INVITED REVIEWS AND META-ANALYSES
A road map for molecular ecology

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Abstract

The discipline of molecular ecology has undergone enormous changes since the journal bearing its name was launched approximately two decades ago. The field has seen great strides in analytical methods development, made groundbreaking discoveries and experienced a revolution in genotyping technology. Here, we provide brief perspectives on the main subdisciplines of molecular ecology, describe key questions and goals, discuss common challenges, predict future research directions and suggest research priorities for the next 20 years.

Keywords: behaviour, community phylogeography, ecological genomics, hybridization, landscape genomics, microbial diversity, molecular adaptation, phylogeography, speciation, trophic ecology

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Introduction

Molecular ecology refers to a diversity of approaches that use molecular genetic techniques to address ecological questions. This nascent discipline has expanded into a field encompassing a broad range of ecological and evolutionary questions, largely shaped since 1991 by the journal *Molecular Ecology*. In the past decade, the field of molecular ecology has been revolutionized, and this revolution is ongoing. Almost daily, new technologies and analytical approaches open up novel ways to address classic questions and enable us to test hypotheses that were previously unanswerable. Today, a typical issue of *Molecular Ecology* may include papers using molecular genetic approaches to investigate the interactions among species (including difficult to culture microbes), the genetics and evolution of ecologically

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important traits, the relatedness among individuals and (based on this information) their dispersal and behaviour, the movement of individuals across landscapes, the formation of new species and the consequences of hybridization between divergent lineages. Here, we examine the current state of the key subdisciplines within the field of molecular ecology and predict possible future directions and ongoing changes.

What will the field of molecular ecology look like in years to come? Much of the current revolution is enabled by new technology, with advances in genomics, satellite imagery, computer hardware and software, and microfluidics leading to major changes in how we can best investigate molecular ecological questions. Molecular ecologists must therefore be masters of integrating these new technological approaches and applying them in innovative ways to key biological questions. And this brings up what is most exciting about the current state and direction of the field—the biological questions remain at the fore.

A classic metaphor relating to many scientific endeavours is 'the drunk under the streetlight', who searches for his lost keys in the area illuminated by a streetlight even though he knows his keys are far away in a darkened area where he cannot see. While many scientific questions are indeed difficult to investigate due to our limited observational abilities, these new technologies are dramatically broadening our horizons. Much of early molecular ecology research was limited by the difficulties and expense in obtaining genetic information, but today we can assess thousands of loci in numerous individuals routinely and relatively cheaply (Davey & Blaxter 2010; Davey *et al.* 2011). We are now at the point where the whole street is illuminated, and many 'key' questions that we have wanted to ask all along can finally be addressed.

This 'road map' paper builds on a symposium about the future of molecular ecology that was held on 6 July 2012 at the First Joint Congress on Evolutionary Biology in Ottawa. The symposium showcased some of the leading research in molecular ecology, mapping out future research priorities with panel discussions. On 24 October 2012, we held an Online Forum to obtain additional feedback from the molecular ecology community about challenges and priorities. These talks and discussions are distilled below into brief perspectives on many of the key research areas in molecular ecology, including discussions of important questions, challenges and priorities.

DNA sequence–based trophic ecology—by Brent C. Emerson

Trophic interactions represent the primary data for investigations ranging from single species conservation through to the resolution of community food webs. However, our capacity to directly acquire data on trophic interactions is limited by our ability to record interactions between consumer and resource species. Monitoring these interactions is challenging when consumers are difficult to observe and becomes more challenging still if a consumer is a resource-use generalist, as opposed to specialist.

In response to these difficulties, efforts have been directed towards the quantification of diet by indirect means such as the analysis of faecal material (morphological remains, plant alkanes, near infrared reflectance spectroscopy), analysis of gut contents (as for faecal analysis, but also including prey-specific antibodies and protein electrophoresis), laboratory feeding experiments and stable isotope analysis of consumer tissues (reviewed in Valentini *et al.* 2009; Pompanon *et al.* 2012).

The expansion of publicly available genomic resources, technical advances in PCR amplification, the development of next-generation DNA sequencing technology and increased understanding of the degradation properties of DNA sequence have been capitalized upon to refine and improve the general approach of developing species-specific primers (Asahida *et al.* 1997). The DNA of resource species can potentially be sampled from the moment the organism is consumed (upper digestive tract) through to the moment it is expelled as faeces, with DNA quality expected to progressively decay with sampling time.

Recent work has shown that regions of chloroplast DNA of more than 500 bp can be amplified from DNA extracted from herbivorous beetles, facilitating the identification of resource species consumed (Jurado-Rivera *et al.* 2009). Similar work on rare weevil species from Mauritius reveals that the upper digestive tract frequently contains plant material from a single resource species, sufficient for co-extraction, cpDNA amplification and sequencing (J. N. J. Kitson, B. H. Warren, V. Florens, C. Baider, D. Strasberg & B. C. Emerson, unpublished data). In such cases where reasonably long DNA regions can be amplified from a single resource species, traditional DNA sequencing approaches can be employed, but degraded samples will most profitably gain from parallel sequencing technology (for reviews see Valentini *et al.* 2009; Pompanon *et al.* 2012). In both cases, the ability to select plant-specific genes (cpDNA) eliminates unwanted co-amplification of consumer DNA, which poses more of a problem for the characterization of predator–prey relationships (for a review see King *et al.* 2008a).

In the case of predators and prey, if there is sufficient prior genomic knowledge and phylogenetic distance between consumer and resource species, resource-

specific primers may be possible, as has been done to compare insectivory among sympatric New World primates (Pickett *et al.* 2012). However, as the genomic distance between consumer and resource species narrows, the challenge of selectively amplifying the resource species increases, but primer blocking does hold some promise for minimizing this problem (Vestheim & Jarman 2008; Shehzad *et al.* 2012).

Promises and challenges

The main goals of DNA sequence–based trophic ecology are to (i) characterize resource species utilization by a focal species and (ii) estimate the proportional representation of each resource species to the focal species' diet. Unfortunately, there is not a one size fits all solution to these goals, because of the inherent variance and complexity of consumer resource systems (Fig. 1). Several of the main challenges and priorities for DNA sequence–based trophic ecology over the next two decades are listed below:

1 Development of methods that provide accurate and unbiased identification of all resource species. Common to all systems is the desire to amplify a DNA sequence region, or regions, across the full range of resource species, in the absence of amplification bias, with each resource species being uniquely identifiable within a fully inventoried DNA sequence reference library of resource species. While this may be considered the gold standard, it may often be difficult to achieve in full.
2 Development of methods that provide accurate and unbiased quantitative estimates of the proportional representation of resource species (see Pompanon

et al. 2012 for a discussion). Current estimates rely upon a quantifiable relationship between the copy number of an amplified DNA sequence region and resource biomass; these estimates also require equal amplification efficiency of the target DNA region across different resource species.
3 Elimination of DNA amplification step (i.e. PCR). Problems arising as a result of PCR amplification bias can potentially be overcome if technology eliminates PCR altogether. This becomes theoretically possible as next-generation sequencing capacity increases and logistically possible as costs come down. If the genomes of each resource species become available as a reference tool, PCR-free sequencing of tissue sampled from the alimentary system of a consumer may permit the estimation of presence, absence and abundance of resource species DNA.
4 Connection of spatial variation in abundance of consumer and resource species with dietary patterns. Combining ecological surveying and sampling with a DNA sequence–based approach to trophic ecology could connect spatial variation in resource species utilization by consumer species to spatial variation in abundance of either group. Such data would contribute to understanding the evolution of diet within groups of evolutionary interest such as plant-feeding insects.
5 Evaluation of diet preferences of consumers. Molecular characterization of resource utilization may be a useful tool for conservation biologists, providing a means to evaluate which resource species are favoured by a consumer species, or whether rarity among consumer species is related to resource species limitations, such as specialization to rare resource species, or competitive exclusion by other consumers.

Even in the absence of quantitative estimates, DNA sequence–based characterization of trophic ecology has already taken us far beyond where we were, and we can expect more to come. The key to success, as always, will be clever questions, careful experimental design and cautious interpretation of data.

Influential passengers: microbial diversity within multicellular organisms—by Graham N. Stone

Cohabiting microorganisms (bacteria, fungi, protists) play diverse roles in the biology of multicellular hosts. Improved methods of molecular detection—and particularly high-throughput sequencing—are driving an explosion of studies detecting bacterial and fungal contributions to the genomic and transcriptomic diversity present within other organisms. It is increasingly clear
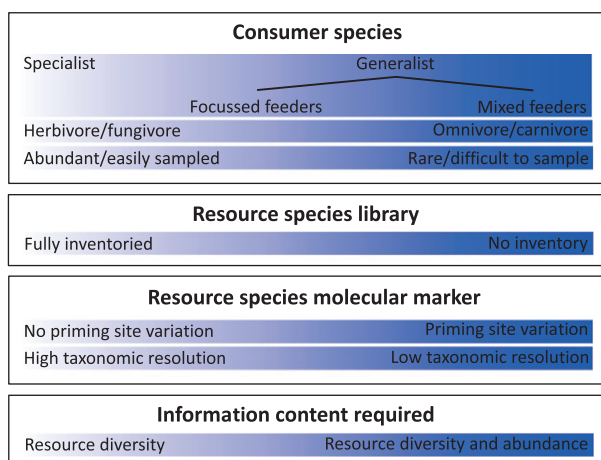

Fig. 1 Four variables of importance for a DNA sequence–based approach to characterize resource use by consumer species. Increased shading from left to right indicates increased technical or logistical difficulty.

that many aspects of organismal phenotypes reflect contributions from a diverse associated microbiome (Zilber-Rosenberg & Rosenberg 2008; Gibson & Hunter 2010).

Some of the newly revealed phenotypic diversity stems from discovering new roles for otherwise well-known symbionts. For example, *Wolbachia* bacteria, primarily known for their influence on the reproductive biology of their hosts (Hilgenboecker *et al.* 2008), are now also known to allow the larvae of leaf-mining moths to maintain 'green islands' of living plant food within fallen leaves (Kaiser *et al.* 2010), and to suppress populations of human pathogens within mosquito vectors (Pan *et al.* 2012). Other new discoveries are of familiar microbial groups in unfamiliar places. Examples include recently demonstrated associations between multiple lineages of nitrogen-fixing bacteria and ants, whether inhabiting the nests of leaf-cutters (Pinto-Tomás *et al.* 2009) or specialized organs within the bodies of honeydew feeders (van Borm *et al.* 2002). Growing numbers of studies, however, reveal unexpected and major roles for microbes in mediating interactions within (Sharon *et al.* 2010) and between other species (Cafaro *et al.* 2011; van der Heide *et al.* 2012; McFrederick *et al.* 2012; Oliver *et al.* 2012; Zhang *et al.* 2012). A new paradigm may be that some of the variation in most host traits can be attributed to such 'influential passengers' (O'Neill *et al.* 1997). However, the microbiomes of the vast majority of organisms remain unsurveyed.

Future promise and challenges

Three general approaches should allow rapid advances in this field in the near future:

1 First, the falling cost of high-throughput sequencing allows large-scale DNA-barcode-based surveys of host-associated microbial diversity. It is now possible to ask how microbial floras vary both across host species (Oliver *et al.* 2010; Anderson *et al.* 2012; Sullam *et al.* 2012) and within them (e.g. Qi *et al.* 2009; Blaalid *et al.* 2012), including our own (Yatsunenko *et al.* 2012).

2 Second, genome and transcriptome libraries for a given focal species inevitably contain contributions from associated microorganisms. Informatics tools used to filter out nonhost contributions during host genome/transcriptome assembly can be also used to focus on host–symbiont associations (Kumar & Blaxter 2011), and differences in base composition and coverage between host and bacterial genomes allow contributions from these sources to be visualized (Fig. 2).

3 Third, sequencing experiments can be designed to distinguish environmental contaminants from microorganisms that are genuinely facultative or obligate symbionts. Given what is known of the genomic relationships between hosts and their symbionts (Dunning-Hotopp *et al.* 2007; The International Aphid Genomics Consortium 2010; Suen *et al.* 2011), it seems
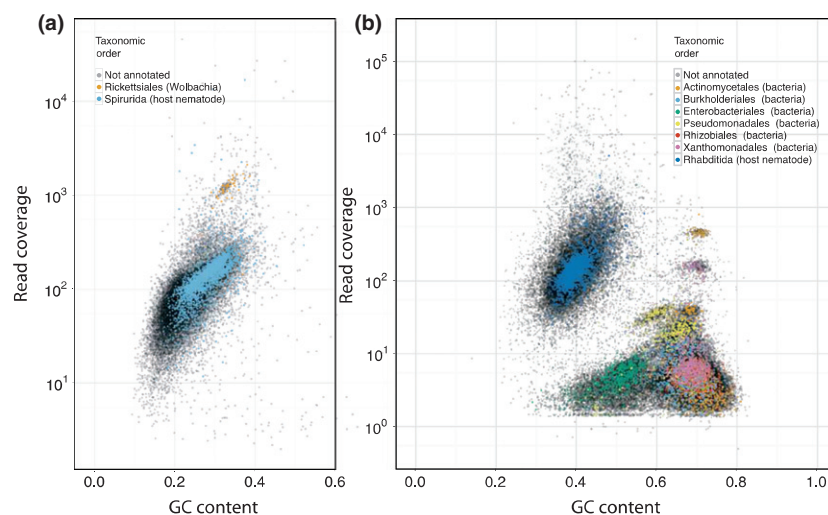

Fig. 2 Visualization of host and associated microbial genomes in two systems. (a) the host nematode *Dirofilaria immitis* and its known *Wolbachia* symbiont and (b) the host nematode (*Caenorhabditis* sp. 5) and its newly revealed microbiome. On each of the two plots, points map individual contigs reconstructed from whole-organism genomic libraries based on variation in GC content and sequence coverage (for full details, see Kumar & Blaxter 2011). In each figure, 10 000 randomly selected contigs were annotated by BLAST comparison with the NCBI nt database and coloured by taxon for the best match identified (non-BLASTed contigs are shown in grey in both figures). In (a), we see clear separation between contigs attributable to the host worm and its symbiont. In (b), the host nematode is clearly separated from microbial contigs attributed to ten distinct clusters from seven taxonomic orders. While some bacterial sequences were expected in this library because the worms had been fed *Escherichia coli*, the roles and origins of the other bacteria are unknown. Modified with permission from Kumar & Blaxter (2011).

likely that some aspects of gene diversity and expression in any host will only make sense when considered at the scale of the combined 'holobiont' (Zilber-Rosenberg & Rosenberg 2008).

Phylogeography—by Bryan C. Carstens

Phylogeographic investigations seek to identify the forces that influence the geographical distribution of genetic variation. As befits a discipline that was developed to conduct phylogenetic investigations within species, studies are commonly concerned with identifying cryptic diversity (e.g. King *et al.* 2008b), estimating lineage divergence (e.g. Evans *et al.* 2011) or understanding species boundaries (e.g. Barrett & Freudenstein 2011; Niemiller *et al.* 2011). However, phylogeography in 2012 bears little resemblance to studies published 20 or even 10 years ago. Changes in the types of data collected by phylogeographers and the analytical methods employed have accelerated in recent years, and the net effect of these changes has been to dramatically improve the quality of phylogeographic inference.

Originally conceived as a mitochondrial bridge between phylogenetics and population genetics (Avise *et al.* 1987), phylogeographic investigations nowadays commonly include multiple nuclear loci (e.g. Amaral *et al.* 2012) or microsatellites (e.g. Zakharov & Hellman 2012). In addition, next-generation sequencing (NGS) has already produced a number of compelling phylogeographic data sets (e.g. Emerson *et al.* 2010; Gompert *et al.* 2010; Zellmer *et al.* 2012). The acquisition of these data is motivated by concerns about selection on the mitochondrial genome (e.g. Kivisild *et al.* 2006), the realization that stochastic forces can lead to incongruence between the history of a population and the history of any single locus (e.g. Maddison 1997), as well as the desire for improved parameter estimates (e.g. Beerli 2006).

The methods used for data analysis have also changed, becoming more reliant on models (e.g. Knowles 2009; Beaumont *et al.* 2010). One exciting development is model comparison, where the probability of multiple models is calculated given the data and subsequently ranked by calculating the relative posterior probability of models (e.g. Fagundes *et al.* 2007; Peter *et al.* 2010) or by using an information theory approach (e.g. Carstens *et al.* 2009; Provan & Maggs 2012). Rather than making qualitative inferences derived from patterns in the data, phylogeographers can now model specific evolutionary scenarios and evaluate the probability of each given the data. This approach is less prone to over-interpretation (Knowles & Maddison 2002) and confirmation bias (Nickerson 1998) and also less likely to be misled by inaccurate parameter estimates.

Future promise and challenges

1 One persistent challenge facing phylogeography is the discovery of cryptic species-level variation. While methods for species delimitation are improving, we lack methods that can accurately discover cryptic diversity from genetic data across a broad range of relevant parameter space. Clustering approaches such as Structurama are broadly applicable (e.g. Rittmeyer & Austin 2012), but methods that utilize species trees are mostly limited to validating proposed species boundaries rather than jointly estimating these boundaries and the species phylogeny (but see O'Meara 2010).

2 A second challenge is identification of optimal sampling design, where the axes of variation are the number of samples and the number of loci. Historically, the discipline has relied largely on sampling that maximized the former because phylogeographic breaks and cryptic diversity are difficult to discover across an undersampled landscape. However, this question needs to be re-examined in the light of our expanded capacity to collect data along the second axis, in order to optimize the sampling design for particular questions.

3 A third challenge is developing methods for comparative phylogeography. To date, most comparative work has proceeded by inferring phylogeographic history on a species-by-species basis and secondarily comparing these results. While integrative community-level approaches to data analysis are generally lacking (but see Hickerson & Meyer 2008), such an approach could dramatically improve our ability to estimate the evolutionary history of ecological communities, particularly those that are coevolved (e.g. Smith *et al.* 2011; and see following section).

Community phylogeography—by Graham N. Stone

As described above, a range of new phylogeographic techniques now allow formal comparison of the support in observed data for alternative scenarios of population history (Bertorelle *et al.* 2010; Hickerson *et al.* 2010; Huang *et al.* 2011). When applied to sets of species, these approaches allow identification of shared routes of range expansion or barriers to gene flow (e.g. Hickerson & Meyer 2008). Data sets for species in the same guild (Bell *et al.* 2011; Dolman & Joseph 2012) or in interacting trophic levels (Smith *et al.* 2011; Stone *et al.* 2012) allow testing of alternative models of community assembly, and hence link population ecology with macroecology (Byrne *et al.* 2011; Ricklefs & Jenkins 2011). It is possible to ask, for example, whether species that
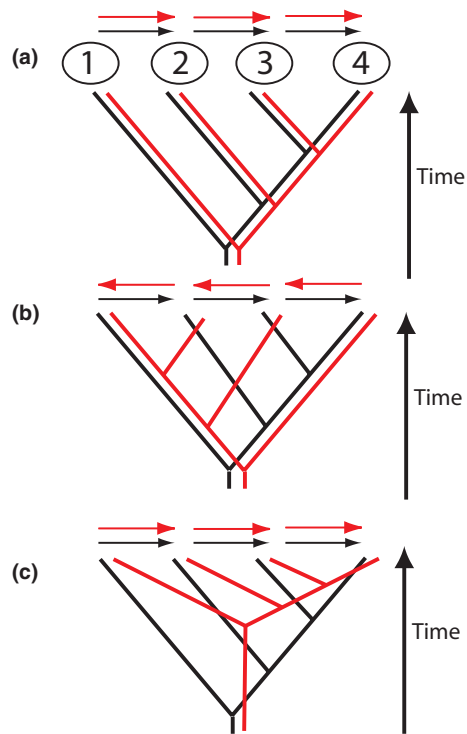
Fig. 3 Examples of alternative population models of community assembly for two lineages (red, black) across four regions (e.g. islands, glacial refugia). (a) Concordant phylogeographic patterns imply that the two lineages spread at the same time in the same direction (shown by arrows) from a shared common origin. This is compatible with ongoing biotic interactions between them (including co-evolution) throughout the range expansion process. (b) Discordant phylogeographies imply that the two lineages dispersed in opposite directions from different origins. There is variation between regions in how long the two lineages have been exposed to each other. In this type of scenario, in contrast to (a), it is possible for the association between the two lineages in each region to have evolved entirely independently. This is a potentially very important contrast when analysing co-evolutionary interactions between lineages. (c) Population histories for the two lineages show the same topology across regions and have spread in the same direction from the same origin. However, dispersal by the red lineage is more recent. This corresponds to delayed tracking of one lineage by the second. While populations of each lineage have a long history of co-occurrence in region 1, populations of the black lineage occupy all other regions before the red lineage. This model would be compatible, for example, with temporary escape of a host or prey species from its parasites or predators, and interruption of any co-evolutionary interactions between lineages.

now form important components of interaction networks (such as food webs or pollination webs) have a long history of co-occurrence, tracking each other through space and time from a shared origin, or instead represent recent associations with discordant phylogeographic histories (Fig. 3; Stone *et al.* 2012).

While discrimination between complex scenarios can be challenging because of the number of parameters that must be estimated, comparative phylogeography research programmes are becoming increasingly accessible. Generation of the data sets required to infer population history in a coalescent framework is becoming increasingly affordable with high-throughput sequencing, making it ever easier to select target species on the basis of their biological interest rather than their tractability with existing markers (Emerson *et al.* 2010). Further, the changing emphasis from a few loci in many individuals to many loci in a few individuals (e.g. Lohse *et al.* 2012) makes it much easier to incorporate species that are either rare, difficult to sample or exist only in museum collections. As an example, Lohse *et al.* (2012) used multilocus data in only a single haploid male individual from each of three populations to infer contrasting times of range expansion across the Western Palaearctic in a guild of parasitoid wasp species.

Future promise and challenges

1 The major challenge in community phylogeography is accurate estimation of the topology of population relationships, and the timing of population splits and dispersal events, for multiple species (such as guilds or trophic levels) (Fig. 3). Even for simple models, this is a very data-hungry problem.
2 Next-generation and third-generation sequencing technologies offer enormous promise for this field, providing increasing power to estimate parameters from population genomic data at low cost. The challenge has been to develop analytical approaches that make best use of many hundreds or thousands of sequences. A major attraction of such population genomic data is that support for alternative simple population models can be estimated in a likelihood framework using very small numbers of individuals per population. Although simple, these models can reasonably be applied to real-world scenarios (Lohse *et al.* 2012; Smith *et al.* 2012).
3 Additional promise is provided by ABC approaches that incorporate data for many loci in many species (Huang *et al.* 2011). Because this approach is simulation based, an ongoing aim for this field is to overcome the computational challenge of extending multispecies models to population genomic-scale data sets.
4 Once phylogeographic relationships for sets of species are determined, it will become possible to use these to control for statistical nonindependence in population-level analyses of interactions between species (Stone *et al.* 2011). This importance of this issue

is increasingly recognized, but remains challenging, in analyses of local adaptation and community genetics (Marko 2005; Evans *et al.* 2008).

Landscape genomics—by Rose L. Andrew and Victoria L. Sork

Landscapes shape gene flow by influencing the ease with which organisms, propagules or gametes move, the production of propagules and the density of receiving populations. The subject of many review articles (e.g. Manel *et al.* 2003; Storfer *et al.* 2007, 2010; Holderegger & Wagner 2008; Sork & Waits 2010), methods papers (Balkenhol *et al.* 2009; Cushman & Landguth 2010a,b; Spear *et al.* 2010), a special edition of *Molecular Ecology* (Waits & Sork 2010) and landscape genetics is an archetypical molecular ecology discipline through its integration of molecular approaches, ecology, population genetics, spatial statistics and geographical tools to address landscape-scale research questions and hypotheses.

Connectivity and the movement of organisms through the landscape matrix are central to landscape genetics (Beier & Noss 1998; Dyer & Nason 2004; McRae & Beier 2007; Braunisch *et al.* 2010). Both individual- and population-level approaches have been developed for modelling how organisms (and genes) actually move across a landscape, rather than as simply isolation by linear distance. These models have valuable applications in conservation and management (Segelbacher *et al.* 2010), allowing practical analysis of whether landscape changes have interfered with gene movement (Braunisch *et al.* 2010) or the presence of corridors facilitated it (Epps *et al.* 2007; Beier *et al.* 2011). In the context of conservation and management, methods such as least cost paths, circuit theory, ocean simulations and population networks have provided tools to assess barriers, corridors and overall patterns of connectivity (Beier & Noss 1998; Dyer & Nason 2004; McRae & Beier 2007; Braunisch *et al.* 2010; Galindo *et al.* 2010).

Other landscape genetic studies have investigated the impact of the local environment on patterns of genetic variation on the landscape, especially with climate variables (Manel *et al.* 2010b; Sork *et al.* 2010; Poelchau & Hamrick 2012). In a study of valley oak (*Quercus lobata*), multivariate genotypes of nuclear microsatellites were significantly associated with climate variables, even after the confounding effects of spatial location were taken into account (Grivet *et al.* 2008; Sork *et al.* 2010). One explanation is that climate shapes migration patterns creating similar gradients. Alternatively, immigrants from localities with different climates may be maladapted to the new location, limiting gene flow in both neutral and adaptive portions of the genome. For

example, in the context of ecological speciation, landscape genetics can be used to generate more realistic null hypotheses when testing whether adaptation reduces gene flow between habitats by eliminating poorly adapted immigrants (e.g. sunflowers, Andrew *et al.* 2012).

As the discipline of landscape genetics extended its focus to adaptive genetic variation (Holderegger *et al.* 2006; Lowry 2010; Manel *et al.* 2010a; Schoville *et al.* 2012), it became clear that patterns of adaptive genetic variation could be distinguished from those created by background demographic processes (Beaumont & Balding 2004; Joost *et al.* 2007). The initial use of AFLPs provided a means of scanning numerous loci across the genome to identify candidate loci, and associations of loci with habitat or climate were a harbinger of the landscape genomics studies we see today. The most compelling of the AFLP-based studies were conducted in concert with other disciplines, such as ecological niche modelling and historical demography (e.g. Freedman *et al.* 2010 and Manel *et al.* 2012). Their spatially explicit models provided excellent opportunity to separate the impacts of gene flow, demographic history and selection on the geographical structure of genetic variation.

The availability of NGS tools (Helyar *et al.* 2011) has facilitated the transition from landscape genetics to landscape genomics. Even for nonmodel systems, it is often possible to link markers to functional genes based on rapidly growing databases of transcriptome sequences (see section on Ecological Genomics and Molecular Adaptation). These tools enable surveys of thousands of genetic variants [single nuclear polymorphisms (SNPs)] found across the genome, greatly facilitating the simultaneous analysis of background genetic structure created by neutral processes, such as population expansion or contraction and gene flow, and identification of candidate genes under natural selection. Similar to the genome-wide association studies (GWAS) that are commonly used for finding genes underlying specific traits in model systems, such as *Arabidopsis thaliana* (Bergelson & Roux 2010; Kover & Mott 2012), several statistical models are available to identify loci that are correlated with environmental gradients while controlling for spatial autocorrelation and demographic effects (Hancock & Di Rienzo 2008; Coop *et al.* 2010; Kang *et al.* 2010). One limitation of these models is that they test a large number of SNPs and climate variables one at a time for phenotypic traits that are often polygenic and for which epistatic interactions may be more important than simple additive effects (Holliday *et al.* 2012; Le Corre & Kremer 2012). Consequently, we are calling for increased use of multivariate statistical approaches (Sork *et al.* 2013).

A spatially explicit perspective is complementary to evolutionary and ecological genomics. Understanding the landscape and habitat factors shaping the distribution of adaptive genetic variation in nature is important to a comprehensive picture of the evolution of locally adapted genes (Lowry 2010), for instance, genes identified from common-garden experiments testing for local adaptation or associations with phenotypes (Anderson *et al.* 2010). Conversely, to understand the relationship between patterns of putatively adaptive genetic variation on the landscape and associated phenotypes under selection, it will be necessary to couple landscape genomics with experimental approaches such as ecological genomics and gene expression studies (Stinchcombe & Hoekstra 2008). Under controlled conditions, it is possible to assess which genes are expressed during drought stress, photoperiod changes or other manipulations. Experimental tests of whether environment-associated genes also affect phenotypes and fitness in reciprocal transplants will provide convincing evidence for their adaptive importance and offer insight into the evolutionary mechanisms maintaining geographical structure (Anderson *et al.* 2013). Increasing cross talk between landscape genomics and evolutionary/ecological genomics is a promising way forward for both fields.

Major challenges

Several challenges have accompanied landscape genetics research from its inception and are now emerging for landscape genomics. Much progress has been made in inferring historical divergence (Gugger *et al.* 2013), gene flow (e.g. Andreasen *et al.* 2012) and molecular demography (e.g. Schoville *et al.* 2012), but incorporating them into landscape genetics and genomics studies remains a challenge. Another big issue pertains to the scale and intensity of sampling, not only for statistical power but also for accurately detecting topographic and environmental effects. Preferably, studies are designed such that an a priori assumption of the important spatial scales is not implicit but can instead be identified (Galpern *et al.* 2012), and downloaded climate variables are at the same scale as the local samples. Historical environmental data are also highly desirable, especially when considering the evolution and spread of locally adapted alleles, and the availability of such data at suitable scales imposes a limit on the scope of landscape genetics and genomics.

The increasing ease of using SNPs as genetic markers has created opportunities, but challenges remain as recently summarized in the study by Helyar *et al.* (2011). A few issues are worth highlighting here. The impact of physical linkage on tests of selection is well known in population genetics through the hitchhiking effect (Barton 2000; Schlötterer 2003). With the increasing marker density created by NGS techniques, statistical techniques that account for linkage are desirable for future landscape genomics. Detecting common variants strongly associated with a given environmental variable is relatively straightforward (Coop *et al.* 2010; Hancock *et al.* 2011); however, rare and population-specific variants are difficult to identify based on global analyses, just as in genome-wide association studies (Buckler *et al.* 2009).

Potential future directions

1 Going beyond identifying loci associated with environments is critical for the ongoing development of landscape genomics as a field. For example, by quantifying connectivity across the ranges of species, landscape genomics can provide a novel perspective on the question of how gene flow promotes or constrains adaptation to new habitats. Essential to such studies will be the coupling of landscape genomics with other approaches, especially experiments, demography and niche modelling.

2 Localized introgression can shape genome-wide genetic structure and is amenable to landscape genomics (Fitzpatrick & Shaffer 2007; Kane *et al.* 2009). In addition to being a potential confounding factor in environmental association analysis, the geographical extent of gene flow between species and the factors driving genome-wide patterns of introgression are important questions (see Hybridization and Speciation).

3 Spatially explicit simulations have made important contributions to the development of landscape genetics (Balkenhol *et al.* 2009; Cushman & Landguth 2010a; Epperson *et al.* 2010) but are not routinely used in empirical studies, especially those concerning adaptation. As real populations represent only a single iteration of the evolutionary process (Buerkle *et al.* 2011), simulations may be essential for gauging the uncertainty around inferences in landscape genomics.

4 The role of epigenetics in plant response to the environment is receiving increased attention (Bossdorf *et al.* 2008; Jablonka & Raz 2009; Becker & Weigel 2012); however, we know little about its prevalence in natural populations. It is now possible to survey DNA sequence variation and epigenetic marks, such as DNA methylation, simultaneously (Feng *et al.* 2011). A landscape genomic analysis could provide first-level evidence for the association of both genetic and epigenetic variation with environmental gradients.

Ecological genomics and molecular adaptation: back to the future—by Sean M. Rogers, Louis Bernatchez, Aurelie Bonin and Jon Slate

From the time when E.B. Ford 'invented' the field of ecological genetics (Ford 1964), followed by the advent of electrophoretic surveys of genomic variation in 1966, molecular ecologists have been challenged to explain the large amounts of standing genetic variation in populations and the degree to which this variation can be explained by adaptive evolution (Lewontin 1991). The field of ecological and evolutionary genomics (EEG) emerged from efforts to understand the genomic mechanisms underlying organismal responses to abiotic and biotic environments (Feder & Mitchell-Olds 2003; Ungerer *et al.* 2008). This framework proposed experimental approaches towards elucidating the genomic architecture of ecologically important traits, how these traits affect fitness and the evolutionary processes by which these traits may arise and persist—the overarching objective of linking genotype to phenotype and ultimately fitness (Dalziel *et al.* 2009).

The merging of genomics with ecology includes more than just the incorporation of a new genomic toolbox. Emerging technologies are providing unparalleled insight into the genomes of species, leading to new questions that need to be tested, while existing questions can be addressed in ways that were not previously possible (Barrett & Hoekstra 2011). In addition, Feder & Mitchell-Olds (2003) predicted that the promises of large-scale genomic data would not change the fact that ecological and physiological knowledge would remain crucial for the interpretation of genomic and postgenomic data. Molecular ecologists have indeed risen to this challenge by (i) demonstrating the significance of standing genetic variation to adaptive evolution (e.g. Colosimo *et al.* 2005); (ii) revealing that even small changes in the sequences of genes (including regulatory regions) may result in striking adaptive evolution (e.g. Hoekstra *et al.* 2006; Chan *et al.* 2010; Rosenblum *et al.* 2010); (iii) measuring selection and validating candidate genes (e.g. Barrett *et al.* 2008, 2011; Bonin *et al.* 2009; Gratten *et al.* 2012); (iv) elucidating the genetic bases of microevolutionary changes in natural populations (e.g. Gratten *et al.* 2008, 2012; Johnstone *et al.* 2011); (v) determining the genomic architecture of adaptive evolution and ecological speciation (e.g. Kane & Rieseberg 2007; Rogers & Bernatchez 2007; Nosil *et al.* 2012); (vi) establishing the importance of plasticity in adaptive evolution (e.g. Ghalambor *et al.* 2007; McCairns & Bernatchez 2010; McCairns *et al.* 2012); and (vii) estimating the role of life history trade-offs in shaping patterns of genome-wide gene expression and linking these trade-offs with adaptive divergence (e.g. Derôme *et al.* 2006; St-Cyr *et al.* 2008; Colbourne *et al.* 2011).

Nonetheless, these studies and increasingly others have revealed additional questions and highlighted significant challenges for the future, including broadening the scope to include a wider range of organismal diversity, especially keystone species. EEG studies should also pay greater attention to relatively undisturbed habitats in the native range of species, unique ecology and behaviours, and long-term synthetic and natural ecological experiments (Feder & Mitchell-Olds 2003; Gratten *et al.* 2008, 2012; Grant & Grant 2011).

Regardless of the organism being studied, the progress over the last 10 years of EEG research highlights at least six priorities that should be considered over the next two decades.

Priority 1: Extended evolutionary theory

1 What does evolutionary theory predict for the consequences of standing genetic variation, dominance, molecular quantitative genetics and nongenetic inheritance (e.g. epigenetic inheritance, parental effects) during adaptive evolution?
2 What does theory predict for the outcome of phenotypic plasticity during adaptive evolution?
3 Beyond single locus traits, what are the predicted consequences of polygenic inheritance during adaptive evolution?

Priority 2: Ecological annotation of genes

1 Annotation of genes is the main limiting factor when making functional inferences for genomic variation, especially in nonmodel species (Pavey *et al.* 2012).
2 There is an urgent need for ecological gene annotation, which will require better data integration and functional analyses.

Priority 3: Phenomics

1 Phenotypes are the variation that selection can see, so greater attention should be paid to the measurement and reporting of phenotype–environment associations.
2 How do different levels of biological organization (from the gene to the different steps of regulation, transcription, signal transduction to networks and pathways) give rise to this variation?
3 Acquiring detailed phenotypic data will be as crucial as molecular data in building genotype–phenotype maps (Houle *et al.* 2010).

Priority 4: Become predictive about organismal response to environmental variation

1 There is a need for further analytical methods development that allows robust discrimination between the consequences of drift and selection.
2 What are the consequences of variation in genetic architecture?
3 Will genome scans of temporal changes in allele frequency reveal targets of selection?

Priority 5: Become predictive about organismal solutions to environmental heterogeneity

1 Under what ecological and evolutionary conditions do organisms respond to environmental heterogeneity by local adaptation (which generates population structure) vs. the maintenance of balanced polymorphisms or a plastic response of phenotypes?
2 How does genetic variation affect population demography and vice versa?
3 Does the stability of communities depend upon ongoing eco-evolutionary feedbacks—or is evolution ecologically trivial?

Priority 6: Experiments

1 Ultimately, we need to move towards a holistic approach and aim to fully integrate multidimensional high-throughput 'omics 'measurements (i.e. ecological systems biology). This should include dynamic, sequential common-garden experiments in the laboratory and in nature.
2 Also needed is validation of the adaptive significance of candidate genes: how repeatable will the findings be?

Given the rise of molecular ecology since the early 1990s, we are optimistic about the future. These priorities should nonetheless serve as a reminder to students of ecological genomics that there is much work to be carried out. Future advances will continue to require re-engineering of scientific attitudes, training and a focus on multidisciplinarity (Feder & Mitchell-Olds 2003).

Speciation and hybridization—by C. Alex Buerkle, Tatiana Giraud, and Alex Widmer

Interest in the evolutionary and genetic processes that lead to the appearance and maintenance of new species has been a driving force in the development of evolutionary theory and genetics. Much of current speciation research involves understanding how isolation between populations might arise and increase as a result of evolutionary processes, in different spatial and ecological settings, and how isolation might be maintained when it is tested by potential hybridization. Presently, there is great interest in 'speciation with gene flow', both in terms of the origin and maintenance of diversity. This includes ecological speciation, that is, the possibility of reproductive isolation arising directly from adaptation to ecological conditions. This work provides a link between ecology and evolution (Egan & Funk 2009; Schluter 2009; Giraud *et al.* 2010; Gladieux *et al.* 2011) and draws attention to the potentially short timescale over which ecological determinants might give rise to evolutionarily relevant isolation.

Until recently (as is true for much of molecular ecology), empirical studies were severely limited by our ability to assay genomic variation in natural populations. This situation has changed dramatically, and studies for any organism can now be based on orders of magnitude more individuals and loci than before. However, access to population genomic data comes with new challenges, in particular for handling and analysing huge data sets. At this stage, we have only just begun to apply these to key questions in speciation and hybridization and to recognize and accommodate the new complexities that arise from sampling the genome at high resolution.

In the last few years, we have begun to document population genomic variation among species and their hybrids, but we have made less progress towards tying patterns of variation to underlying evolutionary processes. Previously, this was a substantial challenge even with single or small sets of loci, because the inferred evolutionary parameters are only applicable to the models that we specify (Excoffier & Heckel 2006; Wegmann *et al.* 2010). This challenge is now compounded when sampling a larger fraction of the genome, as it involves analysis of many loci with potential differences in their evolutionary histories (mutation, recombination, drift and background selection, positive selection, etc.). In the sections that follow, we expand on this overview with more specific examples of how the study of speciation and hybridization might develop further.

Theory

1 Dobzhansky–Muller (DM) incompatibilities have been identified and now dominate discussions of the genetics of reproductive isolation (e.g. Lee *et al.* 2008; Burton & Barreto 2012). However, theory shows that DM incompatibilities can only serve as an effective

isolation mechanism if F1 hybrids have near zero fitness (Gavrilet 1997). Progress towards understanding their role in isolation will come from additional modelling, including using existing theoretical models with high levels of abstraction (e.g. Barton & Rodriguez de Cara 2009) for generating predictions for parameters that can be estimated in experimental and natural populations.

2 We also need to develop models that better predict the evolution of reproductive isolation with genetic distance, depending on the underlying causes and their effects on fitness, for example a snowball effect due to the accumulation of DM incompatibilities (Matute *et al.* 2010; Moyle & Nakazato 2010) vs. a linear increase in reproductive isolation due to ecological isolation (Gourbière & Mallet 2010; Giraud & Gourbière 2012).

Experiments and study of natural cases

1 Estimates of the fitness effect of DM incompatibilities in a larger number and diversity of organisms are needed, as *Drosophila* may not be a good model for all eukaryotes (see data in fungi: Gourbière & Mallet 2010; Giraud & Gourbière 2012). In addition, the generality of the model of accumulation of DM incompatibilities vs. that of reproductive isolation evolution by adaptation to different environments remains to be assessed (Egan & Funk 2009; Schluter 2009). Indeed, while the accumulation of DM incompatibilities with genetic distance has been documented in a variety of organisms (Dettman *et al.* 2007; Anderson *et al.* 2010; Matute *et al.* 2010; Moyle & Nakazato 2010), a whole-genome screen has indicated their absence in yeasts (Kao *et al.* 2010). It would also be interesting to further study cases of ecological speciation and assess their prevalence, and in particular those where adaptation generates reproductive isolation through pleiotropy (Giraud *et al.* 2010; Gladieux *et al.* 2011; Servedio *et al.* 2011).

2 Population genomics of experimental and natural populations may reveal whether and how genomic regions involved in adaptation to a particular environmental factor contribute to isolation. It is, for instance, an open question whether adaptive divergence between species leads to dysfunction and isolation in hybrids or merely to selection against maladapted immigrants (Nosil *et al.* 2009; Giraud & Gourbière 2012).

3 Inferences of the evolutionary history of loci have the potential to indicate the relative times at which isolation was obtained for loci associated with different phenotypic components of isolation (e.g. loci associated with flowering time vs. hybrid sterility: Hey &

Nielsen 2004; Gladieux *et al.* 2011; Cornille *et al.* 2012) and to thereby truly reconstruct the history and initial causes of speciation (but see Gaggiotti 2011; Sousa *et al.* 2011; Strasburg & Rieseberg 2011 for a discussion of the challenges associated with such an approach).

4 NGS may finally allow tests of the genic view of speciation (Wu 2001). Many studies find heterogeneous genomic divergence across the genome (Fig. 4). It remains to be assessed whether this is typical for early stages of divergence and speciation, for example because adaptation has a complex genetic basis that leads to divergence in multiple genomic regions from the beginning. Growing knowledge of genomic variation in recombination rates will play a large role in tying the patterns of heterogeneous population genomic divergence to the underlying evolutionary processes (Via & West 2008; Nosil *et al.* 2009; Nachman & Payseur 2012; Roesti *et al.* 2012; Via 2012). Suppressed recombination can allow divergence to accumulate at a higher than expected rate and to prevent the breakdown of adaptive allelic combinations in the face of gene flow (Rieseberg 2001; Turner *et al.* 2005; Kirkpatrick & Barton 2006; Noor & Bennett 2009; Turner & Hahn 2010; Joron *et al.* 2011).

Empirical study of hybridization and isolating barriers

1 In the coming years, we will have the opportunity to determine the extent to which the fitness of hybrids in natural populations is predicted based on genetic mapping results. Beyond the assessment of the contribution of DM incompatibilities to isolation, taking results from laboratory and controlled crosses into natural populations will probably lead to important advances in the genetics of speciation. Expectations for the transferability of any trait mapping result to a new population must be informed by our knowledge of genetic polymorphism for trait loci, epistatic interactions among genotypes and the effects of environment on organismal trait expression. For example, we already know from mapping studies that polymorphism for isolation traits segregates within populations (Scopece *et al.* 2010; Rieseberg & Blackman 2010; Lindtke *et al.* 2012). Consequently, isolating barriers could be polymorphic among populations with different genetic compositions and ecological settings. A greater understanding of this potential polymorphism, particularly in the context of the timing of origin for different components of isolation, is likely to affect our conception of species' origin and maintenance.

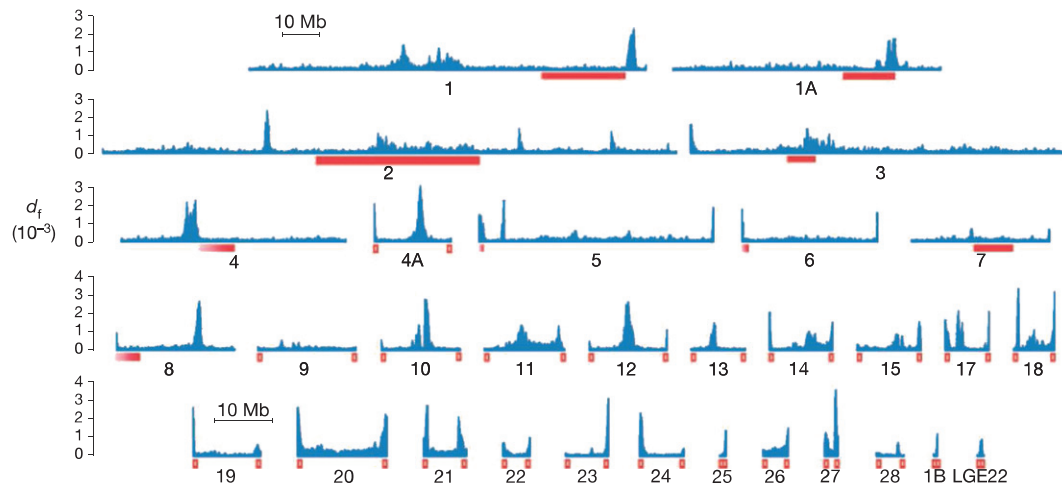2 Similarly, future population genomic studies of hybrid zones will teach us more about how selection

Fig. 4 Example of heterogeneous genomic divergence from a comparison of naturally hybridizing flycatchers. Divergence (d_t) is measured as the density of fixed differences per base pair for 200 kb windows across the genome. Red horizontal bars show the approximate location of centromeres in homologous chromosomes of zebra finch. Reprinted with permission from Fig. 2a in Ellegren *et al.* (2012).

and introgression in hybrids is (or is not) tied to population genomic differentiation between species. Furthermore, advances in analytical methods will provide estimates of the recency of introgression and about the strength and direction of selection.

3 An additional dimension of the genomics of hybridization involves changes in transposable element frequency and activity. At the sequence level, we know that historical, homoploid hybridization has led to TE proliferation and genome expansion (Rieseberg 1991; Baack *et al.* 2005; Ungerer *et al.* 2006; although see Kawakami *et al.* 2011). Hybridization can also directly lead to TE activation and expression, and gene expression misregulation (Parisod *et al.* 2010; Hegarty *et al.* 2011; Buggs 2012; Debes *et al.* 2012). A question little studied so far is whether the mechanisms controlling TE are involved in hybrid inviability and sterility. For instance in fungi, repeat-induced point mutation (RIP) specifically changes repeated sequences, and therefore, aneuploid hybrids may be inviable or sterile because RIP inactivates genes in duplicated chromosome arms (Galagan & Selher 2004; Giraud *et al.* 2008). RNA interference (RNAi) may also have such effects of inactivation of abnormally duplicated sequences in hybrids.

Kinship, parentage and behaviour—by Dany Garant and Lisette Waits

The last 20 years

The field of kinship, parentage and behaviour (henceforth KPB) has been an important component of molec-

ular ecology studies over recent decades. This field has contributed much to our understanding of mating systems, behavioural ecology, sexual selection and the impacts of inbreeding on individual fitness. Yet, the last 20 years have seen major changes in the techniques being applied and the questions being targeted. For instance, studies published two decades ago rarely focused on more than one population and often on only a few families when assessing patterns of relatedness, mate choice and/or reproductive success (see Achmann *et al.* 1992; Patton & Smith 1993; Signer *et al.* 1994 for examples). Development and reliability of techniques were also important in these early studies as allozymes, mtDNA and minisatellites were commonly used (Burke & Bruford 1987; Wetton *et al.* 1987; Chakraborty *et al.* 1988; Lehman *et al.* 1992). Nonetheless, at that time, results provided by such studies were regarded as important breakthroughs in the field of KPB as they improved our understanding of behavioural and ecological processes that were, up until then, based solely on observations.

Since then, the number of KPB studies published in *Molecular Ecology* increased between 1990 and 2005 and has remained fairly constant over recent years with *c.* 10% of papers (between 25 and 35 papers per year) on average over the last 5 years. Microsatellite loci have become the marker of choice for KPB studies (Tautz 1989; Jones & Ardren 2003), and researchers have shown that KPB research can be conducted using low-quality DNA obtained from faecal and hair samples (Bradley *et al.* 2004; Walker *et al.* 2008; DeBarba *et al.* 2010; Stenglein *et al.* 2011), which greatly increases our ability to apply this research to rare and hard-to-capture species. Recent studies have moved on from a focus on tech-

niques to a more informative emphasis on processes and now typically involve the analyses of several populations and thousands of individuals (see references below).

Current state of the field

The field of KPB currently includes studies defining mating systems (Wright *et al.* 2012) and characterizing processes underlying sexual selection (While *et al.* 2011), such as mate choice (Wang & Lu 2011). KPB research also focuses on estimating determinants of reproductive success (Thoß *et al.* 2011) and describing potential inbreeding effects (Nielsen *et al.* 2012) or avoidance (Waser *et al.* 2012), as well as the social structure of populations through assessments of relatedness, dispersal patterns and networking approaches (Rollins *et al.* 2012). Furthermore, the number of studies reconstructing extensive wild pedigrees over multiple generations continues to increase providing a rich resource for KPB studies.

An overview of the studies published in 2011 in *Molecular Ecology* revealed important differences in the methods and approaches between KPB studies and research conducted in other subfields in molecular ecology. Like most molecular ecology studies, microsatellites are widely used, but KPB studies in *Molecular Ecology* on average use a lower number of markers (*c.* 10 loci in 2011) than studies conducted in other subfields (*c.* 20 microsatellite loci in 2011; see Rieseberg *et al.* 2012). Why? Part of the answer may lie in the kind of questions being targeted in this field, which could often be satisfactorily answered using a more limited number of markers. For example, studies of parentage can sometimes reach a good assignment success by using only a handful of highly polymorphic markers (e.g. Oddou-Muratorio *et al.* 2011). However, a low number of markers could be problematic and limit power and resolution of methods, especially when one is interested in both conducting parentage assignments and quantifying genetic diversity of possible parents (Wetzel & Westneat 2009). Most studies should now aim at increasing the number of markers and making good use of the recent developments in NGS techniques that allow rapid and inexpensive development of panels of markers for multiple species (Glenn 2011; Guichoux *et al.* 2011). However, as much of the research in KPB involves matching genotypes, increasing the number of loci will require that more attention be paid to genotyping errors.

The mean number of individuals being analysed in KPB studies (on average >1300 individuals per study in 2011) is higher than in other subfields (on average <500 individuals per study in 2011; see details in Rieseberg *et al.* 2012). This observation is likely related to ques-

tions of interest in KPB, which are now typically addressed across several populations with as many individuals per population as possible. Finally, the statistical methods employed and software packages developed over the years are suitable to most biological systems (reviewed in Blouin 2003; Jones & Ardren 2003) and are also generally accessible (Kalinowski *et al.* 2007; Wang 2007, 2011; Jones & Wang 2010). However, we still lack a comparison of the performance of these different approaches/software in estimating variables of interest in a context-specific manner. Such a comparison should be a priority for KPB, because it would provide useful guidance for assessing the most productive approach/software in a given species/population/environmental context.

Future promise and challenges

Even though many improvements have occurred over the last decades, several critical key elements remain less well defined in studies of KPB:

1 Measuring lifetime fitness in the wild should be a major of focus of KPB studies in the coming decades. While central to evolutionary biology, lifetime reproductive success and longevity as proxies of fitness are still often difficult to assess empirically (Clutton-Brock & Sheldon 2010), despite the advances in molecular techniques and the increasing number of analytical software programs available.

2 Future studies should quantify relevant indicators of human-induced variation on populations and aim at generalizing findings across a broad range of natural environmental conditions. Both human-induced and natural variation in environment should have tremendous impacts on the processes targeted by KPB studies. For example, recent studies have suggested that the extent of social organization could be impacted by changes in population density and ecological conditions (Messier *et al.* 2012; Schradin *et al.* 2012) and hunting pressure (Jedrzejewski *et al.* 2005; Rutledge *et al.* 2010; Onorato *et al.* 2011). Others have shown theoretically (Blyton *et al.* 2012) and empirically (Bergeron *et al.* 2011) that mating system may be variable depending on resource availability. Elucidating the interaction between change in ecological conditions and evolutionary mechanisms will allow researchers to assess the importance of eco-evolutionary dynamics in a broader range of systems (Pelletier *et al.* 2009).

3 More studies should conduct research across multiple habitats to quantify *spatial variation* but also to develop and maintain long-term 'individual-based' studies to accurately describe *temporal variation* (see

Clutton-Brock & Sheldon 2010). Developing such long-term studies will also allow more researchers to reconstruct pedigrees in different wild populations. Pedigrees are valuable tools for obtaining precise inbreeding coefficients and estimating important quantitative genetics parameters (see Dunn *et al.* 2011; Nielsen *et al.* 2012; Richards-Zawacki *et al.* 2012 for recent effective reconstructions). Having several pedigrees available across different biological systems will also help validate/refute the patterns found with neutral molecular markers, as well as document the efficiency of different markers for reconstructing pedigree relationships (Garant & Kruuk 2005).

4 Studies of KPB over the next years will benefit from employing a multitool and multitrait approach (include several traits and different markers) but also from advances in NGS (for instance through the development of SNP markers in nonmodel species—see Van Bers *et al.* 2010 for example), in mapping and gene expression. For example, Laine *et al.* (2012) recently studied nine-spined stickleback (*Pungitius pungitius*) and used available mapping information to separate markers in functional categories. Significant heterozygosity–behaviour correlations were detected with functional markers but not when all markers were combined.

Overall, the field of KPB has provided important advances over the last decades to our understanding of evolutionary, ecological and biological processes and will face stimulating challenges and prospects in the near future.

Conclusions

Molecular ecology represents a spectacularly successful example of cross-disciplinary science, in which the tools and methods of molecular biology, genomics and bioinformatics have been merged with the theory, concepts and approaches of organismal biology, including ecology, evolution, conservation and behaviour. As can be seen from the subdiscipline perspectives outlined above, the questions addressed by molecular ecologists include longstanding discipline-specific problems that can now be investigated with new tools and approaches (e.g. Lodge *et al.* 2012; Malek *et al.* 2012; Orozco-terWengel *et al.* 2012; Parchman *et al.* 2012; Pompanon *et al.* 2012; Tedersoo *et al.* 2012), as well as new questions that have resulted from merging formally disparate disciplines (e.g. Kraaijeveld *et al.* 2012; Nosil & Feder 2012; Ozawa *et al.* 2012; Simms & Porter 2012).

Over the past two decades, molecular ecology has experienced huge advances in genotyping, from allozymes to RFLPs to minisatellites to AFLPs to microsat-

ellites to genotyping arrays to NGS (Mobley 2012; Rowe *et al.* 2012). Likewise, there have been impressive advances in the analytical approaches employed in molecular ecology, with coalescent and landscape genetic approaches providing considerably more robust inferences about the demographic and geographical history of populations than were previously possible (Beaumont *et al.* 2010; Sork & Waits 2010; Storfer *et al.* 2010; Andrew *et al.* 2012; Holderegger & Gugerli 2012; Li *et al.* 2012; Lohse *et al.* 2012; Salzburger *et al.* 2011).

While most molecular ecology studies over this period have addressed questions about the biology of organisms and communities, this is slowly changing, as ecological and evolutionary information are increasingly being employed to identify and functionally characterize ecologically important genes and their products (Blackman *et al.* 2011; Bleuler-Martinez *et al.* 2011; Johnstone *et al.* 2011; Kent *et al.* 2011; Bloomer *et al.* 2012). This general approach includes various types of population genomic scans for 'outlier loci' (Bonin *et al.* 2009; Paris *et al.* 2010; Buckley *et al.* 2012; Collin & Fumagalli 2012; Laurent *et al.* 2012; Midamegbe *et al.* 2011; Prunier *et al.* 2012). In addition, information about geographical location, habitat, phenotype, ecological community and so forth are being used for candidate gene discovery and to make inferences about allelic function (Cox *et al.* 2011; Fischer *et al.* 2011; Johnstone *et al.* 2011; Gratten *et al.* 2012; Orsini *et al.* 2012; Manel *et al.* 2012; Paris & Despres 2012).

Despite the diversity of the questions and problems being addressed by molecular ecologists, many of the challenges are similar. In particular, two themes stand out. One of these concerns the difficulties associated with managing, analysing and integrating the very large data sets that are becoming increasingly commonplace in molecular ecology studies. As noted by Paterson and Piertney (2011):

> 'The challenge will be ensuring that the onslaught of data that accompanies approaches such as NGS, genome scans [and array- and sequence-based analyses of the transcriptome and epigenome] can be coupled to appropriate ecological and phenotypic metadata to allow meaningful analysis to be undertaken.'

A second general challenge concerns the inadequacy of our analytical methods toolbox (despite advances over the past 20 years) for making inferences about the ecology and evolution of organisms, as well as the ecological effects of molecular variation. Frequently discussed needs include better analytical tools for (i) distinguishing the genomic consequences of different ecological and evolutionary processes; (ii) estimating

the timing of gene flow during population divergence; (iii) inferring phylogeography using many genes, populations and species; (iv) distinguishing between contemporary and historical effects of the landscape on patterns of genetic variation; (v) establishing the ecological functions of genes and alleles in natural populations; and (vi) estimating lifetime reproductive success in natural populations from molecular marker data. Because natural selection increasingly appears to play an important role in shaping patterns of molecular variation within and among species (Sella *et al.* 2009), it is important that these analytical tools be robust to non-neutral variation.

Despite these challenges, the future of molecular ecology is bright. New genotyping and analytical tools are allowing us to address key questions and problems with a rigour that was not possible even a decade ago. Of greater importance, however, has been the training of a new generation of molecular ecologists with diverse skills—from fieldwork to computational biology to molecular functional studies. We are confident that this next generation of molecular ecologists has the conceptual and analytical skill sets to successfully respond to the challenges faced by our discipline.

Acknowledgements

References

Achmann R, Heller KG, Epplen JT (1992) Lastmale sperm precedence in the bush cricket *Poecilimon veluchianus* (Orthoptera, Tettigonioidea) demonstrated by DNA fingerprinting. *Molecular Ecology*, **1**, 47–54.

Amaral AR, Beheregaray LB, Bildmann K *et al.* (2012) Influences of past climate changes on historical population structure and demography of a cosmopolitan marine predator, the common dolphin (genus *Delphinus*). *Molecular Ecology*, **21**, 4854–4871.

Anderson JB, Funt J, Thompson DA *et al.* (2010) Determinants of divergent adaptation and Dobzhansky-Muller interaction in experimental yeast populations. *Current Biology*, **20**, 1–6.

Anderson KE, Russell JA, Moreau CS *et al.* (2012) Highly similar microbial communities are shared among related and trophically similar ant species. *Molecular Ecology*, **21**, 2282–2296.

Anderson JT, Lee C-R, Rushworth CA, Colautti RI, Mitchell-Olds T (2013) Genetic trade-offs and conditional neutrality contribute to local adaptation. *Molecular Ecology*, **22**, 699–708.

Andreasen AM, Stewart KM, Longland WS, Beckmann JP, Forister ML (2012) Identification of source-sink dynamics in mountain lions of the Great Basin. *Molecular Ecology*, **21**, 5689–5701.

Andrew RL, Ostevik KL, Ebert DP, Rieseberg LH (2012) Adaptation with gene flow across the landscape in a dune sunflower. *Molecular Ecology*, **21**, 2078–2091.

Asahida T, Yamashita Y, Kobayashi T (1997) Identification of consumed stone flounder, *Kareius bicoloratus* (Basilewsky), from the stomach contents of sand shrimp *Crangon affinis* (De Haan) using mitochondrial DNA analysis. *Journal of Experimental Marine Biology and Ecology*, **217**, 153–163.

Avise JC, Arnold J, Ball RM *et al.* (1987) Intraspecific phylogeography: the mitochondrial bridge between population genetics and systematics. *Annual Reviews of Ecology and Systematics*, **18**, 489–522.

Baack EJ, Whitney KD, Rieseberg LH (2005) Hybridization and genome size evolution: timing and magnitude of nuclear DNA content increases in *Helianthus* homoploid hybrid species. *New Phytologist*, **167**, 623–630.

Balkenhol N, Waits LP, Dezzani RJ (2009) Statistical approaches in landscape genetics: an evaluation of methods for linking landscape and genetic data. *Ecography*, **32**, 818–830.

Barrett CF, Freudenstein JV (2011) An integrative approach to delimiting species in a rare but widespread mycoheterotrophic orchid. *Molecular Ecology*, **20**, 2771–2786.

Barrett RDH, Hoekstra HE (2011) Molecular spandrels: tests of adaptation at the genetic level. *Nature Reviews Genetics*, **12**, 767–780.

Barrett RDH, Rogers SM, Schluter D (2008) Natural selection on a major armor gene in threespine stickleback. *Science*, **322**, 255–258.

Barrett RDH, Paccard A, Healy T *et al.* (2011) Rapid evolution of cold tolerance in stickleback. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **278**, 233–238.

Barton NH (2000) Genetic hitchhiking. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, **355**, 1553–1562.

Barton NH, Rodriguez de Cara MA (2009) The evolution of strong reproductive isolation. *Evolution*, **63**, 1171–1190.

Beaumont MA, Balding DJ (2004) Identifying adaptive genetic divergence among populations from genome scans. *Molecular Ecology*, **13**, 969–980.

Beaumont MA, Nielsen R, Robert C *et al.* (2010) In defense of model-based inference in phylogeography. *Molecular Ecology*, **19**, 436–446.

Becker C, Weigel D (2012) Epigenetic variation: origin and transgenerational inheritance. *Current Opinion in Plant Biology*, **15**, 562–567.

Beerli P (2006) Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. *Bioinformatics*, **22**, 341–345.

Beier P, Noss RF (1998) Do habitat corridors provide connectivity? *Conservation Biology*, **12**, 1241–1252.

Beier P, Spencer W, Baldwin RF, McRae BH (2011) Toward best practices for developing regional connectivity maps. *Conservation Biology*, **25**, 879–892.

Bell RC, MacKenzie JB, Hickerson MJ *et al.* (2011) Comparative multi-locus phylogeography confirms multiple vicariance events in co-distributed rainforest frogs. *Proceedings of the Royal Society of London Series B: Biological Sciences*, **279**, 991–999.

Bergelson J, Roux F (2010) Towards identifying genes underlying ecologically relevant traits in *Arabidopsis thaliana*. *Nature Reviews Genetics*, **11**, 867–879.

Bergeron P, Réale D, Humphries MM, Garant D (2011) Evidence of multiple paternity and mate selection for inbreeding avoidance in wild eastern chipmunks. *Journal of Evolutionary Biology*, **24**, 1685–1694.

Bertorelle G, Benazzo A, Mona S (2010) ABC as a flexible framework to estimate demography over space and time: some cons, many pros. *Molecular Ecology*, **19**, 2609–2625.

Blaalid R, Carlsen T, Kumar S et al. (2012) Changes in the root-associated fungal communities along a primary succession gradient analysed by 454 pyrosequencing. *Molecular Ecology*, **21**, 1897–1908.

Blackman BK, Michaels SD, Rieseberg LH (2011) Connecting the sun to flowering in sunflower adaptation. *Molecular Ecology*, **20**, 3503–3512.

Bleuler-Martinez S, Butschi A, Garbani M et al. (2011) A lectin-mediated resistance of higher fungi against predators and parasites. *Molecular Ecology*, **20**, 3056–3070.

Bloomer RH, Juenger TE, Symonds VV (2012) Natural variation in *GL1* and its effects on trichome density in *Arabidopsis thaliana*. *Molecular Ecology*, **21**, 3501–3515.

Blouin MS (2003) DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *Trends in Ecology and Evolution*, **18**, 503–511.

Blyton MDJ, Banks SC, Peakall R, Lindenmayer DB (2012) Using probability modelling and genetic parentage assignment to test the role of local mate availability in mating system variation. *Molecular Ecology*, **21**, 572–586.

Bonin A, Paris M, Tetreau G et al. (2009) Candidate genes revealed by a genome scan for mosquito resistance to a bacterial insecticide: sequence and gene expression variations. *BMC Genomics*, **10**, 551.

van Borm S, Buschinger A, Boomsma JJ et al. (2002) Tetraponera ants have gut symbionts related to nitrogen-fixing root-nodule bacteria. *Proceedings of the Royal Society of London Series B: Biological Sciences*, **269**, 2023–2027.

Bossdorf O, Richards CL, Pigliucci M (2008) Epigenetics for ecologists. *Ecology Letters*, **11**, 106–115.

Bradley BJ, Doran-Sheehy DM, Lukas D, Boesch C, Vigilant L (2004) Dispersed male networks in western gorillas. *Current Biology*, **14**, 510–513.

Braunisch V, Segelbacher G, Hirzel AH (2010) Modelling functional landscape connectivity from genetic population structure: a new spatially explicit approach. *Molecular Ecology*, **19**, 3664–3678.

Buckler ES, Holland JB, Bradbury PJ et al. (2009) The genetic architecture of maize flowering time. *Science*, **325**, 714–718.

Buckley J, Butlin RK, Bridle JR (2012) Evidence for evolutionary change associated with the recent range expansion of the British butterfly, *Aricia agestis*, in response to climate change. *Molecular Ecology*, **21**, 267–280.

Buerkle CA, Gompert Z, Parchman TL (2011) The $n = 1$ constraint in population genomics. *Molecular Ecology*, **20**, 1575–1581.

Buggs RJA (2012) Monkeying around with ploidy. *Molecular Ecology*, **21**, 5159–5161.

Burke T, Bruford MW (1987) DNA fingerprinting in birds. *Nature*, **327**, 149–152.

Burton RS, Barreto FS (2012) A disproportionate role for mtDNA in Dobzhansky–Muller incompatibilities? *Molecular Ecology*, **21**, 4942–4957.

Byrne M, Steane DA, Joseph L et al. (2011) Decline of a biome: evolution, contraction, fragmentation, extinction and invasion of the Australian mesic zone biota. *Journal of Biogeography*, **38**, 1635–1656.

Cafaro MJ, Poulsen M, Little AEF et al. (2011) Specificity in the symbiotic association between fungus-growing ants and protective *Pseudonocardia* bacteria. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **278**, 1814–1822.

Carstens BC, Stoute HN, Reid NM (2009) An information theoretical approach to phylogeography. *Molecular Ecology*, **18**, 4270–4282.

Chakraborty R, Meagher TR, Smouse PE (1988) Parentage analysis with genetic markers in natural populations. I. The expected proportion of offspring with unambiguous paternity. *Genetics*, **118**, 527–536.

Chan YF, Marks ME, Jones FC et al. (2010) Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a *Pitx1* enhancer. *Science*, **327**, 302–305.

Clutton-Brock T, Sheldon BC (2010) Individuals and populations: the role of long-term, individual-based studies of animals in ecology and evolutionary biology. *Trends in Ecology and Evolution*, **25**, 562–573.

Colbourne JK, Pfrender ME, Gilbert, et al. (2011) The ecoresponsive genome of *Daphnia pulex*. *Science*, **331**, 555–561.

Collin H, Fumagalli L (2012) Evidence for morphological and adaptive genetic divergence between lake and stream habitats in European minnows (*Phoxinus phoxinus*, Cyprinidae). *Molecular Ecology*, **20**, 4490–4502.

Colosimo PF, Hosemann KE, Balabhadra S et al. (2005) Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science*, **5717**, 1928–1933.

Coop G, Witonsky D, Di Rienzo A, Pritchard JK (2010) Using environmental correlations to identify loci underlying local adaptation. *Genetics*, **185**, 1411–1423.

Cornille A, Gladieux P, Smulders MJM et al. (2012) New insight into the history of domesticated apple: secondary contribution of the European wild apple to the genome of cultivated varieties. *PLoS Genetics*, **8**, e1002703.

Cox K, Broeck AV, Van Calster H et al. (2011) Temperature-related natural selection in a wind-pollinated tree across regional and continental scales. *Molecular Ecology*, **20**, 2724–2738.

Cushman SA, Landguth EL (2010a) Scale dependent inference in landscape genetics. *Landscape Ecology*, **25**, 967–979.

Cushman SA, Landguth EL (2010b) Spurious correlations and inference in landscape genetics. *Molecular Ecology*, **19**, 3592–3602.

Dalziel A, Rogers SM, Schulte T (2009) Linking genotypes to phenotypes and fitness: how mechanistic biology can inform molecular ecology. *Molecular Ecology*, **18**, 4997–5017.

Davey JW, Blaxter ML (2010) RADSeq: next-generation population genetics. *Briefings in Functional Genomics*, **9**, 416–423.

Davey JW, Hohenlohe PA, Etter PD, et al. (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics*, **12**, 499–510.

DeBarba M, Waits LP, Garton EO et al. (2010) The power of genetic monitoring for studying demography, ecology, and genetics of a reintroduced brown bear population. *Molecular Ecology*, **19**, 3938–3951.

Debes PV, Normandeau E, Fraser DJ et al. (2012) Differences in transcription levels among wild, domesticated, and hybrid Atlantic salmon (*Salmo salar*) from two environments. *Molecular Ecology*, **21**, 2574–2587.

Derôme N, Duchesne P, Bernatchez L (2006) Parallelism in gene transcription among sympatric lake whitefish ecotypes (*Coregonus clupeaformis* Mitchill). *Molecular Ecology*, **15**, 1239–1250.

Dettman JR, Sirjusingh C, Kohn LM, Anderson JB (2007) Incipient speciation by divergent adaptation and antagonistic epistasis in yeast. *Nature*, **447**, 585–588.

Dolman G, Joseph L (2012) A species assemblage approach to comparative phylogeography of birds in southern Australia. *Ecology and Evolution*, **2**, 354–369.

Dunn SJ, Clancey E, Waits LP, Byers JA (2011) Inbreeding depression in pronghorn (*Antilocapra americana*) fawns. *Molecular Ecology*, **20**, 4889–4898.

Dunning-Hotopp J, Clark M, Oliveira D *et al.* (2007) Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. *Science*, **317**, 1753–1756.

Dyer RJ, Nason JD (2004) Population Graphs: the graph theoretic shape of genetic structure. *Molecular Ecology*, **13**, 1713–1727.

Egan SP, Funk DJ (2009) Ecologically dependent postmating isolation between sympatric host forms of *Neochlamisus bebbianae* leaf beetles. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 19426–19431.

Ellegren H, Smeds L, Burri R *et al.* (2012) The genomic landscape of species divergence in *Ficedula* flycatchers. *Nature*, **491**, 756–760.

Emerson KJ, Merz CR, Catchen JM *et al.* (2010) Resolving postglacial phylogeography using high-throughput sequencing. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 16196–16200.

Epperson BK, McRae BH, Scribner K *et al.* (2010) Utility of computer simulations in landscape genetics. *Molecular Ecology*, **19**, 3549–3564.

Epps CW, Wehausen JD, Bleich VC, Torres SG, Brashares JS (2007) Optimizing dispersal and corridor models using landscape genetics. *Journal of Applied Ecology*, **44**, 714–724.

Evans LM, Allan GJ, Shuster SM, Woolbright SA, Whitham TG (2008) Tree hybridisation and genotypic variation drive cryptic speciation of a specialist mite herbivore. *Evolution*, **62**, 3027–3040.

Evans BJ, Bliss SM, Mendel SA, Tinsley RC (2011) The Rift Valley is a major barrier to dispersal of African clawed frogs (*Xenopus*) in Ethiopia. *Molecular Ecology*, **20**, 4216–4230.

Excoffier L, Heckel G (2006) Computer programs for population genetics data analysis: a survival guide. *Nature Reviews Genetics*, **7**, 745–758.

Fagundes NJR, Ray N, Beaumont M *et al.* (2007) Statistical evaluation of alternative models of human evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 17614–17619.

Feder ME, Mitchell-Olds T (2003) Evolutionary and ecological functional genomics. *Nature Reviews Genetics*, **4**, 651–657.

Feng S, Rubbi L, Jacobsen SE, Pellegrini M (2011) Determining DNA methylation profiles using sequencing. In: *High-Throughput Next Generation Sequencing: Methods and Applications, Methods in Molecular Biology* (eds Kwon YM, Ricke SC), pp. 223–238.

Fischer MC, Foll M, Excoffier L, Heckel G (2011) Enhanced AFLP genome scans detect local adaptation in high-altitude populations of a small rodent (*Microtus arvalis*). *Molecular Ecology*, **20**, 1450–1462.

Fitzpatrick BM, Shaffer HB (2007) Introduction history and habitat variation explain the landscape genetics of hybrid tiger salamanders. *Ecological Applications*, **17**, 598–608.

Ford EB (1964) *Ecological Genetics*, 4th edn. Chapman and Hall, London. 1975.

Freedman AH, Thomassen HA, Buermann W, Smith TB (2010) Genomic signals of diversification along ecological gradients in a tropical lizard. *Molecular Ecology*, **19**, 3773–3788.

Gaggiotti OE (2011) Making inferences about speciation using sophisticated statistical genetics methods: look before you leap. *Molecular Ecology*, **20**, 2229–2232.

Galagan J, Selher E (2004) RIP: the evolutionary cost of genome defense. *Trends in Ecology & Evolution*, **20**, 417–423.

Galindo H, Pfeiffer-Herbert A, McManus M *et al.* (2010) Seascape genetics along a steep cline: using genetic patterns to test predictions of marine larval dispersal. *Molecular Ecology*, **9**, 3692–3707.

Galpern P, Manseau M, Wilson P (2012) Grains of connectivity: analysis at multiple spatial scales in landscape genetics. *Molecular Ecology*, **21**, 3996–4009.

Garant D, Kruuk LEB (2005) How to use molecular marker data to measure evolutionary parameters in wild populations. *Molecular Ecology*, **14**, 1843–1859.

Gavrilet S (1997) Hybrid zones with Dobzhansky-type epistatic selection. *Evolution*, **51**, 1027–1035.

Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, **21**, 394–407.

Gibson CM, Hunter MS (2010) Extraordinarily widespread and fantastically complex: comparative biology of endosymbiotic bacterial and fungal mutualists of insects. *Ecology Letters*, **13**, 223–234.

Giraud T, Gourbière S (2012) The tempo and modes of evolution of reproductive isolation in fungi. *Heredity*, **109**, 204–214.

Giraud T, Refregier G, de Vienne DM *et al.* (2008) Speciation in fungi. *Fungal Genetics and Biology*, **45**, 791–802.

Giraud T, Gladieux P, Gavrilets S (2010) Linking the emergence of fungal plant diseases with ecological speciation. *Trends in Ecology & Evolution*, **25**, 387–395.

Gladieux P, Guérin F, Giraud T *et al.* (2011) Emergence of novel fungal pathogens by ecological speciation: importance of the reduced viability of immigrants. *Molecular Ecology*, **20**, 4521–4532.

Glenn TC (2011) Field guide to next-generation DNA sequencers. *Molecular Ecology Resources*, **11**, 759–769.

Gompert Z, Forister ML, Fordyce JA *et al.* (2010) Bayesian analysis of molecular variance in pyrosequences quantifies population genetic structure across the genome of *Lycaeides* butterflies. *Molecular Ecology*, **19**, 2455–2473.

Gourbière S, Mallet J (2010) Are species real? The shape of the species boundary with exponential failure, reinforcement, and the "missing snowball". *Evolution*, **64**, 1–24.

Grant PR, Grant BR (2011) *How and Why Species Multiply: The Radiation of Darwin's Finches*. Princeton University Press, Princeton, New Jersey.

Gratten J, Wilson AJ, McRae AF *et al.* (2008) A localized negative genetic correlation constrains microevolution of coat color in wild sheep. *Science*, **318**, 319–320.

Gratten J, Pilkington JG, Brown EA *et al.* (2012) Selection and microevolution of coat pattern are cryptic in a wild population of sheep. *Molecular Ecology*, **21**, 2977–2990.

Grivet D, Sork VL, Westfall RD, Davis FW (2008) Conserving the evolutionary potential of California valley oak (*Quercus lobata* Née): a multivariate genetic approach to conservation planning. *Molecular Ecology*, **17**, 139–156.

Gugger P, Makihiko I, Sork VL (2013) Influence of late Quaternary climate change on modern patterns of genetic variation in valley oak, *Quercus lobata* Née. *Molecular Ecology*, **22**, in press.

Guichoux E, Lagache L, Wagner S *et al.* (2011) Current trends in microsatellite genotyping. *Molecular Ecology Resources*, **11**, 591–611.

Hancock AM, Di Rienzo A (2008) Detecting the genetic signature of natural selection in human populations: models, methods, and data. *Annual Review of Anthropology*, **37**, 197–217.

Hancock AM, Brachi B, Faure N *et al.* (2011) Adaptation to climate across the *Arabidopsis thaliana* genome. *Science*, **334**, 83–86.

Hegarty MJ, Batstone T, Barker GL *et al.* (2011) Nonadditive changes to cytosine methylation as a consequence of hybridization and genome duplication in *Senecio* (Asteraceae). *Molecular Ecology*, **20**, 105–113.

van der Heide T, Govers LL, de Fouw J *et al.* (2012) A three-stage symbiosis forms the foundation of seagrass ecosystems. *Science*, **336**, 1432.

Helyar SJ, Hemmer-Hansen J, Bekkevold D *et al.* (2011) Application of SNPs for population genetics of nonmodel organisms: new opportunities and challenges. *Molecular Ecology Resources*, **11**, 123–136.

Hey J, Nielsen R (2004) Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila psudoobscura* and *D. persimilis*. *Genetics*, **167**, 747–760.

Hickerson MJ, Meyer CP (2008) Testing comparative phylogeographic models of marine vicariance and dispersal using a hierarchical Bayesian approach. *BMC Evolutionary Biology*, **8**, 322.

Hickerson MJ, Carstens BC, Cavender-Bares J *et al.* (2010) Phylogeography's past, present, and future: 10 years after Avise, 2000. *Molecular Phylogenetics and Evolution*, **54**, 291–301.

Hilgenboecker K, Hammerstein P, Schlattmann P *et al.* (2008) How many species are infected with *Wolbachia*? A statistical analysis of current data. *FEMS Microbiology Letters*, **281**, 215–220.

Hoekstra HE, Hirschmann RJ, Bundey RJ (2006) A single amino acid mutation contributes to adaptive color pattern in beach mice. *Science*, **313**, 101–104.

Holderegger R, Gugerli F (2012) Where do you come from, where do you go? Directional migration rates in landscape genetics. *Molecular Ecology*, **23**, 5640–5642.

Holderegger R, Wagner HH (2008) Landscape genetics. *BioScience*, **58**, 199–207.

Holderegger R, Kamm U, Gugerli F (2006) Adaptive vs. neutral genetic diversity: implications for landscape genetics. *Landscape Ecology*, **21**, 797–807.

Holliday JA, Wang T, Aitken S (2012) Predicting adaptive phenotypes from multilocus genotypes in Sitka spruce (*Picea sitchensis*) using random forest. *G3: Genes – Genomes – Genetics* **2**, 1085–1093.

Houle D, Diddahally GR, Omhol S (2010) Phenomics: the next challenge. *Nature Reviews Genetics*, **11**, 855–866.

Huang W, Takebayashi N, Qi Y, Hickerson MJ (2011) MTML-msBayes: approximate Bayesian comparative phylogeographic inference from multiple taxa and multiple loci with rate heterogeneity. *BMC Bioinformatics*, **12**, 1.

Jablonka E, Raz G (2009) Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Quarterly Review of Biology*, **84**, 131–176.

Jedrzejewki W, Branicki W, Veit C *et al.* (2005) Genetic diversity and relatedness within packs in an intensely hunted population of wolves *Canis lupus*. *Acta Theriologica*, **50**, 3–22.

Johnstone KA, Lubieniecki KP, Koop BF *et al.* (2011) Expression of olfactory receptors in different life stages and life histories of wild Atlantic salmon (*Salmo salar*). *Molecular Ecology*, **20**, 4059–4069.

Jones AG, Ardren WR (2003) Methods of parentage analysis in natural populations. *Molecular Ecology*, **12**, 2511–2523.

Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources*, **10**, 551–555.

Joost S, Bonin A, Bruford MW *et al.* (2007) A spatial analysis method (SAM) to detect candidate loci for selection: towards a landscape genomics approach to adaptation. *Molecular Ecology*, **16**, 3955–3969.

Joron M, Frezal L, Jones RT *et al.* (2011) Chromosomal rearrangements maintain a polymorphic supergene controlling butterfly mimicry. *Nature*, **477**, 203–206.

Jurado-Rivera JA, Vogler AP, Reid CAM, Petitpierre E, Gomez-Zurita J (2009) DNA barcoding insect-host plant associations. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **276**, 639–648.

Kaiser W, Huguet E, Casas J *et al.* (2010) Plant green-island phenotype induced by leaf miners is mediated by bacterial symbionts. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **277**, 2311–2319.

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**, 1099–1106.

Kane NC, Rieseberg LH (2007) Selective sweeps reveal candidate genes for adaptation to drought and salt tolerance in common sunflower, *Helianthus annuus*. *Genetics*, **175**, 1823–1824.

Kane NC, King MG, Barker MS *et al.* (2009) Comparative genomic and population genetic analyses indicate highly porous genomes and high levels of gene flow between divergent *Helianthus* species. *Evolution*, **63**, 2061–2075.

Kang HM, Sul JH, Service SK *et al.* (2010) Variance component model to account for sample structure in genome-wide association studies. *Nature Genetics*, **42**, 348–354.

Kao KC, Schwartz K, Sherlock G (2010) A genome-wide analysis reveals no nuclear Dobzhansky-Muller pairs of determinants of speciation between *S. cerevisiae* and *S. paradoxus*, but suggests more complex incompatibilities. *PLoS Genetics*, **6**, e1001038.

Kawakami T, Morgan TJ, Nippert JB *et al.* (2011) Natural selection drives clinal life history patterns in the perennial sunflower species, *Helianthus maximiliani*. *Molecular Ecology*, **20**, 2318–2328.

Kent CF, Issa A, Bunting AC *et al.* (2011) Adaptive evolution of a key gene affecting queen and worker traits in the honey bee, *Apis mellifera*. *Molecular Ecology*, **20**, 5226–5235.

King RA, Read DS, Traugott M, Symondson WOC (2008a) Molecular analysis of predation: a review of best practice for DNA-based approaches. *Molecular Ecology*, **17**, 947–963.

King RA, Tibble AL, Symondson OC (2008b) Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. *Molecular Ecology*, **17**, 4684–4698.

Kirkpatrick M, Barton N (2006) Chromosome inversions, local adaptation, and speciation. *Genetics*, **173**, 419–434.

Kivisild T, Shen P, Wall DP *et al.* (2006) The role of selection in the evolution of Human mitochondrial genomes. *Genetics*, **172**, 373–387.

Knowles LL (2009) Statistical phylogeography. *Annual Reviews of Ecology, Evolution and Systematics*, **40**, 593–612.

Knowles LL, Maddison WP (2002) Statistical phylogeography. *Molecular Ecology*, **11**, 2623–2635.

Kover PX, Mott R (2012) Mapping the genetic basis of ecologically and evolutionarily relevant traits in *Arabidopsis thaliana*. *Current Opinion in Plant Biology*, **15**, 212–217.

Kraaijeveld K, Zwanenburg B, Hubert B, et al. (2012) Transposon proliferation in an asexual parasitoid. *Molecular Ecology*, **21**, 3898–3906.

Kumar S, Blaxter ML (2011) Simultaneous genome sequencing of symbionts and their hosts. *Symbiosis*, **55**, 119–126.

Laine VN, Herczeg G, Shikano T, Primmer CR (2012) Heterozygosity-behaviour correlations in nine-spined stickleback (*Pungitius pungitius*) populations: contrasting effects at random and functional loci. *Molecular Ecology*, **21**, 4872–4884.

Laurent R, Toupance B, Chaix R (2012) Non-random mate choice in humans: insights from a genome scan. *Molecular Ecology*, **21**, 587–596.

Le Corre V, Kremer A (2012) The genetic differentiation at quantitative trait loci under local adaption. *Molecular Ecology*, **21**, 1548–1566.

Lee H-Y, Chou J-Y, Cheong L *et al.* (2008) Incompatibility of nuclear and mitochondrial genomes causes hybrid sterility between two yeast species. *Cell*, **135**, 1065–1073.

Lehman N, Clarkson P, Mech LD, Meier TJ, Wayne RW (1992) A study of the genetic relationships within and among wolf packs using DNA fingerprinting and mitochondrial DNA. *Behavioral Ecology and Sociobiology*, **30**, 83–94.

Lewontin RC (1991) Perspectives: 25 years ago in genetics: electrophoresis in the development of evolutionary genetics: milestone or millstone? *Genetics*, **128**, 657–662.

Li J, Li H, Jakobsson M *et al.* (2012) Joint analysis of demography and selection in population genetics: where do we stand and where could we go? *Molecular Ecology*, **21**, 28–44.

Lindtke D, Buerkle CA, Barbara T *et al.* (2012) Recombinant hybrids retain heterozygosity at many loci: new insights into the genomics of reproductive isolation in *Populus*. *Molecular Ecology*, **21**, 5042–5058.

Lodge DM, Turner CR, Jerde CL, et al. (2012) Conservation in a cup of water: estimating biodiversity and population abundance from environmental DNA. *Molecular Ecology*, **21**, 2555–2558.

Lohse K, Barton NH, Melika G, Stone GN (2012) A likelihood-based comparison of populations histories in a parasitoid guild. *Molecular Ecology*, **21**, 4605–4617.

Lowry DB (2010) Landscape evolutionary genomics. *Biology Letters*, **6**, 502–504.

Maddison WP (1997) Gene trees in species trees. *Systematic Biology*, **46**, 523–536.

Malek TB, Boughman JW, Dworkin I *et al.* (2012) Admixture mapping of male nuptial colour and body shape in a recently formed hybrid population of threespine stickleback. *Molecular Ecology*, **21**, 5265–5279.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution*, **18**, 189–197.

Manel S, Joost S, Epperson BK (2010a) Perspectives on the use of landscape genetics to detect genetic adaptive variation in the field. *Molecular Ecology*, **19**, 3760–3772.

Manel S, Poncet BN, Legendre P, Gugerli F, Holderegger R (2010b) Common factors drive adaptive genetic variation at different spatial scales in *Arabis alpina*. *Molecular Ecology*, **19**, 3824–3835.

Manel S, Gugerli F, Thuiller W *et al.* (2012) Broad-scale adaptive genetic variation in alpine plants is driven by temperature and precipitation. *Molecular Ecology*, **21**, 3729–3738.

Marko PB (2005) An intraspecific comparative analysis of character divergence between sympatric species. *Evolution*, **59**, 554–564.

Matute DR, Butler IA, Turissini DA, Coyne JA (2010) A test of the snowball theory for the rate of evolution of hybrid incompatibilities. *Science*, **329**, 1518–1521.

McCairns RJS, Bernatchez L (2010) Adaptive divergence between parapatric freshwater and marine sticklebacks: insights into the role of phenotypic plasticity from an integrated analysis of the ecological genetics of candidate gene expression. *Evolution*, **64**, 1029–1047.

McCairns SRJ, Bourget S, Bernatchez L (2012) Plasticity and heritability of morphological variation within and between parapatric stickleback demes. *Journal of Evolutionary Biology*, **25**, 1097–1112.

McFrederick QS, Wcislo WT, Taylor DR *et al.* (2012) Environment or kin: whence do bees obtain acidophilic bacteria? *Molecular Ecology*, **21**, 1754–1768.

McRae BH, Beier P (2007) Circuit theory predicts gene flow in plant and animal populations. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 19885–19890.

Messier GD, Garant D, Bergeron P, Réale D (2012) Environmental conditions affect spatial genetic structures and dispersal patterns in a solitary rodent. *Molecular Ecology*, **21**, 5363–5373.

Midamegbe A, Vitalis R, Malausa T, Delava E, Cros-Arteil S, Streiff R (2011) Scanning the European corn borer (*Ostrinia* spp.) genome for adaptive divergence between host-affiliated sibling species. *Molecular Ecology*, **20**, 1414–1430.

Mobley KB (2012) Grandfathering in a new era of parentage analysis. *Molecular Ecology*, **20**, 1080–1082.

Moyle LC, Nakazato T (2010) Hybrid incompatibility "snowballs" between *Solanum* species. *Science*, **329**, 1521–1523.

Nachman MW, Payseur BA (2012) Recombination rate variation and speciation: theoretical predictions and empirical results from rabbits and mice. *Philosophical Transactions of the Royal Society of London Series B*, **367**, 409–421.

Nickerson RS (1998) Confirmation bias: a ubiquitous phenomenon in many guises. *Review of General Psychology*, **2**, 175–220.

Nielsen JF, English S, Goodall-Copestake WP *et al.* (2012) Inbreeding and inbreeding depression of early life traits in a cooperative mammal. *Molecular Ecology*, **21**, 2788–2804.

Niemiller ML, Near TJ, Fitzpatrick BM (2011) Delimiting species using multilocus data: diagnosing cryptic diversity in the Southern cavefish, *Typhlichthys subterraneus* (Teleostei: Amblyopsidae). *Evolution*, **66**, 846–866.

Noor M, Bennett S (2009) Islands of speciation or mirages in the desert? Examining the role of restricted recombination in maintaining species. *Heredity*, **103**, 439–444.

Nosil P, Feder JL (2012) Widespread yet heterogeneous genomic divergence. *Molecular Ecology*, **21**, 2829–2832.

Nosil P, Funk DJ, Ortiz-Barrientos D (2009) Divergent selection and heterogeneous genomic divergence. *Molecular Ecology*, **18**, 375–402.

Nosil P, Gompert Z, Farkas T (2012) Genomic consequences of multiple speciation processes in a stick insect. *Proceedings of the Royal Society of London Series B: Biological Sciences*, **279**, 5058–5065.

Oddou-Muratorio S, Klein EK, Vendramin GG, Fady B (2011) Spatial vs. temporal effects on demographic and genetic structures: the roles of dispersal, masting and differential mortality on patterns of recruitment in *Fagus sylvatica*. *Molecular Ecology*, **20**, 1997–2010.

Oliver KM, Degnan PH, Burke GR *et al.* (2010) Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. *Annual Review of Entomology*, **55**, 247–266.

Oliver KM, Noge K, Huang EM *et al.* (2012) Parasitic wasp responses to symbiont-based defense in aphids. *BMC Biology*, **10**, 11.

O'Meara BC (2010) New heuristic methods for joint species delimitation and species tree inference. *Systematic Biology*, **59**, 59–73.

O'Neill SL, Hoffmann AA, Werren JH (eds) (1997) *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*, 2nd edn. Oxford University Press, New York, 226pp.

Onorato D, DeSimone R, White C, Waits LP (2011) Genetic assessment of paternity and relatedness in a managed population of cougars. *Journal of Wildlife Management*, **75**, 378–384.

Orozco-terWengel P, Kapun M, Nolte V *et al.* (2012) Adaptation of *Drosophila* to a novel laboratory environment reveals temporally heterogeneous trajectories of selected alleles. *Molecular Ecology*, **21**, 4931–4941.

Orsini L, Spanier KI, De Meester L (2012) Genomic signature of natural and anthropogenic stress in wild populations of the waterflea *Daphnia magna*: validation in space, time and experimental evolution. *Molecular Ecology*, **21**, 2160–2175.

Ozawa R, Nishimura O, Yazawa S *et al.* (2012) Temperature-dependent, behavioural, and transcriptional variability of a tritrophic interaction consisting of bean, herbivorous mite, and predator. *Molecular Ecology*, **21**, 5624–5635.

Pan X, Zhou G, Wu J (2012) *Wolbachia* induces reactive oxygen species (ROS)-dependent activation of the Toll pathway to control dengue virus in the mosquito *Aedes aegypti*. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, E23–E31.

Parchman TL, Gompert Z, Mudge J *et al.* (2012) Genome-wide association genetics of an adaptive trait in lodgepole pine. *Molecular Ecology*, **21**, 2991–3005.

Paris M, Despres L (2012) Identifying insecticide resistance genes in mosquito by combining AFLP genome scans and 454 pyrosequencing. *Molecular Ecology*, **21**, 1672–1686.

Paris M, Boyer S, Bonin A (2010) Genome scan in the mosquito *Aedes rusticus*: population structure and detection of positive selection after insecticide treatment. *Molecular Ecology*, **19**, 325–337.

Parisod C, Alix K, Just J, et al. (2010) Impact of transposable elements on the organization and function of allopolyploid genomes. *New Phytologist*, **186**, 37–45.

Patton JL, Smith MF (1993) Molecular evidence for mating asymmetry and female choice in a pocket gopher (*Thomomys*) hybrid zone. *Molecular Ecology*, **2**, 3–8.

Paterson S, Piertney SB (2011) Frontiers in host-parasite ecology and evolution. *Molecular Ecology*, **20**, 869–871.

Pavey S, Bernatchez L, Aubin-Horth N, Landry C (2012) What is needed for next-generation ecological and evolutionary genomics? *Trends in Ecology & Evolution*, **27**, 673–678.

Pelletier F, Garant D, Hendry AP (2009) Eco-evolutionary dynamics. *Philosophical Transactions of the Royal Society of London Series B*, **364**, 1483–1489.

Peter BM, Wegmann D, Excoffier L (2010) Distinguishing between population bottleneck and population subdivision by a Bayesian model choice procedure. *Molecular Ecology*, **19**, 4648–4660.

Pickett SB, Bergey CM, Di Fiore A (2012) A metagenomic study of primate insect diet diversity. *American Journal of Primatology*, **74**, 622–631.

Pinto-Tomás AA, Anderson MA, Suen G *et al.* (2009) Symbiotic nitrogen fixation in the fungus gardens of leaf-cutter ants. *Science*, **326**, 1120–1123.

Poelchau MF, Hamrick JL (2012) Differential effects of landscape-level environmental features on genetic structure in three codistributed tree species in Central America. *Molecular Ecology*, **21**, 4970–4982.

Pompanon F, Deagle BE, Symondson WOC *et al.* (2012) Who is eating what: diet assessment using next generation sequencing. *Molecular Ecology*, **21**, 1931–1950.

Provan J, Maggs CA (2012) Unique genetic variation at a species' rear edge is under threat from global climate change. *Proceedings of the Royal Society of London Series B: Biological Sciences*, **279**, 39–47.

Prunier J, Gerardi S, Laroche J, Beaulieu J, Bousquet J (2012) Parallel and lineage-specific molecular adaptation to climate in boreal black spruce. *Molecular Ecology*, **21**, 4270–4286.

Qi W, Nong G, Preston JF *et al.* (2009) Comparative metagenomics of *Daphnia* symbionts. *BMC Genomics*, **10**, 172.

Richards-Zawacki CL, Wang IJ, Summers K (2012) Mate choice and the genetic basis for colour variation in a polymorphic dart frog: inferences from a wild pedigree. *Molecular Ecology*, **21**, 3879–3892.

Ricklefs RE, Jenkins DG (2011) Biogeography and ecology: towards the integration of two disciplines. *Philosophical Transactions of the Royal Society of London Series B*, **366**, 2438–2448.

Rieseberg LH (1991) Homoploid reticulate evolution in *Helianthus*: evidence from ribosomal genes. *American Journal of Botany*, **78**, 1218–1237.

Rieseberg LH (2001) Chromosomal rearrangements and speciation. *Trends in Ecology & Evolution*, **16**, 351–358.

Rieseberg LH, Blackman BK (2010) Speciation genes in plants. *Annals of Botany*, **106**, 439–455.

Rieseberg L, Vines T, Kane N (2012) Editorial 2012. *Molecular Ecology*, **21**, 1–22.

Rittmeyer EN, Austin CC (2012) The effect of sampling on delimiting species from multilocus sequence data. *Molecular Phylogenetics & Evolution*, **65**, 451–463.

Roesti M, Hendry AP, Salzburger W *et al.* (2012) Genome divergence during evolutionary diversification as revealed in replicate lake-stream stickleback population pairs. *Molecular Ecology*, **21**, 2852–2862.

Rogers SM, Bernatchez L (2007) The genetic architecture of ecological speciation and the association with signatures of selection in natural lake whitefish (*Coregonus* sp. Salmonidae) species pairs. *Molecular Biology and Evolution*, **24**, 1423–1438.

Rollins LA, Browning LE, Holleley CE *et al.* (2012) Building genetic networks using relatedness information: a novel approach for the estimation of dispersal and characterization of group structure in social animals. *Molecular Ecology*, **21**, 1727–1740.

Rosenblum EB, Römpler H, Schöneberg T, Hoekstra HE (2010) Molecular and functional basis of phenotypic convergence in white lizards at White Sands. *Proceedings of the National Academy of Sciences*, **107**, 2113–2117.

Rowe HC, Renaut S, Guggisberg A (2012) RAD in the realm of next-generation sequencing technologies. *Molecular Ecology*, **20**, 3499–3502.

Rutledge LY, Patterson BR, Mills KJ *et al.* (2010) Protection from harvesting restores the natural social structure of eastern wolf packs. *Biological Conservation*, **143**, 332–339.

Salzburger W, Ewing GB, von Haeseler A (2011) The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. *Molecular Ecology*, **20**, 1952–1963.

Schlötterer C (2003) Hitchhiking mapping – functional genomics from the population genetics perspective. *Trends in Genetics*, **19**, 32–38.

Schluter D (2009) Evidence for ecological speciation and its alternative. *Science*, **323**, 737–741.

Schoville SD, Bonin A, Francois O *et al.* (2012) Adaptive genetic variation on the landscape: methods and cases. *Annual Review of Ecology, Evolution, and Systematics*, **43**, 23–43.

Schradin C, Lindholm AK, Johannesen J *et al.* (2012) Social flexibility and social evolution in mammals: a case study of the African striped mouse (*Rhabdomys pumilio*). *Molecular Ecology*, **21**, 541–553.

Scopece G, Lexer C, Widmer A, Cozzolino S (2010) Polymorphism of postmating reproductive isolation within plant species. *Taxon*, **59**, 1367–1374.

Segelbacher G, Cushman SA, Epperson BK *et al.* (2010) Applications of landscape genetics in conservation biology: concepts and challenges. *Conservation Genetics*, **11**, 375–385.

Sella G, Petrov DA, Przeworski M, Andolfatto P (2009) Pervasive natural selection in the Drosophila genome? *PLoS Genetics*, **5**, e1000495.

Servedio MR, Van Doorn GS, Knopp M, Frame AM, Nosil P (2011) Magic traits in speciation: 'magic' but not rare? *Trends in Ecology & Evolution*, **26**, 389–397.

Sharon G, Segal D, Ringo JM (2010) Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 20051–20056.

Shehzad W, McCarthy TM, Pompanon F *et al.* (2012) Prey preference of snow leopard (*Panthera uncia*) in South Gobi, Mongolia. *PLoS ONE*, **7**, e32104.

Signer EN, Schmidt CR, Jeffreys AJ (1994) DNA variability and parentage testing in captive Waldrapp ibises. *Molecular Ecology*, **3**, 291–300.

Simms EL, Porter SS (2012) Transcriptomic insights into mechanisms of symbiotic cooperation. *Molecular Ecology*, **21**, 4665–4668.

Smith CI, Tank S, Godsoe W *et al.* (2011) Comparative phylogeography of a coevolved community: concerted population expansions in Joshua trees and four yucca moths. *PLoS ONE*, **6**, e25628.

Smith G, Lohse K, Etges William J, *et al.* (2012) Model-based comparisons of phylogeographic scenarios resolve the intraspecific divergence of cactophilic *Drosophila mojavensis*. *Molecular Ecology*, **21**, 3293–3307.

Sork VL, Waits L (2010) Contributions of landscape genetics – approaches, insights, and future potential. *Molecular Ecology*, **19**, 3489–3495.

Sork VL, Davis FW, Westfall R *et al.* (2010) Gene movement and genetic association with regional climate gradients in California valley oak (*Quercus lobata* Nee) in the face of climate change. *Molecular Ecology*, **19**, 3806–3823.

Sork VL, Aitken SN, Dyer RJ, Eckert AJ, Legendre P, Neale DB (2013) Putting the landscape into the genomics of trees: approaches for understanding local adaptation and population responses to changing climate. *Tree Genetics and Genomics*, doi:DOI 10.1007/s11295-013-0596-x

Sousa V, Grelaud A, Hey J (2011) On the nonidentifiability of migration time estimates in isolation with migration models. *Molecular Ecology*, **20**, 3956–3962.

Spear SF, Balkenhol N, Fortin M-J, McRae BH, Scribner KIM (2010) Use of resistance surfaces for landscape genetic studies: considerations for parameterization and analysis. *Molecular Ecology*, **19**, 3576–3591.

St-Cyr J, Derôme N, Bernatchez L (2008) The transcriptomics of life-history trade-offs in whitefish species pairs (*Coregonus* sp.). *Molecular Ecology*, **17**, 1850–1870.

Stenglein JL, Waits LP, Ausband DE, Zager P, Mack CM (2011) Using noninvasive genetic sampling at grey wolf (*Canis lupus*) rendezvous sites to determine pack size, family relationships, and relatedness. *Journal of Mammalogy*, **92**, 784–795.

Stinchcombe JR, Hoekstra HE (2008) Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. *Heredity*, **100**, 158–170.

Stone GN, Nee S, Felsenstein J (2011) Controlling for non-independence in comparative analysis of patterns across populations within species. *Philosophical Transactions of the Royal Society of London Series B*, **366**, 1410–1424.

Stone GN, Lohse K, Nicholls JA *et al.* (2012) Reconstructing community assembly in time and space reveals enemy escape in a western palearctic insect community. *Current Biology*, **22**, 1–6.

Storfer A, Murphy MA, Evans JS *et al.* (2007) Putting the 'landscape' in landscape genetics. *Heredity*, **98**, 128–142.

Storfer A, Murphy MA, Spear SF, Holderegger R, Waits LP (2010) Landscape genetics: where are we now? *Molecular Ecology*, **19**, 3496–3514.

Strasburg JS, Rieseberg LH (2011) Interpreting the estimated timing of migration events in the IM suite of programs. *Molecular Ecology*, **20**, 2353–2366.

Suen G, Teiling C, Li L *et al.* (2011) The genome sequence of the leaf-cutter ant *Atta cephalotes* reveals insights into its obligate symbiotic lifestyle. *PLoS Genetics*, **7**, e1002007.

Sullam KE, Essinger SD, Lozupone CA *et al.* (2012) Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. *Molecular Ecology*, **21**, 3363–3378.

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research*, **17**, 6463–6471.

Tedersoo L, Bahram M, Toots M *et al.* (2012) Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. *Molecular Ecology* **21**, 4160–4170.

The International Aphid Genomics Consortium (2010) Genome sequence of the Pea Aphid *Acyrthosiphon pisum*. *PLoS Biology*, **8**, e100031.

Thoß M, Ilmonen P, Musolf K, Penn DJ (2011) Major histocompatibility complex heterozygosity enhances reproductive success. *Molecular Ecology*, **20**, 1546–1557.

Turner TL, Hahn MW (2010) Genomic islands of speciation or genomic islands and speciation? *Molecular Ecology*, **19**, 848–850.

Turner T, Hahn M, Nuzhdin S (2005) Genomic islands of speciation in *Anopheles gambiae*. *PLoS Biology*, **3**, e285.

Ungerer MC, Strakosh SC, Zhen Y (2006) Genome expansion in three hybrid sunflower species is associated with retrotransposon proliferation. *Current Biology*, **16**, R872–R873.

Ungerer MC, Johnson LC, Herman MA (2008) Ecological genomics: understanding gene and genome function in the natural environment. *Heredity*, **100**, 178–183.

Valentini A, Miquel C, Nawaz MA *et al.* (2009) New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the *trnL* approach. *Molecular Ecology Resources*, **9**, 51–60.

Van Bers NEM, van Oers K, Kerstens HHD *et al.* (2010) Genome-wide SNP detection in the great tit *Parus major* using high throughput sequencing. *Molecular Ecology*, **19**, 88–98.

Vestheim H, Jarman SN (2008) Blocking primers to enhance PCR amplification of rare sequences in mixed samples - a case study on prey DNA in Antarctic krill stomachs. *Frontiers in Zoology*, **5**, 12.

Via S (2012) Divergence hitchhiking and the spread of genomic isolation during ecological speciation-with-gene-flow. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, **367**, 451–460.

Via S, West J (2008) The genetic mosaic suggests a new role for hitchhiking in ecological speciation. *Molecular Ecology*, **17**, 4334–4345.

Waits L, Sork VL (2010) Contributions of landscape genetics - approaches, insights, and future potential. *Molecular Ecology*, **19**, 3489–3495.

Walker FM, Taylor AC, Sunnucks P (2008) Female dispersal and male kinship-based association in southern hairy-nosed wombats (*Lasiorhinus latifrons*). *Molecular Ecology*, **17**, 1361–1374.

Wang J (2007) Triadic IBD coefficients and application to estimating pairwise relatedness. *Genetical Research*, **89**, 135–153.

Wang J (2011) COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular Ecology Resources*, **11**, 141–145.

Wang C, Lu X (2011) Female ground tits prefer relatives as extra-pair partners: driven by kin-selection? *Molecular Ecology*, **20**, 2851–2863.

Waser PM, Berning ML, Pfeifer A (2012) Mechanisms of kin discrimination inferred from pedigrees and the spatial distribution of mates. *Molecular Ecology*, **21**, 554–561.

Wegmann D, Leuenberger C, Neuenschwander S, Excoffier L (2010) ABCtoolbox: a versatile toolkit for approximate Bayesian computations. *BMC Bioinformatics*, **11**, 116.

Wetton JH, Carter RE, Parkin DT, Walters D (1987) Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature*, **327**, 147–149.

Wetzel DP, Westneat DF (2009) Heterozygosity and extra-pair paternity: biased tests result from the use of shared markers. *Molecular Ecology*, **18**, 2010–2021.

While GM, Uller T, Wapstra E (2011) Variation in social organization influences the opportunity for sexual selection in a social lizard. *Molecular Ecology*, **20**, 844–852.

Wright LI, Fuller WJ, Godley BJ *et al.* (2012) Reconstruction of paternal genotypes over multiple breeding seasons reveals male green turtles do not breed annually. *Molecular Ecology*, **21**, 3625–3635.

Wu C (2001) The genic view of the process of speciation. *Journal of Evolutionary Biology*, **14**, 851–865.

Yatsunenko T, Rey FE, Manary MJ *et al.* (2012) Human gut microbiome viewed across age and geography. *Nature*, **486**, 222–227.

Zakharov EV, Hellman JJ (2012) Genetic differentiation across a latitudinal gradient in two co-occurring butterfly species: revealing population differences in a context of climate change. *Molecular Ecology*, **17**, 189–208.

Zellmer AJ, Hanes MM, Hird S, Carstens BC (2012) Deep phylogeographic structure and environmental differentiation in the carnivorous plant *Sarracenia alata*. *Systematic Biology*, **61**, 763–777.

Zhang T, Luan J, Qi J *et al.* (2012) Begomovirus–whitefly mutualism is achieved through repression of plant defenses by a virus pathogenicity factor. *Molecular Ecology*, **21**, 1294–1304.

Zilber-Rosenberg I, Rosenberg E (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Review*, **32**, 723–735.

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