

Developmental buffering: how many genes?

James S. Patterson and Christian Peter Klingenberg*

Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT, UK

*Author for correspondence (email: cpk@manchester.ac.uk)

The question of how phenotypic variation is regulated has attracted an increasing amount of attention in recent years (Debat and David 2001; Flatt 2005). In particular, the discovery of the intriguing effects of mutations of heat-shock protein 90 (Hsp90) on phenotypic variability (Rutherford and Lindquist 1998; Queitsch et al. 2002) sparked a great deal of interest in the question of whether there are specific genes with key roles in the regulation of phenotypic variation and evolvability (e.g., Wagner et al. 1999).

A number of studies have been published in the past several months that addressed a range of questions concerning the buffering role of Hsp90 in flies (Carey et al. 2006; Debat et al. 2006; Milton et al. 2006; Gibert et al. 2007), fish (Yeyati et al. 2007) and plants (Sangster et al. 2007). Together with some other recent studies, these articles offer some new perspectives on the genetic basis of developmental buffering.

HSP90 AND ITS EFFECTS ON DEVELOPMENTAL BUFFERING

One clear conclusion from reading these recent articles may be that the Hsp90 story has become very complicated. Whereas the broad range of different phenotypic traits affected by inhibition of Hsp90 activity initially suggested that this protein might have a very general effect on developmental buffering, the recent studies in *Drosophila* have yielded a fairly lengthy list of traits that are and are not affected. Of the six different bristle counts studied by Milton et al. (2006), buffering by Hsp90 appeared to be limited to those that are normally invariant. Wing size did not respond to the inhibition of Hsp90, but had a low degree of variability for all genotypes (Milton et al. 2006). Analyses of shape variation yielded inconsistent results, as neither pharmacological inhibition of Hsp90 nor one of two mutations increased the amount of shape variation, whereas a second mutation only did so in one of two genetic backgrounds (Debat et al. 2006). In those situations where Hsp90 did have an effect, it tended to affect both the variation in the individual averages of left and right wing shapes and the fluctuating asymmetry of shape (Debat et al.

2006). Moreover, Carey et al. (2006) showed that selection for an abnormality of the eye that is buffered by Hsp90 has no correlated effects on several fitness correlates, and is therefore limited to the selected trait itself.

Overall, therefore, the buffering by Hsp90 seems to be limited to some specific morphological traits, such as particular bristle counts, but appears not to affect others. Milton et al. (2006) discuss the effects of Hsp90 in the context of a model of the control of specific signalling pathways involved in bristle formation, which may be the mechanistic basis for the switch between different numbers of bristles. Similarly, experimental evidence indicates that Hsp90 can affect the degree of pigmentation of the abdomen by genetic interactions with specific components of a regulatory network (Gibert et al. 2007).

This emphasis on specific molecular interactions also sheds new light on the relatively minor effects of Hsp90 on traits resulting from the aggregate activity of many developmental and physiological processes, such as shape and life-history traits (Carey et al. 2006; Debat et al. 2006). Even though Hsp90 may also be involved in some of these processes, its effects may be diluted by the action of many other regulatory processes.

Although most studies of Hsp90 and phenotypic variability have been conducted in *Drosophila*, similar patterns appear also to hold for other organisms. In zebrafish, pharmacological inhibition and knockdown of Hsp90 induced a range of specific abnormalities depending on the genotype (Yeyati et al. 2007). These experiments showed that, depending on the genotype, inhibition of Hsp90 can either enhance or reduce the penetrance of specific phenotypes. Furthermore, the regular occurrence of various unilateral defects and of inversion of heart looping suggests a link of Hsp90 to the control of symmetry. Similarly, in *Arabidopsis*, inhibition of Hsp90 by drug treatment or RNAi resulted in a range of specific abnormalities (Sangster et al. 2007). In addition, the reduction of Hsp90 activity affected the expression levels of numerous genes, particularly genes that are involved in the response to environmental stimuli, as well as the resistance to herbivory. These results mostly correspond

to findings in *Drosophila*, as far as the comparison can be made despite the differences in the organisms, experimental set-up and statistical methods.

PHENOTYPIC PLASTICITY: HSP90 AND DEVELOPMENTAL “UNBUFFERING”

Many organismal traits show phenotypic plasticity, the ability to respond to environmental conditions, which can be viewed as the opposite of buffering, because developmental processes are sensitive to specific environmental stimuli. If phenotypic plasticity is simply a developmental “unbuffering,” this raises the question of whether the underlying mechanisms are the same, but just operating in reverse.

Because effects of Hsp90 are temperature dependent (Rutherford and Lindquist 1998; Sangster et al. 2007), it seems natural to ask whether the ability to respond to temperature might rely on Hsp90. In a study of plasticity of abdominal colouration in *Drosophila*, a major temperature-dependent effect of Hsp90 has indeed been shown, but it is only part of a more complex regulatory system in which other components, in particular the Hox gene *Abdominal-B*, play a major role (Gibert et al. 2007).

Other cases of phenotypic plasticity may rely on different systems not involving Hsp90, such as hormonal control, which may even behave in a way that is very similar to the role of Hsp90 as a “capacitor” for genetic variation (Suzuki and Nijhout 2006). It appears, therefore, that Hsp90 may be just one factor among others controlling phenotypic plasticity.

GENETIC CONTROL OF DEVELOPMENTAL BUFFERING

These results suggest that Hsp90 is an important factor in developmental buffering, but it is only one among others. Moreover, the responses are trait specific, which further limits the possibility of generalization. Therefore, the challenge will be to investigate how many processes are involved and what their relative effects are.

For instance, a comparison of 115 fully controlled genotypes of *Drosophila*, which carried deficiencies in different regions of the genome, revealed substantial differences in the variability of size and shape of the wing, suggesting that many

genes are involved in the control of morphological variation (Breuker et al. 2006). For these strains, the amounts of individual variation of the average shape of the left and right wings and fluctuating asymmetry of wing shape were correlated, suggesting a shared control of both levels of variability.

Overall, these recent empirical studies support the view that numerous mechanisms are involved in developmental buffering and that their effects are trait specific. This is consistent with the view that developmental buffering and its genetic control may be an intrinsic property of developmental processes (Klingenberg and Nijhout 1999; Bergman and Siegal 2003). Phenotypic effects and evolutionary consequences of buffering will therefore depend on the specific developmental mechanisms that produce the traits of interest.

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