Response to Comment on "A Test of the Snowball Theory for the Rate of Evolution of Hybrid Incompatibilities"

Daniel R. Matute,* David A. Turissini, Jerry A. Coyne

Barbash claims that deficiency mapping of inviability regions cannot distinguish hybrid lethality from haploinsufficiency, the phenomenon whereby a single functional copy of a gene cannot maintain normal function in a hybrid genetic background. Although we acknowledge that his hypothesis deserves careful experimental testing, we argue against his conclusions and provide evidence that our methodology is suitable to study the evolution of Dobzhansky-Muller incompatibilities.

Our study (*1*) tested the snowball theory for the rate of evolution of hybrid incompatibilities (HI). We identified potential Dobzhansky-Muller interactions (DMIs)—interactions between two or more genes that have evolved independently in isolated populations and contribute to inviability or sterility in a hybrid individual—using deficiency mapping in two interspecific crosses: *Drosophila melanogaster*/*D. santomea (mel/san)* and *D. melanogaster*/*D. simulans* (*mel/sim).* We compared the viabilities of F_1 female hybrids who carried a chromosome deficiency from *D. melanogaster*, *df/san*, with those of F_1 hybrid females who carried a *D. melanogaster* nondeficient balancer chromosome (*Bal*), *Bal/san,* (or *sim*) hybrids. Because *df/san* hybrids carry only one copy of the chromosomal region under study, Barbash (*2*) questions the ability of our methods to identify potential loci involved in DMIs. Here, we acknowledge that Barbash's hypothesis deserves a formal test but then present analyses and facts that argue against his conclusions that our methodology is not suitable to study the evolution of DMIs.

Barbash argues that our power to detect DMIs is substantially higher in the *mel/san* than in the *mel/sim* crosses and that we thus underestimated the number of DMIs in the latter hybridization. However, the power calculation he presents is not pertinent to our experimental design. To establish whether there was a snowball effect, we included only the subset of deficiencies that were tested in both studies ($n = 224$), not "453 crosses with *D. santomea*" as Barbash states, so the genomic coverage and the number of deficiencies tested is the same for both crosses.

To find out whether the difference in total flies scored per cross affected our likelihood of detecting a snowball effect, we repeatedly drew samples of size 53 (the 40th percentile on the *mel/sim* data set) from the empirical distribution of the data for both crosses to establish how many *san* regions would be categorized as lethals if the sample sizes of both data sets were identical [1000 replications per deficiency tested (*3*)]. Under this protocol, the probability of detecting a quadratic increase over a linear increase—if the two crosses had the same power—remains significant and just as high as in our initial study [Akaike Information Criterion linear (AIC_{linear}) = 20.16, $AIC_{quadratic}$ = 14.21; weighted $AIC_{quadratic}$ = 0.951]. This analysis shows that the number of HI alleles in *D. santomea* was not inflated by counting more total progeny than in the *mel/san* cross. Our experimental design has sufficient statistical power to show that the number of HI snowballs with time.

Barbash (*2*), citing four cases of genes causing HI, argues that in "the best-characterized *Drosophila* HI loci" the presence of the gene rather than its absence causes HI (*4*–*7*). This argument is flawed in several ways. First, the aim of detecting HI alleles through deficiency mapping is not to detect alleles that cause lethality by their absence, but to uncover those alleles that act recessively. Second, three of these genes—*odsH*, *lhr*, and *zhr*—are dominant alleles that cause hybrid breakdown in *Drosophila* hybrids, and we expect them to cause HI by their presence (*5*–*7*). This observation thus cannot be used as evidence that deficiency mapping cannot uncover recessive alleles involved in DMIs. The fourth case, *hmr* in *mel/sim* hybrids, seems to involve dose-dependent lethality, but this effect is seen only when genotypes have a dosage different from that of wild-type hybrids [i.e., *mel/sim* and *sim/mel* female hybrids carrying two copies of hmr^{mel} have reduced viability (*7*)]. Thus, *hmr* does not constitute evidence for Barbash's haploinsufficiency hypothesis.

The only recessive allele that has been identified as part of a DMI and has been tested for the haploinsufficiency hypothesis is $nup160^{sim}$, a nuclear pore protein involved in the hybrid inviability of *mel/sim* hybrid males (*8*, *9*). $nup160^{sim}$

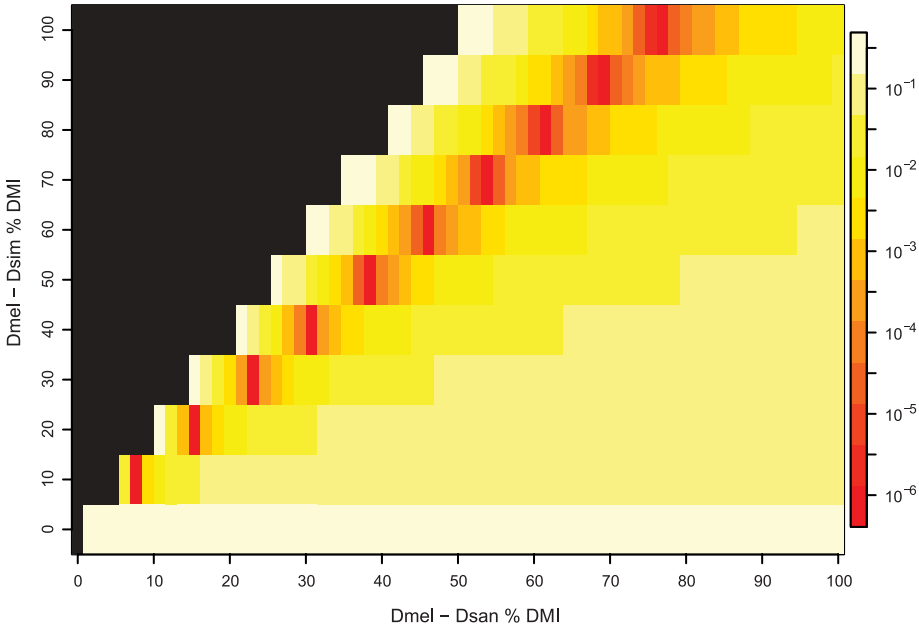

Fig. 1. The snowball effect holds even if some of the candidate regions cause HI through haploinsufficiency rather than by negative epistatic interactions. The heat map shows the probability of inferring a snowball assuming that a proportion of the candidate DMIs cause HI because they are haploinsufficient. Axes represent the percentage of lethals that are bona fide DMIs (the rest would be haploinsufficiencies) in the two crosses studied. The red color represents the highest weighted AIC for the quadratic model; the black represents the highest weighted AIC for the linear model (i.e., no snowball effect). Red: Fit of a quadratic model is higher than in (*1*). Yellow: A quadratic model is strongly preferred over a linear model. Black: A linear model is preferred over a quadratic model.

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behaves identically in both pure species and hybrid backgrounds in both hemizygous and homozygous form [i.e., homozygous and hemizygous *nup160*^{sim} are both lethal in *mel/sim* hybrid males (*8*, *9*)]. Given that this hypothesis is based on only one study with only one gene, it remains to be seen whether the "pattern" will hold the test of time.

Using pole cell transfers, Sanchez and Santamaria (*10*) were able to obtain viable F_1 *yak/mel* hybrids of both sexes. Barbash argues that these results are a challenge to our own, implying that X-linked lethal regions from *san* are not expected. This argument, however, is weak. First, Barbash compares the viability of *mel/san* hybrids with that of *yak/mel* hybrids. Given that these two hybrids have different cytoplasmic elements (e.g., the mitochondrial genome), it is not valid to compare the epistatic interactions that take place in these two hybrids (*11*, *12*). Second, Barbash claims that, because there are at least 13 HI genes in the *san* X chromosome, it would be necessary to have 13 partners in the *mel* X chromosome to explain HI in *mel/san* females; he argues the probability of this event as 8.8×10^{-10}. This calculation is misleading because we don't know how many *mel* alleles that interact with *san* lethal recessives reside in the X chromosome. Additionally, the calculations made by Barbash do not take into account the large effect of the X chromosome in hybrid breakdown (*13*). Given that the genomic distribution of bona fide DMIs and hybrid-specific haploinsuficiencies remains unknown, it is not possible to calculate the probability of the events described by Barbash.

To assess the robustness of our conclusions (i.e., the existence of a snowball effect), we made a model assuming that a proportion of the candidate DMIs cause HI because they are haploinsufficient (but only in hybrid backgrounds) and then calculated the likelihood of misinferring a snowball (nonlinear) effect. We calculated the probability of inferring a snowball effect assuming that a certain proportion of the lethal regions were not bona fide DMIs but haploinsufficiencies in both crosses. Our results (Fig. 1) demonstrate that the snowball effect can be misinferred only if two conditions apply simultaneously: (i) if more than 50% of the genes causing inviability in the *mel-san* cross are haploinsufficients and (ii) if the number of *mel-sim* DMIs is close to, or higher than, the real number (i.e., there is no haploinsufficiency in *mel/sim* hybrids). These results suggest that for haploinsufficiencies to be a confounding factor and lead to a spurious finding of a snowball effect, it would be necessary for the hybrid genetic background in *mel/san* hybrids to be diverging faster than linearly. To our knowledge, there is no way to explain why the hybrid genetic background is "sensitized" at a rate that increases quadratically with divergence time unless one invokes negative epistatic interactions in hybrids. Either way, counting these alleles constitutes a good proxy for the number of DMIs in hybrids.

Barbash does not explain why the number of haploinsuffiencies should increase faster than linearly with divergence time, but such an increase, even if lethality reflects haploinsufficiency rather than recessive lethality, is an explicit prediction of the DMI theory. For these reasons, we consider that the haploinsufficiency hypothesis needs to be formally tested, but the arguments made by Barbash do not alter any of the conclusions made by Matute *et al*. (*1*).

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