

Evolutionary Ecological Genomics

The recognition that evolution can happen on ecological timescales (Hairston *et al.* 2005; Pelletier *et al.* 2009; Ellner *et al.* 2011; Becks *et al.* 2012) has prompted the integration of ecology and evolution, while easier access to high-throughput sequencing technologies has increased the number of genetic nonmodel species entering the 'omics' era (e.g. Turner *et al.* 2010; Colbourne *et al.* 2011; Jones *et al.* 2012). We are now in a position to identify the genetic basis of adaptation and the mechanisms of adaptive responses in the wild. It nonetheless remains a challenge to go beyond descriptive measures of patterns of genetic variation and to identify the evolutionary processes driving species adaptation and evolution. This special issue represents a broad cross-section of research into evolutionary adaptation at the genetic level. The approaches used vary from classic QTL studies to RAD sequencing and RNAseq. Given the rapid advance of sequencing technology, we fully expect that 'genomic' as defined here will be merely 'genetic' in a few years, but we nonetheless hope that the results and methods described in this special issue will serve as a blueprint for future work in this field.

Contributions

This special issue began with a symposium at the 2011 European Society of Evolutionary Biology (ESEB) meeting in Tübingen, Germany, and has grown to include contributions from many other groups. Most papers focus on nonmodel species, suggesting that the 'genomicization' of ecological model organisms (Stapley *et al.* 2010) is a popular approach among evolutionary biologists for exploring species evolution and adaptation in natural environments. Two trends are evident from the contributions to this special issue: (i) approaches that use a reduced representation of the genome, such as RAD-tag and SNP-chips, are more common than whole-genome sequencing in population-based analyses, and (ii) a bottom-up approach that screens the genome or the transcriptome of natural populations for signatures of selection is more common in the analysis of natural population of vertebrates and invertebrates. The contributions can be grouped into three main themes: (i) processes underlying patterns of variation in the wild, (ii) mechanisms of adaptation and (iii) speciation and hybridization.

Genomic variation in the wild: from patterns to processes

Neutral and adaptive processes

The decreasing cost of 'omics' technologies has allowed us to study genome-wide patterns of variation at large geographic scales and the targeting of multiple gradients of selection. Contrasting neutral and adaptive genomic variation is a cornerstone of studies inferring evolutionary processes driving present-day population genomic/genetic structure. A number of studies in this issue adopted a bottom-up approach involving screens for adaptive and neutral genetic variation in natural landscapes. Bourret *et al.* (2013) compared neutral and adaptive genome-wide patterns of variation in natural populations of the Atlantic salmon (*Salmo salar*) across its natural range. They showed that complex interactions between the environment and endogenous barriers to gene flow in secondary contact zones can complicate the identification of genomic signatures of adaptive divergence. Campitelli & Stinchcombe (2013) compared variation in an ecologically relevant Mendelian leaf shape polymorphism with genome-wide neutral variation in the ivyleaf morning glory. The results of this comparison suggest that the latitudinal cline in leaf lobation for this plant is maintained by spatially varying natural selection. Further, using a genome reduced representation approach, Bruneaux *et al.* (2013) studied genome-wide patterns of variation in the nine-spined stickleback contrasting populations from marine and freshwater habitats. The close phylogenetic relationship of this species to the three-spined stickleback, a model species with a sequenced genome, allowed the authors to identify MAP kinase pathways and elements underlying osmoregulation as prime elements of adaptation to the freshwater systems. Focusing on the link between genomic and environmental variation, Orsini *et al.* (2013) identified correlations between biotic and abiotic variables occurring in the natural landscape and patterns of neutral and adaptive genetic variation in the waterflea *Daphnia magna*. These results, combined with the analysis of markers with different mutation rates, allowed the identification of demographic and selective processes driving population genomic structure in *D. magna*.

Heritability of fitness traits

Heritable genetic variation and covariation mediate the rate and direction of a population's response to selection, crucial for natural populations to adapt to new environmental regimes. It is thus important to study the heritability of adaptive phenotypes. Previous studies identified differences in gene expression between adult butterflies of the Glanville fritillary butterfly (*Melitaea cinxia*), underlying putative functional variation in individual life history traits. In the study presented here, Kvist *et al.* (2013) showed that metapopulation dynamics in heterogeneous environments, represented by three different temperatures, maintain heritable gene expression variation that affects the regulation of life history traits.

Bioinformatic developments

The continuous production of high-throughput data has stimulated both the development of new sequence analysis algorithms and the need for guidance for the identification of the 'right' tools to use. In this issue, Vijay *et al.* (2013) present a comparative analysis of methods used for *de novo* assemblies and offer practical and methodological guidance in the design and analysis of RNA-seq experiments. The guidance presented by Vijay and co-workers will be particularly useful in the study of organisms with no genomic background information.

Mechanisms of adaptation*Evolutionary mechanisms*

Understanding the mechanisms of adaptation is a central question in evolutionary biology (e.g. Barrett & Hoekstra 2011) as it allows us to identify genes underlying adaptive responses in the wild and to determine the source of the genetic changes behind adaptive responses (i.e. standing genetic variation or *de novo* mutations following environmental changes). Feulner *et al.* (2013) focused on identifying and quantifying existing genetic variation in a natural population of the three-spined stickleback (*Gasterosteus aculeatus*). By comparing six complete genomes with high coverage (15x), the authors revealed that *c.* 10% of the genome is polymorphic, consisting of a complex make-up of SNPs, indels and structural variation. This study contributes to the understanding of the role of standing genetic variation in parallel adaptive evolution.

The mechanisms underlying parallel or convergent evolution have received much attention (e.g. Arendt & Reznick 2008) because of the advantage of predicting the evolution of the same phenotypes in similar

environments. In this issue, Manousaki *et al.* (2013) and Colombo *et al.* (2013) used the Midas cichlid species complex to study the evolution of thick lips. Both studies found supporting evidence and identified candidate genes underlying parallel adaptive evolution of the thick lip-related traits. Interestingly, some ecological and transcriptomic traits seem to have evolved in parallel, whereas others did not (Manousaki *et al.* 2013), suggesting that cases of phenotypic parallel evolution in nature can be a complex mosaic of parallel and nonparallel genomic evolution. Thurber *et al.* (2013) showed that the evolution of distinct populations of weedy rice mainly involved different quantitative trait loci, despite parallel trait changes and a close evolutionary relationship. A similarly complex picture of the mutations underlying parallel phenotypic evolution is emerging in other systems (Manceau *et al.* 2010; Elmer & Meyer 2011).

Molecular and genetic mechanisms

Studies of local adaptation provide important insights into the role of natural selection relative to gene flow and other evolutionary forces in the response of natural populations to environmental factors. In absence of other selective forces, each local population evolves traits that provide an advantage under its local environmental conditions (Kawecki & Ebert 2004). Genetic mechanisms underlying local adaptation were investigated in an elegant study by Anderson *et al.* (2013). Using recombinants inbred lines derived from different populations of *Boechera stricta* (Brassicaceae) planted in field experiments, the authors demonstrated that local adaptation stems from a fine-tuned balance between antagonistic pleiotropy and conditional neutrality. In the former, natural selection favours alternate alleles in distinct habitats, whereas in the latter specific alleles are favoured in one environment but neutral in other environments. Anderson *et al.* (2013) found strong evidence for both, including antagonistic pleiotropy at a large-effect flowering time quantitative trait locus. Identifying the genetic basis of local adaptation was also the goal of Leinonen *et al.* (2013) who used greenhouse common garden experiment with *Arabidopsis lyrata*. Consistent with the findings on *Boechera stricta* (Anderson *et al.* 2013), beneficial dominance effects of conditionally neutral quantitative trait loci were identified.

Olsen *et al.* (2013) identified the genomic basis of widespread variation in cyanogenesis, a classic presence-absence polymorphism of chemical defence against herbivores in white clover (*Trifolium repens*). Long-term balancing selection had been hypothesized to maintain the polymorphism (Olsen *et al.* 2008); however, in the study presented here, the authors found no evidence

supporting this mechanism. Instead, they provide evidence that cyanogenesis in white clover has evolved through recurrent gene deletions, most likely in response to directional selection in multiple populations.

Schwartz & Bronikowski (2013) investigated the genetic basis of temperature stress tolerance in divergent ecotypes of the garter snakes (*Thamnophis elegans*). The authors used physiological and genetic assays to test whether laboratory-born juveniles from natural populations of the garter snakes vary concomitantly in candidate nodes of the stress response network, both under induced stress and in stress-free conditions. This study showed that life history phenotypes diverged at the molecular level in how they respond to stress, particularly in nodes regulating oxidative stress. Brown *et al.* (2013) identified the molecular mechanisms driving adaptive responses to parasite burden in natural populations of Soay sheep, using diverse genomic/genetic resources developed for cattle, an important economic species. The advantage of working with a species phylogenetically related to a genetic model species is clear in this contribution.

Experimental manipulations in controlled laboratory conditions are invaluable to investigate molecular mechanisms of adaptation. In this respect, the contribution of Lenz *et al.* (2013) is exemplary. Using laboratory-reared populations of the three-spined sticklebacks from lake and river ecotypes, Lenz *et al.* showed differences in the adaptive responses to parasite infection, where different transcriptomic pathways are activated upon parasite exposure, both at the global expression and at the functional level.

Speciation and hybridization

The greater capacity to analyse genome-wide patterns of variation has improved our ability to delineate inter-specific relationships and assess the levels of gene flow among taxa, allowing us to clarify issues relative to speciation, hybridization and introgressions Andrew *et al.* (2013). In this issue, Wagner *et al.* (2013) produce a well-resolved phylogeny showing the monophyly of the Lake Victoria cichlids, refuting previous studies that showed no resolution of species or higher-level relationships (e.g. Takahashi *et al.* 2001). They present a compelling case study on the value of large genome-wide data sets for elucidating the phylogenetic relationships among species and testing for monophyly. By progressively adding loci to a matrix of markers, they examined the trade-off between missing data and the number of loci, finding that resolution improved with increasing matrix size. This study represents a breakthrough in the analysis of recent adaptive radiations,

considering that previous patterns have generally been attributed to the recent origin of the radiation and ongoing hybridization between species. Using a dense RAD marker panel, Nadeau *et al.* (2013) studied the recent radiation of *Heliconius* butterflies, comprising multiple levels of divergence with ongoing gene flow between species. The authors showed that the previous unresolved phylogenetic relationships within the *Heliconius melpomene* species group can be explained with the lower resolution of previously used markers. In addition, they showed that divergence among races involves a very small part of the genome, in agreement with the concept of genic speciation (Wu 2001).

The availability of genome-wide data for a large number of species offers improved means of identifying hybrids and examining the genetic and evolutionary consequences of species hybridization. Hamilton *et al.* (2013) showed that exogenous selection is likely to play a strong role in maintaining the hybrid zone of spruce species, and identified frost tolerance as possible basis for hybrid superiority in intermediate environments. Despite a wealth of results supporting the expectation that sex chromosomes are likely to play a strong role in species divergence, Stolting *et al.* (2013) found that a putative incipient sex chromosome harbours a large region of allele sharing between two ecologically divergent European poplar hybridizing species (*P. alba* and *P. tremula*). This region and the observed genome-wide autocorrelation of divergence support recurrent gene flow between the two species rather than shared ancestral polymorphism as a possible explanation for allele sharing. The genomic distribution of shared alleles also provided evidence of introgression from domesticated pig to wild boar (Goedbloed *et al.* 2013). Goedbloed and co-workers proposed a novel approach to investigate genetic introgression using the case study of the Northwest European wild boar and showed that introgression was much higher than previously thought in this species. *In silico* simulations of hybridization further revealed that the level of introgression of domesticated pigs in wild boar stretched back five or more generations.

Challenges in evolutionary ecological genomics

In this momentum of major technological developments, one of the biggest challenges we face is producing more data than we can analyse at the expense of the scientific questions we can address. Genomic data can provide useful new insights only if investigations are hypothesis driven with clear sampling and experimental design to guide in data generation. As data-gathering technology continues to advance, our ability to understand selection, adaptation and speciation will

be limited more by conceptual and analytical weaknesses than by the amount of molecular data produced. Comprehensive data on the environmental context and phenotype of organisms adapting to new environments may also become a limiting factor.

Replicated designs that analyse the same environmental/ecological contrasts in multiple comparisons or gradients are ideal for confidently identifying processes driving adaptive responses in nature (e.g. see Bourret *et al.* 2013). However, this requires a good knowledge of the environmental and ecological context of natural systems. Even with nearly complete genomes, our ability to establish a functional link between genotypes and phenotypes depends upon the characterization of phenotypes under selection. At the moment, high-throughput phenotyping and environmental association analyses represent a major bottleneck for many natural systems. Further, following the identification of signature of selection underlying relevant phenotypes, the functional link between phenotype and genotype has to be validated. This validation can be done using experimental populations in laboratory-controlled experiments or reverse genetic techniques, which are commonly not available for nonmodel species. Importantly, validation of functional links in laboratory-controlled experiments also requires genomic tools (e.g. chromosomal maps), in which molecular ecologists should invest. The work of Anderson *et al.* (2013), Leinonen *et al.* (2013) and Schwartz & Bronikowski (2013) is exemplary in showing that chromosomal maps can lead to the identification of the genomic mechanisms underlying adaptive phenotypes even in the absence of a genome sequence.

Future avenues of research

The 'genomicization' of ecological model systems has opened new avenues for the study of natural systems. The thorough ecological and environmental background knowledge we can acquire for ecological model species (e.g. Orsini *et al.* 2013) combined with an increasing number of genetic/genomic tools generated for non-model taxa (e.g. Anderson *et al.* 2013; Hamilton *et al.* 2013; Kvist *et al.* 2013) will finally allow us to link patterns of phenotypic and genotypic variation to patterns of environmental variation. Establishing these links will give us unprecedented insights into the evolutionary dynamics of natural populations to demographic and adaptive processes. The next step towards our understanding of adaptive responses to natural selection will involve population and community sequencing. With the accessibility of NGS technologies now extending to nonmodel taxa, complex communities of microorganisms can be efficiently sequenced to obtain insights into species composition and gene content (Coelho *et al.*

2013). Meta- and environmental genomics are expected to have an increasing impact in biology in the coming years. As the number of sequenced genomes of non-model species grows, we predict that an increasing number of phylogenomic studies will be undertaken in the near future with improved methodological approaches (e.g. Nadeau *et al.* 2013; Wagner *et al.* 2013).

These are exciting times for biologists! At last, we have tools to answer fundamental questions in evolutionary biology that we did not even dare to ask a few years ago.

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