

Dobzhansky–Muller model of hybrid dysfunction supported by poor burst-speed performance in hybrid tiger salamanders

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Abstract

Speciation may result from ‘complementary’ genetic differences that cause dysfunction when brought together in hybrids despite having no deleterious effects within pure species genomes. The theory of complementary genes, independently proposed by Dobzhansky and Muller, yields specific predictions about the genetics of hybrid fitness. Here, I show how alternative models of hybrid dysfunction can be compared using a simple multivariate analysis of hybrid indices calculated from molecular markers. I use the approach to fit models of hybrid dysfunction to swimming performance in hybrid tiger salamander larvae. Poor burst-speed performance is a dysfunction suggesting low vigour and could translate directly into low survival. My analyses show that the Dobzhansky–Muller model fits these data better than heterozygote disadvantage. The approach demonstrated here can be applied to a broad array of nonmodel species, potentially leading to important generalizations about the genetics of hybrid dysfunction.

Hybrid dysfunction was once seen as a paradox, posing species and speciation as major challenges to Darwin’s theory of natural selection (Darwin, 1859 Ch. 8; Huxley, 1908 p. 463; Orr, 1996; Coyne & Orr, 2004 Ch. 7). The problem is that natural selection should not allow the evolution of unfit offspring. The paradox was resolved independently by Dobzhansky (1936) and Muller (1940, 1942), who showed that independent evolution within isolated populations can result in genetic incompatibilities between populations without any population having to pass through a low-fitness transitional state (also see Poulton, 1908; Bateson, 1909; Orr, 1996). The simplest formalization of the Dobzhansky–Muller model begins with a species fixed for an A_1A_1 genotype at one locus and B_1B_1 at another. The species becomes split into two isolated populations. In one isolate, an A_2 allele becomes fixed (either by drift or selection; the point is that the genotypes $A_1A_2B_1B_1$ and $A_2A_2B_1B_1$ are not less fit than the ancestral $A_1A_1B_1B_1$). In the other population, B_2 becomes fixed. Hybrid genotypes combining the two derived alleles A_2 and B_2 may suffer low fitness, even

sterility or inviability because of interactions between these alleles that evolved without deleterious effects within their native genetic backgrounds. That is, hybrid dysfunction results from ‘complementary’ epistasis between loci A and B (Orr, 1995).

This model is a great example of a theoretical ‘oversimplification’ that has proven extremely useful (e.g. Box, 1976). The Dobzhansky–Muller model has stimulated several advances in the theory of evolution of postzygotic reproductive isolation (e.g. Orr, 1995; Orr & Orr, 1996; Gavrillets, 1997, 2004; Turelli & Orr, 2000; Orr & Turelli, 2001; Kondrashov, 2003). For example, the widespread phenomenon known as Haldane’s rule can be explained if incompatibilities generally occur as recessive or partially recessive effects. Haldane’s rule is the empirical observation that, in animals with sex chromosomes, hybrids of the heterogametic sex are more likely to suffer dysfunction than hybrids of the homogametic sex (Haldane, 1922; Orr, 1997). Under the dominance theory (Muller, 1940, 1942; Orr, 1997; Turelli & Orr, 2000), Haldane’s rule occurs when sex-linked Dobzhansky–Muller incompatibilities are fully or partially recessive. That is, incompatibilities are not fully expressed when one or both loci are heterozygous for alleles from the different species, but they are expressed strongly in the heterogametic sex because loci on the sex

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chromosome are hemizygous. The general prediction of the dominance theory (in the notation introduced in the previous paragraph) is that later generation hybrids with genotype $A_2A_2B_2B_2$ should suffer the greatest dysfunction, genotypes $A_1A_2B_2B_2$ and $A_2A_2B_1B_2$ should suffer less than half as much, and the double heterozygote $A_1A_2B_1B_2$ less than half as much dysfunction as the heterozygous-homozygous genotypes (Turelli & Orr, 2000). If this prediction is upheld across different kinds of organisms, it would represent one of the most exciting generalities in evolutionary genetics (Coyne & Orr, 2004).

The Dobzhansky–Muller model was proposed as an alternative to single-gene speciation in which allele A_1 is incompatible with allele A_2 . This scenario is unlikely because the A_2A_2 genotype cannot evolve from the A_1A_1 genotype if the heterozygote A_1A_2 is sterile or inviable. That is, hybrid dysfunction should not be caused by low fitness of heterozygotes, because the evolutionary pathway between the two species is blocked by natural selection ('Darwin's dilemma'; Coyne & Orr, 2004, p. 267). There are two solutions that do allow single-gene speciation to occur. First, if A_1A_2 is not completely inviable or sterile, genetic drift may allow lineages to pass through 'adaptive valleys' created by low-fitness genotypes (Carson & Templeton, 1984). The primary criticism of this idea is that traversable adaptive valleys must be fairly shallow, that is A_1A_2 cannot have very low fitness, therefore hybrid dysfunction is not likely to be strong enough to isolate the incipient species (Barton & Charlesworth, 1984). Alternatively, single-gene speciation can occur unopposed by selection if multiple substitutions occur at the same locus (Nei *et al.*, 1983). If the ancestral genotype was A_1A_1 , and the heterozygotes A_1A_2 and A_1A_3 are viable and fertile, then one daughter species may become fixed for the A_2A_2 genotype while the other becomes fixed for A_3A_3 . Coyne & Orr (2004, pp. 267–268) argued that this latter scenario is unlikely because multiple substitutions at a single locus accumulate slowly. This criticism depends on the assumptions that: (i) hybrid dysfunction evolves rapidly relative to molecular evolution and (ii) the probability that mutations within the same gene are incompatible is not high relative to the probability that mutations in different genes are incompatible. In fact, the rate of evolution of hybrid inviability varies widely (Prager & Wilson, 1975; Fitzpatrick, 2004), and salamanders (including the subjects of this study) often retain the ability to hybridize for millions of years. In addition, the importance of intra-locus vs. inter-locus incompatibility depends on the extent of interaction (epistasis) across the genome, which has yet to be quantified (Malmberg & Mauricio, 2005). Thus, the importance of heterozygote disadvantage vs. Dobzhansky–Muller incompatibilities remains an empirical issue.

Quantitative trait locus (QTL) studies of hybrid dysfunction have generally supported the Dobzhansky–

Muller model (Nairn *et al.*, 1996; Orr & Presgraves, 2000; Presgraves, 2003; Coyne & Orr, 2004; Sun *et al.*, 2004; Malmberg & Mauricio, 2005; Brideau *et al.*, 2006; Noor & Feder, 2006; Sweigart *et al.*, 2006; Willett, 2006; Payseur & Place, 2007). No studies have supported a simple heterozygote disadvantage model, except in cases of F1 sterility caused by chromosome rearrangements (Coyne & Orr, 2004). On the other hand, support for the dominance theory is equivocal. Tao & Hartl (2003) found strong support for recessivity of incompatibilities between *Drosophila simulans* and *D. mauritiana*, but the *Lhr-Hmr* interaction in *D. simulans* × *D. melanogaster* hybrids is dominant (Brideau *et al.*, 2006), and incompatibilities involving electron transport proteins in *Tigriopus* show more complex patterns of additive × dominance epistasis (Willett, 2006). To date, QTL studies have been limited to a taxonomically restricted set of model organisms and, in order to be conclusive, require the detection of genes with large effects. To evaluate the theory more broadly, we need a more general formulation of the Dobzhansky–Muller model with parameters estimable from data in a broader group of organisms.

Turelli & Orr (2000) provide such a formulation. If incompatibilities are limited to pairwise interactions, there are three kinds of incompatibilities possible within an F2 or later generation hybrid. H_0 incompatibilities are due to interactions between complementary loci that are both heterozygous ($A_1A_2B_1B_2$), H_1 incompatibilities are between a homozygous and a heterozygous locus ($A_1A_2B_2B_2$ or $A_2A_2B_1B_2$), and H_2 incompatibilities are between loci that are both homozygous for incompatible alleles ($A_2A_2B_2B_2$). Suppose there are n pairs of complementary genes with dysfunction-causing differences between two species, and they are scattered randomly across the genome. The expected number of each type of incompatibility suffered by a particular hybrid genotype can be expressed in terms of the fraction of loci (or fraction of the genome) that are homozygous for alleles from species 1, p_1 , the fraction homozygous for alleles from species 2, p_2 , and the fraction that are heterozygous, p_H . If incompatibilities are asymmetric (e.g. A_2 and B_2 are incompatible, but the alleles A_1 and B_1 are not), then a given hybrid is expected to have np_1p_2 H_2 incompatibilities, $np_H(p_1 + p_2)$ H_1 incompatibilities, and np_H^2 H_0 incompatibilities. Turelli & Orr (2000) assumed that each incompatibility contributes additively to a hybrid breakdown score:

$$E(S) = n[h_2p_1p_2 + h_1p_H(p_1 + p_2) + h_0p_H^2], \quad (1)$$

where h_i is the average effect of an H_i incompatibility, and that this breakdown score is translated into fitness by a monotone decreasing function. We do not need to know the identities of the genes contributing to hybrid breakdown in order to fit this model to data and estimate the relative magnitudes of the h_i values. In this paper, I illustrate how the Turelli–Orr formulation of the Dobzhansky–Muller model can be evaluated via statistical

analysis of the relationship between individual hybrid phenotypes (such as performance, fertility and viability) and descriptions of individual hybrid genotypes based on ancestry-informative molecular markers (AIMs).

My study system included an array of hybrid tiger salamander larvae from wild populations. I used molecular data and burst-speed trials to evaluate the relationship between the Turelli–Orr hybrid breakdown score (eqn 1) and hybrid performance. In particular, I compared the Dobzhansky–Muller model vs. heterozygote disadvantage as descriptions of hybrid dysfunction in tiger salamanders, and I estimated the relative magnitudes of the parameters in the Turelli–Orr model to evaluate the predictions of the dominance theory.

Methods

Study system

The salamanders *Ambystoma californiense* and *A. tigrinum mavortium* hybridize in California, where *A. t. mavortium* was introduced into the range of *A. californiense* (Riley *et al.*, 2003). A diversity of hybrid genotypes can be found in wild populations, and persistent linkage disequilibrium and habitat-dependent invasion success indicate that incomplete reproductive isolation constrains admixture between the two lineages (Fitzpatrick & Shaffer, 2004). These factors make the *Ambystoma* hybrid zone a good system for studying variation among hybrid genotypes.

Theoretical discussions of hybrid dysfunction have usually focused on inviability or sterility. However, sub-lethal and condition-dependent dysfunction are common in hybrids and hybrid zones (e.g. Sage *et al.*, 1986; Grant & Grant, 1998; Schluter, 1998; Ramsey *et al.*, 2003). These negative phenotypes may have important effects on the outcome of natural selection in the wild, even though they do not cause outright death or sterility. There is no reason to think that the genetic basis of such quantitative hybrid dysfunction is qualitatively different from that of hybrid inviability or sterility. Furthermore, sub-lethal dysfunction can be observed, and the individuals expressing it can be genotyped. In contrast, outright inviability must often be inferred from the absence of certain genotypes.

Poor escape performance is a dysfunction that can directly increase the probability of death in the presence of predators, and more generally reflects lack of vigour that may influence successful feeding, interference competition and mating. Associations between burst swimming speed and survival in the face of predators have been demonstrated in sticklebacks (Swain, 1992), tadpoles (Watkins, 1996), and Guppies (Walker *et al.*, 2005). I used burst speed as a measure of escape performance in tiger salamander larvae, and tested for hybrid dysfunction in performance among individuals collected from wild populations as embryos.

Burst-speed performance

Tiger salamander eggs were collected in February 2001 from four breeding ponds in the hybrid zone between *Ambystoma californiense* and *A. t. mavortium* (Riley *et al.*, 2003). Pond 1 (32 eggs) is site 52 of Fitzpatrick & Shaffer (2007); pond 2 (36 eggs) is site 56, pond 3 (eight eggs) is site 21, and pond 4 (21 eggs) is site 55. Eggs were maintained in captivity until hatching and hatchlings were raised in a common laboratory environment as described by Fitzpatrick *et al.* (2003). When larvae were of 2–3 cm total length, they were tested for fast-start performance, measured, and killed for DNA analysis. This size range was large enough to be convenient for filming but not so large that the animals would no longer be vulnerable to predators in the field (primarily predaceous aquatic insects that themselves become prey for larger tiger salamander larvae).

Methods for measuring performance were described by Fitzpatrick *et al.* (2003). Briefly, each animal was placed in a dish over a 1 cm grid in 3 cm of water. As in nature, all larvae rested on the bottom and therefore ‘ground effects’ were an important part of the environment in all cases. Startle response was elicited by tapping the side of the dish. Escape responses were filmed at 500 frames s^{-1} and each sequence digitized. After smoothing estimates of position over time, I estimated velocity ($cm s^{-1}$) by differentiating each time series.

I used the maximum velocity attained by each individual as its measure of propulsive performance. Each individual was measured only once, leaving within-individual variance as an uncontrolled source of error. I assume that this error is unbiased with respect to genotype so that it may reduce statistical power, but is unlikely to produce erroneous conclusions. It is generally assumed that the startle response of naïve larvae or fry elicits an escape ‘fast start’ at maximal effort; however burst speed might be affected by both physical capability and motivation or fearfulness (Irschick & Losos, 1998; Domenici & Blake, 2000). Thus realized performance (which provides the direct link to the fitness consequences of a particular action) results from morphological, physiological and behavioural factors.

Molecular markers

Each larva was assayed for the eight unlinked nuclear markers used by Fitzpatrick & Shaffer (2004, 2007), derived from Voss *et al.* (2001). These are single-nucleotide differences that distinguish *A. californiense* from *A. t. mavortium* alleles of eight mapped nuclear genes. Each individual was characterized as being homozygous for either species or heterozygous at each marker. I used these marker genotypes to estimate P_T , the proportion of each genome derived from introduced *A. t. mavortium*, P_{CT} , the proportion of each genome heterozygous, P_{TT} , the proportion of the genome homozygous for

A. t. mavortium alleles, and P_{CC} , the proportion of the genome homozygous for *A. californiense* alleles.

These markers represent a small sample of the genome and should not be thought of as candidates or QTL markers. Because of the high level of linkage disequilibrium in hybrid tiger salamander populations (Fitzpatrick & Shaffer, 2004), they can be expected to provide a good estimate of the overall genomic ancestry of an individual because the sampled loci are correlated with other unlinked loci across the genome (Parra *et al.*, 1998; Redden *et al.*, 2006). Estimating genomic heterozygosity based on a few markers is unreliable in large panmictic populations where unlinked loci segregate independently (DeWoody & DeWoody, 2005; but see Aparicio *et al.*, 2007). However, in admixed populations, statistical associations (linkage disequilibria) between unlinked loci decay slowly and can be maintained by immigration and by selection against hybrids (Long, 1991; Barton & Gale, 1993; Barton, 2000; Pritchard *et al.*, 2000). When linkage disequilibrium is high across the genome, a few randomly chosen markers can be used to estimate genome-wide ancestry and heterozygosity (Parra *et al.*, 1998; Redden *et al.*, 2006). For example, a principal components analysis of allele frequencies for this set of markers in the tiger salamander hybrid zone (Fitzpatrick & Shaffer, 2007) found that 94.2% of the variance among ponds could be explained by the first principal component and all unlinked nuclear markers were strongly correlated with that component (variable-variate correlations > 0.97). Linkage disequilibria within ponds in this system are probably maintained by a combination of selection and dispersal between ponds with disparate allele frequencies (Fitzpatrick & Shaffer, 2004, 2007).

Statistical analyses

The predictor variables in the Turelli–Orr model (eqn 1) are products of estimated proportions from multinomial distributions (each individual has potentially unique P_{TT} , P_{CT} , and P_{CC}). As multinomial proportions covary (they must sum to 1), estimates of their products must be corrected for bias. From the definition of covariance, the expectation of a product of random variables is the product of the means plus the covariance (Lynch & Walsh, 1998, p. 817). Similarly, the expectation of the square of a random variable is the square of the mean plus the variance. From the covariances and variances of multinomial proportions, estimators for $P_2 = P_{TT}P_{CC}$, $P_1 = P_{CT}(P_{CC} + P_{TT})$ and $P_0 = P_{CT}^2$, are as follows:

$$\begin{aligned} \hat{P}_2 &= \hat{P}_{CC}\hat{P}_{TT} + \frac{-\hat{P}_{CC}\hat{P}_{TT}}{k} \\ \hat{P}_1 &= \hat{P}_{CT}\hat{P}_{TT} + \hat{P}_{CT}\hat{P}_{CC} + \frac{-\hat{P}_{CT}\hat{P}_{TT} - \hat{P}_{CT}\hat{P}_{CC}}{k} \\ \hat{P}_0 &= \hat{P}_{CT}^2 + \frac{\hat{P}_{CT}(1 - \hat{P}_{CT})}{k} \end{aligned} \quad (2)$$

where k is the number of markers (eight in this case).

I used Akaike's information criterion (AIC and Δ_i ; Burnham & Anderson, 2004) to compare the fit of six models to the data:

$$\text{Model 1: } BS_{\text{hybrid}} = \text{Constant} - (B_2\hat{P}_2 + B_1\hat{P}_1 + B_0\hat{P}_0)$$

$$\text{Model 2: } BS_{\text{hybrid}} = \text{Constant} - (B_2\hat{P}_2 + B_1\hat{P}_1 + B_0\hat{P}_0) + B_T\hat{P}_T$$

$$\text{Model 3: } BS_{\text{hybrid}} = \text{Constant} - B_H\hat{P}_{CT}$$

$$\text{Model 4: } BS_{\text{hybrid}} = \text{Constant} - (B_H\hat{P}_{CT} + B_T\hat{P}_T)$$

$$\text{Model 5: } BS_{\text{hybrid}} = \text{Constant} - (B_H\hat{P}_{CT} + B_{H2}\hat{P}_{CT}^2)$$

$$\text{Model 6: } BS_{\text{hybrid}} = \text{Constant} - (B_H\hat{P}_{CT} + B_{H2}\hat{P}_{CT}^2) + B_T\hat{P}_T$$

(3)

Model 1 is a simple Turelli–Orr model. The coefficients, B_i , estimate the composite parameters nh_i in the Turelli–Orr model (eqn 1), that is, the product of the number of pairwise incompatibilities and the average effect of each type of incompatibility. Model 2 allows for an additive difference between the parental species by estimating a coefficient, B_T , for the relationship between maximum burst-speed velocity (BS) and the proportion of the genome derived from *A. t. mavortium* (P_T). Model 3 allows burst speed to depend only on heterozygosity (P_{CT}), with model 4 including the additive term. Models 5 and 6 fit a quadratic function of heterozygosity. The quadratic term could represent dominance \times dominance epistasis or a nonlinear relationship between the number of underdominant loci and performance. Models 1 and 2 correspond to the Dobzhansky–Muller model where hybrid performance is a function of interactions between genes. Models 3 and 4 correspond to a nonepistatic model where hybrid performance is influenced by heterozygosity (dominance interactions between alleles of the same locus). Note that in the Turelli–Orr model, a simple effect of heterozygosity is inseparable from interactions involving heterozygous loci because $P_{CT}(P_{TT} + P_{CC}) = P_{CT} - P_{CT}^2$. That is, the epistatic Turelli–Orr model includes a nonlinear function of heterozygosity. An alternative treatment, using linear and quadratic functions of heterozygosity (P_{CT}) and ancestry (P_T) following Lynch (1991), is shown in Appendix S1 (see Supplementary material).

I fitted the models via iteratively reweighted least squares using the generalized linear model (glm) function in R 2.3.0 (<http://www.r-project.org>). I included terms for the source pond and the snout–vent length (SVL) of each individual to account for potential effects of these factors on burst speed. After choosing a model based on AIC, I used bootstrap distributions to estimate confidence limits for the fitted parameter values.

Traditional estimates of confidence intervals for regression coefficients do not account for error in the independent variables. The genomic proportions, P_{TT} , P_{CC} , P_T and P_{CT} , were estimated with error due to sampling the genome. Therefore, I used two bootstrap approaches to incorporate this sampling variance into assessments of confidence in my parameter estimates. The standard nonparametric bootstrap (Efron, 1979) simulates the

sampling distribution of estimators, and the Bayesian bootstrap (Rubin, 1981) simulates the posterior distribution of parameters given the data. The standard nonparametric bootstrap resamples the data with replacement, effectively drawing random data sets from a multinomial distribution. The Bayesian bootstrap draws posterior probabilities for each observation in the data set from a Dirichlet distribution (the Bayesian conjugate prior of the multinomial distribution). Operationally, each Bayesian bootstrap replicate is the full data set with each observation assigned a random weight drawn from a Dirichlet distribution.

In both cases, I bootstrapped across molecular markers and across individuals for each of 10,000 replicates. Bootstrapping across markers addresses error in individual estimates of P_{CC} , P_{CT} and P_{TT} due to sampling only a fraction of each salamander's genome. This is in the same spirit as bootstrapping across characters in phylogenetic analysis (Felsenstein, 1985) and results in each individual having associated with it a distribution (bootstrap or posterior) rather than a single-point estimate of the summary statistics P_{ij} . Bootstrapping across individuals addresses errors caused by sampling a finite number of salamanders from each population. As each standard or Bayesian bootstrap replicate includes both sampling processes (sampling markers and sampling individuals) the resulting distributions incorporate uncertainty from both kinds of sampling error. The typical frequentist interpretation of nonparametric bootstrap distributions is that an estimate is significantly different from zero if 95% of its bootstrap distribution is greater or less than zero. The fraction of the Bayesian bootstrap distribution that is greater than zero is taken as an estimate of the posterior probability that the parameter is greater than zero relative to an uninformative prior (i.e. that the parameter is equally likely to be greater or less than zero).

Graphical interpretation

Beyond comparing the fit of the Dobzhansky–Muller model (models 1 and 2) vs. heterozygote disadvantage (models 3 and 4), more detailed inferences about the genetics of hybrid dysfunction can be obtained from the relative magnitudes of the fitted parameters (hence the importance of rigorous evaluation of confidence intervals). The easiest way to see this is by comparing performance surfaces. First, it is important to note that the space of possible genotypes described by genomic proportions is triangular. Because $P_{CC} + P_{CT} + P_{TT} = 1$, these proportions define a ternary coordinate system. The same triangular space can be described by P_T and P_{CT} ; pure native ($P_T = 0$) and pure introduced ($P_T = 1$) individuals have zero heterozygosity, individuals such as F1 hybrids with $P_{CT} = 1$ must have $P_T = 0.5$, but individuals with $P_T = 0.5$ may have any value of heterozygosity. Plotting performance or fitness as a surface over this triangular genotype space allows us to visualize different

models of hybrid fitness (Fig. 1). If fitness or performance is a linear function of ancestry or heterozygosity, then the surface is a triangular section of a plane (Fig. 1a–c). If dysfunction is caused by incompatibilities, the surface is curved (Fig. 1d–f). If incompatibilities are recessive (h_1 and $h_0 = 0$ in eqn 1), only recombinant hybrids with $P_{TT} \neq 0$ and $P_{CC} \neq 0$ express dysfunction (Fig. 1d). If incompatibilities are additive ($h_0 = 1/2h_1 = 1/4h_2$), then all hybrids with intermediate ancestry have the same expected breakdown (Fig. 1e). This occurs because even though heterozygous incompatibilities are less severe than homozygous incompatibilities, maximally recombinant homozygotes ($P_{CT} = 0$, $P_{CC} = P_{TT} = 0.5$, so maximum $P_{TT}P_{CC} = 0.25$) express, on average, only 1/4 of the incompatibilities expressed in the F1 ($P_{CT} = P_{CT}^2 = 1$). By the same logic, F1 hybrids will have the greatest expected dysfunction if incompatibilities are dominant ($h_0 = h_1 = h_2$) because they fully express all possible incompatibilities, but curvature of the surface still distinguishes this case from simple heterozygote disadvantage (Fig. 1c,f).

Results

Model 1, the simple Dobzhansky–Muller model (including terms for SVL and source pond), was the best description of the data according to AIC (Table 1). Adding an extra term for an additive species difference (model 2) did not significantly increase the likelihood, and models 3 and 4, invoking heterozygote disadvantage rather than interlocus incompatibilities, had substantially lower likelihoods (Table 1). According to the Δ_i statistics in Table 1, the models invoking heterozygote disadvantage (3 and 4) had significantly less support than the models invoking epistasis (1 and 2). Models 5 and 6, with quadratic functions of heterozygosity, were also unsupported (Table 1). Table 2 and Fig. 2 illustrate the fit of the Dobzhansky–Muller model to the data. The fitted performance surface is most consistent with additivity of Dobzhansky–Muller incompatibilities (Figs 1e and 2d). The B_i estimates in Table 2 support this interpretation: $B_0 \approx 1/2B_1 \approx 1/4B_2$. However, the estimated B_0 is not statistically distinguishable from zero (with 5% type I error), therefore we cannot formally reject a recessive Dobzhansky–Muller model where heterozygous–heterozygous incompatibilities cause no dysfunction. Nevertheless, almost 95% of the posterior (Bayesian bootstrap) distribution of B_0 is greater than zero, leading me to prefer the hypothesis that $B_0 > 0$ if there is no *a priori* reason to consider complete recessivity the preferred or null hypothesis. An alternative Bayesian perspective on recessivity vs. dominance of incompatibilities is to compare the fraction of the posterior density with B_0/B_1 greater or less than 1/2. From the Bayesian bootstrap distribution, the fractions are 0.43 and 0.57, respectively, providing little reason to prefer either hypothesis.

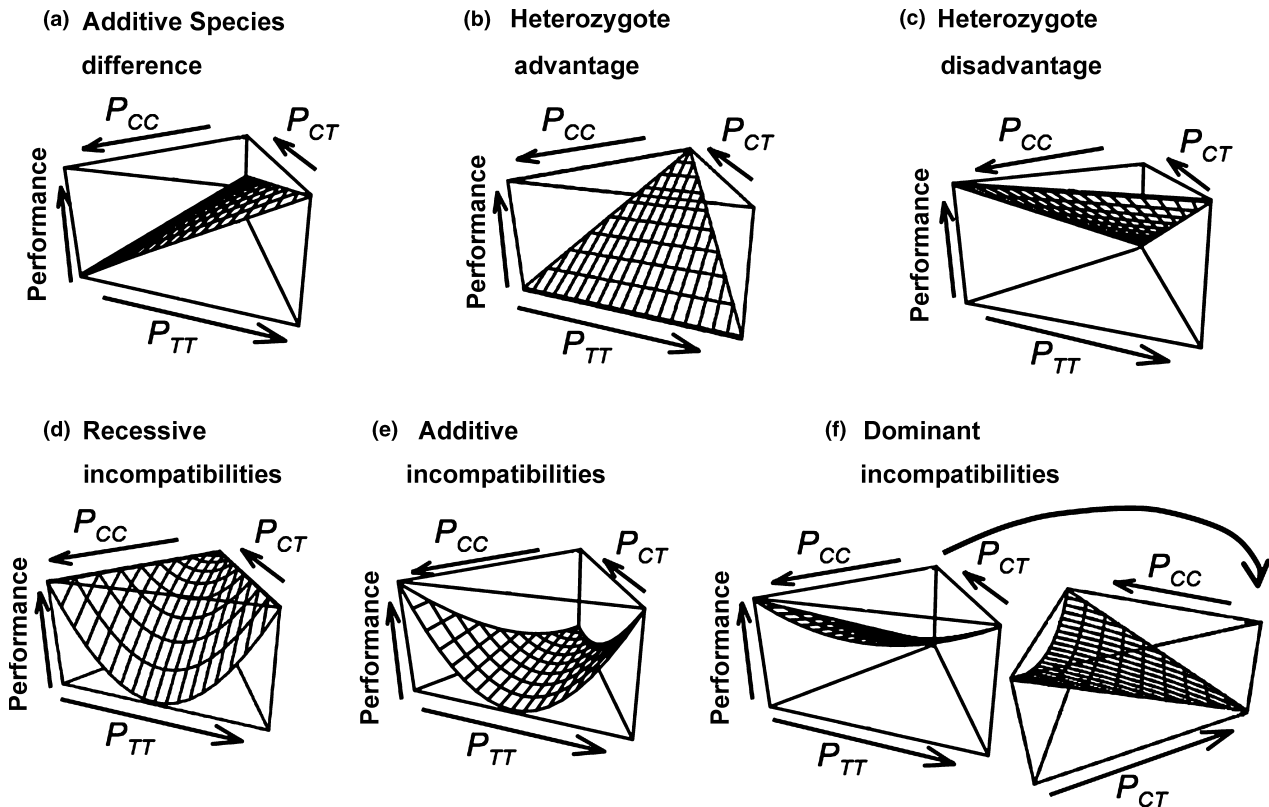


Fig. 1 Hypothetical performance surfaces on the ternary coordinate system defined by the proportion of the genome homozygous for *Ambystoma tigrinum mavortium* alleles (P_{TT}), the proportion homozygous for *A. californiense* alleles (P_{CC}), and the proportion of the genome that is heterozygous (P_{CT}).

Table 1 Comparison of alternative models for the genetics of variation in burst speed performance among hybrid tiger salamander larvae.

Model	R^2	F_{DF}	P	Log-likelihood	AIC	Δ_i
1	0.3419	6.606 _{7,89}	< 0.0001	379.498	-740.996	0
2	0.3476	5.860 _{8,88}	< 0.0001	379.917	-739.833	1.113
3	0.2362	5.628 _{5,91}	0.00015	372.271	-730.542	10.454
4	0.2365	4.645 _{6,90}	0.00037	372.289	-728.578	12.418
5	0.2525	5.066 _{6,90}	0.00016	373.317	-730.633	10.363
6	0.2534	4.316 _{7,89}	0.00037	373.378	-728.757	12.239

F -statistics and associated P -values test each model against the null model of no association between maximum burst speed and any independent variable.

AIC is the Akaike information criterion; a lower (i.e. more negative) AIC represents a better fit for the data. Δ_i is the rescaled AIC (AIC of model i - AIC of best model in the set); models with $\Delta_i \leq 2$ have substantial support and models with $\Delta_i > 10$ have essentially no support (Burnham & Anderson, 2004).

There was no statistically significant variation among source ponds not accounted for by variation among genotypes (Table 2). The relationship between SVL and maximum burst speed was significant (longer larvae

tended to be faster, Table 2), however, leaving SVL out of the model did not greatly affect the parameter estimates for the genetic variables ($B_2 = 0.0414$, $B_1 = 0.0188$, $B_0 = 0.0103$), nor their statistical significance.

Bootstrapping over markers only or over individuals only allowed an assessment of the relative influence of sampling markers vs. individuals on overall standard errors. Standard errors of the coefficients among bootstrap replicates when only markers were resampled (SE_K ; Table 2) were much smaller than standard errors among bootstrap replicates when only individuals were resampled (SE_N ; Table 2). Furthermore, bootstrap standard errors when both markers and individuals were resampled were not much different from the individuals-only standard errors (Table 2). The similarity between inferences from parametric P -values (which do not account for error caused by sampling markers) and bootstrap support values (Table 2) also indicate that the sampling error caused by using a few markers to estimate genome-wide summary statistics is not very large for this data set.

There were no significant associations between burst speed and individual marker genotypes. Separate one-way ANOVAs of burst speed among genotypes never supported rejecting the null hypothesis (with $\alpha = 0.05$)

Table 2 Fitted model for maximum burst speed in hybrid tiger salamander larvae.

Term	B_i	SE	SE_K	SE_N	d.f.	F	Parametric P-value	Bootstrap support	
								NP	Bayesian
Intercept	0.0225	0.0065	0.00136	0.0064	1	0.0004	0.9998	0.9998	
$B_2: P_{TT}P_{CC}$	0.0349	0.0099	0.00658	0.0096	1	12.09	0.0008	0.9970	0.9985
$B_1: P_{CT}[P_{TT}+P_{CC}]$	0.0191	0.0088	0.00494	0.0074	1	5.27	0.0241	0.9550	0.9827
$B_0: P_{CT}^2$	0.0075	0.0047	0.00187	0.0045	1	2.31	0.1325	0.9259	0.9467
SVL	0.0010	0.0004	0.00007	0.0004	1	6.85	0.0104	1.0000	1.0000
Pond	Na				3	0.88	0.4556		

B_i is the fitted regression coefficient.

SE is the standard error of the coefficient estimated from the nonparametric bootstrap distribution (bootstrapping across both markers and individuals).

SE_K and SE_N are bootstrap standard errors when bootstrapping across only markers and only individuals respectively. Bootstrap support values are the proportions of each distribution in which the regression coefficient was greater than zero.

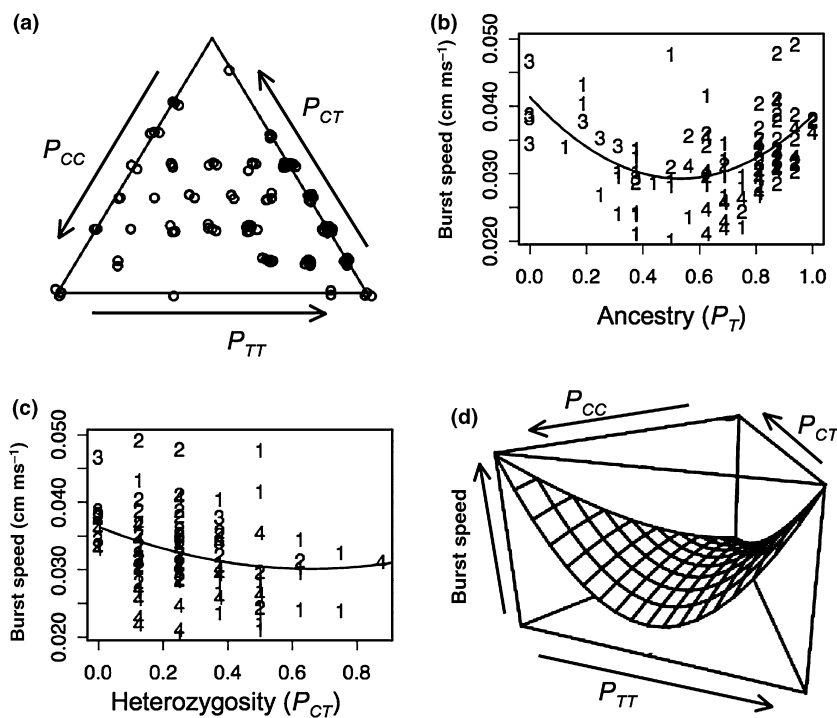


Fig. 2 (a) The distribution of observations on the ternary coordinate system as in Fig. 1. (b) The relationship between burst speed and individual ancestry (P_T). Pure *Ambystoma californiense* have $P_T = 0$, pure *A. tigrinum mavortium* have $P_T = 1$, and hybrids with mixtures of alleles from each parental lineage fall in between. The line is a quadratic regression: $y = 0.041 - 0.045P_T + 0.043P_T^2$ ($r^2 = 0.25$, $F_{2,94} = 16.1$, $P = 9.7 \times 10^{-7}$). Each data point is represented by a numeral corresponding to its source population. (c) The relationship between burst speed and individual heterozygosity (P_{CT}). Pure parentals both have $P_{CT} = 0$, F1 hybrids have $P_{CT} = 1$, and other hybrids fall in between. The line is a quadratic regression: $y = 0.036 - 0.019P_{CT} + 0.014P_{CT}^2$ ($r^2 = 0.095$, $F_{2,94} = 4.9$, $P = 0.009$). (d) The full Dobzhansky–Muller model fitted surface (Table 2) over the ternary coordinate system for comparison with Fig. 1.

that *CC* homozygotes, *TT* homozygotes and *CT* heterozygotes had the same average burst speed (detailed results available from the author). Taken together with the nonparametric bootstrap results, this shows that the patterns in Table 2 and Fig. 2 are unlikely to be driven by a single locus. Rather, genotypes for a single marker are uninformative outside the context of the multilocus genotype.

Discussion

My analyses show that the Dobzhansky–Muller model explains slow burst speed of hybrid tiger salamander

larvae better than heterozygote disadvantage. Though rejection of heterozygote disadvantage may not be surprising in the light of existing data (Coyne & Orr, 2004), its theoretical plausibility (Nei *et al.*, 1983) means that it cannot be dropped from consideration. The Dobzhansky–Muller model has been studied primarily in the context of hybrid sterility and inviability of model organisms such as *Drosophila* (Coyne & Orr, 2004). My approach in this study illustrates how the Dobzhansky–Muller model can be evaluated in nonmodel systems and applied to sub-lethal and condition-dependent hybrid dysfunction in addition to sterility and inviability. The power of the Dobzhansky–Muller model to account for

poor swimming performance in hybrid tiger salamander larvae strongly supports the generality of 'conspecific epistasis' as an important outcome of genome evolution and cause of reproductive isolation (Orr & Presgraves, 2000).

The apparent additivity of incompatibilities contributing to hybrid dysfunction in burst speed is somewhat at odds with previous data and theory on the Dobzhansky–Muller model. Inviability and sterility are thought to result most often from recessive or partially recessive incompatibilities in *Drosophila* and *Lycopersicon* (Presgraves, 2003; Tao & Hartl, 2003; Coyne & Orr, 2004; Moyle & Graham, 2005), though dominant incompatibilities are not unheard of (Brideau *et al.*, 2006; Sweigart *et al.*, 2006) and Willett (2006) showed complex patterns of additive \times dominance epistasis among three electron transport loci in *Tigriopus*. Recessivity of incompatibilities is an important part of the dominance theory explanation of Haldane's rule (the tendency for the heterogametic sex to suffer greater hybrid dysfunction in the F1 generation; Turelli & Orr, 2000). Tiger salamanders do not have degenerate sex chromosomes (Cuny & Malacinski, 1985), therefore they are not subject to Haldane's rule. However, the importance of additive, recessive and dominant incompatibilities is general as it relates to the relative fitness of highly heterozygous genotypes such as F1 hybrids vs. highly recombinant genotypes with many homozygous–homozygous interactions (Fig. 1). As noted by Coyne & Orr (2004), the value of the dominance theory and other hypotheses is not simply in explaining Haldane's rule *per se*, but in suggesting broad generalities about the evolution of hybrid dysfunction in very different kinds of organisms.

Two issues that may affect the reliability of inferences based on the statistical approach used in this study are the relationship between individual genotype and the Turelli–Orr hybrid breakdown score, and the relationship between the breakdown score and the phenotype of dysfunction. First, recall that $E(S)$ is the *expected* breakdown score for genotypes sharing the same P_{TT} , P_{CC} and P_{CT} . The variance around that expectation depends on the diversity of genotypes sharing the same summary statistics and the distribution of effects of incompatibilities. The h_i parameters in the Turelli–Orr model are average effects per pairwise incompatibility, and their use in calculating $E(S)$ may be justified only if their magnitudes and variances are small, and if most dysfunction is due to pairwise incompatibilities rather than more complex interactions (Turelli & Orr, 2000). The fact that hybrid dysfunction accumulates gradually with genetic divergence in most groups of animals studied (Coyne & Orr, 2004; Bolnick & Near, 2005) suggests that many incompatibilities of relatively small effect usually contribute to hybrid breakdown, and therefore Turelli & Orr's (2000) $E(S)$ is probably valid. The relative importance of pairwise vs. more complex incompatibles is unknown, but pairwise incompatibilities are probably

most common in the early stages of divergence, whereas more complex incompatibilities are likely to arise as more differences accumulate (Orr, 1995).

Secondly, Turelli & Orr (2000) did not specify a function linking $E(S)$ to fitness or performance, requiring only that it be monotonic. I have reported results for the simplest, linear function. However, it is easy to fit an exponential or logistic relationship by \log_e and logit transformations, respectively, of the dependent variable (for the logit, the variable should be first transformed so that it ranges from just over zero to just less than one). Applying those transformations to my data did not substantially affect the fit between model and data; the best fit in both cases was model 1 with $R^2 = 0.353$ for the exponential and $R^2 = 0.303$ for the logistic. Further, in both cases the fitted performance surface was indistinguishable from Fig. 2d, and parameter estimates were consistent with $B_0 \approx \frac{1}{2}B_1 \approx \frac{1}{4}B_2$. Thus, at least for the range of genotypes and burst speeds in this study, the main conclusions are not sensitive to the function linking hybrid dysfunction to the Turelli–Orr breakdown score; the Dobzhansky–Muller model explains the data better than heterozygote disadvantage and incompatibilities appear to be additive, on average.

An alternative framework for studying the genetics of hybrid fitness was described by Lynch (1991); also see Hill, 1982; Lynch & Walsh, 1998; Rundle & Whitlock, 2001). Lynch related phenotypes of hybrids to the quantitative genetic concepts of additive effects, dominance, additive \times additive epistasis, dominance \times dominance epistasis and additive \times dominance epistasis using a polynomial function of ancestry and heterozygosity. As shown in Appendix S1, the Turelli–Orr model can be expressed as a special case of Lynch's model and estimates of B_i (eqn 2) calculated from fitted coefficients of Lynch's model. Comparison of the Lynch and Turelli–Orr frameworks leads to two interesting insights. First, when Dobzhansky–Muller incompatibilities are additive, hybrid dysfunction is attributed to additive \times additive epistasis in Lynch's framework. Secondly, the 'dominance effect' in Lynch's framework is affected by between-locus incompatibilities unless incompatibilities are exactly additive, i.e. 'dominance' is not necessarily a simple combination of single-locus dominance effects. In any case, analysis of my data using Lynch's framework agrees with the results in Table 2 (Appendix S1).

I have illustrated a simple approach to estimating parameters of the Turelli–Orr formulation of the Dobzhansky–Muller model using variables estimable from molecular marker data from natural hybrids. This allows the study of the genetics of hybrid dysfunction to extend to nonmodel organisms and components of dysfunction other than inviability and infertility. My results from tiger salamander burst-speed performance support the Dobzhansky–Muller model of genic incompatibilities as the cause of hybrid dysfunction (or at least abnormal trait expression) and suggest that most incompatibilities are

approximately additive in their effects on burst speed. Extension of this approach to other taxa, particularly those subject to Haldane's rule, is likely to yield valuable generalizations regarding the genetics of hybrid dysfunction. To link these results to the dynamics of hybridization between *A. californiense* and *A. t. mavortium*, it will be necessary to understand the relationship between burst speed and survival in the field and to apply genetic analyses to other components of tiger salamander fitness.

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Supplementary material

The following supplementary material is available for this article:

Appendix S1 The Tuvelli–Orr model as a special case of Lynch's model of hybrid fitness.

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1420-9101.2007.01448.x>

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