analyses show that the *SD* is a multilocus gene complex involving two key loci—the driver, *Segregation distorter* (*Sd*), and the target of drive, *Responder* (*Rsp*)—and at least three upward modifiers of distortion. Molecular analyses show that *Sd* encodes a truncated duplication of the gene *RanGAP*, whereas *Rsp* is a large pericentromeric block of satellite DNA. The Sd–RanGAP protein is enzymatically wild type but mislocalized within cells and, for reasons that remain unclear, appears to disrupt the histone-to-protamine transition in drive-sensitive spermatids bearing many *Rsp* satellite repeats but not drive-insensitive spermatids bearing few or no *Rsp* satellite repeats. Evolutionary analyses show that the *Sd–RanGAP* duplication arose recently within the *D. melanogaster* lineage, exploiting the preexisting and considerably older *Rsp* satellite locus. Once established, the *SD* haplotype collected enhancers of distortion and suppressors of recombination. Further dissection of the molecular genetic and cellular basis of *SD*-mediated distortion seems likely to provide insights into several important areas currently understudied, including the genetic control of spermatogenesis, the maintenance and evolution of satellite DNAs, the possible roles of small interfering RNAs in the germline, and the molecular population genetics of the interaction of genetic linkage and natural selection.

> *Mendelian inheritance is a marvelous device for making evolution by natural selection an efficient process.... The Mendelian system works with maximum efficiency only if it is scrupulously fair to all genes. It is in constant danger, however, of being upset by genes that subvert the meiotic process to their own advantage*. James F. Crow (1979)

SEGREGATION Distorter (*SD*) is a selfish, coadapted gene complex on chromosome *2* (an autosome) found at low frequency in nearly all natural populations of the fruit fly, *Drosophila melanogaster*. In heterozygous males carrying *SD* and a typical wild-type second chromosome (SD/SD^+), most SD^+-bearing spermatid nuclei fail to complete the histone-to-protamine transition during spermiogenesis, so that primarily *SD*-bearing spermatids develop properly and go on to fertilize eggs. SD/SD^+ males thus sire almost exclusively *SD*-inheriting progeny. This distortion of classic Mendelian ratios has intrigued geneticists and evolutionary biologists for more than 50 years—and for good reason. As we describe below, *SD* is a newly evolved system that subverts one of the fundamental laws of inheritance by exploiting an ancient molecular pathway. In this review, we describe how *SD* was discovered and first characterized; the genetic components and features of the *SD* system; the molecular basis by which *SD* is thought to achieve its transmission advantage; theoretical and molecular population genetics analyses of the dynamics and evolutionary history of the *SD* system; and, finally, some of the still-unsolved questions concerning *SD*. The answers, we believe, have important implications for our understanding of spermiogenesis and the regulation of a ubiquitous, but still poorly understood, class of selfish sequences—genomic satellite DNAs.

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Six Strange Chromosomes

In 1956, a first-year graduate student in James F. Crow's laboratory, Yuichiro Hiraizumi, tackled a straightforward, if

laborious, population genetics problem of the day: in natural populations of *D. melanogaster*, "recessive" lethal mutations were found to segregate at lower frequencies than expected, raising the possibility of nonnegligible heterozygous fitness effects (Hiraizumi and Crow 1957, 1960; Hiraizumi *et al.* 1960). Hiraizumi set out to estimate those heterozygous effects for second chromosomes extracted from a natural population in Madison, Wisconsin. For one collection, this involved testcrossing 183 +/*cn bw* males to *cn bw* females (where + is a wild-derived chromosome, *cn bw* is a standard tester chromosome, and *cn* and *bw* are recessive eye color markers that together produce white eyes when homozygous). For these crosses, healthy wild-derived second chromosomes will produce half wild-type progeny (+/*cn bw*) and half white-eyed progeny (*cn bw*/*cn bw*). Sickly wild-derived second chromosomes might, however, produce a few less wild-type progeny than otherwise expected. In a footnote to the article, Hiraizumi and Crow (1960) made reference to an altogether unexpected class of second chromosomes:

> Among the chromosomes examined, six (five lethal and one control) behaved in a strange way, giving 0.80–0.95 wildtype flies. This turned out to depend on distorted segregation ratios in heterozygous males. . . .

These six strange chromosomes were the first *SD* chromosomes, the starting materials for analyses to be undertaken together by Hiraizumi and a new postdoc, Larry Sandler. Just before arriving in Crow's lab, Sandler had published a seminal paper with his Ph.D. advisor, Ed Novitski, "Meiotic drive as an evolutionary force" (Sandler and Novitski 1957). Biased transmission, they argued, may be a pervasive, underappreciated cause of allele frequency change in natural populations over evolutionary time scales. (Although Sandler originally defined meiotic drive in the strict sense to refer to biased transmission resulting from disturbance of meiosis *per se* (Sandler and Novitski 1957), he would later use meiotic drive in the broad sense to refer to biased transmission resulting from disturbances at any stage of gametogenesis (Zimmering *et al.* 1970).) With Hiraizumi, Sandler had a chance to tackle the basis of a naturally occurring meiotic drive system in a well-studied genetic model species. In the few years that followed, the two would publish eight articles on *SD* together (reviewed in Crow 1991; Ganetzky 1999).

Initial Characterization of *SD*

In the first article on *SD*, Sandler *et al.* (1959) got most essential facts about the system right. They showed that the original six *SD* chromosomes fell into two classes. The five "lethal" *SD-5*-type chromosomes were allelic for the same recessive lethal mutation and carried two nonoverlapping paracentric inversions on *2R*, one proximal and one distal; the one "control" *SD-72* chromosome was lethal free and had only the distal inversion in common with *SD-5* (Figure 1). All six *SD* chromosomes caused very strong distortion, with $k \geq 0.95$ (where k is the proportion of *SD*-bearing progeny sired by heterozygous SD/SD^{+} males), when *SD*-bearing males were heterozygous against *cn bw*, or heterozygous against other marked or wild-derived second chromosomes. Distortion was male specific, as transmission through heterozygous females was normal. As recombinant *SD* chromosomes lacking the inversions still caused distortion, *SD*, they concluded, must be a form of genic meiotic drive rather than chromosomal drive. Finally, preliminary mapping placed the *SD* locus in or near the centromeric heterochromatin of chromosome *2*.

Two findings from this early work led to reasonable but ultimately incorrect conclusions. First, Sandler *et al.* (1959) found that *SD* chromosomes failed to distort when heterozygous against two different pericentric inversion-bearing chromosomes, *In(2LR)Cy* and *In(2LR)Pm*^{2}. From this, they inferred that chromosomal synapsis—at the centromeric *SD* region in particular—was required for distortion. [It would later be shown, however, that the *In(2LR)Cy* and *In(2LR) Pm*^{2} chromosomes are simply insensitive to distortion (Hartl 1975a).] Second, *SD* is not a single locus. While Sandler and Hiraizumi (1960) and Hiraizumi and Nakazima (1967) would later infer that the *SD* locus involved two separable loci, their results were complex. Part of the problem was that Sandler and Hiraizumi (1960) had been unlucky in choosing *SD-72* (rather than *SD-5*) for genetic mapping, unaware that it carried a pericentric inversion that prevented recombination in the critical region (Lewis 1962).

Together, three articles ushered in the modern understanding of the genetics of the *SD* complex. First, Sandler and Carpenter (1972) settled a key question concerning the action of *SD*: Was the presence of *SD* essential for spermatid development in *SD* males? Or did *SD* somehow alter SD^{+} homologs in a way that caused SD^{+} spermatid lethality? Using clever genetic trickery, they showed that spermatids bearing SD^{+} chromosomes are gamete lethal. This finding led them to infer that *SD* is a *trans*-acting factor that acts at a *cis*-acting "*sensitive-receptor*" site on the SD^{+} chromosomes. *SD* chromosomes must therefore possess an *insensitive-receptor.* Taking the pericentric inversion of *SD-72* into account, the old mapping data of Sandler and Hiraizumi (1960) now made sense. The *SD* system can indeed be decomposed into two loci: *SD*, the distorter, resides on chromosome arm *2L* between 37B2 and 39–40 and the *receptor*, the target of distortion, resides between 39–40 and 42A but is not the centromere itself.

Second, Dan Hartl (1974), who began work on *SD* as a student in Crow's lab, used recombination to cleanly partition the *SD* region of a *SD-5*-type chromosome, which lacks the pericentric inversion, into two major loci—a leftmost distorter locus, which he termed *Segregation distorter* (*Sd*), and a rightmost sensitivity locus, which he named *Responder* (*Rsp*). Thus *SD* refers to chromosomes that have the genotype, *Sd* Rsp^{i}, whereas SD^{+} chromosomes have the genotype Sd^{+} Rsp^{s}, where Rsp^{i} and Rsp^{s} are insensitive and sensitive to

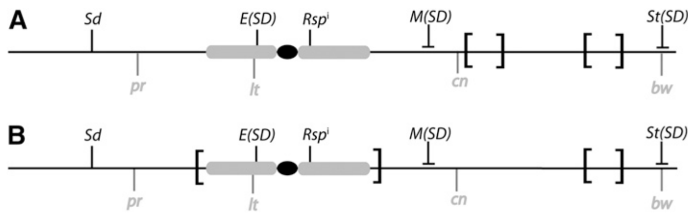

Figure 1 The *SD* complex. (A) Schematic of the *SD-5* chromosome showing the location of the distorting gene, *Sd*, the major enhancer of distortion, *E(SD)* on *2L* (the black dot is the centromere), the target locus, *Rsp*^{i}, and the strong upward modifiers *M(SD)* and *St(SD)*, on *2R* above the chromosome. Markers commonly used to dissect the genetics of *SD* are diagramed below the chromosome: *pr* (2-54.5), *lt* (2-55), *cn* (2-57.5) and *bw* (2-104.5). *SD-5* has two paracentric inversions on *2R* (brackets). (B) Same as (A) except for the *SD-72* chromosome. *SD-72* has a pericentric inversion (brackets) and a paracentric inversion on *2R*.

segregation distortion, respectively. Heterozygous *SD/SD*$^{+}$ males thus transmit *Sd Rsp*^{i}-bearing sperm to >95% of progeny as the action of *Sd* disrupts development of *Sd*$^{+}$ *Rsp*^{s}-bearing sperm. As a final proof of principle, Hartl (1974) constructed suicidal *Sd Rsp*^{s} recombinant chromosomes: in heterozygous *Sd Rsp*^{s}*/Sd*$^{+}$ *Rsp*^{i} males, *Sd Rsp*^{s} are unable to distort against their insensitive homologs and instead distort against themselves.

Third, Barry Ganetzky (1977), who began work on *SD* as a student in Sandler's lab, used X rays to mutagenize *SD-5* chromosomes. For the first time, the chromosomal locations of the *SD* loci would begin to be known with precision. The X-ray-induced lesions would also reveal important properties of the *SD* loci (discussed below). Ganetzky's experiments showed that *Sd* falls between cytological regions 37D2–7 and 38A6–B2 on chromosome arm *2L*, and *Rsp* resides in the centric heterochromatin of chromosome arm *2R*. These findings are consistent with those of Sandler and Carpenter (1972) but much refined. But then came a surprise. Ganetzky (1977) discovered a third major locus on *SD* chromosomes, one only hinted at in previous analyses (Hartl 1975a): a strong upward modifier of distortion, which Ganetzky named *Enhancer of Segregation Distorter*, *E(SD)*, exists in the centric heterochromatin of chromosome arm *2L* of *SD* chromosomes. After more than 15 years, the genetic structure of the *SD* complex finally emerged (Figure 1).

Segregation Distorter

Ganetzky's (1977) experiments not only mapped *Sd* but provided information on its properties. In particular, he distinguished two possibilities: if *Sd* represents a loss of wild-type *Sd*$^{+}$ function, then mutagenesis ought to be able to induce wild-type chromosomes to become distorters; conversely, if *Sd* is a gain-of-function mutation, then mutagenesis ought to be able to revert *SD* chromosomes to nondistorters. While Ganetzky was unable to induce distorter mutations on wild-type chromosomes, he recovered four *SD*-5 chromosome revertants—*SD* chromosomes that lost the ability to distort due to deletion or inactivaton of the *Sd* locus. *Sd* behaves as a neomorph.

The discovery that *Sd* is neomorphic, and hence dispensable for wild-type function, likely helped perpetuate the sense that there was something foreign about it. Historical context was also a factor. *SD* was discovered at a time when transposable elements were just coming into focus, in particular with McClintock's maize work, which had a profound and lasting effect on Sandler's (and others') thinking about *SD*:

> The phenomenon of segregation–distortion is, directly, a case in which a genetic entity located on some chromosome influences the behavior of an element or elements located on a different chromosome. The system is thus analogous to "*Ac-Ds*-like" systems in maize (McClintock 1951, 1956).... The obvious analogy is that *SD* is an *Ac*-like element and the point or points on the *SD*$^{+}$ chromosome which are subject to (the action of *SD*) are *DS*-like elements (Sandler *et al.* 1959).

> While this complexity may simply mean that segregation–distortion is polygenic, it may also very well be that the elements involved are not conventional, and therefore, that standard genetic interpretations, such as those we have employed here may be misleading (Sandler and Carpenter 1972).

> To call these "mutations" is the conservative assumption: there is no specific evidence against the possibility that they are minute chromosomal rearrangements or deletions or insertions of a virus-like or foreign element.... (Hartl 1974).

> The answer may lie in the hints that the elements of distortion are extrinsic and perhaps transposable (Sandler and Golic 1985).

In 1983, Brittnacher and Ganetzky would do another X-ray mutagenesis screen on *SD* chromosomes other than *SD-5*, including an *SD* chromosome collected in Italy (Brittnacher and Ganetzky 1983). Two advances would come from this work. First, the experiments showed that other *SD* chromosomes, even ones from geographically disparate populations, had the same *Sd* locus with the same properties in the same chromosomal position. Second, the experiments precisely narrowed the location of the *Sd* locus to a small interval 37D2–6 (Figure 2A; Brittnacher and Ganetzky 1983), which made feasible the molecular analyses that would finally dispel any notion that *Sd* is a foreign element.

The precise localization of *Sd* to such a small chromosomal interval allowed for the molecular dissection of the region and the discovery that *SD* flies from around the world all share a ~5-kb tandem duplication in the *Sd* region (Powers and Ganetzky 1991). In transformation experiments, a ~12-kb genomic DNA fragment that includes the duplication can cause full-strength segregation distortion in

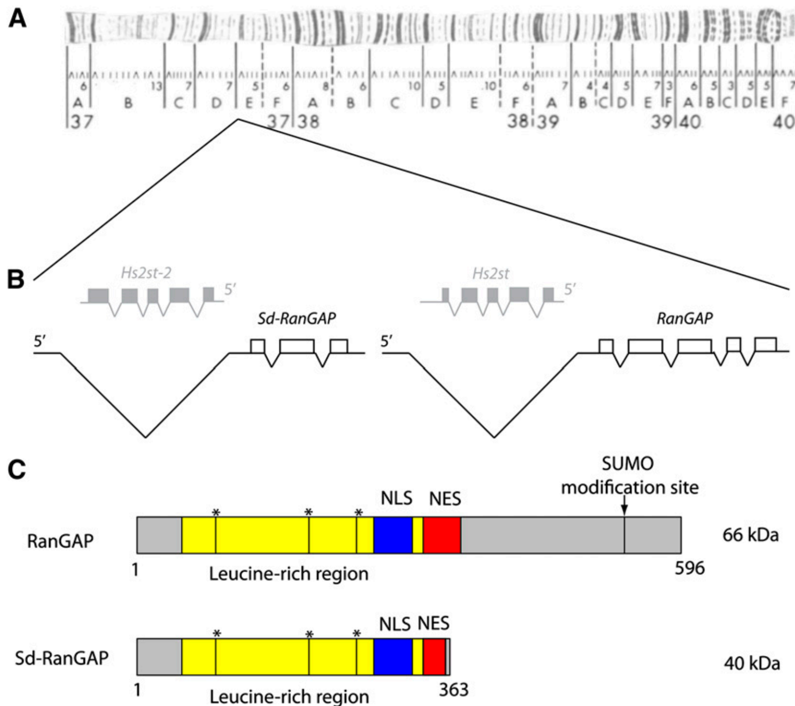

Figure 2 Structure of the *SD* region. (A) A polytene map showing the location of the *Sd* locus at band 37D2-6. (B) The structure of the *Sd* locus showing that *Sd–RanGAP* is a partial duplication of the *RanGAP* locus. *Hs2st* (shaded in gray) is a gene occurring in the intron of *RanGAP* that is also duplicated in *Sd–RanGAP* (*Hs2st-2*). (C) A schematic of RanGAP (66 kD) and Sd–RanGAP (40 kD) proteins. RanGAP contains a large leucine-rich domain with a nuclear localization signal (NLS, blue) and two adjacent nuclear export signals (NES, in red). The * denotes some of the sites required for RanGAP activity. RanGAP contains a SUMO modification site at its C terminus that is required for tethering RanGAP to the cytoplasmic side of the nuclear pore. Sd–RanGAP is missing 234 amino acids at its C terminus including a NES and the SUMO modification site. Sd–RanGAP retains the NLS and sites required for activity.

the appropriate genetic background (McLean *et al.* 1994). *Sd* thus involves extra *endogenous* DNA.

Two nested genes are duplicated within the 12-kb fragment: one encodes *heparan sulfate 2-O-sulfotransferase* (*Hs2st*) and the other *Ran GTPase activating protein* (*RanGAP*; Figure 2B). *Sd* and Sd^+ are structurally equivalent in the proximal half of the duplication, but the duplication junction cleaves the 3′-end of the distal copy of *RanGAP*, introducing a nonsense mutation that results in a truncated RanGAP protein that lacks at most 234 amino acids from its COOH-terminus (Figure 2C; Merrill *et al.* 1999). Expression of this truncated RanGAP alone is sufficient to reproduce segregation distortion in the appropriate genetic backgrounds (Merrill *et al.* 1999). The duplicate gene encoding the truncated distorting protein is hereafter called *Sd–RanGAP*.

The hope of course is that the discovery of *Sd–RanGAP* might, after 40 years, finally reveal the cellular basis of segregation distortion. Wild-type RanGAP functions in many cellular venues but is known best for its role in nuclear transport. Below we consider how production of a truncated Sd-RanGAP protein might affect nuclear transport and other Ran-mediated systems, specifically during spermatogenesis, to induce dysfunction of sensitive *Rsp*-bearing spermatids. But first, we turn to the question of the genetic and molecular nature of the *Rsp* locus itself.

Responder

Being buried in the heterochromatin of *2R*, *Rsp* has proved to be a challenge to study. *Rsp* changed names several times during its first 14 years. Sandler and Hiraizumi (1960) first designated *Rsp* as *Activator of SD* [*Ac(SD)*] and hypothesized that *Ac(SD)* was required in *cis* to "activate" *SD* function to cause distortion (here *Ac(SD)* is the genotypic equivalent of Rsp^i). They proposed that *SD* induced breakage on its homolog during synapsis and speculated that *Ac(SD)* and *SD* resided within a chromosome aberration, an insertion or duplication, that was later shown to be a pericentric inversion (Lewis 1962). Hiraizumi and Nakazima (1967) later posited that *Ac(SD)* was the locus conferring insensitivity to the *SD* chromosome. The experiments of Sandler and Carpenter (1972) revealed *Ac(SD)* as the direct target of distortion, which they renamed *receptor: SD* chromosomes have an *insensitive-receptor* allele and SD^+ have a *sensitive-receptor* allele. Finally, Hartl (1973) renamed the locus to *Responder* to fit his hypothesis that *Sd* encodes a regulator protein that binds *Rsp*.

Rsp is located in the proximal *2R* heterochromatin (Brittnacher and Ganetzky 1989) but is not the centromere (Lyttle 1989). Sensitive *Rsp* alleles, like *Sd*, behave as a neomorphs: deletions of *2R* heterochromatin containing *Rsp* convert sensitive chromosomes into insensitive chromosomes (Ganetzky 1977). Sensitive *Rsp* alleles act in *cis* to cause spermatid dysfunction: moving *Rsp* to a different chromosome makes that chromosome sensitive to *SD* (Brittnacher and Ganetzky 1989; Lyttle 1989). To explain its frequency in populations, it is usually assumed that sensitive *Rsp* alleles have some function in spermatogenesis, although that function must be nonessential as homozygous deletions of *Rsp* are viable and fertile (Ganetzky 1977). As we discuss below, the *Rsp* locus does not correspond to a conventional protein-coding gene but instead corresponds

to a class of DNA sequences whose function we know little about: satellite DNA.

Responder corresponds to a block of satellite DNA repeats

The first clue that *Rsp* might have atypical genetic properties came from the discovery that most natural populations harbor a continuous range of sensitivities to segregation distortion (Sandler and Carpenter 1972; Martin and Hiraizumi 1979; Hiraizumi *et al.* 1980; Hiraizumi and Thomas 1984; Temin and Marthas 1984; Lyttle 1986). Three other kinds of data, appearing nearly simultaneously, then converged on the finding that *Rsp* comprises a repetitive satellite DNA locus in the proximal heterochromatin of *2R*.

Cytological evidence: In cytological preparations of mitotic chromosomes from Rsp^{s} and Rsp^{i} strains, Pimpinelli and Dimitri (1989) discovered a large size difference in the heterochromatic band *h39* on chromosome *2* (Figure 3A). The size of the *h39* band correlated with sensitivity to *SD*: the *h39* band is largest on super-sensitive Rsp^{ss} chromosomes, intermediate sized on sensitive Rsp^{s} chromosomes, and small or absent on Rsp^{i} chromosomes (Pimpinelli and Dimitri 1989; Figure 3B).

Molecular evidence: Guided by the assumption that *Rsp* was repetitive (and by Pimpinelli and Dimitri's cytological evidence; see above), Wu and colleagues screened a λ-phage library for DNA fragments that hybridized to Rsp^{s} flies better than Rsp^{i} flies (Wu *et al.* 1988). One of the clones, called H_0, contained a characteristic *Xba*I restriction site. Digestion with *Xba*I revealed a 240-bp repeat. Sequencing this clone indicated that the *Xba*I repeat was a dimer of two related 120-bp A+T-rich repeats, corresponding to a satellite repeat (Figure 3C). The sensitivity to segregation distortion correlates with the number of *Rsp* repeats: Rsp^{ss} alleles have ~2500 copies, Rsp^{s} alleles have ~700 copies, Rsp^{i} alleles associated with SD^{+} chromosomes have 100–200 copies, and Rsp^{i} alleles associated with *SD* chromosomes have $<$20 copies of the dimeric repeat (Wu *et al.* 1988; Lyttle 1991).

Genetic evidence: By moving blocks of *2R* heterochromatin to the Y chromosome, Lyttle was able to shift the sensitivity to *SD* to the Y chromosome (Lyttle 1989). Importantly, however, in one translocation, both chromosome *2 and* the Y chromosome had some degree of sensitivity indicating that the breakpoint occurred within the *Rsp* locus and, therefore, that the sensitivity of *Rsp* was itself physically divisible (Lyttle 1989), consistent with *Rsp* being identified as a large block of satellite DNA at heterochromatin band *h39* (Wu *et al.* 1988; Pimpinelli and Dimitri 1989).

Lyttle's translocations were particularly informative as he was able to construct males that produced sperm with zero, one, or two copies of a Rsp^{s} allele by using males carrying two different Rsp^{s} alleles: one on the Y chromosome (the $Dp(2;Y)Rsp^{s}$ allele) and one on chromosome *2* (*cn bw*), in an SD/SD^{+} background (Lyttle 1989). Rather than measuring the frequency of *SD* sperm as *k*, Lyttle measured the proportion of Rsp^{s}-bearing spermatids escaping the action of *SD* as a measure of Rsp^{s} survival probability. While the survival probability correlated with the number of Rsp^{s} alleles carried by a spermatid, it appeared that the survival of a sperm with a single Rsp^{s} allele is increased if another Rsp^{s} allele is present in the genome. These findings imply that Sd corresponds to some limiting gene product that gets diluted in the presence of multiple sensitive targets (but see Hiraizumi 1990). However, sperm bearing two copies of Rsp^{s} made up a smaller fraction of sperm than expected if each Rsp^{s} affects spermatid dysfunction independently, suggesting an epistatic effect of multiple Rsp^{s} copies in the same spermatid (Lyttle 1989).

The molecular structure of the Rsp locus

Rsp repeats are organized as dimers with "left" and "right" 120-bp A+T-rich repeats that have a *Xba*I restriction site (TCTAGA) between each dimer (Figure 3C; Wu *et al.* 1988). Canonical *Rsp* repeats (*i.e.*, those identified by the H_0 clone of Wu *et al.* 1988) occur as head-to-tail tandem repeats dispersed throughout region *h39*; but these comprise only ~15% of the total size of the Rsp^{s} locus (Cabot *et al.* 1993; Houtchens and Lyttle 2003). Rather than being interspersed with transposable elements and other kinds of satellite sequences, the canonical *Rsp* repeats are interspersed with other, more divergent variants of *Rsp* repeats (Houtchens and Lyttle 2003; Figure 3C). The left and right *Rsp* repeats each form monophyletic clades with ~4% divergence within each repeat type and ~16% between the two repeat types (Cabot *et al.* 1993; Houtchens and Lyttle 2003). The noncanonical repeat sequences are considerably more variable than the canonical ones, with sequence divergence among noncanonical variants ranging from 8 to 32% (Houtchens and Lyttle 2003).

Recombination at Rsp and variability in sensitivity to distortion

The large variability in *Rsp* repeat number, and hence sensitivity, between individuals could be generated by unequal exchange events (Wu and Hammer 1991). Cabot *et al.* (1993) found evidence for recombination events between left and right copies of *Rsp* and all of the exchanges were restricted to a 29-bp region, where left and right copies are the most similar. Cabot *et al.* (1993) compared *Rsp* repeats cloned and sequenced from three chromosomes: *lt pk cn bw* (Rsp^{ss}, ~2500 copies), *cn bw* (Rsp^{s}, ~700 copies), and Canton-S ($Rsp^{semi\text{-}sensitive}$, ~300 copies). To their surprise, *Rsp* repeats within a chromosome differed more than *Rsp* repeats between chromosomes (Cabot *et al.* 1993). There are two possible explanations for this result: recombination between homologs at the *Rsp* locus may have homogenized *Rsp* repeats between chromosomes; alternatively, recent selection near the centromere may have dragged a *Rsp* allele to high frequency. The latter explanation would lead to the

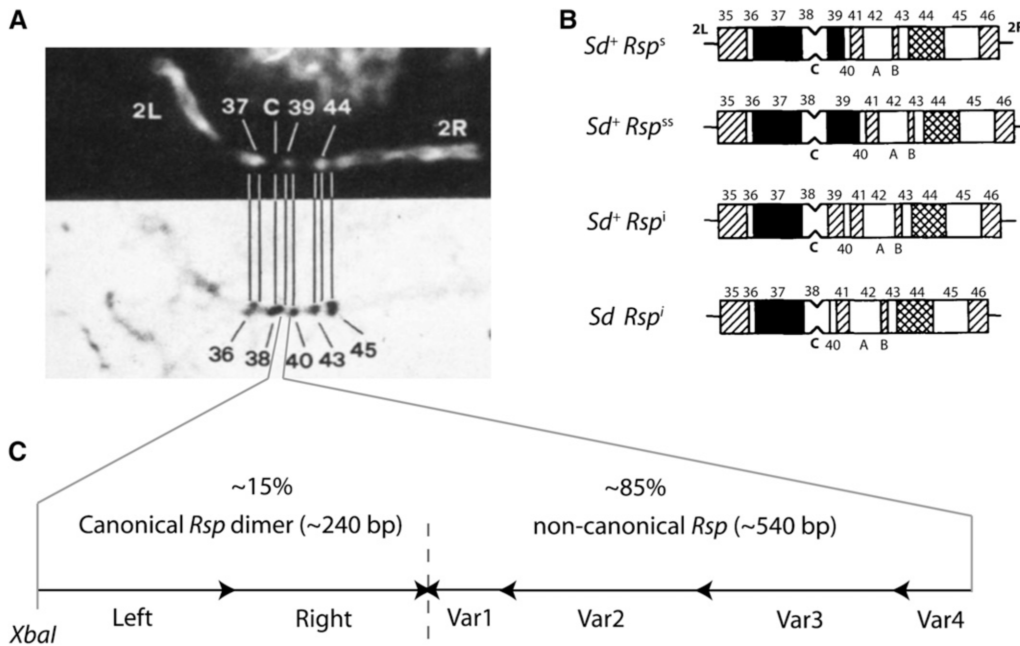

Figure 3 Structural organization of the *Rsp* locus. (A) Hoeschst fluoresence (above) and N-banding (below) of mitotic chromosome *2* from larval neuroblasts (modified from Figure 1B of Pimpinelli and Dimitri 1989). (B) Thick bars represent chromosome *2* heterochromatin and thin lines represent euchromatin. The shading represents the intensity of Hoeschst fluorescence in mitotic chromosomes of larval neuroblasts (modified from Figure 5, A, C–E of Pimpinelli and Dimitri 1989, with permission from the Genetics Society of America). (C) Schematic of a canonical *Rsp* dimer and non-canonical *Rsp* repeats (Houtchens and Lyttle 2003) comprising ~15% and ~85% of the ~600-kb *cn bw Rsp* locus, respectively. Canonical *Rsp* dimers consist of related left and right repeats and a characteristic *XbaI* restriction site.

homogenization of *Rsp* repeats between chromosomes because different *Rsp* alleles would share a recent common ancestor.

Enhancer of Segregation Distorter

Genetic deletion of *Sd* typically eliminates distortion altogether, whereas deletion of the enhancer, *E(SD)*, reduces the strength of distortion [from $k = 0.99$ to ~ 0.70; (Ganetzky 1977; Brittnacher and Ganetzky 1984; Sharp *et al.* 1985)]. These observations imply that *Sd* is the major distorting locus, while *E(SD)* functions as a secondary modifier. But as it turns out, *E(SD)* has its own capacity for distortion, even in the absence of *Sd*. Sharp *et al.* (1985) discovered that recombinant *SD-5* chromosomes lacking *Sd*—*i.e.*, Sd^{+} *E(SD)* Rsp^{i} —cause modest distortion against sensitive Rsp^{s} alleles ($k = 0.6$) and stronger distortion against supersensitive Rsp^{ss} alleles ($k = 0.85$). Temin (1991) further showed that *E(SD)* distorts against sensitive *Rsp* chromosomes in a dose-dependent manner. Furthermore, *Sd* and *E(SD)* are both suppressible by the same genetic modifiers (see below), suggesting that they distort via the same mechanism (Temin 1991).

The independent distorting ability of *E(SD)* raises an intriguing possibility: Was *E(SD)* the first distorter in the system to evolve on a Rsp^{i} chromosome, followed by the later acquisition of *Sd* (Lyttle 1993)? To address this question directly, the molecular identity of *E(SD)* must be determined. We can, however, at least exclude the possibility that *E(SD)* and *Sd* are both recent duplicates of the *RanGAP* gene. For one, their genetic properties differ: *E(SD)* has a dose-dependent effect on the strength of distortion; *Sd* does not. More directly, Southern blot analysis reveals no evidence for sequence homology between *Sd–RanGAP* and *E(SD)* (Powers and Ganetzky 1991).

Other Modifiers of the *SD* System

For a segregation distorter to invade a population it must be genetically linked to its target, with insensitive alleles occurring in *cis* and sensitive target alleles in *trans* (Prout *et al.* 1973; Charlesworth and Hartl 1978). Free recombination between distorter and target would regularly generate suicide combinations. Nonrecombining sex chromosomes and the low-recombination centromeric regions are thus particularly susceptible to the invasion of multilocus drive systems. Once a drive system is established, however, it may recruit further modifiers of recombination (see below).

Genetic enhancers and suppressors of segregation distortion have different linkage requirements: enhancers will evolve in *cis* with the distorter, whereas suppressors can evolve anywhere in the genome in *trans* with the distorter (Thomson and Feldman 1974; Hartl 1975b; Crow 1991). The regions of the genome under strongest pressure to evolve suppressors are those directly targeted by the distorter (*e.g.*, the target locus should evolve from a sensitive to an insensitive state). But when a distorter like *SD* is at equilibrium frequency, even unlinked suppressors are expected to increase in frequency (Hartl 1975b; Crow 1991). To explain why, Crow (1991) offered the "grandchild" argument. Imagine two scenarios, one in which *SD* is challenged by a suppressor, reducing k, and one in which *SD* is not challenged by a suppressor, leaving k close to 1. The number of progeny sired by SD/SD^{+} males is the same with or without the suppressor—only the ratio of *SD* to SD^{+}

alleles changes. As *SD* chromosomes (being usually homozygous sterile or lethal) come with a fitness cost, any modifier that reduces *k* will increase the fraction of fitter SD^+ alleles in the population and thus leave more grandchildren (Crow 1991). Unlinked modifiers that suppress distortion will therefore increase in frequency in the population because they contribute more progeny to future generations.

As we discuss next, the *SD* complex and its system of recombination modifiers, enhancers, and suppressors provide evidence consistent with the rich population genetic theory of drive systems. We argue later that the molecular identities and functions of these enhancers and suppressors may prove essential to solving the problem of the mechanistic and molecular basis of *SD*.

Chromosomal inversions on SD

The three major *SD* loci are clustered about the centromeric region of chromosome *2*, where the rate of crossing over per physical unit is strongly reduced (Figure 1). In addition to exploiting this centromeric effect on crossing over, most *SD* chromosomes have also recruited genetic modifiers of recombination—chromosomal inversions that further tighten the linkage among its interactors (Sandler and Hiraizumi 1960; Hartl and Hiraizumi 1976; Crow 1988; Lyttle 1991; Lyttle 1993). *SD-72* chromosomes (and its derivatives), for example, carry a pericentric inversion that captures *E(SD)* and Rsp^i and facilitates their linkage disequilibrium with *Sd*, thereby preventing wasteful production of nondriving or worse, suicidal, recombinants.

Most *SD* chromosomes also carry one or more inversions on chromosome arm *2R* (Figure 1 and Table 1). *SD-72* and *SD-5* carry *In(2R)NS* (52A;56F)—a common cosmopolitan inversion—and *SD-5* carries an additional inversion, *In(2R) 45C-F;49A* (Lewis 1962). In Africa, the locally common *SD* chromosome, *SD-Mal*, carries the two overlapping inversions, *In(2R)44F3-12;54E3-10* and *In(2R)51B6-11;55E3-12* (Presgraves *et al.* 2009). On *SD-5* and *SD-Mal* chromosomes, paracentric inversions prevent crossing over along ~40% and ~50% of the length of arm *2R*, respectively. None of these paracentric inversions overlap the three major *SD* loci, and none are required for distortion. Indeed, recombinant *SD-5* like chromosomes fail to provide any evidence that its two paracentric inversions (or the alleles therein) contribute to distortion intensity (Sandler *et al.* 1959; Hiraizumi and Nakazima 1967).

What, then, is their function? In addition to the three major loci, *SD* chromosomes have collected numerous, mostly uncharacterized enhancers along *2R* (discussed below). The commonly accepted hypothesis is that the *2R* inversions help to hold *SD* and its multiple enhancers together as a single distorting haplotype. There is, however, considerable physical distance between the major *SD* loci clustered about the centromere and the paracentric inversions on *2R* (Figure 1): the inversions can be readily separated from the three major *SD* loci. Their strong association with *SD* therefore suggests that strong epistatic selection—*i.e.*, selection favoring a particular multilocus genotype and disfavoring recombinants—also reinforces the long-distance linkage disequilibrium that spans from *Sd* on *2L*, across the centromeric heterochromatin, and (in many cases) across most of *2R*.

Stabilizer of Segregation Distorter

Ordinarily, there is little variation in segregation distortion strength from male to male among SD/SD^+ heterozygotes. In analyses of *SD-5* and *SD-72* recombinants, Sandler and Hiraizumi (1960) discovered a major modifier of *SD*, the loss of which reduces the mean strength of distortion, from $k = 1.0$ to 0.71 and, interestingly, increases the variance in *k* among males of the same genotype. As the loss of this modifier makes segregation distortion ratios "unstable," it was dubbed, *Stabilizer of Segregation Distorter* [*St(SD)*]. Sandler and Hiraizumi (1960) showed that *St(SD)* must reside beyond the distal-most breakpoint of the inversion (56F), at the tip of *2R*. Without the inversions on *2R*, *St(SD)* would be effectively unlinked from the major *SD* loci near the centromere.

Modifier of Segregation Distorter

Hiraizumi *et al.* (1980) revealed evidence of an additional modifier of segregation distortion in a genetic mapping analysis of a recombinant *SD-36* chromosome (an *SD-5*-like chromosome; Figure 1) for which the two paracentric inversions on *2R* and *St(SD)* were removed (Hiraizumi and Nakazima 1967). A locus outside of the *SD* complex, somewhere in the euchromatic region proximal to 43E on arm *2R*, has a major effect on the strength of segregation distortion, boosting it from $k = 0.66$ to 0.92 (Hiraizumi *et al.* 1980). They named the locus *Modifier of Segregation Distortion* [*M(SD)*].

Unlinked modifiers: Suppressors of Segregation Distorter

Unlinked suppressors of *SD* [*Su(SD)*] can segregate at high frequencies in natural populations on both the X (Kataoka 1967; Hiraizumi and Thomas 1984) and third chromosomes (Trippa and Loverre 1975). *Su(SD)* loci also occur in laboratory strains such as the X chromosome balancers, *Muller-5* and *FM6* (Sandler and Rosenfeld 1962; Nicoletti and Trippa 1967) and the third chromosome balancer, *TM6* (Lyttle 1986). Curiously, the action of the *Su(SD)3* (on chromosome *3*) appears specific to particular *SD* chromosomes: it enhances the distortion of some *SD* chromosomes but suppresses distortion of others (Trippa and Loverre 1975). It is unclear, if these suppressors represent adaptations to the presence of *SD* in natural populations or incidental genetic variation that happens to affect the distortion phenotype. The frequency of *Su(SD)X*, for instance, does not correlate with *SD* frequency (*e.g.*, no *SD* chromosomes were identified in a Texas population, but *Su(SD)X* was found at a frequency of ~75%; Hiraizumi and Thomas 1984). Once the *Su(SD)* loci are identified, molecular screens will allow more

Table 1 Representative *SD* chromosomes from natural populations of *D. melanogaster* sampled worldwide

SD chromosome	Origin	Chromosome 2 inversions	References
Mediterranean			
SD–Roma, *SD–VO17*	Italy	None	1,2,3
SD–Las Arenas	Spain	None	2
North America			
SD–5, *SD–36*, *SD–79*	Wisconsin	*In(2R)45C–F;49A* + *In(2R)NS* [52A2–52B1;56F9–56F13]	4
SD–72, *SD–Mad*	Wisconsin	*In(2LR)39D;42A* + *In(2R)NS* [52A2–52B1;56F9–56F13]	4,5,6
SD–200, *SD–201*, *SD–202*, *SD–203*, *SD–204*, *SD–205*, *SD–206*	Baja, Mexico	[Presence of inversion(s) inferred from suppressed crossing over on 2R]	7
South America			
SD–EC49	Ecuador	*In(2R)NS* [52A2–52B1;56F9–56F13]	8
Pacific–Australia–Japan			
SD–NH2	Odate, Japan	*In(2LR)39D;42A* + *In(2R)NS* [52A2–52B1;56F9–56F13] + *In(2R)55E;60E*	9
SD–Kona871	Hawaii	*In(2LR)39D;42A* + *In(2R)NS* [52A2–52B1;56F9–56F13] + *In(2R)50CD;59D*	10
SD–Kona873	Hawaii	*In(2L)25EF;29EF* + *In(2LR)39D;42A* + *In(2R)NS* [52A2–52B1;56F9–56F13]	10
SD–Kona877	Hawaii	*In(2LR)39D;42A* + *In(2R)47EF;55C* + *In(2R)NS* [52A2–52B1;56F9–56F13]	10
Africa			
SD–BN19[a]	Benin	None	11
SD–MD31[a]	Cameroon	None	11
SD–MD21, *SD–NK04*	Cameroon	*In(2R)Ken* [44F3–12;54E3–10 + 51B6–11;55E3–12]	11
SD–GN09	Gabon	*In(2R)Ken* [44F3–12;54E3–10 + 51B6–11;55E3–12]	11
SD–KN20, *SD–KN91*, *SD–MK92*	Kenya	*In(2R)Ken* [44F3–12;54E3–10 + 51B6–11;55E3–12]	11
SD–KM87, *SD–KY38*	Kenya	*In(2L)t* [22D3–22E1;34A8–34A9] + *In(2R)Ken* [44F3–12;54E3–10 + 51B6–11;55E3–12]	11
SD–ZK178, *SD–ZK216*	Zimbabwe	*In(2L)t* [22D3–22E1;34A8–34A9] + *In(2R)Ken* [44F3–12;54E3–10 + 51B6–11;55E3–12]	11

This list is not comprehensive. The representative *SD* chromosomes are grouped by geographic locality and inversion status. References: 1, Nicoletti and Trippa (1967); 2, Wu *et al*. (1988); 3, Powers and Ganetzky (1991); 4, Sandler *et al*. (1959); 5, Lewis (1962); 6, Temin *et al*. (1990); 7, Mange (1961); 8, T. Lyttle and D. Presgraves, unpublished results; 9, Hiraizumi and Nakazima (1965); 10, Lyttle and Haymer (1992); 11, Presgraves *et al*. (2009).
[a] These chromosomes, while genotypically *SD*, do not cause segregation distortion (Presgraves *et al.* 2009)

comprehensive (and less labor intensive) surveys of suppressor allele frequencies.

Mechanism of *Segregation Distortion*

While the identities of the distorter, *Sd*, and the target, *Rsp*, have been established for the *SD* system, the mechanism of distortion has remained elusive. In the following sections, we first review the pertinent aspects of normal spermatogenesis, discuss the segregation distortion phenotype, and then present current models of *SD* function.

Normally during *Drosophila* spermatogenesis, each diploid spermatogonial cell undergoes four mitotic divisions and then grows substantially before entering meiosis as a primary spermatocyte (Figure 4A; reviewed in Fuller 1993). Each of the 16 primary spermatocytes undergoes two meiotic divisions to produce 64 round spermatids that then differentiate into mature sperm. Because each cell division has incomplete cytokinesis, cytoplasmic bridges connect the developing germ cells. During spermiogenesis, the nucleus must undergo a radical remodeling as it shrinks ~200-fold from round spermatid to the needle-shape of mature sperm (reviewed in Fuller 1993). To accomplish this hypercompaction of chromatin, preelongation spermatids replace canonical histones with sperm-specific protamines, a process known as the histone-to-protamine transition (Rathke *et al.* 2007; Miller *et al.* 2010). The spermatids then elongate and individualize and move to the seminal vesicle as mature sperm (see Fuller 1993).

Sandler and colleagues originally proposed that *SD* causes distortion by rendering SD^+-bearing spermatids dysfunctional or, in their specific hypothesis, dead (Sandler *et al.* 1959). While effectively correct, the particulars of the model were later refuted, as early cytological studies would reveal that meiosis in SD/SD^+ testes was normal, at least at the resolution of light microscopy (Peacock and Erickson 1965; Hartl *et al.* 1967; Nicoletti *et al.* 1967), ruling out hypotheses of *SD*-induced chromosome breakage during synapsis (Sandler *et al.* 1959; Crow *et al.* 1962). Peacock and Erickson (1965) next proposed the "functional pole" hypothesis, building on Novitski and Sandler's suggestion that only half of male meiotic products become functional (analogous to the polar bodies of female meiosis; Novitski and Sandler 1957). Finding no gross abnormalities in SD/SD^+ testes, Peacock and Erickson reasoned that *SD* must affect the orientation of chromosomes at metaphase I so that *SD* chromosomes orient to the functional pole to the exclusion of SD^+ chromosomes (Peacock and Erickson 1965). This model predicts that *SD* males suffer no sperm dysfunction and hence no reduced fertility. But two subsequent studies would show unequivocally that *SD* males sire fewer progeny than wild-type males, refuting

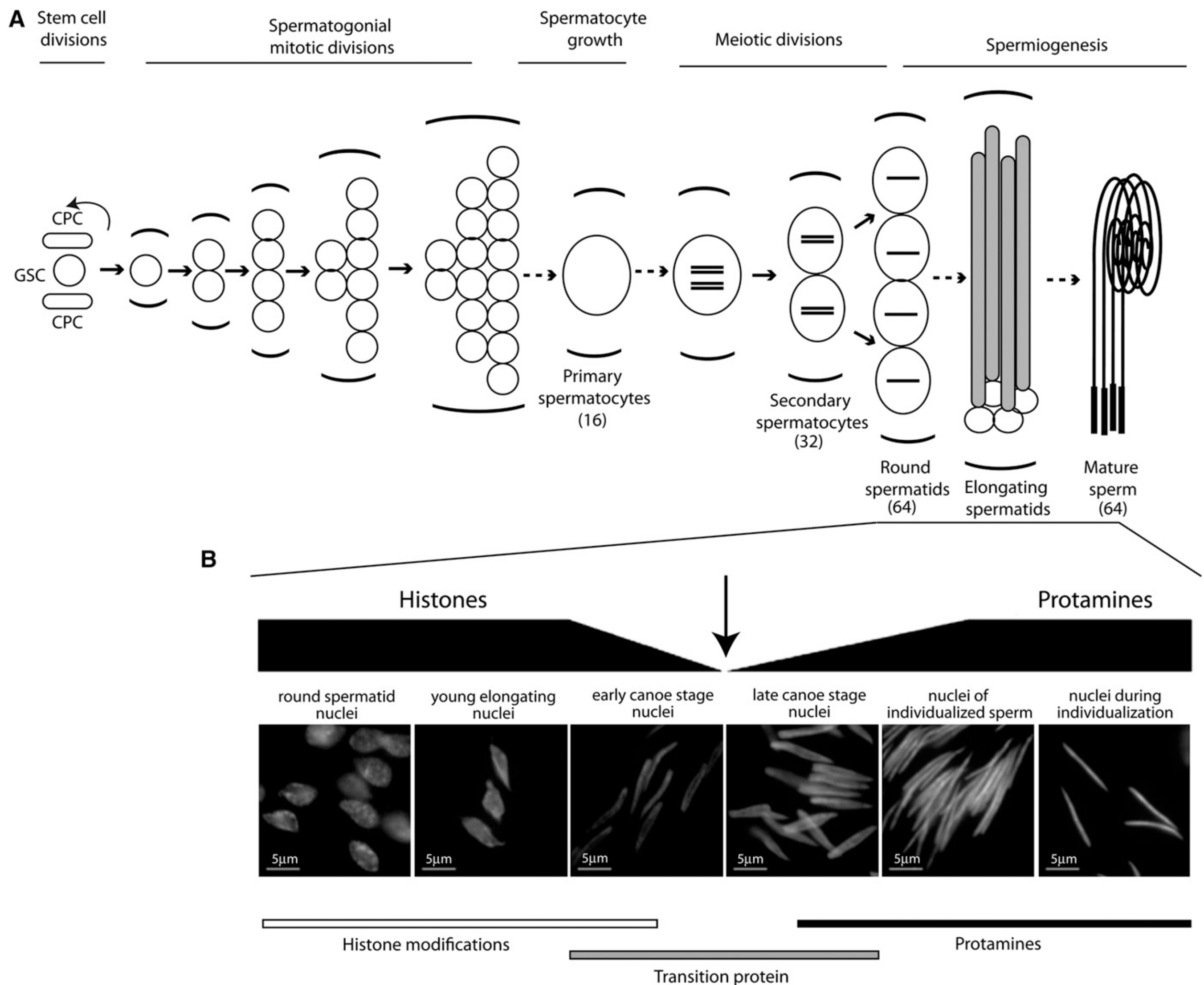

Figure 4 Stages of spermatogenesis in *D. melanogaster*. (A) Cell divisions are represented with a solid arrow and differentiation events without cell divisions are represented by a dotted arrow. Germline stem cells (GSC) are surrounded by cyst progenitor cells (CPC), which give rise to the cyst cells encapsulating developing germ cells. Each germline stem cell divides to produce another stem cell or a daughter spermatogonial cell. The spermatogonia undergo four rounds of mitotic divisions to create 16 primary spermatocytes. Only 1 of the 16 primary spermatocytes is shown. Most of the transcription during spermatogenesis occurs in primary spermatocytes prior to entering meiosis. Primary spermatocytes undergo two rounds of meiotic divisions to create 64 spermatids. Spermatids differentiate and individualize as mature sperm, which are coiled and deposited in the seminal vesicle. All divisions have incomplete cytokinesis so that cells in a cyst are connected through cytoplasmic bridges. Prior to individualization, all cells develop within a cyst (Fuller 1993). (B) Spermiogenesis showing the histone-to-protamine transition. The arrow corresponds to the first visible difference between *SD* and *SD*$^+$ spermatids in *SD*/*SD*$^+$ heterozygotes. Beneath the images of nuclei during spermiogenesis is a schematic showing the primary chromatin component during spermiogenesis (modified from Figure 7 of Rathke *et al.* 2007 (DOI: 10.1242/jcs.004663), with permission from the Journal of Cell Science).

the functional pole hypothesis and suggesting instead some form of sperm dysfunction (Hartl *et al.* 1967; Nicoletti *et al.* 1967). Later, Sandler and Carpenter (1972) were able to generate males that produced individual spermatids bearing *both SD and SD*$^+$ chromosomes; their experiments showed that *SD* will destroy itself when in the same spermatid as *SD*$^+$, confirming the sperm dysfunction hypothesis.

The nature of the sperm dysfunction was finally revealed by ultrastructure analyses of *SD/SD* and *SD/SD*$^+$ testes. The first ultrastructural studies showed that roughly half of the developing spermatids in the cysts of *SD/SD*$^+$ heterozygotes had abnormal sperm tail formation (Nicoletti 1968). Later studies showed that the first visible difference between *SD* and *SD*$^+$ spermatids occurs in early postelongation cysts as a difference in chromatin condensation (Tokuyasu *et al.* 1977; Figure 5, A–C) and that, usually, these spermatids fail to individualize or coil (Peacock *et al.* 1972; Tokuyasu *et al.* 1977). The large, uncondensed *SD*$^+$ spermatid nuclei that fail to individualize end up in the waste bag (Peacock *et al.*

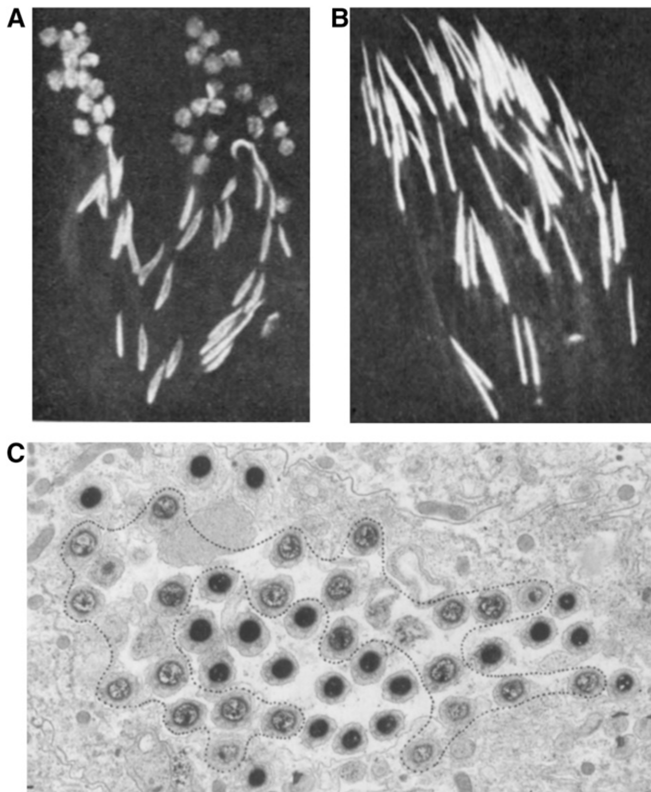

Figure 5 SD^+ spermatid dysfunction is due to a failure to proper condense chromatin after meiosis. (A) A fluorescent image of a cyst of elongating spermatids in an SD/SD^+ heterozygote. About half of the spermatids (those corresponding to SD^+) are not elongating. (B) A comparable cyst in a wild-type testis showing a cyst of elongating spermatids. (Images in A and B are from figure 3, B and C of Hauschteck-Jungen and Hartl 1978; reprinted with permission from the Genetics Society of America.) (C) An ultrastructure image of SD/SD^+ testes at the coiling stage showing that approximately half of the spermatids (again corresponding to SD^+) have abnormal condensation (spermatids within dotted line). (Image in part C is reprinted from figure 2 of Tokuyasu *et al.* 1977, with permission from Elsevier.)

1972; Tokuyasu *et al.* 1977), and those few SD^+ spermatids that do individualize break down later in the testicular duct, the seminal vesicle (Peacock *et al.* 1972), or the female reproductive tract (Hartl 1969; Childress and Hartl 1972). *SD* is a sperm killer (and thus causes meiotic drive in the broad sense; Zimmering *et al.* 1970).

Motivated by the observation that *Sd* genetically mapped near the histone locus and might therefore correspond to the histones themselves, Kettaneh and Hartl (1976) examined the histone-to-protamine transition in *SD* homozygotes and inferred that the transition does not take place in these testes. *SD* is temperature sensitive—both heat and cold treatment reduce the intensity of distortion in most cases (Mange 1968; Denell *et al.* 1969; Hihara 1971; Matthews and Mortin 1983)—allowing temperature-shift experiments to pinpoint the timing of *SD* action during spermatogenesis. As the developmental time course of spermatogenesis in *D. melanogaster* is known (Khishin 1955), the timing of *SD* action can be inferred by shifting SD/SD^+ males to different temperatures at various development stages and measuring its effect on the strength of distortion. The first temperature-shift assays suggested that the critical action of *SD* occurs during early meiosis I (Mange 1968), but the response to temperature varies among different *SD* chromosomes (Mange 1968; Hihara 1971, 1974) and inconsistencies exist in the inferred timing of *SD* action, with some inferring a later, postmeiotic critical stage (*e.g.*, *SD-72* acts postmeiotically; Matthews and Mortin 1983). Temperature shift experiments on X-linked suppressors of *SD* found the critical stage to be postmeiotic, in early spermatids (Hihara 1971; Hiraizumi 1993). A postmeiotic critical stage, if correct, would correspond neatly with the histone-to-protamine transition.

Models of *Segregation Distortion*

> The *SD* system has been a merciless destroyer of otherwise elegant hypotheses, as the pages of this review recount, and it would be too much to expect that the present hypothesis will fare much better. (Hartl and Hiraizumi 1976)

> With the recent identification of the *Sd* gene product as a mutated version of one of the key players in nuclear transport, the end game may at last be in sight. . . . But it would be a mistake to underestimate this wily prey. (Ganetzky 1999).

We have already seen the chromosome breakage and functional pole hypotheses for *SD* falsified. In the following sections, we present a brief summary of other hypotheses on the mechanism of segregation distortion, including our own speculations.

Direct interaction between Sd and Rsp

On the basis of a complementation analysis of 11 *SD* chromosomes, Hartl (1973) proposed that *Sd* encodes a multimeric protein (a "regulator") that, during normal spermatogenesis, complexes at the *Rsp* locus. In SD/SD^+ males sperm dysfunction results from the inability of Sd hetero-multimers (*Sd* and Sd^+ products) to complex at the *Rsp* locus on the SD^+ chromosome (Hartl 1973). As spermatid dysfunction in SD/SD^+ testes happens after meiosis, when only limited transcription occurs, Hartl (1973) proposed that the function of *Rsp* might be to silence transcription of a gene or genes after meiosis.

Ganetzky's 1977 discovery that *Sd* and *Rsp* were neomorphic—deletions of *Sd* fail to distort and deletions of *Rsp* create insensitive *Rsp* alleles—refuted the idea that different *Sd* alleles correspond to deletions (Peacock and Miklos 1973). His discovery also required a modification of Hartl's model. Ganetzky (1977) proposed that the *Sd* product and the *Rsp* locus do not interact during normal spermatogenesis (as both loci are dispensable) but rather sperm dysfunction is a specific consequence of their interaction. Both Ganetzky's and Hartl's models imply that sensitive and insensitive *Rsp* alleles compete for binding with a limited amount of *Sd* product. But Hartl's model predicts that insensitive Rsp^i alleles bind with higher affinity to Sd, whereas

Ganetzky's model predicts that sensitive *Rsp*^{s} alleles bind with higher affinity to Sd. Ganetzky's model was later modified by Hiraizumi *et al.* (1980), who suggest instead that the product of *Sd* prevents a direct, essential interaction between the product of *M(SD)* and *Rsp*.

Disrupted nuclear transport in SD males

The discovery that *Sd* is in fact *Sd-RanGAP*, which encodes a truncated RanGAP protein, naturally led to the proposal that segregation distortion disrupts nuclear transport, one of the well-known functions of wild-type RanGAP. Normally, the RanGAP protein is tethered to the cytoplasmic side of the nuclear pore complex (Figure 6, A and B), where it stimulates hydrolysis of Ran–GTP to Ran–GDP; its cofactor, Ran guanine exchange factor (RanGEF) is chromatin bound in the nuclear compartment where it converts Ran–GDP back to Ran–GTP by nucleotide exchange. The RanGAP–RanGEF system thus maintains a steep Ran–GTP concentration gradient across the nuclear envelope (high in nucleus, low in cytoplasm) that stimulates and provides directionality to nuclear transport (Gorlich and Kutay 1999).

Sd–RanGAP is enzymatically wild type but has an abnormal subcellular distribution (Figure 6, C and D). Unlike wild-type RanGAP, Sd–RanGAP is found inside the nucleus, at least for a subset of primary spermatocytes, presumably owing to the loss of one of its nuclear export sequences (NES; Figure 2C; Kusano *et al.* 2001). Sd–RanGAP is also diffusely distributed in the cytoplasm of some primary spermatocytes (Figure 6, C and D), perhaps because the sites responsible for tethering wild-type RanGAP to the cytoplasmic face of the nuclear pore by SUMOylation are missing in the truncated protein (Figure 2C; Kusano *et al.* 2001).

Kusano *et al.* (2001) hypothesize that the mislocalization of enzymatically active Sd–RanGAP to the nucleus affects nuclear transport, thereby preventing some factor required for chromatin condensation from entering the nucleus and ultimately causing the dysfunction of *SD*$^{+}$ spermatids. Sd–RanGAP requires enzymatic activity *and* nuclear localization to cause segregation distortion: mutating sites required for enzymatic activity or appending a NES to Sd–RanGAP both disrupt segregation distortion (Kusano *et al.* 2001). Two lines of evidence suggest that Sd–RanGAP causes segregation distortion by perturbing Ran-mediated nuclear transport. First, if Sd–RanGAP localization in the nucleus causes excess Ran–GDP to accumulate in the nucleus, then overexpressing Ran and/or RanGEF should increase the concentration of Ran–GTP in the nucleus, thus restoring the gradient of Ran–GTP/Ran–GDP across the nuclear membrane. Second, Kusano *et al.* (2001) showed that, indeed, overexpression of either Ran or RanGEF in the germline suppresses segregation distortion in *Rsp*^{s} and *Rsp*^{ss} backgrounds. Second, Kusano *et al.* (2001) used GFP–NLS–NES reporters of nuclear transport activity to show that, at least in salivary glands, nuclear export is disrupted in *SD* flies.

This functional work on Sd–RanGAP and its role in nuclear transport is groundbreaking. There are, nevertheless, several caveats concerning the disrupted nuclear transport model of distortion. The first concerns the protein in question: RanGAP features in a wide range of functions in the cell (see below), and it is difficult to know whether nuclear transport is the critical phenotype or correlated with the critical phenotype. Second, the results of the nuclear transport assay are not entirely consistent with segregation distortion phenotypes (*e.g.*, the NES–GFP was still "trapped" in the nucleus in some *SD* revertants but not others; Kusano *et al.* 2001). The fact that Kusano *et al.* (2001) performed the nuclear transport assay in salivary glands presents another difficulty, as *SD* affects postmeiotic spermatid development. The apparent inconsistencies between segregation distorter phenotypes and the results of the transport assay might be attributable to differences in nuclear transport between salivary glands and testes.

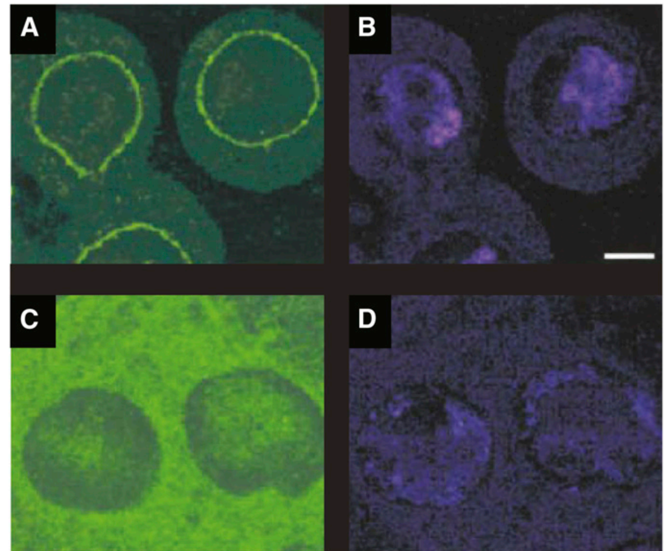

Figure 6 *Sd–RanGAP* is mislocalized in some primary spermatocytes. (A) Immunolocalization of RanGAP (green) in primary spermatocytes of *SD*$^{+}$/*SD*$^{+}$ testes. (B) Propidium Iodide staining showing the location of DNA (blue) in the nucleus. RanGAP normally localizes to the cytolplasmic side of the nuclear envelope. (C and D) The same as A and B except showing the localization of Sd–RanGAP (with an anti-HA antibody in a Sd–RanGAP-HA transgenic fly showing segregation distortion). Sd–RanGAP-HA diffusely stains the cytoplasm and shows aberrant expression in the nucleus (A–D are reprinted from Kusano *et al.* 2001, with permission from Elsevier).

Some experiments suggest that segregation distortion is due to the mislocalization of Sd–RanGAP rather than some functional difference between wild-type RanGAP and Sd–RanGAP (Kusano *et al.* 2002). Kusano *et al.* (2002) show that even wild-type RanGAP can cause segregation distortion: a 10- to 20-fold overexpression of wild-type RanGAP in the germline causes segregation distortion and shows aberrant localization similar to Sd–RanGAP. Interestingly, extra doses of *E(SD)*, known to cause segregation distortion (Sharp *et al.* 1985; Temin 1991), do not affect *Sd–RanGAP* or *RanGAP* expression but instead lead to an accumulation of RanGAP in the nucleus of primary spermatocytes. Its

cytoplasmic signal around the nuclear membrane, however, looks normal (Kusano *et al.* 2002) unlike Sd–RanGAP (Kusano *et al.* 2001). The mislocalized RanGAP appears to affect the Ran–GTP/Ran–GDP gradient: overexpression of Ran or RanGEF suppresses segregation distortion caused by both wild-type RanGAP and extra doses of *E(SD)* (Kusano *et al.* 2002).

Unanswered questions under the nuclear transport model

There are two unanswered questions under the nuclear transport model. First, why do only *Rsp*^{s} spermatids die? It is possible that nuclear Sd–RanGAP preferentially accumulates in *Rsp*^{s} nuclei, perhaps because Sd–RanGAP binds *Rsp* and so the nuclear transport defect occurs only in these spermatids (Pimpinelli and Dimitri 1989; Kusano *et al.* 2001; Kusano *et al.* 2002). Kusano *et al.* (2001, 2002) report on the distribution of Sd–RanGAP in premeiotic primary spermatocytes, but the subcellular localization of Sd–RanGAP in postmeiotic spermatids is unknown, leaving an important question unanswered: Is Sd–RanGAP mislocalized in only half of the developing spermatids—those with *Rsp*^{s} (Figure 7A)? Alternatively, Sd–RanGAP could be mislocalized in all spermatids, disrupting the Ran–GTP/Ran–GDP gradient, but *Rsp*^{s}-bearing spermatids are especially sensitive to this perturbation (Kusano *et al.* 2001, 2002). For example, *Rsp* may bind some limited chromatin condensation factor. Therefore, in spermatids with many *Rsp* repeats (*Rsp*^{s} and *Rsp*^{ss}), the *Rsp* locus may act as a sink for the chromatin factor preventing the proper condensation of other chromatin (Figure 7B).

The second difficulty, and one of the most critical questions of all, for the nuclear transport model is this: Why is the only observable phenotype in *SD/SD*$^+$ heterozygotes specific to the postmeiotic stages of spermatogenesis? The NLS–NES–GFP assays show disrupted nuclear transport in salivary gland cells, but how could all of the cells of the fly function normally with such a defect in an essential nuclear transport pathway? Spermiogenesis is unlike any other developmental process. Given the radical nature and rapid pace of spermatid nuclear remodeling, one can easily imagine that developing spermatids are especially sensitive to perturbations in the Ran cycle (Kusano *et al.* 2001, 2002, 2003).

What else does RanGAP do?

The cycling of Ran–GTP and Ran–GDP does more than facilitate nuclear transport. Ran is involved in mitotic spindle assembly, nuclear envelope assembly, chromosome segregation, and processes occurring during anaphase and cytokinesis (Joseph *et al.* 2002; reviewed in Quimby and Dasso 2003; Renshaw and Wilde 2011; Figure 8). RanGAP and RanGEF are critical in establishing the gradient of Ran–GDP and Ran–GTP for these other cellular functions and not only across the nuclear envelope (Figure 8A**)**. During interphase, the Ran gradient functions after the breakdown of the nuclear envelope to spatially organize spindle assembly, directing microtubule growth toward chromatin (Figure 8B). A high level of Ran–GTP at chromatin also directs nuclear envelope reassembly after mitosis (reviewed in Renshaw and Wilde 2011; Figure 8C). If RanGAP is mislocalized and causes a reduction in nuclear Ran–GTP, it could plausibly affect any one of these other functions as well. In *Schizosaccharomyces pombe*, RanGAP has a role in the nucleus in addition to the cytoplasm: nuclear RanGAP interacts with histones (H3) and histone methyltransferases (*e.g.*, Clr4, the yeast ortholog of *D. melanogaster* Su(var)3–9) to mediate heterochromatin assembly (Nishijima *et al.* 2006). Given that the establishment of a Ran–GTP/Ran–GDP gradient has many critical functions throughout the cell cycle, segregation distortion may be caused by something other than disruption of nuclear transport.

It is also possible that Sd–RanGAP interferes with some other cellular process specific to male germ cells. The gene *ran-like* is a testis-specific retroduplicate of *Ran*, encoding a testis-specific GTPase whose functions are currently unknown. *ran-like* originated at least 5 million years ago and has a history of rapid evolution in the *melanogaster* group, but appears to have functionally degenerated in some species—observations that led Tracy *et al.* (2010) to speculate that *ran-like* is a gene that spread to fixation by distortion. We suggest the possibility that, at least in *D. melanogaster*, *ran-like* may have acquired an important male germline-specific function—one that *SD* may disrupt to cause spermatid dysfunction. In particular, if the mislocalization of Sd–RanGAP inappropriately hydrolyzes ran-like-GTP in the testis to cause segregation distortion, it could explain why the effects of *SD* are specific to spermatogenesis.

Segregation distortion and small RNAs

A truncated Sd–RanGAP makes it easy to imagine how a disrupted Ran (or ran-like) gradient might affect spermatid development, but the connection to the repetitive satellite DNA locus, *Rsp*, remains unclear. Considering the still newly discovered roles of small RNAs and RNA interference (RNAi) in the germline may provide important insights. The well-studied *Stellate* (*Ste*)–*Suppressor of Stellate* [*Su(Ste)*] system of *D. melanogaster*, which some have argued is an ancient cryptic X–Y meiotic drive system (Hurst 1996), involves RNAi (Aravin *et al.* 2004). In this system, the X-linked products of the *Ste* locus are silenced by small antisense RNAs corresponding to Y-linked repeats of *Su(Ste)*. Another study has implicated RNAi in the Winters *sex-ratio* distortion system of *Drosophila simulans*, for which most Y-bearing spermatids are dysfunctional owing to a postmeiotic chromatin condensation problem reminiscent of *SD* (Tao *et al.* 2007). Ordinarily the X-linked driver, called *Distorter on the X* (*Dox*), is suppressed by an autosomal gene, called *Not much yang* (*Nmy*). *Nmy* is a retrogene that originated from *Dox* and that now gives rise to a dsRNA transcript that Tao *et al.* (2007) hypothesize silences *Dox* via RNAi. Tao *et al.* (2007) speculate that the *SD* system might also involve RNAi.

Some repetitive sequences like satellite DNAs and transposable elements produce 23- to 30-bp repeat-associated

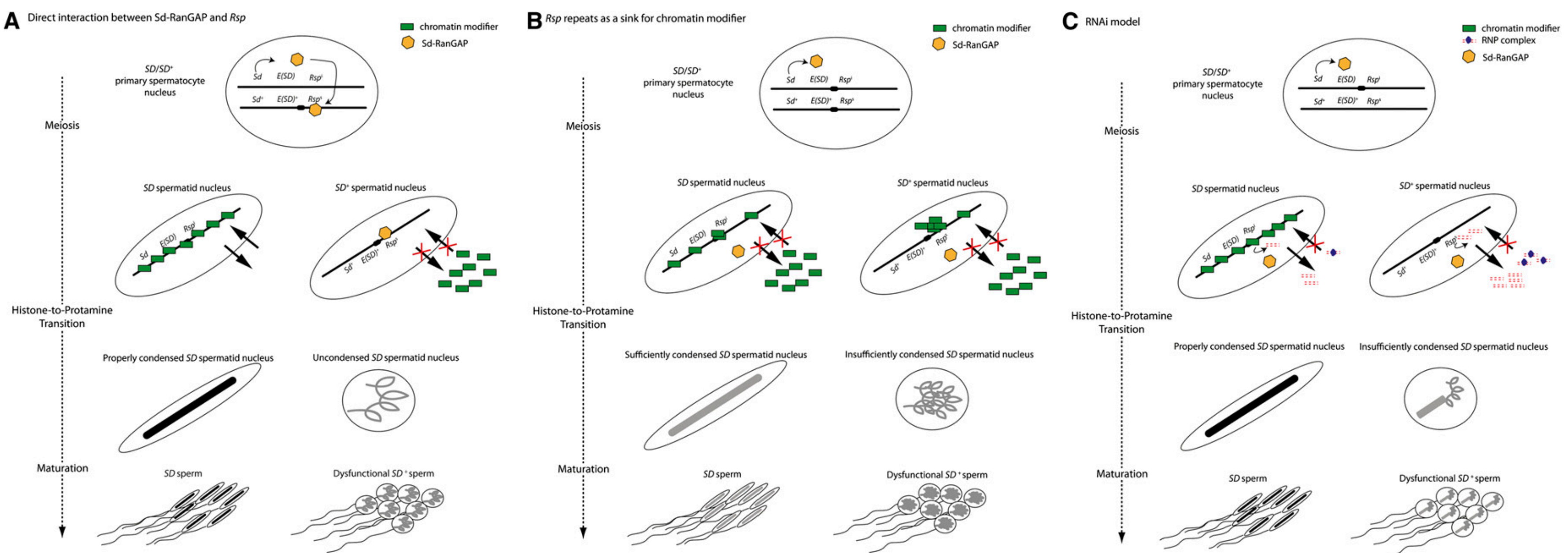

Figure 7 Current models of *SD* action. (A) Sd–RanGAP directly binds *Rsp* repeats, disrupting chromatin condensation in *Rsp*^{s}-bearing spermatids causing spermatid dysfunction either as a consequence of disrupted nuclear transport or some other cellular function of RanGAP. (B) Sd–RanGAP disrupts nuclear transport globally, but *Rsp*^{s}-bearing spermatids are disproportionately sensitive to this disruption because large blocks of *Rsp* act as a sink for chromatin modifiers when their access to the nucleus is limited. (C) *Rsp* rasiRNAs, presumably required for postmeiotic chromatin condensation, are exported from the nucleus, where they form ribonucleoprotein (RNP) complexes; however, the RNP complexes fail to target chromatin modifiers to the genomic *Rsp* satellite because of some disrupted RanGAP, or ran-like, function (see text). Although the disruption is shown as a failure to reenter the nucleus due to disrupted transport, a disrupted Ran-GTP/Ran-GDP (or ran-like-GTP/ran-like-GDP) gradient could affect chromatin condensation more directly.

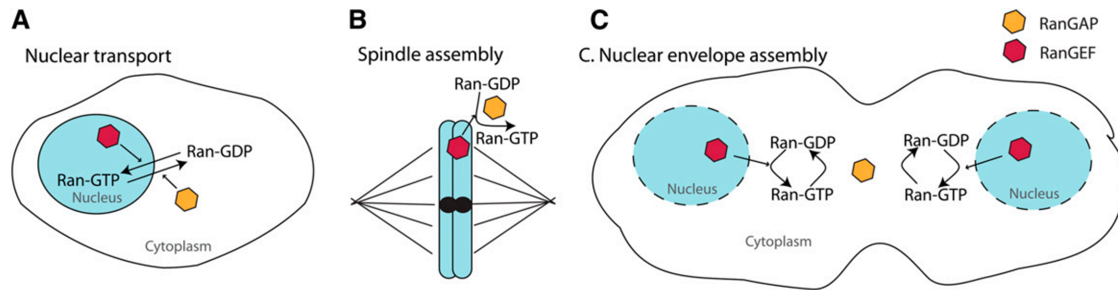

Figure 8 The role of the Ran cycle throughout the cell cycle. (A) The Ran cycle during interphase aids in nuclear transport. A gradient of Ran-GTP/Ran-GDP is established by RanGAP and RanGEF located in the cytoplasm and nucleus, respectively. (B) The Ran cycle during mitosis, prior to metaphase. A gradient of Ran-GTP/Ran-GDP aids in spindle assembly. (C) The Ran cycle at telophase during cell division. After mitosis, the Ran cycle is involved in reassembling the nuclear envelope.

small interfering RNAs or rasiRNAs (Aravin *et al.* 2003). Some rasiRNAs are implicated in silencing heterochromatin in plants, fungi, and *Drosophila*, where ribonucleoprotein (RNP) complexes associated with rasiRNAs recruit silencing factors to the chromatin (Pal-Bhadra *et al.* 2004; Verdel *et al.* 2004; Buhler and Moazed 2007; Kavi and Birchler 2009; Wang and Elgin 2011). The most famous rasiRNA pathway in the germline is the Piwi pathway including *P-element-induced wimpy testis* (*Piwi*), *Aubergine* (*Aub*), and *Argonaute 3* (*Ago3*). While the role of rasiRNAs associated with the Piwi pathway (called piRNAs) in controlling transposable elements is well studied (Brennecke *et al.* 2007), the role of rasiRNAs in controlling satellite DNAs is less clear. *Rsp* generates rasiRNAs in *Drosophila* ovaries (Saito *et al.* 2006), and consistent with the involvement of RNAi in *SD*, we have recently determined that among rasiRNAs bound by Ago3 and Aub in the testis (Nishida *et al.* 2007; Nagao *et al.* 2010), *Rsp* rasiRNAs are enriched (A. Larracuente and D. Presgraves, unpublished results).

If SD/SD^{+} heterozygotes suffer disrupted nuclear transport during spermatogenesis, then *Rsp* rasiRNAs, after associating with RNP complexes in the cytoplasm, may not be efficiently imported back into the nucleus for targeting to the *Rsp* satellite (Figure 7C). Presumably the rasiRNAs direct chromatin remodeling complexes after meiosis in the testis, similar to RNAi-dependent heterochromatin formation in somatic tissues (Pal-Bhadra *et al.* 2004; Kavi and Birchler 2009). Because Rsp^{s} alleles have many (and Rsp^{i} alleles have few) repeats, Rsp^{s}-bearing spermatids might be disproportionately affected by the dearth of *Rsp* rasiRNAs in the nucleus and as a result fail to condense their pericentric heterochromatin (Ferree and Barbash 2007; Tao *et al.* 2007). While the Ran cycle is involved in nuclear export of miRNAs (Ohrt *et al.* 2006; Mtango *et al.* 2009), its role in rasiRNA transport is unknown. It is possible then that neither Ran nor ran-like are responsible for the postmeiotic import of rasiRNAs to their target satellites. One alternative that relies on the Ran (or perhaps ran-like) cycle, but not nuclear transport *per se*, involves the Ran-GTP/Ran-GDP gradient present during cell division. During mitosis, chromosomes "signal" their location in the cell with high concentrations of Ran-GTP (Renshaw and Wilde 2011; Kiyomitsu and Cheeseman 2012; Figure 8C). The initiation of postmeiotic chromatin condensation might similarly require high Ran-GTP (or ran-like-GTP) concentrations at chromosomes.

The simple fact is that we still lack a cogent model for how *Sd* and *Rsp* interact to cause distortion. The key problems are that: (1) *RanGAP* is likely pleiotropic, raising the possibility that nuclear transport is not the critical phenotype but rather correlated with the critical phenotype; (2) we are surprisingly ignorant about the genetic control of spermiogenesis in *Drosophila*; and (3) we are ignorant about the molecular basis of the maintenance of satellite DNAs in the *Drosophila* male germline.

Population Dynamics and Evolution of *SD*

The frequency of SD components in natural populations

SD is found at low frequencies (1–5%) in most natural populations of *D. melanogaster* surveyed throughout the world, including North America, South America, Europe, the Pacific islands, Australia, Asia, and Africa (Hiraizumi and Nakazima 1967; Hartl 1975a; Hiraizumi and Thomas 1984; Temin and Marthas 1984; Hao *et al.* 2000; Presgraves *et al.* 2009). The remarkably consistent frequency of *SD* among different geographic populations strongly suggests a stable deterministic equilibrium between drive and negative selection, one that is robust to variation in local climate and population demography. However, given the strength of *SD*'s distortion ($\geq$95%), its low population frequency seems puzzling.

Several factors may hold *SD* back. First, *SD* chromosomes tend to be homozygous sterile and, often, lethal. But when *SD* is at low frequency these effects are easily swamped out by the strength of meiotic drive as homozygotes are so rarely formed (Temin and Marthas 1984). Indeed, the predicted frequency of a strong distorter that is homozygous sterile is $\sim$50%— much higher than that observed (Crow 1979). Second, as SD/SD^{+} males have reduced fertility (Hartl *et al.* 1967), it seems likely that they suffer reduced sperm competitive ability. Third, the appreciable frequency of Rsp^{i} alleles segregating in some natural populations may also keep *SD* in

check (Kataoka 1967; Hartl 1970, 1977; Hihara 1974; Hartl and Hartung 1975; Trippa and Loverre 1975; Charlesworth and Hartl 1978; Hiraizumi and Thomas 1984; Temin and Marthas 1984). The frequency of *Rsp*^{i} alleles ranges between 3% (Kataoka 1967), ~12–30% (Temin and Marthas 1984), 45% (Hartl and Hartung 1975), and even up to 86% (Trippa *et al.* 1980), depending on both the population and the screen used to identify these alleles. Simple population models predict that *SD* chromosomes can be stably maintained at low frequency (Charlesworth and Hartl 1978; Crow 1979). The models make plausible assumptions about the strength of distortion and the fitness of the *Sd Rsp*^{i} chromosome; they further assume that *Sd*$^{+}$*Rsp*^{i} chromosomes suffer a small reduction in fertility compared to *Sd*$^{+}$*Rsp*^{s} chromosomes to facilitate maintenance of sensitive alleles at *Rsp*. As these fitness measures are not known with any precision for this potentially broad range of genotypes (see Hauschteck-Jungen and Hartl 1978), the extent to which these assumptions hold in natural populations remains to be determined (Temin and Marthas 1984).

Early inferences about the history of SD

Studies on the evolutionary history of *SD* have led to conflicting notions about its age. Two of the earliest observations seemed to support the idea that *SD* must be old (*e.g.*, Sandler and Hiraizumi 1960; Lyttle 1991; Lyttle 1993). First, the intrinsic complexity of the *SD* system—with its multiple enhancers, modifiers, stabilizers, and (usually) inversions—seemed to imply a history long enough for its piecemeal evolution. Indeed, wherever *SD* is found, it seems to have acquired local chromosome *2* inversions (Hartl and Hiraizumi 1976; Crow 1979; Presgraves *et al.* 2009). Second, the worldwide distribution of *SD* implies that the system has been with *D. melanogaster* throughout much of its dispersal history.

Other kinds of observations, however, suggested the possibility of a more recent origin. While most *SD* chromosomes are associated with inversions, both inversion-bearing and inversion-free—and thus the presumed ancestral type—*SD* chromosomes occur in Spain and Italy, suggesting that *SD* may have originated in the Mediterranean basin and subsequently spread throughout the world (Temin *et al.* 1990; Lyttle 1991; Wu and Hammer 1991). As *D. melanogaster* is a sub-Saharan species thought to have a single out-of-Africa expansion into Europe ~15,000 years ago (Baudry *et al.* 2004; Li and Stephan 2006; Thornton and Andolfatto 2006; Hutter *et al.* 2007), the Mediterranean origins hypothesis would seem to place a ~15,000-year upper limit on the age of *SD*.

Molecular evolution and population genetics of Sd–RanGAP

Sd–RanGAP appears to be specific to *D. melanogaster* (Powers and Ganetzky 1991), raising two possibilities. Did *Sd–RanGAP* originate after the split between *D. melanogaster* and its sister species of the *D. simulans* species complex, ~3 MYA? Or did *Sd–RanGAP* originate before the species split only to be lost from other (non-*melanogaster*) lineages? With the molecular identification of *Sd–RanGAP*, the molecular evolutionary history of the gene responsible for distortion can be studied directly. The amount of nucleotide divergence between *Sd–RanGAP* and its parent gene, *RanGAP*, provides information on the timing of the origin of the duplicate and, hence, the age of the distorter. Within *D. melanogaster*, *Sd–RanGAP* and *RanGAP* divergence is more than an order of magnitude less than the divergence observed between the *RanGAP* orthologs in *D. melanogaster* and *D. simulans*. *Sd–RanGAP* thus arose *within* the *D. melanogaster* lineage well after its split from *D. simulans*.

The first molecular population genetic analyses of *SD* chromosomes revealed two haplotypes, one represented by the inversion-free *SD* chromosomes and the other by all other *SD* chromosomes (Wu and Hammer 1991). Population genetic analyses at four loci on chromosome *2*, however, found little or no nucleotide differentiation between *SD* and *SD*$^{+}$ chromosomes, consistent with a recent Mediterranean origin and rapid geographic expansion of *SD* (Palopoli and Wu 1996). Either the *SD* complex evolved so quickly that nucleotide differentiation has not had time to accumulate (Palopoli and Wu 1996) or recombination (gene conversion) between *SD* and *SD*$^{+}$ chromosomes has prevented their differentiation. Recently, *SD* chromosomes were found in *D. melanogaster* populations distributed throughout Africa at frequencies typical of other worldwide populations, 1–5%, raising doubts about Mediterranean origins model and suggesting that *SD* may have originated earlier than ~15,000 years ago (Presgraves *et al.* 2009).

Rapid turnover among SD chromosomes

The Charlesworth and Hartl (1978) theory shows that one kind of *SD* chromosome can displace another, while the overall population frequency of *SD* remains unchanged. Two different studies provide evidence for precisely this kind of rapid replacement. The first was observed directly in a longitudinal study in Madison, Wisconsin. In 1956, among the original six *SD* chromosomes sampled by Hiraizumi in Madison, five were lethal-bearing *SD-5* chromosomes and one was a lethal-free *SD-72* chromosome. In 1979, among 44 *SD* chromosomes sampled in the same Madison populations by Temin and Kreber (1981), one was a *SD-5* chromosome and 19 were *SD-72* chromosomes. Thus, in ~25 years, *SD-72*, with its pericentric inversion acting to tighten the genetic linkage among *Sd*, *E(SD)*, and *Rsp*^{i}, replaced *SD-5* as the predominant *SD* chromosome.

The second replacement event was inferred from molecular population genetic data. In Africa, a new class of *SD* chromosomes, *SD-Mal*, causing perfect distortion (k = 1.0, for over 10,000 progeny scored) and bearing two overlapping paracentric African-endemic inversions on *2R*, swept across the continent very recently (Presgraves *et al.* 2009). The expansion of *SD-Mal* chromosomes across east and west

Africa was so recent and rapid that a region spanning *Sd–RanGAP* at 37E on *2L* to region 55B on *2R* comprises a single haplotype lacking any nucleotide variability (95% upper confidence limit on the age of the sweep ~3400 years). An alternative class of *SD* chromosomes still segregates in Africa, one lacking inversions and, surprisingly, lacking the ability to cause detectable distortion (Presgraves *et al.* 2009). Why these latter *SD* chromosomes fail to distort remains unclear, but they will, presumably, soon be lost from the population.

Molecular evolution of Responder

The DNA sequence divergence between the left and right halves of the *Rsp* repeats is greater than the average divergence between *D. melanogaster* and its sibling species *D. simulans*, strongly suggesting the dimeric structure of the *Rsp* repeats formed before the speciation event (Wu *et al.* 1988; Cabot *et al.* 1993). The *Rsp* repeats thus appear to have been in place when the *Sd* duplication arose. The dearth of *Rsp* repeats from the sibling species of *D. melanogaster* (at least using hybridization-based approaches; Moschetti *et al.* 1996; reference to C. I. Wu, unpublished results, in Lyttle 1991; and Cabot *et al.* 1993) is most likely due to either their rapid divergence, or loss, rather than *Rsp* being a novelty in the *D. melanogaster* genome. Understanding the evolutionary history of the *Rsp* repeats is, however, somewhat complicated by the discovery that these repeats also occur outside of the *h39* locus, near the pericentromeric region of the third chromosome at cytological band *80C* (Moschetti *et al.* 1996; Houtchens and Lyttle 2003) and elsewhere (A. Larracuente, unpublished results).

Is Responder a functional element maintained by natural selection?

> *The only function that can be attributed to Rsp^{sens} is that it causes a chromosome carrying it to be distorted by SD, a peculiar reason to exist*. (Ganetzky 1977).

Why do *Rsp*^{s} alleles persist in natural populations? *SD* frequencies of 3–4% are sufficient to cause the fixation of *Rsp*^{i} alleles because of their insensitivity to segregation distortion (Charlesworth and Hartl 1978; Crow 1979). Large blocks of *Rsp* repeats might provide some benefit to flies that outweighs the negative effects of being distorted by *SD*. To test for such a fitness benefit, Wu *et al.* (1989) used large cage experiments to compete *Sd*$^{+}$*Rsp*^{i} flies bearing a deletion for the *Rsp* locus (*R16*; Ganetzky 1977) against the standard *Rsp*^{s} strain (*cn bw*) in the presence of *SD* and, separately, in the absence of *SD*. Not surprisingly, in the presence of *SD*, the *R16* chromosome outcompeted *cn bw*, due to its insensitivity to *SD*. In the absence of *SD*, however, *cn bw* outcompeted *R16*— the frequency of *R16* decreased each generation. The fitness effect of the *R16* deletion was on viability, not male fertility (Wu *et al.* 1989). If correct, these experiments would provide some of the first evidence for the functional significance of a satellite DNA.

However, while the fitness detriment of the *R16* deletion could reflect the deletion of the *Rsp* locus, the details of the construction of the *R16* chromosome raise doubts. *R16* was constructed by irradiating *cn bw* chromosomes (Ganetzky 1977), which could have induced other, deleterious, mutations. Cytological inspection confirms that the deleted region of *2R* heterochromatin is confined to band *h39*—the site of the *Rsp* locus (Pimpinelli and Dimitri 1989). While the *R16* deletion is unlikely to remove unique DNA sequences corresponding to conventional genes (Wu *et al.* 1989), it does remove a cluster of *Bari-I* repeats. The *Bari-I* repeats encode transposon-like elements found in euchromatin and a single unique heterochromatic location at *h39*, adjacent to the *Rsp* repeats (Caizzi *et al.* 1993). The *Bari-I* repeats may evolve under evolutionary constraints, as the repeats at *h39* have intact open reading frames and invariant array length (Caizzi *et al.* 1993). Therefore, this region of *2R* heterochromatin is likely to have several functional elements that may account for the results of the cage experiments.

While the cage experiments may not satisfactorily demonstrate functionality of the *Rsp* repeats (Wu *et al.* 1989), it is still likely that *Rsp* has a function outside of its role in segregation distortion. *Rsp* is a type of satellite DNA— tandemly repeated sequences originally recognized as forming a "satellite" band on a CsCl gradient owing to their A+T- or G+C-rich nucleotide composition. Although once thought to be junk DNA, it seems that satellite DNA may have functional roles that are still not well understood (*e.g.*, Karpen *et al.* 1996; Aravin *et al.* 2003; Pal-Bhadra *et al.* 2004; Brennecke *et al.* 2007). Similar to other satellite repeats, *Rsp* repeat DNA is bent, as revealed by its slowed mobility on polyacrylamide gels (Doshi *et al.* 1991). Additionally, nucleosome spacing at the *Rsp* locus is ~240 bp compared to ~190 bp of bulk heterochromatin (Doshi *et al.* 1991). The bending of satellite DNA (Radic *et al.* 1987) and nucleosome spacing differences may have a role in facilitating the compaction of heterochromatin (Doshi *et al.* 1991). Satellite sequences also generate rasiRNAs, whose role is not fully understood (Aravin *et al.* 2003; Brennecke *et al.* 2007); however, one likely role is in directing heterochromatin formation (Pal-Bhadra *et al.* 2004; Kavi and Birchler 2009).

Unexplained Phenomena

Several early articles report that *SD* induces a heritable X-linked suppressor effect ("conditional distortion"; Sandler and Hiraizumi 1959; Sandler and Hiraizumi 1961; Sandler and Rosenfeld 1962); that certain insensitive second chromosomes can reduce the sensitivity of previously sensitive chromosomes ("translocal modification"; Sandler *et al.* 1959); and that there is a high "mutability " revealed by *St(SD)* (Sandler and Hiraizumi 1959; Sandler and Hiraizumi 1960). These phenomena may be explained by the failure to control for the many then-unappreciated modifiers of *SD*, of both small and large effect, segregating in the genetic background (Miklos and Smith-White 1971; Hartl 1975a).

A few observations, however, lack satisfactory explanation under any of the proposed models. First, negative distortion has been reported, in which the *SD* chromosome is recovered at a frequency significantly *less* than 50% (Hiraizumi 1989, 1990, 1993; Hiraizumi *et al.* 1994). This negative distortion appears to be restricted to two genotypes: a weak recombinant *SD* chromosome (*Sd E(SD) Rsp*^{i} *M(SD)*$^{+}$ *St(SD)*$^{+}$) in the presence of an X-linked suppressor of segregation distortion (*Su(SD)*X; Hiraizumi 1989, 1990) or a strong *SD* chromosome (*SD-72*) in the presence of the *Su(SD)*X chromosome but at high temperature. Second, *SD* has an age effect (older males exhibit reduced segregation distortion), an observation that, like any other, requires explanation. What is especially intriguing, however, is that the age effect appears to be heritable: the sons of old *SD/SD*$^{+}$ males also exhibit reduced distortion, an effect that persists for multiple generations (Sandler and Hiraizumi 1961; Hiraizumi and Watanabe 1969). Some RNAi-mediated phenomena show heritable age effects, including P-M hybrid dysgenesis. Specifically, the age-related recovery of fertility in P-M dysgenic hybrid females is mediated by novel *P*-element insertions into piRNA clusters. These novel, heritable piRNA cluster insertions give rise to piRNAs that silence *P*-elements, protecting against further dysgenesis (Khurana *et al.* 2011). It is unclear what parallels, if any, exist between piRNA-mediated silencing of transposons and the heritable age effect of *SD*. Third, among *SD*$^{+}$ progeny escaping the action of *SD*, there is a female-biased sex ratio (Hiraizumi and Nakazima 1967; Denell *et al.* 1969). The success of a particular *SD*$^{+}$ spermatid appears to depend on whether or not it cosegregates with an X or Y chromosome. This sex-ratio effect suggests either that sensitive *Rsp*-like sequences occur on some Y chromosomes or that *SD* has off-target effects on non-*Rsp* sequences on the Y (consistent with observations of Enns 1970). Any complete model of the molecular basis of *SD* will have to account for these phenomena.

Conclusions

The >50 years of continuous, intensive study of the genetics, molecular biology, and evolution of *SD* has made it one of our best known meiotic drive systems. The genetic properties of *Sd*, *E(SD)*, *Rsp*, *M(SD)*, and *St(SD)* have been established and the molecular identities of both the major driver, *Sd–RanGAP*, and the target, *Rsp*, have been determined. The early work on *SD* provided an important example at a time when the idea of selfish genetic systems was still unfamiliar. Its discovery and characterization helped establish the real and persistent susceptibility of Mendelian segregation to selfish genetic elements (Sandler and Novitski 1957) and, at the same time, the impetus for the evolution of genetic modifiers that restore fair segregation (Crow 1991). The work on *SD* further shows that, far from requiring some foreign genetic invader of the genome, even an ancient endogenous molecular pathway (*i.e.*, Ran cycling) with multiple essential functions in the cell can be coopted by selfish gene systems. *SD* has also provided some of the most striking examples of the interaction between selection and recombination: the core distorting *Sd E(SD) Rsp*^{i} genotype was able to only invade natural populations of *D. melanogaster* because of its fortuitous clustering around the low-recombination centromeric region of chromosome *2*, and its subsequent evolution involved the rapid recruitment of secondary modifiers of recombination (chromosomal inversions) that further tighten the genetic linkage among its interactors. The short-term evolutionary benefit of reduced crossing over on *SD* chromosomes, however, comes with a long-term evolutionary cost: most inversion-bearing *SD* chromosomes have accumulated recessive lethal mutations that they cannot now shed.

Despite these insights into the evolution of fair segregation, selfish genes and coadapted gene complexes, many questions about *SD* remain. First, despite having the molecular identities of both the driver and the target, a basic understanding of how *Sd* and *Rsp* interact to cause segregation distortion continues to elude. Second, we know little about *Rsp* and its function in spermatogenesis. Why is the *Rsp* satellite in particular, and not others, sensitive to *Sd*-mediated distortion? And why do sensitive alleles of *Rsp* persist in populations? Third, how is it that the presence of mislocalized, enzymatically active Sd–RanGAP seems to affect only spermiogenesis without inducing other major phenotypic consequences? Fourth, the molecular identities of the many modifiers of *SD* in the genome are still unknown. *E(SD)* is a strong enhancer, has the capacity to drive on its own and, in extra doses causes wild-type RanGAP to be mislocalized to the nucleus. The two other major modifiers, *M(SD)* and *St(SD)*, are less well characterized, having been largely neglected despite their large effects on the expression of segregation distortion. Identifying and characterizing these modifiers and the segregating suppressors of *SD* may well provide the clues necessary to pin down the molecular basis of segregation distortion and some of the unexplained phenomena, like negative distortion (see above Hiraizumi 1989, 1990, 1993; Hiraizumi *et al.* 1994).

Going forward, work on *SD* seems certain to provide important insights into the genetics, genomics, evolution, and basic biology of spermatogenesis in *Drosophila*. So far *SD*'s mode of action has remained obscure, in part, because we know surprisingly little about the genetic control of spermiogenesis, nuclear remodeling, the role of small RNAs during spermatogenesis, and the maintenance, function, and evolution of genomic satellite DNAs. The *SD* system thus presents an entrée into these notoriously difficult problems. Determining, for instance, the connection between *Sd* and *Rsp*, as well as the molecular identities and functions of the various modifiers of *SD*, will provide information on the genetic control of spermiogenesis, including the role of RanGAP. Determining how large blocks of *Rsp* satellite (*Rsp*^{s} alleles) lead to spermatid dysfunction in the presence of *SD* will undoubtedly have implications for how pericentromeric heterochromatin is regulated during

spermatogenesis and, possibly, for the role of small RNAs in the male germline.

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