Does genetic conflict drive rapid molecular evolution of nuclear transport genes in *Drosophila*?

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**Summary**
The Segregation Distorter (*SD*) system of *Drosophila melanogaster* is one of the best-characterized meiotic drive complexes known. *SD* gains an unfair transmission advantage through heterozygous *SD/SD* males by incapacitating *SD*-bearing spermatids so that virtually all progeny inherit *SD*. Segregation distorter (*Sd*), the primary distorting locus in the *SD* complex, is a truncated duplication of the *RanGAP* gene, a major regulator of the small GTPase Ran, which has several functions including the maintenance of the nucleocytoplasmic RanGTP concentration gradient that mediates nuclear transport. The truncated Sd-RanGAP protein is enzymatically active but mislocalizes to the nucleus where it somehow causes distortion. Here I present data consistent with the idea that wild-type *RanGAP*, and possibly other loci able to influence the RanGTP gradient, has been caught up in an ancient genetic conflict that predates the *SD* complex. The legacy of this conflict could include the unexpectedly rapid evolution of nuclear transport-related proteins, the accumulation of chromosomal inversions, the recruitment of gene duplications, and the turnover of repetitive sequences in the centric heterochromatin. *BioEssays* 29:386–391, 2007. © 2007 Wiley Periodicals, Inc.

**Introduction**
The Segregation Distorter (*SD*) system of *Drosophila melanogaster* gets a transmission advantage by, in effect, subverting Mendel’s first law: although heterozygous *SD/SD* females transmit *SD* and *SD* equally, heterozygous *SD/SD* males produce almost exclusively *SD*-bearing progeny. Distorted transmission occurs because chromatin remodeling is disrupted in *SD*+-bearing spermatid nuclei so that only *SD*-bearing sperm survive spermatogenesis. After nearly fifty years of intensive study, the genetic, molecular and cellular bases of this textbook case of meiotic drive are finally becoming clear. Here I suggest that the molecular properties of *SD*—a genetic curiosity carried by just 1–5% of flies in the wild—hint at a previously unrecognized genetic conflict that predates *SD* and that may have far-reaching evolutionary consequences. First, I present a brief overview of the *SD* system (for detailed reviews, see Refs 2–7).

*SD* is a multi-locus selfish gene complex

The *SD* complex consists primarily of three interacting loci clustered around the centromere of chromosome 2: (1) the main distorter, Segregation distorter (*Sd*), (2) an upward modifier of distortion, Enhancer of *SD* (*E(SD)*), and (3) the cis-acting target locus, Responder (*Rsp*), which has allelic forms ranging from distortion-insensitive to super-sensitive (Fig. 1a). (Note that *Sd* refers to the distorting locus, whereas *SD* refers to the distorting *chromosome* carrying the full complex of relevant loci.) *Rsp*, the first component of the *SD* system to be molecularly characterized, consists of a large array of 120 bp long AT-rich repeats, and *Rsp* sensitivity correlates with repeat copy number: *Rsp* alleles with <200 copies—or indeed no copies at all—are insensitive, those with ~700 are sensitive, and those with ~2500 are supersensitive. (4–9) *SD* chromosomes carry insensitive *Rsp* alleles (*Rsp*), whereas most wild-type *SD*+ chromosomes (60–80%; Ref. 10) carry sensitive ones (*Rsp*). Thus, *SD* chromosomes are *Sd E(SD) Rsp* and *SD*+ chromosomes are *Sd+ E(SD)+ Rsp* (Fig. 1a). It is convenient to think of *Sd* as producing a toxin in *SD/SD*+ males that incapacitates *Rsp*+-bearing sperm.

The evolutionary persistence of the *SD* complex hinges on its ability to maintain linkage disequilibrium among the appropriate alleles. This linkage disequilibrium is achieved in three ways. (1) The *SD* loci, being clustered around the centromere of chromosome 2, reside in a region of reduced crossing-over in the *D. melanogaster* genome. (2) Most *SD* chromosomes have acquired chromosomal inversions that further restrict recombination throughout the region. (3) When *Sd* is recombined onto a *Rsp* chromosomal background, it forms a “suicide combination” that can distort against itself and is quickly eliminated. Thus, the *SD* system

Abbreviations: *SD*, Segregation Distorter; RanGAP, Ran GTPase Activator Protein; E(SD), Enhancer of *SD*; Rsp, Responder; RanGEP, Ran Guanine Exchange Factor; NES, Nuclear export signal; Mya, Million years ago; MK, McDonald-Kreitman; Dntf-2r, *Drosophila* nuclear transport factor-2 related.
can only invade and persist in natural populations if $Sd$ and $Rsp$ loci are sufficiently tightly linked. (13)

**Molecular and cellular bases of segregation distortion**

In recent years, Barry Ganetzky’s group at Wisconsin has identified both the molecular genetic and the cellular bases of segregation distortion. (3, 14–17) They showed that $Sd$ corresponds to a partial tandem duplication of the $RanGAP$ gene, which encodes the $Ran$ GTPase Activator Protein. (17) The left half of the tandem duplication includes only 3.5 of $RanGAP$’s 6 exons and thus encodes a truncated Sd-RanGAP protein (234 amino acids) which lacks the C-terminal 362 amino acids of wild-type RanGAP (596 amino acids). Transgenic assays confirm that the truncated Sd-RanGAP protein per se causes segregation distortion. (17)

Finding that $Sd$ encodes a truncated RanGAP provided the first clue to the cellular basis of segregation distortion. RanGAP plays an essential and evolutionarily conserved role in several Ran-mediated cell biological processes, including cell-cycle progression, spindle assembly, nuclear envelope assembly and heterochromatin packaging (reviewed in Refs 18–21). Here, however, we focus on its role in nuclear transport (Fig. 2). The small GTPase protein Ran is recycled between the nucleus, where it is bound to GTP, and the cytoplasm, where it is bound to GDP Ran cycling is driven by the action of two proteins, RanGAP and RanGEF ($Ran$ Guanine Exchange Factor), which largely reside on separate sides of the nuclear envelope (Fig. 2). The cytoplasmic protein RanGAP stimulates hydrolysis of RanGTP to yield RanGDP; RanGDP then enters the nucleus where the nuclear protein RanGEF converts it to RanGTP by exchanging GDP for GTP; RanGTP then cycles back to the cytoplasm, and the Ran cycle begins again (Fig. 2). As a result of the stable RanGAP-RanGEF system, a concentration gradient of RanGTP is maintained across the nuclear envelope (low RanGTP concentration in the cytoplasm, ~1000-fold higher in the nucleus, Ref. 22), which simultaneously provides thermodynamic impetus and directionality for nuclear transport. (20)

How does the Sd-RanGAP protein cause distortion? Sd-RanGAP possesses the same enzymatic activity as wild-type RanGAP but, because it is truncated, lacks critical intracellular localization domains, including at least one nuclear export signal (NES) and a sumoylation site that normally tethers RanGAP to the cytoplasmic side of the nuclear pore complex. (14) Consequently, in $SD/SD^+$ males, the active Sd-RanGAP enzyme mislocalizes to the nucleus and, presumably via downstream effects on the nucleocytoplasmic RanGTP concentration gradient, causes segregation distortion. (3, 14) Multiple experimental manipulations show that the distorting ability of Sd-RanGAP is abolished if either its enzymatic activity or its ability to localize to the nucleus is compromised, confirming that segregation distortion is caused by inappropriate RanGAP activity in the nucleus.

Kusano et al. later showed that, aside from its mislocalization, there is, in fact, nothing special about Sd-RanGAP—it is

![Figure 1. a: The chromosomal organization of the SD complex in D. melanogaster. The critical primary loci—$Sd$, $E(SD)$, and $Rsp$—are clustered near the centromere of chromosome 2. For completeness, two secondary modifiers of segregation distortion, Modifier of SD (M(SD)) and Stabilizer of SD (St(SD)), are shown, but are not considered further in the text.) Note that this figure is not to scale. b: The Sd locus corresponds to a tandem partial duplication of the RanGAP gene and encodes a C-terminally truncated Sd-RanGAP protein. Only SD chromosomes carry Sd.

![Figure 2. The compartmentalized regulation of Ran cycling. Normally, in the cytoplasm, RanGAP stimulates the GTP hydrolysis by Ran (RanGTP $\rightarrow$ RanGDP); in the nucleus, RanGEF facilitates exchange of Ran-bound GTP for GDP (RanGDP $\rightarrow$ RanGTP). The compartmentalization of these reactions leads to a stable gradient across the nuclear envelope with low RanGTP concentrations in the cytoplasm and high concentration in the nucleus. This concentration gradient drives proper nuclear transport. In SD/SD' flies, the truncated but active Sd-RanGAP enzyme mislocalizes to the nucleus, where it presumably disrupts the RanGTP concentration gradient, causing segregation distortion. NPC = nuclear pore complex.](image-url)
simply performing its enzymatic function in the wrong place.\(^{(15)}\)

To prove the point, Kusano et al. showed that wild-type RanGAP can also induce segregation distortion when experimentally mislocalized to the nucleus. This was done in two ways. First, overexpressing wild-type RanGAP by some 20-fold in the testis alters its normal subcellular distribution to one that is similar to that seen in SD-bearing males. Second, Kusano et al.\(^{(5)}\) took advantage of the sometimes overlooked fact that \(E(SD)\) can cause segregation distortion independent of \(S_d\).\(^{(4,23,24)}\) They showed that, in flies bearing an extra dose of \(E(SD)\), wild-type RanGAP mislocalizes to the nucleus and distortion results. Interestingly, \(E(SD)\), whose molecular identity remains unknown, does not alter RanGAP expression levels (as a transcription factor might) but instead appears to facilitate nuclear import of RanGAP or impede its normal export from the nucleus.\(^{(15)}\)

These studies demonstrate that segregation distortion can be induced by several means—by \(S_d\)-RanGAP, by overexpression of wild-type RanGAP and by extra doses of \(E(SD)\)—that have the same effect: each causes the inappropriate nuclear localization of RanGAP activity, which in turn affects nuclear RanGTP concentrations, and induces segregation distortion. This is, of course, only half of the story. Precisely how altered RanGTP concentrations affect the \(Rsp\) locus to bring about distortion is still unclear.\(^{(3)}\)

**Evolutionary evidence for ancient genetic conflict?**

The molecular basis of RanGAP-mediated segregation distortion immediately raises an evolutionary question: What would happen to newly arising mutations in wild-type RanGAP that cause the protein to partially localize to (or linger in) the nucleus? These could be replacement mutations modifying, for instance, the efficacy of RanGAP’s nuclear export signals. If such mutations arose on a relatively insensitive \(Rsp\) background, they could cause segregation distortion and quickly spread in the population. Even a weak transmission advantage of, say, 1% amounts to very strong natural selection. If such mutations have repeatedly arisen and spread to fixation during RanGAP’s history, then we should see evidence of recurrent “selfish substitutions” at wild-type RanGAP. The signature of such recurrent selfish sweeps would be indistinguishable from that of recurrent positive selection (see also Ref. 25).

To test this possibility, I used a standard molecular population genetics approach. I collected DNA sequence polymorphism and divergence data by re-sequencing the protein-coding region of wild-type RanGAP from 11 \(D.\ melanogaster\) and 11 \(D.\ simulans\) chromosomes, two species that diverged 3–5 Mya.\(^{(26,27)}\) All of the chromosomes sampled originated from natural populations in Zimbabwe, the putative ancestral range of these species (and all \(D.\ melanogaster\) lines are \(SD^+\)). Under a standard neutral model of molecular evolution, in which only neutral changes contribute to polymorphism and divergence (and where we assume that synonymous changes are neutral, or very nearly so), the ratio of replacement to synonymous fixed differences between species ought to be roughly equal to that of polymorphisms.\(^{(28,29)}\) A McDonald-Kreitman test using the RanGAP data strongly rejects this model—there is a highly significant excess of replacement fixations between the two species (Table 1). Typically, this pattern is taken as strong evidence of a history of adaptive evolution. But given what is

| Table 1. MK tests of RanGAP in \(D.\ melanogaster\) and \(D.\ simulans\) |
|------------------|------------------|------------------|------------------|
| **Species pair** | **Polymorphisms** | **Fixations** | **Tachida’s** |
|                  | \(R^a\) | \(S^b\) | \(R/S\) Ratio | \(R^a\) | \(S^b\) | \(R/S\) Ratio | **Z^c** | **P-value** |
| \(D.\ melanogaster\)–\(D.\ simulans\) | 9     | 24    | 0.375     | 58   | 45   | 1.289     | 0.536 | 0.0048 |
| \(D.\ simulans\)–\(D.\ yakuba\) | 6     | 20    | 0.300     | 107  | 92   | 1.163     | 0.588 | 0.0034 |
| \(D.\ melanogaster\)–\(D.\ yakuba\) | 3     | 5     | 0.600     | 95   | 101  | 0.941     | 0.195 | 0.7228 |
| \(D.\ melanogaster\) lineage | 1     | 4     | 0.250     | 14   | 22   | 0.636     | 0.406 | 0.3870 |
| \(D.\ simulans\) lineage | 5     | 15    | 0.333     | 22   | 12   | 1.833     | 0.740 | 0.0052 |

\(^a\)Replacement changes.

\(^b\)Synonymous changes.

\(^c\)Tachida’s \(Z = \log_{10}\left(\frac{R/S \text{ fixed differences}}{R/S \text{ polymorphisms}}\right)\); positive values are consistent with an excess of fixed replacement differences.\(^{(45)}\)

Eleven chromosomes each were sampled from \(D.\ melanogaster\) and from \(D.\ simulans\). \(D.\ yakuba\) RanGAP was obtained by BLAST against the \(D.\ yakuba\) genome sequence (Washington University Genome Sequencing Center). \(D.\ yakuba\) diverged from the \(D.\ melanogaster\)–\(D.\ simulans\) common ancestor ~12 Mya.\(^{(27)}\) Tachida’s \(Z^c\), a summary statistic of MK data, is positive in all comparisons, indicating an excess of replacement fixations. However, MK tests using only polymorphism data from \(D.\ melanogaster\) have limited statistical power as few polymorphic mutations segregate at these loci. \(D.\ melanogaster\) and \(D.\ simulans\) have limited statistical power as few polymorphisms segregate at these loci. \(D.\ melanogaster\) and \(D.\ simulans\) were obtained by re-sequencing the protein-coding region of wild-type RanGAP from 11 \(D.\ melanogaster\) and 11 \(D.\ simulans\) chromosomes, two species that diverged 3–5 Mya.\(^{(26,27)}\) All of the chromosomes sampled originated from natural populations in Zimbabwe, the putative ancestral range of these species (and all \(D.\ melanogaster\) lines are \(SD^+\)). Under a standard neutral model of molecular evolution, in which only neutral changes contribute to polymorphism and divergence (and where we assume that synonymous changes are neutral, or very nearly so), the ratio of replacement to synonymous fixed differences between species ought to be roughly equal to that of polymorphisms.\(^{(28,29)}\) A McDonald-Kreitman test using the RanGAP data strongly rejects this model—there is a highly significant excess of replacement fixations between the two species (Table 1). Typically, this pattern is taken as strong evidence of a history of adaptive evolution. But given what is...
known about RanGAP and its properties, I suggest that this excess of amino acid substitutions between species reflects a history of recurrent selfish sweeps. These would represent amino acid changing fixations that do not increase the fitness of the flies but rather spread for no other reason than their intrinsic transmission advantage. The RanGAP coding region also shows four fixed in-frame length changes between species—one deletion in D. melanogaster, one insertion and two deletions in D. simulans—that may also be consequential. (D. simulans has 601 codons, D. melanogaster has 596). The fact that many of the fixed replacement and length differences in RanGAP occurred in the D. simulans lineage and the rest in the D. melanogaster lineage before the Sd-RanGAP duplication arose implies that a long evolutionary history of RanGAP-mediated conflict over transmission predates the existence of Sd.

Under this genetic conflict scenario, not all of the changes in RanGAP are necessarily selfish substitutions; some could be compensatory substitutions. As a selfish substitution reaches fixation (dragging a relatively insensitive Rsp allele with it via hitchhiking and epistatic selection), variation for sensitivity will be exhausted and distortion will dissipate. The population may, however, be left with a compromised RanGAP protein and Rsp array. Establishing conditions that favor compensatory changes in RanGAP (or in other proteins that influence nuclear transport; see below) and the replenishment of variability at the repetitive Rsp locus. The recurrent substitutions at wild-type RanGAP might therefore reflect past cycles of distortion and compensation.

**Implications of ancient conflict for the Responder locus**

The significant excess of amino acid changing fixations at RanGAP, in the D. simulans lineage in particular, is surprising because the target locus, Rsp, is believed to be restricted to D. melanogaster. How could selfish sweeps occur in D. simulans, a species supposedly lacking a sensitive target locus? There are at least two possibilities.

First, it is possible that the repetitive Rsp array, while sufficient for sensitivity to distortion, is not necessary for sensitivity to distortion—i.e., other distortion-sensitive repetitive DNAs may occur in the vicinity of the chromosome 2 centromere of D. simulans. The second and more compelling possibility, however, is that Rsp once existed in D. simulans but was subsequently eliminated. Indeed, the level of divergence among canonical Rsp repeat copies within D. melanogaster reaches up to 25%, making some canonical Rsp repeats older than the D. melanogaster–D. simulans species split (average silent divergence across loci between D. melanogaster and D. simulans is ~12%; Ref. 33). Notably, canonical Rsp sequences account for just 10–20% of the ~600 kb Rsp locus. In recent work, Houtchens and Lyttle showed that the remainder comprises even more diverged, non-canonical Rsp sequences. These diverged sequences push the time of origin for Rsp repeats even further back in the history of the D. melanogaster-D. simulans common ancestor. It appears, then, that Rsp was eliminated in the D. simulans lineage, consistent with a history of RanGAP-mediated distortion.

Importantly, the non-canonical Rsp repeats discovered by Houtchens and Lyttle would not have been identified in earlier hybridization-based screens for Rsp in non-melanogaster species. The existence of distortion-sensitive sequences in species other than D. melanogaster therefore remains an open question.

**The genomic legacy of nuclear transport-mediated genetic conflict**

If selfish sweeps have occurred at RanGAP, then other loci may also be caught up in the genetic conflict. In principle, any gene able to directly alter the localization of RanGAP (e.g., E(SD)) or, more indirectly, the RanGTP concentration gradient, could influence transmission, either as a suppressor of distortion or even as another distorter. Indeed, E(SD)'s ability to cause distortion independently of Sd<sup>34</sup> suggests that, like wild-type RanGAP, it too will have a history of rapid evolution driven by recurrent selfish sweeps.

In addition to E(SD) and wild-type RanGAP, I suggest that at least one other gene, the recently discovered new gene duplication, Dntf-2r (Drosophila nuclear transport factor-2 related; Refs 36,37), may have historically caused segregation distortion. The parent gene of Dntf-2r is located on the X chromosome and encodes Drosophila Ntf-2 whose function is to escort RanGDP into the nucleus via direct interactions with the nuclear pore complex. The new duplicate gene, Dntf-2r, which only occurs in four species of the D. melanogaster subgroup (D. melanogaster, D. simulans, D. sechellia, and D. mauritiana), exhibits several characteristics that implicate it in past distortion: (1) At its origin, Dntf-2r would act like an extra dose of Ntf-2 and thus influence the RanGTP concentration gradient—much like Sd-RanGAP and extra doses of E(SD). (2) Unlike its parent gene, Dntf-2r is expressed exclusively in the testis—where segregation distortion occurs. (3) Like wild-type RanGAP, Dntf-2r has experienced a significant excess of amino acid substitutions—consistent with a history of recurrent selfish sweeps. (4) Dntf-2r is located near the base of chromosome arm 2L (cytological position 36F), just one cytological division distal to RanGAP (position 37E)—thus, like Sd-RanGAP, wild-type RanGAP and E(SD), the new Dntf-2r gene is well-positioned to exploit opportunities for linkage disequilibrium with Rsp (or its functional equivalent). Indeed, it seems possible that the Dntf-2r duplication initially arose on a chromosome with a relatively insensitive Rsp and then rose to fixation through its intrinsic transmission advantage.

The legacy of this ancient conflict over transmission may be inscribed in the Drosophila genome in several ways. For one,
Hypothesis

this conflict could explain the accelerated protein evolution of a class of housekeeping genes (those affecting nuclear transport) that seem unlikely candidates for recent and recurrent adaptive evolution: in a recent population genetic analysis six of six nucleoporins—protein constituents of the nuclear pore complex—also show histories of recurrent adaptive evolution between D. melanogaster and D. simulans, particularly in the D. simulans lineage.\(^{(38)}\) It is tempting to speculate that these nucleoporins evolved in response to \(\text{RanGAP}\) or \(\text{Dntf-2r}\)-mediated conflict over transmission (for other possible causes for the rapid evolution of nucleoporins, see Ref. 38). For another, this conflict could contribute to several larger-scale phenomena, including the accumulation of inversions on the second chromosome, the turnover of repetitive sequences in the centric heterochromatin and the recruitment of gene duplications, like \(\text{Dntf-2r}\). Indeed, gene duplications appear to be prevalent features of every well-characterized segregation distortion system studied so far, including \(\text{SD}\) in \(D. \text{melanogaster, sex ratio in D. simulans}^{(39)}\) and the \(\text{t}\)-complex of the house mouse \(\text{Mus musculus}^{(40)}\).

Conclusions

When \(\text{RanGAP}\) activity is mislocalized to the nucleus—either by \(\text{Sd-RanGAP}\) or by extra doses of \(\text{E(SD)}\) or by overexpression of wild-type \(\text{RanGAP}\)—distortion occurs against sensitive \(\text{Rsp}\) alleles. The nuclear transport pathway in \(Drosophila\) therefore appears to be intrinsically susceptible to selfish mutations that provide no fitness benefit but instead exploit nuclear transport to gain a transmission advantage. These mutations could take the form of gene duplications (e.g. \(\text{Sd-RanGAP, Dntf-2r}\)), amino acid substitutions (e.g. wild-type \(\text{RanGAP}\), Table 1), noncoding substitutions that cause over-expression of \(\text{Ran-GAP}\), or secondary modifiers, like chromosomal inversions. The key requirement for the spread of such selfish mutations is their linkage disequilibrium with relatively insensitive \(\text{Rsp}\)-like target alleles. If such a conflict has taken place, possibly compromising nuclear transport, then compensatory substitutions at nuclear transport-related genes distributed throughout the genome should occur (e.g. the rapid evolution of nucleoporins; Ref. 38).

As with any evolutionary arms races, past genetic conflicts can be hidden in extant species. For instance, in \(D. \text{pseudoobscura bogatana}\), there is no evidence for \(X\)-linked segregation distortion. But when the \(D. \text{p. bogatana}\) \(X\) chromosome is introgressed onto the naive genetic background of its sister species \(D. \text{p. pseudoobscura}\), it causes strong distortion in males leading to production of >95% daughters (i.e. \(Y\)-bearing sperm are eliminated; Ref. 41). In \(D. \text{p. bogatana}\), then, it appears that an \(X\)-linked distorter evolved and later came under the control of genetic suppressors. \(D. \text{p. pseudoobscura}\), however, lacks those suppressors and consequently cannot stop the \(D. \text{p. bogatana}\) \(X\) from causing distortion. The growing number of examples of similar cryptic distorters that become released in hybrid genetic backgrounds suggests that the fixation of drivers and suppressors within species may not be uncommon.\(^{(42–44)}\)

Testing the hypothesis that \(\text{RanGAP, Dntf-2r}\), and six nucleoporins have been caught up in such an ancient (and now possibly masked) genetic conflict over transmission is not trivial. But the conflict hypothesis makes some testable predictions. First, the conspicuous concentration of rapid evolution at these genes in \(D. \text{simulans}\) requires that \(\text{Rsp}\)-like targets exist (or at least once existed) in this species. Functional evidence for such susceptibility to \(\text{RanGAP}\)-mediated distortion in \(D. \text{simulans}\) would at least confirm the possibility of this conflict in this lineage. Second, association tests demonstrating a correlation between weak segregation distortion and variation at wild-type \(\text{RanGAP}\) variants (e.g. amino acid polymorphisms or quantitative variation in \(\text{RanGAP}\) expression) would confirm that genetic variation for distortion exists at essential genes in the nuclear transport pathway. Finally, it would be most interesting to test whether the \(D. \text{simulans RanGAP}\) can induce distortion on a naive \(D. \text{melanogaster}\) genetic background, and vice versa.

In concluding, it is important to remember that we have considered the potential impact of just one segregation distorter system here. How many other loci are involved in similar but independent conflicts over transmission, each having its own impact on genome evolution?

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