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NEWS AND VIEWS

PERSPECTIVE

A truly ecological epigenetics study

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Until a few years ago, epigenetics was a field of research that had nothing to do with ecology and that virtually no ecologist had ever heard of. This is now changing, as more and more ecologists learn about epigenetic processes and their potential ecological and evolutionary relevance, and a new research field of ecological epigenetics is beginning to take shape. One question that is particularly intriguing ecologists is to what extent epigenetic variation is an additional, and hitherto overlooked, source of natural variation in ecologically important traits. In this issue of *Molecular Ecology*, Herrera & Bazaga (2011) provide one of the first attempts to truly address this question in an ecological setting. They study variation of DNA methylation in a wild population of the rare, long-lived violet *Viola cazorlensis*, and they use these data to explore interrelations between environmental, genetic and epigenetic variation, and in particular the extent to which these factors are related to long-term differences in herbivore damage among plants. They find substantial epigenetic variation among plant individuals. Interestingly, this epigenetic variation is significantly correlated with long-term differences in herbivory, but only weakly with herbivory-related DNA sequence variation, which suggests that besides habitat, substrate and genetic variation, epigenetic variation may be an additional, and at least partly independent, factor influencing plant–herbivore interactions in the field. Although the study by Herrera & Bazaga (2011) raises at least as many new questions as it answers, it is a pioneering example of how epigenetics can be incorporated into ecological field studies, and it illustrates the value and potential novel insights to be gained from such efforts.

Keywords: DNA methylation, ecological epigenetics, herbivory, natural variation

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Ecological epigenetics, the study of epigenetics in an ecological context, is a young field of research. It has been catalysed, among others, by some early empirical studies (e.g., Das & Messing 1994; Fieldes 1994; Cubas *et al.* 1999) that demonstrated the existence of heritable epigenetic variation, and its potential environmental causes and phenotypic consequences, and in particular by a recent series of review articles (e.g., Kalisz & Purugganan 2004; Rapp & Wendel 2005; Bossdorf *et al.* 2008) that speculated about the potential ecological and evolutionary relevance of epigenetic processes and laid the conceptual foundations for ecological and evolutionary epigenetics. After a period of several years where conceptual papers seemed to outnumber such that contained data, empirical research is recently gaining some momentum (e.g., Whittle *et al.* 2009; Bossdorf *et al.* 2010; Gao *et al.* 2010; Herrera & Bazaga 2010; Lira-Medeiros *et al.* 2010; Verhoeven *et al.* 2010). Still, with a few exceptions, most empirical research so far has been performed on model organisms, often under laboratory conditions, and the new study of Herrera & Bazaga (2011) is one of the first attempts to truly take epigenetics to the field and to incorporate epigenetics questions in an ecological field study of a non-model species.

Their study builds on a long-term investigation into the violet *Viola cazorlensis*, a perennial plant endemic to mountainous habitats in southeastern Spain (Fig. 1). Carlos Herrera and his coworkers have studied the evolutionary ecology of this species over many years, and the present study takes advantage of several existing data from this long-term study to explore interrelations between environmental, genetic, epigenetic and phenotypic variation within a population, in particular the extent to which long-term differences in herbivore damage can be explained by environment, genotype and epigenotype. A key innovative aspect of the study is that it explicitly links epigenetic variation to ecologically important phenotypic variation in the field. Another strength is that the authors are very careful in addressing alternative explanations and the complexity of their study system. They test for epigenetic effects only after correcting for substrate and microhabitat, and they use structural equation modelling (SEM) to compare alternative models of the possible causal relationships between genetic, epigenetic and phenotypic variation. The latter is particularly important because, with the exception of natural epimutants (e.g., Cubas *et al.* 1999) or species that are genetically uniform (e.g., Verhoeven *et al.* 2010), we should generally expect plants in natural populations to vary simultaneously at the genetic and epigenetic level, and it is thus necessary, particularly in field studies, to attempt statistical solutions for disentangling genetic from epigenetic effects. Herrera & Bazaga (2011) chose to boil down their genetic and epigenetic data to a few principal components and used these in structural equation models. Other solutions are conceivable, e.g., the use of model selection instead of SEM, or the linking of epigenetic and

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Fig. 1 The endemic violet *Viola cazorlensis* in one of its typical habitats in the limestone mountain ranges of southeastern Spain.

phenotypic data with methods developed for quantitative genetics of wild populations (e.g., Ritland 2000; Garant & Kruuk 2005), but the approach of Herrera & Bazaga (2011) certainly is one possibility, and it can serve as a model for others.

An epigenetic field study such as the one by Herrera & Bazaga (2011) faces some inevitable challenges, in particular the fact that the observed variation in herbivory, just as any other phenotype measured in the field, likely reflects both heritable variation as well as phenotypic plasticity (e.g., if substrate differences influence plant palatability). As epigenetic processes are involved in almost all growth and differentiation processes, such phenotypic plasticity should be associated with some degree of epigenetic change, and this alone could cause epigenetic–phenotypic correlations in the field, even in the complete absence of any heritable variation. Herrera & Bazaga (2011) tried to account for this as much as possible by correcting for substrate and habitat influences before testing for epigenetic effects, but it nevertheless remains a tricky issue. Another difficulty is that herbivore damage is known to induce biochemical responses in plants, with corresponding epigenetic changes, which means that the direction of the causal relationship between epigenetic and herbivory variation must remain to some degree unclear, which is something also pointed out by Herrera & Bazaga (2011), who suggest that the true causal relationship might in fact be bidirectional. Ultimately, only manipulative experiments in a common environment will be able to tease apart these different causal hypotheses – all interesting in themselves – for explaining epigenetic-phenotypic correlations. Still, for many long-lived organisms such as the violet studied by Herrera & Bazaga (2011) such experiments may not be feasible, and for these species the insights from field studies may be the best we can get.

A comprehensive research programme in ecological epigenetics must include molecular studies and controlled experiments, but also field studies that test whether epigenetic patterns in natural populations are consistent with theoretical predictions and the results of more controlled, but less realistic, experiments. Field studies such as the one by Herrera & Bazaga (2011), notwithstanding their challenges, are tests of ecological relevance and therefore important pieces of the ecological-epigenetic puzzle.

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