

NEWS AND VIEWS

PERSPECTIVE

Triangulating the genetic basis of adaptation to multifarious selection

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Understanding how natural populations adapt to their local environments is a major research theme for ecological genomics. This endeavour begins by sleuthing for shared genetic similarities among unrelated natural populations sharing adaptive traits to documented selective pressures. When the selective pressures have low dimensionality, and the genetic response is localized to a few genes of major effect, this detective work is relatively straightforward. However, in the real world, populations face a complex mixture of selective pressures and many adaptive responses are the result of changes in quantitative traits that have a polygenic genetic basis. This complex relationship between environment and adaptation presents a significant challenge. How can we begin to identify drivers of adaptation in natural settings? In this issue of *Molecular Ecology*, Orsini *et al.* (2012) take advantage of the biological attributes of the freshwater microcrustacean *Daphnia* (Fig. 1) to disentangle multidimensional selection's signature on the genome of populations that have repeatedly evolved adaptive responses to isolated selective pressures including predation, parasitism and anthropogenic changes in land use. Orsini *et al.* (2012) leverage a powerful combination of spatially structured populations in a geographic mosaic of environmental stressors, the historical archive of past genotypes preserved in lake-bottom sediments and selection experiments to identify sets of candidate genomic regions associated with adaptation in response to these three environmental stressors. This study provides a template for future investigation in ecological genomics, combining multiple experimental approaches with the genomic investigation of a well-studied ecological model species.

Keywords: adaptation, *Daphnia*, experimental evolution, genome scans, paleogenetic, selection

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A common objective for evolutionary biologists and ecologists is the ability to infer the consequences of changing

environments to natural populations. How do populations respond to novel selective pressures? How do anthropogenic alterations to the environment interact with existing demographic and selective forces? Developing a prospective view of adaptation requires an understanding of the selective forces at play, the genetic underpinnings of adaptive traits and the origins of genetic variation. Unfortunately, the complexity of both selection and the genetic basis of adaptation alone forms a daunting barrier to progress in many systems. Often researchers are much like real-life versions of Detective Hercule Poirot in Agatha Christie's novels, sifting through clues using 'order and method' to establish causal connections without a conventional path to this goal. Progress at finding causality requires a detailed understanding of the ecological context that natural populations are exposed to and a combination of empirical approaches and analytical tools to link local environmental conditions to genes. Because of these inherent complexities, many of the most well-documented studies demonstrating the genetic basis of adaptation come from discovering a direct connection between strong low-dimensional selection and an adaptive response mediated by a few genes of major effect (e.g. Storz *et al.* 2007; Feldman *et al.* 2009; van't Hof *et al.* 2011). In many cases, selection and the genetic basis of adaptation are often far more complicated. Fortunately, we are now entering an arena where solving this type of mystery is increasingly tractable. The recent development of genomic tools in traditionally non-model systems, and the increasing availability of high-throughput sequencing, has spurred ecological genomic studies. This area of investigation and studies combining genome-wide genetic markers are now a significant component of recent articles in *Molecular Ecology* (Rieseberg *et al.* 2012).

Disentangling the various elements of natural selection is one of the primary challenges. Orsini *et al.* (2012) tackle this central problem in a multifaceted study of *Daphnia* populations taking advantage of extensive previous work documenting patterns of land-use intensity (Michels 2008) and rapid micro-evolutionary responses to fish predation (Cousyn *et al.* 2001) and parasite exposure (Decaestecker *et al.* 2007). By examining a spatially structured set of populations embedded in a geographic mosaic of environmental gradients and implementing a replicated design, they are able to isolate orthogonal environmental factors. This analysis, using a genome scan strategy (Storz 2005) based on a combination of 84 microsatellite loci (Jansen *et al.* 2011) and 82 SNPs (Orsini *et al.* 2011), allows the authors to identify a set of candidate markers linked to the environmental stressors.

By itself, this spatially structured study with a genome scan demonstrating repeated local adaptation would be

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Fig. 1 Adult *Daphnia magna* carrying a resting egg in the brood pouch. The water flea *Daphnia* is a renowned ecological model system and rapidly developing as an ecological and environmental genomics model species. Photo credit Joachim Mergeay.

noteworthy. However, not satisfied with the inferential power of this approach alone, Orsini *et al.* (2012) take two additional measures to validate these results. These validation steps set this study apart from others. Their first approach leverages a unique feature of *Daphnia* biology, the preservation of genotypes from the past in lake-bottom sediments. A number of recent studies demonstrate that diapausing eggs trapped in these sedimentary layers yield DNA suitable for genetic analysis and in some cases are viable and can be hatched in the laboratory allowing a direct comparison of contemporary and historical genotypes (Hairston *et al.* 1999; Cousyn *et al.* 2001, Decaestecker *et al.* 2007). Orsini *et al.* (2012) use this powerful paleogenetic assay of legacy genotypes to show rapid genetic evolution in response to changing environments. In addition, for one of the stressors (fish predation) they conduct a selection experiment. Taken together, these data sets confirm the results of the genome scans. Similar loci are identified as outliers in all three data sets. One important consequence of this data set triangulation is the increased power and reduction of false positives. Orsini *et al.* (2012) find a larger proportion of outlier loci in the contemporary structured populations than is typical, and they are able to validate many of these outliers with their paleogenetic and experimental evolution analysis.

So, where does the genomics part of the ecological genomics come into play? All these results could well be achieved with access to a reasonable number of genetic markers and the appropriate sampling strategies. However, the identification of sets of candidate genetic markers is only a first step at identifying the genetic basis of adaptation and leaves us hanging in suspense. For many non-

model systems, we would remain mystified about the functional implications of these putative linked markers associated with adaptation, unable to delve any deeper into the nature of adaptation. Fortunately, for a growing number of species, *Daphnia* in particular, this barrier is rapidly yielding. The first genome sequence and gene annotation of a *Daphnia* species was recently published (Colbourne *et al.* 2011). This paper describing the genome biology of *Daphnia pulex*, along with a set of *c.* 40 companion papers, reveals a compact, gene-rich genome that is replete with genes that are responsive to environmental stressors. Many of these genes are orthologous to annotated genes on other organisms and their function can be inferred by homology while many others (nearly half of the gene set!) are previously unknown. Orsini *et al.* (2012) use the draft sequence of a second species, *Daphnia magna*, to search in genomic regions closely linked to their candidate markers. As expected from the analysis of the *D. pulex* genome, many of the genes in these regions are orphans lacking homology to genes of known function, but many do have identifiable orthologs with known function. Among these, orthologs are genes associated with proteolysis, metabolic processes and neuronal development. While the picture is far from complete, and a map between genotype and adaptive phenotype is nascent, the fuel for further investigation is considerably enriched by this connection to genome content.

The significance of this study is multifaceted. In one dimension, Orsini *et al.* (2012) establish a powerful investigative approach combining naturally occurring environmental gradients and adaptation, with historical paleogenetic and experimental evolution validation. They show with a paleogenetic analysis (perhaps for the first time) that the repeated patterns of evolution among spatially structured contemporary populations are linked to evolutionary change over short ecological timescales. This insight connects microevolutionary process and pattern and reinforces the recent emphasis on studying coupled evolutionary and ecological dynamics (Matthews *et al.* 2011). Beyond this advance, they demonstrate the value in genomic information to empower our ability to tackle the challenges of understanding the consequences of natural selection in the wild. Inferential studies establishing a statistical link between the genome and selection are an important step towards establishing the causal linkage between genes, phenotype and fitness (Barrett & Hoekstra 2011). As we struggle to understand the impacts of anthropogenic alterations to the environment and attempt to develop a predictive ability to forecast the responses of natural populations, ecological genomic studies following the template of Orsini *et al.* (2012), conducted in diverse species and ecological contexts, and forging the links between organism and environment will be critical.

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M.E.P. is interested in the relationship between genome structure and function, phenotypic plasticity, and the process of adaptive evolution in changing environments..

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