

Valladares F, Niinemets U. 2008. Shade tolerance, a key plant feature of complex nature and consequences. *Annual Review of Ecology & Systematics* 39: 237–257.

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Understanding natural epigenetic variation

Recently, there has been increased interest in understanding the role of epigenetic processes in ecology and evolution (e.g. Richards, 2006; Bossdorf *et al.*, 2008; Johannes *et al.*, 2008; Richards *et al.*, 2010). We now know that some epigenetic marks are not reset each generation, but are faithfully transmitted across generations (Jablonka & Raz, 2009), that natural variation can exist not only in the DNA sequence but also at the epigenetic level (e.g. Vaughn *et al.*, 2007) and that epigenetic variation can cause significant heritable variation in phenotypic traits (e.g. Johannes *et al.*, 2009). Moreover, heritable epigenetic modifications can be triggered by exposure to different environmental conditions (e.g. Verhoeven *et al.*, 2010). If we put these different pieces of evidence together, then this clearly suggests that epigenetic mechanisms could add an additional layer of complexity to heritable phenotypic variation, and thus to the diversity and evolutionary potential of natural populations. However, in spite of abundant speculation about the potential ecological and evolutionary implications of epigenetic processes, most previous work has been carried out on only a few types of agricultural crops and on model species such as *Arabidopsis thaliana*, frequently under artificial conditions, and we therefore still have no idea of the true importance of epigenetic processes in natural populations. Because of this, several authors have argued for expanding research efforts into ecologically relevant circumstances across model and nonmodel organisms and have outlined experimental and statistical approaches that would facilitate the merging of molecular-based insight with sound evolutionary ecology (Bossdorf *et al.*, 2008; Johannes *et al.*, 2008; Richards, 2008). In this issue of *New Phytologist* (pp. 867–876), Herrera & Bazaga provide an intriguing example of how researchers are now beginning to respond to this call.

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Herrera & Bazaga measured natural epigenetic variation in *Viola cazorlensis*, a long-lived violet endemic to south-eastern Spain. They surveyed 14 natural populations across a 1000-m elevation gradient in the limestone mountains of the Sierra de Cazorla, sampled leaves from multiple individuals in each population and analysed the variation in DNA methylation among these plants using methylation-sensitive amplified fragment-length polymorphism (MS-AFLP) markers. One of the strengths of this study was that the same individuals had previously been analysed for DNA-sequence variation using standard AFLP markers (Herrera & Bazaga, 2008), which allows genetic and epigenetic data to be linked. It was found that epigenetic differences between populations and individuals are strongly correlated with the presence of specific AFLP loci that were previously implicated in divergent selection and adaptive differentiation in floral traits between the populations. They conclude that the observed epigenetic variation may thus be involved in population differentiation in ecologically important traits.

To our knowledge, the study of Herrera & Bazaga is the first attempt of a true population epigenetics study, and the authors are to be commended for their pioneering effort. They show how epigenetic variation is partitioned within and between *Viola* populations and search for indications that epigenetic variation may be involved in population adaptation. Studies like the one by Herrera & Bazaga are essential first steps for assessing the ecological and evolutionary relevance of epigenetics. However, observational epigenetic studies in natural populations do have some limitations, and these reflect some of the main current challenges in ecological and evolutionary epigenetics. Here, we discuss some of these challenges and the possible next steps towards understanding natural epigenetic variation.

One of the fundamental differences between genetic and epigenetic variation is that the latter is, to some extent, environmentally labile and reversible. Many of the developmental processes that underly the phenotypic plasticity of plants in response to different environments involve epigenetic changes (e.g. the plasticity of flowering time, Sung & Amasino, 2004). Therefore, patterns of epigenetic differentiation among field populations that are measured in different environments – like the ones observed in the study of Herrera & Bazaga – will probably include a reversible component as a result of phenotypic plasticity and a nonreversible or relatively stable component as a result of heritable epigenetic differentiation. Clearly, epigenetic contributions to phenotypic plasticity and to heritable variation are interesting from both an ecological and an evolutionary perspective. But in this respect, analyses of epigenetic variation are similar to analyses of phenotypic variation, and common garden experiments are necessary to firmly establish inheritance and to differentiate between plastic and heritable components of variation. For this reason, an informative

next step would be to conduct a common garden study with offspring from the plants analysed by Herrera & Bazaga, and repeat the MS-AFLP analyses under these conditions.

Another important issue is the degree to which epigenetic variation is independent from DNA sequence variation. Epigenetic variation that is unrelated to DNA-sequence variation has the potential to explain phenotypic variation beyond that already explained by DNA sequence. To examine correlations between genetic and epigenetic variation, previous studies have largely limited themselves to the visual comparison of multivariate patterns of genetic and epigenetic similarity, or they have used Mantel tests to identify a correlation between genetic and epigenetic similarity matrices (e.g. Cervera *et al.*, 2002). Here, Herrera & Bazaga have taken a novel approach and correlated epigenetic variation with the variation in outlier AFLP loci that show striking differentiation between populations and may thus reflect adaptive population divergence. They find that epigenetic variation is strongly correlated with 10 of these outlier loci and therefore could be involved in adaptive differentiation. Given that 90% of the epigenetic variation was explained by these outlier loci, epigenetic population differentiation in this system may be largely a direct consequence of DNA-sequence variation. Alternative interpretations remain possible, but are difficult to distinguish based solely on observational AFLP and MS-AFLP data. For instance, as mentioned by Herrera & Bazaga, population-specific selection could act on both genetic and epigenetic variation independently. Also, random epigenetic drift could result in epigenetic population differences that develop in parallel to adaptive genetic differences, causing a correlation between genetic and epigenetic population differentiation without a functional link between the two.

Finally, demonstrating adaptation is a challenge that is not unique to ecological and evolutionary epigenetics, but in fact has a rich history in ecological genetics, where researchers have long recognized the need to disentangle genetic and environmental contributions to phenotypic variation, and the fitness consequences of adaptation vs maladaptation. Truly demonstrating adaptation requires reciprocal transplant studies in the field or studies in a controlled environment (reviewed in Kawecki & Ebert, 2004). Outlier locus analysis (e.g. using AFLP; Herrera & Bazaga, 2008) and McDonald–Kreitman tests of neutrality (e.g. in flowering-time genes; Flowers *et al.*, 2009) can suggest selection on ecologically important genes – as strong selection will lead to fixation of adaptive sequence variants or reduced polymorphism at those loci – but demonstrating that the observed variation is adaptive still requires association with actual fitness or performance of whole organisms when evaluated in relevant environments. In the case of epigenetic effects, which can be environmentally labile, a common environment approach will be even more critical to rule out the possibility that any association of epialleles is

not merely a transient and environmentally induced association.

In the end, we wish to understand the ecological and evolutionary relevance of observed epigenetic variation. Herrera & Bazaga have contributed to this quest by demonstrating that epigenetic variation is correlated with outlier DNA-sequence loci, which, in turn, have been shown to be correlated with ecologically important floral traits (Herrera & Bazaga, 2008). This is an interesting, but indirect, approach linking natural epigenetic variation to ecologically important phenotypic variation. Building on this and the few other pioneering studies that are emerging, we can think of several possibilities for future studies in ecological epigenetics that would contribute to our understanding of ecological processes, and ultimately adaptation. Studies that are conducted in a common environment or field reciprocal transplants will be critical to minimize the environmentally induced phenotypic and epigenetic effects and will allow us to:

- extend the matrix-correlation approach to phenotypic similarity matrices and test for a correlation between epigenetic similarity and univariate or multivariate phenotypic similarities, possibly after correcting for genetic influences;
- examine the relationship between epigenetic variation and environmental variables that describe the ecological characteristics of the habitat, again by using a matrix approach, or by correlating individual MS-AFLP loci with environmental variables
- extend the population-genomics approach to the epigenetic level and identify MS-AFLP outlier loci, which can then, in turn, be related to either phenotypic or environmental data.

All of these approaches would benefit from advancement of population epigenetic theory which is currently little developed.

At this time in the science of epigenetics, we can still only speculate about the adaptive significance of heritable epigenetic effects in natural populations. The study by Herrera & Bazaga provides one of the first glimpses into how epigenetic variation is distributed within and among natural plant populations. Soon, more studies will follow. We encourage these to also discriminate between plastic and heritable variation (through contrasting patterns of epigenetic differentiation between field and common garden conditions), and to further explore the relationships among genetic, epigenetic and phenotypic variation. However, until a solid theory of population and quantitative epigenetics has been developed (see Slatkin, 2009; and Tal *et al.*, 2010, for first attempts), unravelling the ecological and evolutionary interpretation of these relationships will remain a challenge.

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Letters

Potentially immortal?

Reductions in photosynthetic and growth rates, enhanced foliar photo-oxidative stress and decreased vigour of flower organs are processes associated with aging at the organ level in perennial plants. However, such events are not necessarily indicative of physiological deterioration at the organism level, as occurs in humans and other animals. The combination of modular growth and dormancy in perennial plants, two features individually shared with some animals (Fig. 1), increases their plasticity and strongly reduces the potential damage caused by aerobic life, to the extreme that the likelihood of dying from aging is, at best, negligible.

Modular structure and meristem dormancy

In contrast to most animals, plants have a tremendous plasticity in the form and function of their organs (Walbot,

1996). This is particularly relevant in perennials, in which the apical meristem of at least one of their shoots remains indeterminate beyond its first phase of growth and development. Perennial plants organize their body growth in the vertical plane, based on the division and differentiation of meristems. This structure allows perennial plants to explore the environment vertically in the search for light, and enables some of them to be very large and to have a very long life span. Some trees such as sequoias and pines, among others, can grow up to 100 m in height and survive for centuries and even millennia (Peñuelas, 2005; Munné-Bosch, 2007, 2008). Other species, such as some herbaceous perennials, can also survive for centuries by keeping their meristems intact (García *et al.*, 2008). This lifestyle is determined already at the embryo stage, in which apical (root and shoot) meristems are formed but remain inactive. After some days, weeks, or even years, seeds break dormancy and the seedling germinates. During postembryonic development, modular