

NEWS AND VIEWS

MEETING REVIEW

Advances in Ecological Speciation: an integrative approach

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The role of natural selection in promoting reproductive isolation has received substantial renewed interest within the last two decades. As a consequence, the study of ecological speciation has become an extremely productive research area in modern evolutionary biology. Recent innovations in sequencing technologies offer an unprecedented opportunity to study the mechanisms involved in ecological speciation. Genome scans provide significant insights but have some important limitations; efforts are needed to integrate them with other approaches to make full use of the sequencing data deluge. An international conference ‘Advances in Ecological Speciation’ organized by the University of Porto (Portugal) aimed to review current progress in ecological speciation. Using some of the examples presented at the conference, we highlight the benefits of integrating ecological and genomic data and discuss different mechanisms of parallel evolution. Finally, future avenues of research are suggested to

advance our knowledge concerning the role of natural selection in the establishment of reproductive isolation during ecological speciation.

Keywords: adaptive evolution, ecology, genome scan, natural selection, parallel evolution, reproductive isolation

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In the seminal work of Charles Darwin, natural selection was proposed as the main driver of diversification (Darwin 1859). Yet, during most of the last century, speciation was essentially viewed as a result of random processes, where genetic drift plays an important role (e.g. founder events) (Wright 1940; Mayr 1954, 1963; Templeton 1980).

In the last two decades, the consensus view of speciation has evolved dramatically and is now centred on identifying the mechanisms responsible for the evolution of reproductive isolation, especially when gene flow is present during the process (Smadja & Butlin 2011; The Marie Curie SPECIATION Network *et al.* 2012). This change in focus has resulted in a renewed recognition of the role of selection, in particular of ecologically driven selection, as a powerful cause of speciation (Schluter 2000; Elias *et al.* 2012; Nosil 2012).

In addition, the recent advent of next-generation sequencing (NGS) has offered unprecedented power to infer genome-wide patterns of divergence during speciation, at high resolution even in nonmodel organisms (Feder *et al.* 2012; Nosil 2012; Nosil *et al.* 2012; Roesti *et al.* 2012; Mateus *et al.* 2013). The increasing availability of high-density genetic maps and reference genomes initiated an important shift from the identification of ‘speciation genes’ in interspecific crosses (Noor & Feder 2006; Nosil & Schluter 2011) towards ‘the genetic architecture’ of reproductive isolation during the speciation process (The Marie Curie SPECIATION Network 2012; Rogers *et al.* 2013).

In this context, an international conference entitled ‘Advances in Ecological Speciation’ (AES) was organized at the Research Centre in Biodiversity and Genetic Resources (CIBIO), University of Porto (Vairão, Portugal), on 29–30 April 2013. The main goals were to (i) present recent advances in ecological speciation research; (ii) identify the major current challenges; and (iii) propose integrative approaches and new avenues of research in the field. The conference was divided into four scientific sessions covering relevant themes in ecological speciation (adaptation, genomics, adaptive radiations and hybridization), each led by a plenary speaker. In this meeting review, we discuss the state of the field using the plenary talks as a starting point and propose some directions to move research forward, hoping to inspire the design of new research

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programmes and to contribute to a more comprehensive framework for the study of speciation.

Evidence for ecological speciation

During the opening talk, entitled 'Quest for the origin of stickleback species', Dolph Schluter started by highlighting two key conditions that need to be verified before concluding that any given pair of species originated through ecological speciation: (i) phenotypic differentiation must be driven by natural selection and (ii) reproductive isolation must evolve as a consequence of divergent natural selection. Establishing the crucial link between adaptation and reproductive isolation is a major challenge, and many studies claiming to provide empirical evidence in support of ecological speciation have important methodological limitations that restrict their ability to draw such a conclusion (reviewed in Hendry 2009). Therefore, although data are often consistent with ecological speciation, unequivocal evidence is still limited to a relatively small number of case studies (e.g. see Tables 1 and 2 from Hendry 2009).

Genome scans and ecological speciation

Although early studies in ecological speciation had a strong ecological focus (e.g. Nagel & Schluter 1998), genetic data have increasingly been used to infer the evolutionary forces involved in this process (Nadeau & Jiggins 2010; Stapley *et al.* 2010). In her talk 'On the genomics of adaptive divergence in sticklebacks', Felicity Jones identified the most relevant questions to understand the genetic basis of ecological speciation: What types of mutations are involved? How many loci? What is their function? and How do they affect phenotypes and contribute to reproductive isolation?

The screening of thousands of loci throughout the genome (genome scans) by means of sequencing restriction-site-associated DNA (RADs, e.g. Hohenlohe *et al.* 2010), genotyping-by-sequencing (GBS, e.g. Narum *et al.* 2013), SNP genotyping (e.g. Neafsey *et al.* 2010) or even full-genome sequencing (e.g. Jones *et al.* 2012) enables the identification of loci with exceptionally high levels of differentiation ('outliers') between populations or ecotypes. These are often interpreted as signs of adaptation and/or reproductive isolation (Storz 2005; Nosil *et al.* 2009; Via 2009; Nadeau & Jiggins 2010; Narum *et al.* 2013). Most genome scans have focused on cases in which divergence is very recent and gene flow is ongoing (the 'magnifying glass' approach; Via 2009), because outliers stand out from relatively low levels of background divergence. Describing the genome-wide pattern of genetic divergence during speciation ('islands' vs. 'continents' of divergence; divergence hitchhiking vs. genomic hitchhiking *sensu* Feder *et al.* 2012; among others) has become one of the most active areas in speciation research (e.g. Michel *et al.* 2010; Feder *et al.* 2012; Nosil *et al.* 2012; Renaut *et al.* 2012). However, only a few high-resolution genome scans exist for different stages of

the speciation continuum (i.e. different degrees of reproductive isolation) within the same system (e.g. *Heliconius* butterflies—Martin *et al.* 2013), so we remain ignorant about how these patterns progress during divergence. We do know that ancient alleles present as standing variation can be important contributors to local adaptation (e.g. Colosimo *et al.* 2005), and this means that we may need to look at long timescales and wide spatial scales to get a full picture.

One perplexing result of genome scans is the relatively high proportion of outlier loci detected very early in speciation. While this suggests a pervasive effect of divergent natural selection acting on a large fraction of the genome (e.g. Parchman *et al.* 2013), this conclusion probably oversimplifies the view of genomic divergence. Indeed, our capacity to identify signatures of natural selection can be affected by multiple factors, potentially leading to a high false-positive rate. For example, correlated coancestry in highly structured populations (Bierne *et al.* 2013) as well as neutral mutations that arise in the front of a wave of expansion (Excoffier & Ray 2008; Bierne *et al.* 2011) will create outliers purely due to demographic processes. Also, Roesti *et al.* (2012) showed that recombination rate strongly affects the heterogeneity of divergence across the genome. This may reflect the impact of background selection on within-population variation more than the effect of local adaptation on differentiation. The results presented by Sébastien Renaut in his talk 'Genomics of adaptive divergence and speciation in wild sunflowers' suggest that the functional architecture of genomes (e.g. variation in mutation and recombination rates, and gene density across the genome) plays a greater role in shaping genomic patterns of divergence among species than does gene flow (Renaut *et al.* 2013). Even if selection is implicated, it can take various forms: a genomic region can be highly divergent among populations during the progression of a selective sweep that plays no part in reproductive isolation or because it contains alleles implicated in intrinsic incompatibilities that tend to form clines coincident with those for adaptive alleles at ecological boundaries (Bierne *et al.* 2011). Finally, as Roger Butlin stressed during his closing lecture entitled 'Questions in (ecological) speciation', drawing the line between outlier and nonoutlier loci can be a very speculative enterprise that attempts to impose categories on a continuum. By itself, a genome scan alone carries insufficient information to enable distinctions between different speciation scenarios (Seehausen *et al.* 2014). Indeed, part of the problem is that many genome scan studies attempt to infer divergent natural selection without relating the observed genomic patterns to the ecology of the species, with the risk that the patterns of divergence they detect may have little to say about ecological speciation. The power of genome scans will be truly realized only when they are combined with other approaches, especially those that directly address the connections from gene to phenotype to environment, linking genomics to ecology.

A general consensus among conference attendees was that ecological speciation studies based on genome scans need to address additional questions: What is the demo-

graphic history of the populations? What is the genomic context of outlier loci (recombination and mutation rates along the genome, gene density, etc.)? How do outlier loci relate to the phenotypic differences between populations? How are these functional effects linked to ecologically mediated reproductive isolation? How are other components of isolation, such as assortative mating, coupled to these ecologically mediated barriers? and What are the genomic signatures left by these different processes? It is crucial to consider these factors to avoid premature conclusions about the role of divergent selection in putative examples of ecological speciation. More refined analyses using other features of diversity and divergence in addition to F_{ST} may help (see recommendations in Noor & Bennett 2009). As in any emerging field, the development of statistical and computational tools to tackle these questions is lagging behind the generation of data, slowing down advances in ecological speciation research. Thus, an enormous pressure exists for the development of new statistical and bioinformatics methods, user-friendly computational packages and guidelines for best practice so that we can improve the quality of our interpretation of genomic data. The use of population/evolutionary replicates and comparisons between multiple populations or with closely related taxa are also important to make inferences about the loci underlying adaptation/reproductive isolation more robust, and new models are needed to provide a more appropriate theoretical framework to detect them (Bierne *et al.* 2013). However, all these improvements may still be insufficient because, as mentioned previously, multiple factors can bias our results. Genome scans need to be combined with alternative lines of evidence (Abbott *et al.* 2013), especially about the effect of individual genes on adaptive phenotypes.

Beyond genome scans: an integrative approach

Several approaches used to complement genome scans were highlighted: candidate genes, comparative transcriptomics, QTL mapping, admixture mapping, functional analysis and experimental manipulation.

Roger Butlin presented an example of a candidate gene approach that was used to identify the molecular mechanisms behind the formation of host races in pea aphids. His research group used targeted sequencing to analyse candidate families of genes (150 genes in total) coding for odorant and gustatory receptors as well as for olfactory binding proteins. Through a standard F_{ST} approach, they were able to reduce the list to <20 genes putatively involved in host acceptance between different races (Smadja *et al.* 2012). Importantly, some of these candidates present copy number variation (CNV) contributing to the fixed differences between host races (L. Duvaux, unpublished), suggesting that CNV can have a role in ecological speciation (Feulner *et al.* 2013). However, it should be noted that candidate gene approaches neglect novel loci that may be important to the speciation process and so may give a biased picture.

Despite technical difficulties for some species, less-biased forward genetic approaches such as QTL mapping are also

being used increasingly in ecological speciation studies (e.g. Hawthorne & Via 2001; Rogers & Bernatchez 2005, 2007; Baird *et al.* 2008; Malek *et al.* 2012). Dolph Schluter presented a set of manipulative experiments combined with QTL analysis to infer the genetic basis of ecologically based reproductive isolation between benthic and limnetic sticklebacks. F1 hybrids are known to have similar fitness to the parental forms in laboratory conditions but lower fitness in the wild, suggesting an ecological mechanism of selection against hybrids (extrinsic postzygotic isolation, Hatfield & Schluter 1999). In a follow-up experiment, F1 hybrids were introduced into large artificial ponds simulating the natural environment, and the resulting F2 offspring individuals were analysed for phenotypic traits related to feeding, which were mapped to stickleback chromosomes using a QTL approach. The results showed that a considerable proportion of F2 individuals had mismatched combinations of parental traits that made them less fit in terms of niche use efficiency (M.E. Arnegard, unpublished). The fact that this kind of epistasis is only noticeable in an appropriate ecological context (Schluter & Conte 2009) highlights the importance of performing manipulative experiments in the wild or in seminatural environments. Evaluating the overlap between the QTLs identified during such manipulative experiments with the loci involved in assortative mating and those detected as outliers in genome scans will be highly informative about the importance of these traits in speciation.

Another example of the power of QTL mapping to identify genes (or genomic regions) involved in ecological speciation is the study performed by Colosimo *et al.* (2005), in which they were able to identify the gene (*EDA*) controlling lateral plate number between the marine and freshwater forms of sticklebacks. A whole-genome sequence analysis presented by Felicity Jones showed that this locus indeed maps to a region of high differentiation between the two forms (Jones *et al.* 2012) and that 80 other similar highly differentiated regions exist across the genome.

In addition, *EDA* was identified by another complementary approach—admixture mapping (F. Jones, unpublished). This approach takes advantage of the existence of admixed individuals in hybrid zones to map phenotypic traits (Buerkle & Lexer 2008) and presents three major advantages relative to QTL mapping. First, it avoids the need to perform crosses in the laboratory; second, it is informative about reproductive isolation in natural conditions, as interactions between loci explaining differential survival of hybrids in the wild can be identified; and third, if samples are collected along a transect crossing a hybrid zone, it can be coupled with cline analyses to quantify and map the strength of selection in different parts of the genome (Barton & Hewitt 1985; Barton & Gale 1993; Gompert & Buerkle 2009, 2011; Lindtke *et al.* 2013).

Once genes putatively involved in adaptation or reproductive isolation have been identified, the ultimate demonstration of their role comes from functional tests (Barrett & Hoekstra 2011; Nowick *et al.* 2013). Walter Salzburger, in his talk 'Evolution in Darwin's Dreamponds: Adaptive

radiation and explosive speciation in East African cichlid fishes', presented preliminary functional evidence based on transgenesis experiments in zebrafish, associating the formation of dummy egg-spots, characteristic of haplochromine cichlid anal fins and supposedly involved in multiple aspects of mating and courtship behaviour, with the expression of an androgen receptor cofactor identified by comparative transcriptomics (M.E. Santos, unpublished). The ability to establish functional links from genotypes to phenotypes, and from phenotypes to fitness and reproductive isolation by combining sophisticated genetic, transgenic, cellular and developmental studies in the laboratory, with studies in the wild of natural contact zones and admixture between divergent populations promises an exciting future for the field of speciation. Although the use of functional tests in nonmodel species is still very challenging, new molecular techniques for custom genome editing (TALEN, CRISPR/Cas9—Bogdanove & Voytas 2011; Jinek *et al.* 2012) will certainly contribute to extending functional studies beyond traditional model species (e.g. Lo *et al.* 2013).

The study of repeated evolution

The repeated evolution of phenotypic traits as a consequence of adaptation to similar environments provides one of the strongest forms of evidence for the role of natural selection in diversification (Rundle *et al.* 2000; Schluter 2000; Nosil 2012).

When two or more ecotypes from a single taxon are present in more than one location, clustering of individuals from the different populations, based on neutral markers, by location rather than by ecotype is generally interpreted as a signature of parallel evolution, implying that ecotypes have repeatedly originated *in situ* (Fig. 1—parallel origins scenarios). Unfortunately, such data leave open the possibility that the ecotypes have a single, common origin (Fig. 1—single origin scenario), without parallel evolution, but with the genetic pattern produced by introgression between ecotypes within locations (Butlin *et al.* 2008; Johannesson *et al.* 2010; Nosil 2012). A way to overcome this problem, based on coalescent simulations using approximate Bayesian computation (ABC) and given sufficient information from multiple neutral markers of different types, was presented by Roger Butlin. Comparing the probability of different models of migration during divergence between ecotypes in the marine snail, *Littorina saxatilis*, that have been described in separate geographical locations (Britain, Sweden and Spain), Butlin *et al.* (2014) found strong support for parallel independent evolution of ecotypes.

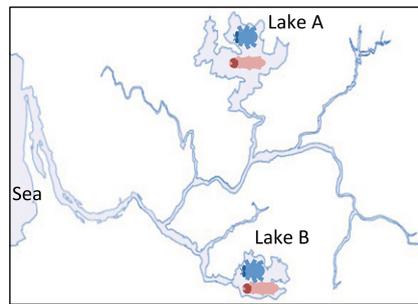
Whether or not the genes underlying adaptive differentiation are the same or different is a separate question. Preliminary results from a follow-up study also presented by Roger Butlin showed that a higher number of outliers in *L. saxatilis* are shared between localities (Britain/Sweden/Spain) than expected by chance (A.M. Westram, unpublished), which is similar to the pattern reported for independent species pairs of sunflowers by Sébastien Renaut

(Renaut *et al.* 2014). Although the haplotypes involved in *L. saxatilis* ecotype differentiation are unknown as yet, if future analyses show they are the same across different localities, these haplotypes must have originated either locally through repeated *de novo* mutations (Fig. 1—scenario I) or have been derived from standing genetic variation (Fig. 1—scenario IV). In this case, assessing the age of the haplotypes would be very informative to distinguish between these two alternative scenarios. Nevertheless, these preliminary results also show that most outlier loci are different among localities, suggesting that parallel genetic changes, that is, different mutations underlying that same phenotypic change in different populations, may underlie parallel ecotype formation in *L. saxatilis* (Fig. 1—scenario II).

A better understanding is available for the genetic basis of the repeated evolution of freshwater forms of sticklebacks from a marine ancestor. Based on 21 whole-genome sequences from marine and freshwater sticklebacks from 13 different locations across the Northern Hemisphere, Felicity Jones showed that approximately 35% of loci underlying parallel marine–freshwater divergence are shared among distinct freshwater populations, suggesting that repeated use of the same standing genetic variation is important during adaptive evolution (Jones *et al.* 2012). This shared adaptive variation among freshwater populations seems to result from repeated events of gene flow, mediated by the marine form, into freshwater environments (Jones *et al.* 2006).

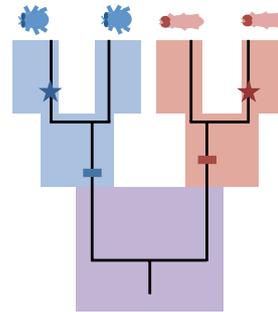
Parallel phenotypic evolution resulting from shared variation due to gene flow among ecotypes inhabiting different locations was referred to by Roger Butlin as evolution in concert (Fig. 1—scenario III) (following Morjan & Rieseberg 2004; Johannesson *et al.* 2010). However, the distinction between the two scenarios (evolution in concert vs. evolution from standing variation) is not always simple, as standing variation at the time of the colonization of a different location can result from gene flow from existing ecotype populations. In many cases, it may be that the genetic basis of parallel phenotypic divergence involves a mix of the scenarios presented in Fig. 1.

Convergent phenotypic evolution where similar phenotypes evolve from different evolutionary starting points can also be interpreted as a signature of natural selection. One of the most emblematic cases is in the cichlid fishes in African lakes, as highlighted by Walter Salzburger, where the amount of convergence for several different phenotypes is remarkable, both between (Kocher *et al.* 1993) and even within lakes (Muschick *et al.* 2012). For example, in Lake Tanganyika, convergent evolution of pharyngeal teeth associated with diet occurred in distantly related tribes within the same adaptive radiation, suggesting that adaptation to ecological conditions was a key component in these explosive radiations (Muschick *et al.* 2012). Nevertheless, whether repeated phenotypic evolution in this case is truly convergent, or has evolved according to some of the scenarios discussed above, remains to be explicitly tested. A recent analysis by Conte *et al.* (2012) based on data from



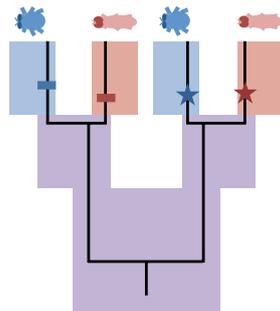
Repeated evolution of ecotypes

Geographic distribution of two divergent water beetle ecotypes.



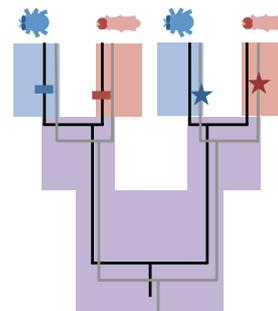
Single origin of ecotypes

Some alleles will be shared due to mutations in the ancestral ecotype populations but unique alleles may have arisen more recently (at the same locus, as shown here, or at different loci).



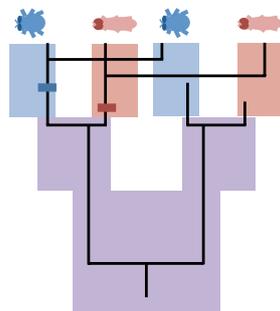
Parallel origins of ecotypes I – Parallel mutations in the same gene

Parallel mutations in the same gene contribute to ecotype formation (may be the same or different substitutions, deletions, etc., as shown here).



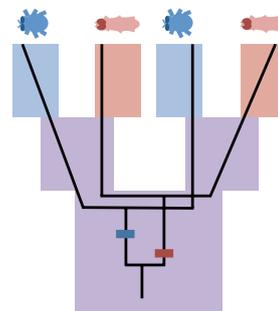
Parallel origins of ecotypes II – Parallel mutations in different genes

Parallel mutations at different loci contribute to ecotype formation.



Parallel origins of ecotypes III – Evolution in concert

Adaptive alleles are shared between localities as a result of gene flow among ecotypes from different lakes.



Parallel origins of ecotypes IV – Shared ancestral polymorphism

Adaptive alleles are shared due to independent fixation from a common polymorphic gene pool.

Fig. 1 Different genetic paths to a pattern of repeated phenotypic divergence. Two ecotypes of an imaginary water beetle, both inhabiting two semi-isolated lakes (A and B), are shown. The different boxes represent populations, while the lines denote gene trees. Adaptive mutations (small rectangles) underlying the formation of each ecotype are represented (with the corresponding colour) upon the gene trees, with different mutations presented by stars. The origin of the ecotypes could have followed one of two main evolutionary trajectories, single versus parallel origin, but the histories of adaptive loci do not fall simply into these two classes. Although in the top right panel, the water beetle ecotypes have a single origin and some underlying alleles are shared among lakes, more recent non-shared adaptive mutations may have occurred in each lake. Scenarios where ecotypes have evolved in parallel include various possible gene histories: (i) repeated *de novo* adaptive mutations in the same gene; (ii) parallel adaptive mutations in different genes; (iii) evolution in concert, when adaptive alleles (at least one) originated in one lake and subsequently colonized the other lake due to gene flow between them; and (iv) adaptive divergence from standing genetic variation existing in the ancestral population that colonized the two lakes. The plausibility of each scenario depends mostly on the levels of gene flow between lakes, effective population sizes, mutation rate and numbers of genes underlying the adaptive phenotypic traits. Multiple types of adaptive gene histories could be found in the same ecotype pair.

various published studies showed that although gene reuse during repeated phenotypic evolution is relatively frequent in nature, its proportion is lower in cases of phenotypic convergence when compared with parallel evolution.

As emphasized earlier, for parallel evolution to be considered as evidence of ecological speciation, we have to consider the independent evolution of not only the adaptive traits, but also reproductive isolation itself (Schluter & Nagel 1995). This was precisely the focus of a study presented by Dolph Schluter, where mating preference was evaluated for benthic and limnetic sticklebacks from different lakes (Enos and Paxton), showing that females mate more often with males from the same ecotype, even if from another lake, than with males of the other ecotype (Rundle *et al.* 2000). This provides crucial evidence that reproductive isolation is a consequence of divergent natural selection because, in this case, assortative mating depends on the adaptive phenotypes regardless of origin. If these ecotypes have parallel origins, as suggested by genetic data (Taylor & McPhail 2000), the mechanisms that make individuals preferentially mate with individuals of the same ecotype must have also evolved in parallel.

Ecological speciation: where next?

Ecological speciation presumes the existence of a direct link between ecologically based divergent selection and reproductive isolation. Despite the enormous advances, our knowledge on the subject is far from complete. Here, we will propose some major guidelines for current ecological speciation research, which were reviewed by Roger Butlin during his closing lecture.

- 1 Phenotypes, the raw material for natural selection, need to be rigorously characterized (e.g. high-throughput phenotyping—Sozzani & Benfey 2011). We are also largely ignorant about pleiotropy, phenotypic constraints and correlations between traits (Seehausen *et al.* 2014). This may be particularly important in the study of repeated evolution, as similar phenotypes may have originated in independent lineages because of strong evolutionary, genetic or developmental constraints rather than due to natural selection alone.
- 2 Transcriptome and genome sequencing are highly revealing about numerous aspects of ecological speciation (e.g. importance of regulatory vs. coding regions—Jones *et al.* 2012; differences in divergence between allopatric vs. sympatric species pairs—Renaut *et al.* 2013) and will be even more informative when linked to information on phenotypes, fitness and reproductive isolation by combining genetic mapping, transgenics and studies of cellular and developmental biology with studies of reproductive barriers and fitness in natural populations.
- 3 The role of chromosomal rearrangements and their importance in maintaining the associations between genes involved in adaptation and reproductive isolation needs further investigation (Kirkpatrick & Barton 2006; Faria *et al.* 2011). Felicity Jones showed that inversions

harbour clusters of loci showing high divergence between marine and freshwater sticklebacks, providing additional support for the importance of these regions in speciation (Rieseberg 2001; Faria & Navarro 2010). This is particularly important because chromosomal rearrangements promote coupling between the genes involved in intrinsic and extrinsic reproductive barriers, thus facilitating speciation (Faria *et al.* 2011).

- 4 Without a high-quality reference genome, many inferences are limited and likely to change when complete information becomes available. This is especially relevant when CNVs have been suggested to play a role in ecological speciation. However, genome assembly and annotation are still challenging in nonmodel organisms, particularly for large or highly duplicated genomes or when short reads are used (Schatz *et al.* 2012).
- 5 Although a remarkable degree of reproductive isolation is often observed in early stages of speciation, this may represent a bias in the way the research community selects target species for study, which tends to be driven towards taxa already presenting marked phenotypic differences and some degree of reproductive isolation. Therefore, we need to correct this bias in future studies on the early stages of speciation by widening the range of study systems to include comparisons between populations or taxa without prior evidence of differentiation, isolation or hybridization.
- 6 Perhaps the most important contribution to make the field move forward is to focus on approaches that ask how each type of barrier (e.g. ecological, intrinsic incompatibility, assortative mating) forms and how barriers come to be associated with one another (e.g. by reinforcement—Smadja & Butlin 2011; Abbott *et al.* 2013), fostering progress towards complete reproductive isolation. Classifying speciation (e.g. allopatric, sympatric, mutation order, chromosomal, ecological) tends to detract from this holistic view.
- 7 Finally, as suggested before, the 'rush to genomes' must not make us lose sight of one of the most important components of ecological speciation: ecology. Comparative analyses and manipulative experiments in the laboratory or field are needed to test mechanisms of selection on traits. Documenting the interplay between genes and ecology via phenotypes is essential for understanding the origin of reproductive isolation.

Dedication

The AES conference was dedicated to Professor Godfrey Hewitt, one of the founders of molecular ecology, a very influential evolutionary geneticist (2005 Molecular Ecology Prize) and, most importantly, an exceptional mentor (2006 Nature award for Creative Mentoring in Science) who leaves a remarkable legacy to future generations of evolutionary biologists. He was much loved and all of us remember his engaging and inspiring personality. Many of the suggestions we have presented here were greatly

inspired by him, and we recognize that this manuscript would have been substantially improved with his helpful criticisms.

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