

too many discrepancies known to affect cofactor binding? The answer resides in the widespread phenomenon of genetic redundancy, in the existence of mechanisms of genomic turnover that can slowly homogenize new mutations through redundant genetic elements, and in the cohesive manner by which a sexual population can be changed by such mechanisms from one genetic composition to another. The detailed arguments on how 'molecular coevolution' might become established are given elsewhere^{2,3}. Clearly the buffering provided by redundancy and the slow homogenization experienced by redundant copies, uniformly across a sexual population, ensures that selection has the time and relaxed conditions to seek out variant alleles at the cofactor genes whose proteins are better able to effectively bind with the emerging set of newly homogenized cofactor binding sites within promoters.

Now, I do not know if the *est6/5* promoters contain redundant multiple binding sites; however, I would be surprised if they didn't, given what is known about the multiplicity of binding sites in the promoters of many other eukaryotic genes, including, of course, the important hierarchy of genes controlling *Drosophila* development. Indeed, many of these genes (e.g. *Kruppel*, *engrailed*, *hairy*) have multiple binding sites for different kinds of transcription factors with varying levels of binding affinities, such that the level of gene expression is the net result of several, redundant, combinatorially interacting, regulatory elements. Our own recent studies of suggestive changes between *D. melanogaster* and *Musca domestica* regarding the homeobox of the *bicoid* gene and the multiple *bicoid* binding sites in front of the segmentation gene *hunchback* could be a case of molecular coevolution in the making (C. Fazakerley, unpublished). Furthermore, the 5' region upstream of *Drosophila Adh* is known to be riddled with the fine-grained repetitive products of slippage-like turnover mechanisms⁴. Not only does this mean that it is illegitimate to compare divergence in such regions with point-mutational differences in the third positions of codons (the P/S ratio in Oakeshott *et al.*, and similar comparisons in *Adh*⁴), but such turnover in binding site number and composition could result, ultimately, in eliciting a compensatory, molecular coevolutionary response by the cofactors, if function is not to be lost.

Hence, a biological function can be maintained between species, but the specific molecular basis by which the requisite molecules interact to bring about the function has changed. This in turn leads to a testable incompatibility between say molecule A from one species and molecule B in another, in an A-B interaction. This is precisely what is observed in several genetic systems^{3,5}, starting with the highly redundant rDNA promoters and the RNA polymerase I complex of cofactors where 'molecular coevolution' was first described². 'Molecular coevolution' is best viewed as an outcome of the interaction between genomic homogenizing systems and natural selection.

Redundancy ensures that there is a much higher degree of tolerance between functionally interacting molecules than the usual lock-and-key imagery of natural selection, acting alone, would lead us to believe. If a cofactor is hardwired by selection into a single binding site, then any mutational change in the latter would cause problems for the former. Yet, as Oakeshott *et al.*

rightly point out, this is not what we see in the case of *est5/6* between two particular *Drosophila* species. This is not to suggest that every mutational change elicits a corresponding change in the interacting molecules (indeed, areas of relative sequence conservation are observed between the *est6* promoters of sibling species of *D. melanogaster*); nevertheless, greater than expected flexibility is at the heart of the matter.

There are clear functional tests and analytical DNA approaches capable of dissecting the internal structure of the *est5/6* promoters and revealing whether 'molecular coevolution', based as it is on a degree of inevitable turnover among redundant genetic elements, can explain the 'esterase enigma'.

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Reply from J.G. Oakeshott *et al.*

Dover's model of molecular coevolution could explain the enigma that a majority of *Est6* expression sites are conserved across *Drosophila* species despite wholesale changes in the sequence of the *Est6* promoter across these species¹. In support of Dover's argument, there are indeed several short (6 to 10 bp) repeats along the promoter that might provide the redundancy of binding sites for tissue-specific transcription factors that his model seems to require². As Dover points out, it is now important to carry out a fine-scale functional dissection of the *Est6* promoter in order to determine whether or not the repeated sequences lie within particular tissue-specific control elements.

Our major question about Dover's model concerns his assumption that a promoter will contain multiple functionally redundant control elements for tissue-specific expression. Dover cites a few promoters where there is indeed evidence for some functional redundancy. However, as he acknowledges, there is also evidence from a multitude of promoters that tissue-specific control elements act coordinately through a complex set of epistatic interactions. Different promoter elements are not simply independent and additive in their effects. Other models of promoter evolution, such as Dickinson's^{3,4} for example, point to these 'combinatorial networks' among promoter elements as a major constraint on regulatory change. Such networks may be compatible

with Dover's model but it is not clear to us that newly duplicated copies of individual promoter elements would be likely to provide a functional template for further regulatory evolution.

One study of *Est6* illustrating potential constraints on promoter-sequence evolution is about to appear in *Genetics*⁵. This study compares the sequence and function of several examples of the *Est6* promoter isolated from a field population of *D. melanogaster*. It finds, essentially, just two allelic forms of the promoter. These forms differ in their effects on *Est6* expression. However, they differ from each other at not one, but 14 nucleotide sites, and these sites are distributed over about a third (325 bp) of the *Est6* promoter (coordinates –494 to –819 in Box 2 of Oakeshott *et al.*¹). In this case it appears that a substantial block of *Est6* promoter sequences are not evolving independently.

Finally, we wish to correct two typographical errors in Table 2 of Oakeshott *et al.*¹. The divergence in glycosylation sites among *D. melanogaster* alleles should be 0.04, not 10.04 as shown, and footnote (a) should say that variation was calculated as the average pairwise divergence.

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Species diversity and stability in grassland

In his comment on our work on the Park Grass Experiment, Scott Collins¹ seriously misrepresents our results and consequently comes to the erroneous conclusion that there is a contradiction and/or an artefact in our data. He suggests that the relatively low biomass variability (CV) we found in plots with high biomass, and the relatively high variability of plots with low species richness is contradictory because biomass and species richness are negatively correlated. In fact, there is no contradiction here because we used multiple regression to simultaneously include the effects of biomass and species richness on CV. This model demonstrates² that biomass and species richness had opposing effects on CV, and for a given plot

biomass there was a tendency for more species-rich plots to be less variable than species-poor plots. We were careful not to offer a biological interpretation of the relationship between biomass and biomass variability (CV) because these two variables are not independent of each other. Hence, none of our conclusions was based on the relationship between biomass and CV, and Collins is wrong to impute that an artefact in this relationship could account for our results. The problem he tried to explain as the product of an artefact simply doesn't exist. Our data are in agreement with those of Tilman and Downing³ about the positive relationship between diversity and stability.

In further pursuing a solution to a non-existent problem Collins makes another error in suggesting that Tilman and Downing measured variability over time, while we measured variability over space. In fact both studies^{2,3} compared biomass variability over time for plots with different species-richness. The chief difference between the two studies was the way in which temporal variability was measured and the fact that we were able to test for a relationship between species-richness and CV on 42 occasions between 1862–1991. It is regrettable that Collins spent so much time chasing these will-o'-the-wisps when there were genuine puzzles revealed by our analysis. One of our most intriguing findings was the discovery that almost a quarter of the species on non-acidified plots showed outbreaks near the middle of a 60-year time series that we analysed⁴. This was quite unexpected in communities that are otherwise stable and suggests that the concept of the stable plant community must be re-evaluated.

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Reply from S.L. Collins

Silvertown *et al.* are concerned that I misrepresented their research methods in such a way that I created a non-existent problem. If I have misrepresented their research, I am only too happy to have that information corrected.

I disagree, however, with two statements in their reply. First, they noted as intriguing that species abundances fluctuate over time, and that the concept of the stable plant community must be re-evaluated. Re-evaluation of the stable plant community is not newsworthy. Such results have been reported previously in undisturbed vegetation (e.g. Ref. 1), and the concept of the stable plant community has long since given way to the non-equilibrium paradigm². What is important in their research is analysis of the relationship between stability and diversity. Dodd *et al.*³ measure biomass stability as the CV of biomass over an 11-year window, centered on the year in question. They use this approach 'to avoid prejudging the causal relationship between species richness and biomass variability'. It also implies considerable influence of the year in question on the relationship five years into the future or five years into the past. Nevertheless, if I understand their approach, these CVs in a given year are then compared among the different plots in space. This is not a direct temporal measurement of the effect of richness on biomass as found in Tilman and Downing⁴. Thus, I still contend that Dodd *et al.* use a spatial approach, whereas Tilman and Downing use a temporal approach to the problem.

Overall, the purpose of my article was to highlight what I believed to be an important attempt to understand the relationship between richness and biomass stability using long-term data from permanent plots. Issues of spatial and temporal scales, and the associated mechanisms involved in this relationship, still require attention.

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The fitness of hybrids

In their recent *TREE* article on the relative fitness of hybrids to their progenitors, Arnold and Hodges¹ draw attention to the often underemphasized role of hybridization in the process of evolutionary diversification. Their compilation of several studies involving both natural and manipulative estimates

of relative hybrid fitness reveals that a general pattern of reduced hybrid fitness is not supported by the available data. The authors discuss potential caveats with regard to this interpretation but the breadth of taxa represented in their review, as well as the variety of fitness components quantified, seem to support this overall conclusion. Our purpose here is to mention two additional interpretive caveats not addressed in their article. One involves a potential bias in the inclusion of taxa that are studied, and the other involves the importance of environment-dependent fitness.

Studies of hybrid fitness are often motivated by a desire to understand how hybrid zones remain stable, as well as a desire to understand how hybridizing species maintain their integrity in the face of interspecific gene flow. Thus, it seems likely that organisms used in hybridization studies often will have been chosen because hybrids of the two parental species are relatively common. It is perhaps not surprising, therefore, that many of these hybrids do not suffer any major reduction in fitness (i.e. unfit hybrids would be likely to be rare in nature). Consequently, the generality of conclusions about hybrid fitness that are based on the evaluation of currently available data may be questionable.

Secondly, as suggested by Arnold and Hodges, natural hybrids may exist in one of the parental niches or they may occupy a novel, third niche. This latter possibility is embodied in the 'bounded hybrid superiority' model² and is undoubtedly important in the maintenance of many hybrid populations. When hybrids must 'make a living' in either or both of the parental niches, however, manipulative studies are more difficult to interpret. For example, when hybrid performance has been evaluated in both habitats, intermediate fitness relative to the parents in each of these habitats can still translate into overall selection against hybrids. If reduced hybrid performance in one habitat (relative to that parent) is not adequately compensated for by performance in the other habitat, hybrids will be at a selective disadvantage, despite them exhibiting intermediate performance in each habitat separately. This idea is most clearly depicted as a concave fitness set³. Results of hybrid performance in *Gasterosteus*^{4,5} are suggestive of this pattern of environment-dependent fitness. Further experiments of this type such as the reciprocal introductions proposed by Arnold and Hodges would greatly clarify the relevance of this last remark.

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