corresponding sequence from rap1 GAP, which appears superficially to be the most similar in this region to *pn* according to Fig. 3*d* of ref. 1, no significant alignment was produced; in fact neither did the rap1 GAP subsequence when compared to the Ras GAP subsequences (contrary to the implications of Fig. 3*d*). Indeed, it has also been the impression of others that rap1 GAP is not significantly similar to any other protein, either compared globally, or using this subsequence[8].

Consequently, we find that there is no similarity between *pn* and GAPs — *pn* is as likely to be a GAP as is any randomly chosen protein. In the absence of confirmatory biochemical or genetic data concerning the model proposed by Teng *et al.*[1] must therefore be regarded currently as untenable. Because their model fails to explain the known biochemical defect in eye pigment production in *pn* flies[9,10], the simplest and not very unusual explanation would be that *pn* encodes a novel protein of unknown function.

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VENKATESH AND TENG REPLY — It is evident using computer comparison programs that the deduced TcD37 protein of *Drosophila* does not exhibit a high degree of homology with GAP or GAP-like proteins and might therefore be a novel protein with an unknown function. However, by these very same criteria (as noted by Barnes and Bürglin and others[8]), computer analysis would not predict that mammalian rap1 GAPs are related to Ras GAPs. Yet both of these subclasses of proteins have been shown to function as GTPase activating proteins[8,11–13]. In addition, other proteins, such as the products of JC99 and JC265, which have marginal similarity to GAPs also appear to participate in the biochemical pathway involving Ras[14]. Thus, based on the available sequences of GAP and GAP-like proteins, it seems that these proteins are quite divergent and that the assessment of their function solely on the basis of significant homology would be inadequate.

The model we have proposed to explain the lethal interaction between *pn* and $awd^{k\text{-}pn}$ is not only based on sequence similarity but also draws upon our current understanding of these two loci. The lethality of *pn* $awd^{K\text{-}pn}$ double mutants is apparently due to the expression of $Awd^{K\text{-}pn}$ in combination with a lack of Pn (ref. 15). The *awd* locus encodes an NDP kinase[3] and $awd^{K\text{-}pn}$ is a missense mutation that appears to yield a protein product with a neomorphic function (A. Shearn, personal communication). It has been suggested that NDP kinases (like Awd and Nm23) regulate some critical biological processes, such as development and tumour metastasis, via a GTP-binding protein[16,17]. This hypothesis is supported by studies that implicate NDP kinases in the effector activation of certain G proteins[18–20], and three different NDP kinases can activate the small G protein, ADP-ribosylation factor (Arf), by the direct phosphorylation of Arf-GDP to Arf-GTP (ref. 21). Furthermore, contrary to Barnes and Bürglin's statement that Awd is principally microtubule-associated, immunological and biochemical studies indicate that only a small proportion of the enzyme appears to be associated with cytoskeleton while the most of it is found in other subcellular locations, such as the cytosol and nucleus (A. Shearn, personal communication). Because much of the non-microtubule-associated NDP kinase is probably also Awd protein (as homozygous *awd* mutants have less than 2% of the total enzyme activity of wild-type larvae[3]), this subcellular distribution suggests that Awd may provide NTPs for more than one biological process. In the light of this information, we have proposed a new model as one possible explanation of the *pn* $awd^{K\text{-}pn}$ lethality. As with any model, its validity is subject to the rigours of testing by molecular, biochemical and genetic approaches.

Finally, Barnes and Bürglin state that our model fails to account for the pigment biosynthesis defect in the eyes of *pn* flies. But, as noted by Ruggieri and McCormick[2], it is conceivable that the hypothetical Ras-like G protein modulated by Awd and Pn is also involved in regulating the biosynthesis of pteridine pigments in *Drosophila*.

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Are *Anolis* lizards evolving?

SIR — Since Hurlbert[1] published his paper on experimental design in 1984, ecologists have been particularly careful to replicate their experiments. However, experiments without adequate replication are still occasionally performed, and the recent report of selection in *Anolis* lizards[2] is an example. This experiment involved measuring differential survival of lizards with respect to their morphology. Morphologically distinct lizards from four populations from different habitats (ecotypes) were maintained in separate enclosures in one habitat. There was significant differential survival of both males and females in just one of the enclosures. Because each ecotype was maintained in only one enclosure there was no replication within ecotypes. The key result was that selection (differential survival) occurred in the ecotype derived from the most distinct habitat.

The significant selection in the one enclosure could be due to this ecotypic difference, as the authors argue, but it may also be due to density (much lower than in other enclosures), or average body size (much higher than in other enclosures). More seriously, it could be random. This can be illustrated using a thought experiment, such as the following 'fox-test': if one rogue fox that ate lizards and led to differential survival of different morphologies is dropped into the experiment at random, one-quarter of the time it will land in the enclosure that contains the ecologically most different morphotype, and all results follow. The probability of the association between intense selection and location is thus 0.25, which is clearly not significant. By this I do not mean to imply that foxes are the selective agent, but rather that something peculiar to one enclosure can cause the apparent selection. Replication of treatments over experimental units is the only way to estimate and control for such random variation. Inferential statistics such as ANOVA, which test for treatment effects, require an estimate of error within treatments. In this case, the treatment variable is ecotype, for which there is no replication (only one enclo-