Response to Comment on "Evolutionary Paths Underlying Flower Color Variation in *Antirrhinum*"

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Rausher's assertion that divergent selection across a hybrid zone is incompatible with adaptive ridges, although intuitively reasonable, is incorrect on several fronts. His alternative hypothesis of a single moveable peak predicts a sharp environmental transition across the hybrid zone that is not supported by observations. Thus, a high fitness path still provides the most straightforward explanation of our data.

We welcome the opportunity to respond to Rausher's comment (*1*) because it allows us to discuss in greater detail some of the issues raised by our report (*2*). Rausher argues that our results do not support the view that a path of high fitness connects species having recently diverged from a common ancestor because if such a path existed, steep clines could not be maintained at hybrid zones. Consider species or subspecies X and Y, which differ at two loci, *A* and *B*. X has high activity of gene *B* (genotype *aaBB*), whereas Y has high activity of gene *A* (genotype *AAbb*). We can represent this situation by a two-dimensional (2D) genotypic space, with increasing activity of gene A along one axis and gene B along the other (Fig. 1A). Heterozygotes would occupy intermediate positions along each axis, depending on the degree of dominance (for simplicity, we show the

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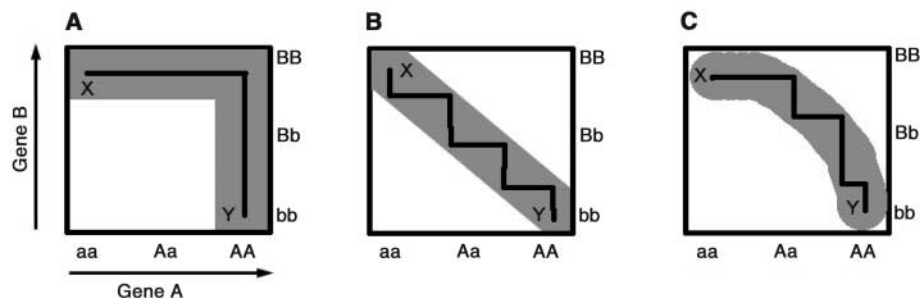*To whom correspondence should be addressed. E-mail: enrico.coen@bbsrc.ac.uk

codominant case in Fig. 1). According to the traditional Dobzhansky-Muller (DM) model, an L-shaped ridge of high fitness (gray area in Fig. 1A) would allow the evolution of reproductive isolation between X and Y without the populations having to pass through an adaptive valley. Rausher asserts that because of well-fit intermediate genotypes along the ridge, there would be no barrier to gene flow to oppose introgression of the *A* allele into X or the *B* allele into Y. This intuition, while reasonable, goes against theoretical predictions of DM models, which show that steep clines can indeed be maintained, depending on population structure and migration rates (*3*).

Rausher (*1*) also assumes that the high fitness path is L-shaped rather than diagonal or curved as in Fig. 1B and 1C. In the latter cases, multiple genetic steps are required to traverse the species gap while staying in the high fitness region, forming a zigzag path. Each element of the zigzag can be thought of as a mini DM step in which allele activity is changed to some degree. Accumulation of several such steps would result in alleles that differ substantially in function. When populations separated by several of these steps meet (i.e., X and Y in Fig. 1, B or C),

there will be selection against spread of both alleles into the parental populations. For example, in the case of species X, introgression of either the *A* or *b* allele would generate lower fitness genotypes (taking the phenotypes toward the low fitness combinations *AABB* or *aabb*). Thus, there would be an even stronger barrier to introgression than for an L-shaped path, allowing a steep cline to be maintained. We found several DNA sequence differences between *ros1* and *ROS1* alleles, as well as a functional difference in the tightly linked *EL* gene (which can be considered as part of the same locus for these purposes), consistent with multiple steps being involved. Rausher's central claim that a fitness ridge is incompatible with a steep cline therefore seems to be incorrect on several fronts.

We would also like to correct Rausher's assertion that we assume that the adaptive landscape is identical in all populations and species. We only propose that the adaptive landscape (or fitness space, as we prefer to call it), as defined by the alleles of the three color genes, may be broadly similar across the species range. We recognize that the adaptive landscape may vary because of differences along genetic dimensions not captured in our 3D genotypic space, as well as environmental differences. Thus, we would be surprised if the detailed profile of the fitness path we propose were to be precisely the same across the species range.

Rausher's favored hypothesis is that flower color in each species tracks a single peak that moves according to the environment. Thus, there would have to be an environmental difference across the hybrid zone to maintain the observed cline, giving a landscape with a peak corresponding to yellow flowers on one side and a different landscape with a peak corresponding to magenta on the other. We have found no evidence to support this view. In particular, there is no obvious transition in habitat, and the same pollinator species are found on both sides of the contact zone and visit both types of flower. The lack of obvious habitat differentiation and differences in pollinator abundance and behavior is in contrast to the situation reported in reference 10 cited in (*1*). In our view, the notion of a high fitness path still provides the most straightforward explanation for all our data.

References
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Fig. 1. 2D genotypic space with the region of high fitness shown in gray and the internal black lines indicating possible genetic paths taken during evolution.