

# Comment on “A Test of the Snowball Theory for the Rate of Evolution of Hybrid Incompatibilities”

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Matute *et al.* (Reports, 17 September 2010, p. 1518) tested the theory that the number of genes involved in hybrid incompatibility increases faster than linearly. However, the method they used is inappropriate because it detects genes that are haploinsufficient in a hybrid background but that would not contribute to lethality in wild-type hybrids, thus overestimating the frequency of hybrid inviability.

Matute *et al.* (1) tested the prediction that the number of genes involved in hybrid incompatibility increases faster than linearly with the divergence time between species. They used *Drosophila melanogaster* strains heterozygous for multigene deletions to screen for chromosomal regions that reduce viability in female F1 hybrids. They found many more regions that reduce viability in *D. santomea* hybrids (71) compared with *D. simulans* hybrids (10) and concluded that their data support the theory that Dobzhansky-Muller (DM) hybrid incompatibility (HI) genes accumulate exponentially (or snowball) with respect to the time of divergence. However, one confounding factor in their study is that the sample sizes were much larger in crosses with *D. santomea* compared with *D. simulans* [from table S1 in (1):  $n = 453$  crosses with *D. santomea*, mean number of balancer females = 87.4, SD = 70.6; from table S2 in (1):  $n = 226$  crosses with *D. simulans*, mean number of balancer females = 53.5, SD = 51.8;  $P = 4.04 \times 10^{-12}$  by 2-tailed  $t$  test]. There was thus substantially more power to detect deviations from a 1:1 ratio of progeny with *D. santomea* than with *D. simulans*.

More fundamentally, Matute *et al.*'s method of screening will potentially detect regions that reduce hybrid viability due to haploinsufficiency rather than because they contain DM HI genes. The authors searched for regions that, when hemizygous for either *D. simulans* or *D. santomea* genes, reduce viability of F1 female hybrids compared with normal (fully diploid) hybrid females. Because wild-type female hybrids are never hemizygous for chromosomal regions, it is essential to consider how the putative DM HI regions identified in their screen would function in normal hybrids. If the reported viability effects only occur in hemizygous deletion hybrids, then they would never contribute to incompatibility in normal hybrids and thus do not contain DM HI genes.

Matute *et al.* described their screen as having “uncovered” or “exposed” recessive hybrid lethality genes from either *D. simulans* or *D. santomea*. If so, then these putative recessive HI genes must also cause HI when homozygous in normal hybrids but not when heterozygous in hybrids (since F1 female hybrids would then be lethal). For a gene to cause HI when both homozygous and hemizygous, but not when heterozygous, strongly implies that the HI gene is behaving like a loss-of-function mutation in a hybrid background. It is clear, however, that many HI genes do not behave as loss-of-function mutations, because it is not how four of the best-characterized *Drosophila* HI loci function. These loci have the opposite property whereby the presence, not the absence, of the locus causes HI (2–5).

Nevertheless, HI genes may have properties that are not easily modeled from intraspecific genetics and thus need to be directly tested in hybrid backgrounds. A vast amount of experimental work using interspecific introgressions has defined most hybrid incompatibility effects as being recessive (e.g., 6, 7). Relevant introgressions have not been performed between the species used by Matute *et al.*, but X-linked regions can be easily tested for viability effects by examining hemizygous males. If the X chromosomes of *D. simulans* and *D. santomea* contain recessive inviability genes based on assays of hemizygous females, then hybrid males carrying these X chromosomes should have reduced viability or be lethal. This is an extremely sensitive test, because these male hybrids are hemizygous for their entire X chromosome and thus will be exposed to the full load of all X-linked recessive inviability genes.

Such tests have been described and demonstrate that there are no recessive hybrid lethal genes on the *D. simulans* X chromosome (8). These data therefore contradict the report of Matute *et al.* that the *D. simulans* X chromosome contains one region causing full lethality in *D. melanogaster* hybrids. Such tests have not been performed with *D. melanogaster*/*D. santomea* hybrids. *D. yakuba*, however, is a close sibling species of *D. santomea* that is estimated to have

diverged from *D. santomea* within the past ~0.4 million years (9). Because the *D. melanogaster*–*D. santomea* divergence is estimated at ~12.8 million years ago (1), the vast majority of *D. santomea* genes causing lethality in *D. melanogaster* hybrids must be shared with *D. yakuba*. Pole-cell transplants were used to generate F1 hybrids that are essentially equivalent to a mating of *D. yakuba* females to *D. melanogaster* males (10). Amazingly, F1 hybrid males carrying the *D. yakuba* X chromosome were comparable in viability to F1 hybrid females. There are thus no recessive alleles on the *D. yakuba* X chromosome that cause lethality or even reduce viability in *D. melanogaster* hybrids. Because the maternal germline was from *D. yakuba*, these F1 hybrids were not susceptible to incompatibilities between the maternal cytotypic and the X. It seems unlikely, however, that all of the 13 X-linked regions identified by Matute *et al.* involve such maternal effects.

There are two ways in which hemizygous males might fail to detect hybrid lethality effects seen in hemizygous females. One is that all 13 X-linked regions in *D. santomea* reduce viability by interacting with X-linked genes in *D. melanogaster*. Such effects would not occur in males because they have only a single X chromosome. Assuming that the X chromosome contains ~20% of the genome (11), the probability that all 13 interactions are X-X is  $[0.2]^{13} = 8.19 \times 10^{-10}$ . The second possibility is that the X-linked viability effects are female-specific. However, only five X-linked genes are known that can produce female-specific lethality when mutated, and most also have pleiotropic vital functions in males (12).

I therefore suggest that many of the regions identified by Matute *et al.* (1) do not contain DM HI genes. Some may, but identifying them will require further experimental tests performed in a fully diploid genotype. What then is causing the reduced viability and lethal effects identified by Matute *et al.*? Hybrids are highly sensitive to gene dosage effects that cannot be predicted from phenotypes in pure species. For example, increased dosage of the wild-type *D. melanogaster* *Hmr* gene reduces hybrid female viability, and under some conditions *Hmr* can cause dominant sterility and lethality (8). In contrast, within *D. melanogaster*, *Hmr* has the opposite genetic properties: Its absence reduces viability and fertility, and these effects are purely recessive (13). I propose that the results of Matute *et al.* reveal that hybrids are more sensitive to haploinsufficiency than pure species. The reasons are unclear, but it may reflect the fact that even viable F1 hybrids have many nonadditive changes in gene expression compared with their parental species [e.g., (14)]. Hybrids should therefore be considered as sensitized genetic backgrounds with distinct properties, compared with pure species. For example, in sensitized backgrounds, loss-of-function mutations that are purely recessive in an otherwise wild-type background can become dominant (15).

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Because genetic properties are so different in sensitized backgrounds, it is impossible to predict the frequency of different types of genetic effects between hybrid and pure-species backgrounds. The relative rarity of lethal haploinsufficiencies within *D. melanogaster* (*11*) therefore does not speak to their frequency in hybrids. In fact, the results of Matute *et al.* interestingly suggest that sensitivity to haploinsufficiency in hybrids is not constant but rather increases with time of divergence. However, the observed inviability effects cannot be assumed to reflect DM incompatibil-

ities and thus cannot support or refute the snowball theory.

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