Population Genomics Provides Key Insights in Ecology and Evolution

Paul A. Hohenlohe, Brian K. Hand, Kimberly R. Andrews, and Gordon Luikart

Abstract Population genomic tools have revolutionized many aspects of biology, as detailed throughout the chapters of this volume. In particular, population genomics has provided key insights into ecological and evolutionary processes in natural and managed populations. These studies address a wide range of questions, including demography, phylogeny, genetics of ecologically relevant traits, and adaptation. They have also facilitated the conservation and management of biodiversity and harvested populations. Rather than exhaustively document the applications of population genomics in ecology and evolution, in this chapter we provide perspectives on a few key issues confronting researchers seeking to use population genomic tools in non-model systems. A wide variety of molecular and computational genomic approaches are available and have been used in ecological and evolutionary studies. There is no single best approach; rather, the genomic approach used should be tailored to best address the particular study goals and guided by the biology of the system. A large number of trade-offs, costs, and benefits distinguish genomic approaches, which we discuss below. To illustrate these issues, we focus on several published case studies and assess how the research questions were addressed.

Keywords Genetics of adaptation · Inbreeding · Next-generation sequencing · Phylogenomics · Population genetic structure · Population genomics

P. A. Hohenlohe (✉)
Department of Biological Sciences, Institute for Bioinformatics and Evolutionary Studies, University of Idaho, Moscow, ID, USA
e-mail: hohenlohe@uidaho.edu

B. K. Hand · G. Luikart
Flathead Lake Biological Station, Conservation Genomics Group, Division of Biological Sciences, University of Montana, Polson, MT, USA

K. R. Andrews
Department of Fish and Wildlife Sciences, University of Idaho, Moscow, ID, USA

Genetics and Genomics Group, NOAA Pacific Marine Environmental Lab, University of Washington JISAO, Seattle, WA, USA

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1 Introduction

1.1 Defining Population Genomics in Ecology and Evolution

Population genomic approaches are applied to a wide and growing range of questions in ecology and evolution (Table 1). Some of these questions are long-standing subjects of traditional population genetic studies, but genomic tools provide greatly improved accuracy or the ability to use far fewer sampled individuals. Other questions in ecology and evolution, particularly those that involve identifying specific loci with functional importance, are newly accessible with genomic approaches. The experimental design, molecular techniques, and analytical tools used also vary widely, and a major challenge of applying genomic tools in ecology and evolution is choosing among all of these options. We discuss these considerations in detail below, highlighting a number of published studies that provide illustrative examples of population genomics in ecology and evolution.

There are multiple ways to define the term "genomics" and to distinguish population genomics from population genetics. Traditional population genetics has a long and rich history over the past century, and much of the classical theory of population genetics (e.g., Fisher 1958; Wright 1978) was developed before there was

Table 1 Examples of research issues in ecology and evolution that are addressed with population genomic approaches

Issue in ecology and evolution	Analytical methods and metrics
Broad-sense genomics	
Estimation of genetic diversity	Heterozygosity, allelic diversity, nucleotide diversity
Effective population size	Linkage disequilibrium (LD), two-sample methods
Population structure, admixture	Bayesian clustering, principal component analysis (PCA)
Source population assignment	Clustering methods
Inbreeding	Identity-by-descent methods
Narrow-sense genomics	
Mapping phenotypic traits	Genome-wide association studies (GWAS)
Fine-scale demographic history	Coalescent, diffusion approximation methods
Fine-scale estimates of current historic hybridization	Phylogenetic, haplotype-based methods
Loci for local adaptation	Outlier methods, genotype-environment association (GEA), multilocus covariance
Loci for inbreeding depression	GWAS
Loci for adaptive introgression	Outlier, cline analysis
Defining population units on local adaptation	Outlier, GEA

These are split into "broad-sense" and "narrow-sense" genomic studies (see text for definitions of these terms). Also shown are some of the classes of analytical approaches used to address each issue, illustrated by examples given in the text. For all of these questions, many different genomic approaches may be used, from reduced representation to whole-genome sequencing

a large body of empirical data against which to test it. Molecular population genetic studies in natural populations began in the 1970s with allozyme methods (e.g., Lewontin 1974), and early empirical discoveries led to fundamental changes in our understanding of the forms and amount of genetic variation present in natural populations and the evolutionary forces influencing it (Kimura 1983). From there, the development of new techniques continued to spur the field forward (Allendorf 2017). The advent of PCR (Mullis and Faloona 1987) and Sanger sequencing (Sanger et al. 1977) opened the way to investigating DNA sequence variation at specific loci to address ecological and evolutionary questions, notably the field of molecular systematics (Moritz and Hillis 1996).

A number of other genetic marker types have been developed in recent decades, such as short regions of mitochondrial DNA (Avise 1994) and microsatellites (Selkoe and Toonen 2006), which have become widely used and facilitated studies of genetic variation within natural populations in a wide range of organisms. However, these techniques are limited to a relatively small number of loci, and most require some prior identification of loci, for example, in order to develop PCR primers. In most cases, such as microsatellites, these genetic markers are assumed to represent a random sample of genetic variation across the genome, and are often assumed to reflect neutral evolutionary forces that affect genomes as a whole, such as demography or population structure. Traditional genetic markers like microsatellites have been used to identify functionally important loci (e.g., quantitative trait loci [QTL] in studies of laboratory crosses; Cresko et al. 2004). Nonetheless, because of their sparse distribution across the genome, these loci have had limited utility for addressing a core issue in ecology and evolution: the genetics of adaptation in natural populations.

The current revolution in genomics has been driven by next-generation sequencing technologies that allow heterogeneous pools of DNA fragments – i.e., pools of DNA fragments that differ in sequence and come from multiple locations across the genome – to be sequenced in parallel and in very large numbers (Mardis 2008). This changes the scaling relationship between the number of markers and the workload required for data generation. So, for example, increasing the number of microsatellite or Sanger-sequenced loci in a traditional study may require a concomitant increase in the number of primers to be validated or the number of PCRs to be conducted; in contrast, with next-generation sequencing, large increases in marker number can be achieved simply by adjusting the protocol or increasing the total amount of sequencing (see discussion of these trade-offs below). A simple definition of the term "population genomics" could rely solely on this technological advance, encompassing any study that uses next-generation sequencing and related recent technological advances to assay a large number of loci across the genomes of individuals sampled from one or more populations. This is the "broad-sense" definition of genomics of Garner et al. (2015).

Many population genomic studies under the broad-sense definition address questions that were tractable with traditional markers such as microsatellites, but the increase in number of loci sampled may improve precision and accuracy of the results. We discuss examples of such studies below and also the question of when to

use next-generation sequencing (i.e., broad-sense genomic tools) to address questions that can still be answered with traditional genetic methods. In some cases, the questions addressed with genomic tools are long-standing in ecology and evolution, but the dense sampling of the genome with genomic approaches provides novel insight by revealing much finer-scale patterns. These include estimation of phylogeny, where the different evolutionary histories among regions of the genome can be distinguished, and demographic history, where much finer time scales of inference are possible.

Narrower definitions of population genomics as distinct from genetics emphasize the novel concepts or questions addressed in genomic studies that were previously intractable with traditional methods (Black et al. 2001; Luikart et al. 2003; Allendorf 2017). In ecology and evolution, a central goal is to detect particular loci associated with selection, adaptation, or ecologically relevant traits and to distinguish these from a genome-wide background (Luikart et al. 2003). When a physical or linkage map of the genome is available, sequence or marker data can be placed in a genomic context along chromosomes or linkage groups, and particular regions of the genome that are influenced by evolutionary forces like selection can be identified (Luikart et al. 2018). Even in the absence of a reference map, however, the number of genetic markers possible in studies of non-model organisms allows a qualitative shift in the inferences that can be drawn regarding adaptive processes. As we discuss in more detail below, these inferences do not always require complete sampling of all functionally important parts of the genome.

Here we propose a narrow-sense definition for population genomics in ecology and evolution: *a population genomic study is one in which genetic loci are sampled to a sufficient density across the genome that there is an appreciable likelihood of detecting any genomic regions that are associated with fitness or ecologically relevant traits and distinguishing these factors from background evolutionary forces that affect the genome as a whole*. Below we describe some examples of such "narrow-sense" population genomics.

1.2 Overview of Approaches

Molecular techniques for population genomics in ecology and evolution fall into a few broad categories (Box 1; see also Luikart et al. 2018; Holliday et al. 2018). The range of techniques presents a number of trade-offs in the density and distribution of genetic variation that is sampled across the genome, as well as the number of individual and population samples that may be included given a study's budget, the computational resources required, and the types of inferences that can be made from the data. Importantly, many of the techniques are applicable in cases where little or no prior genomic information is available. This has democratized the field of genomics, opening vast areas of biodiversity to detailed genomic study that was previously impractical.

Box 1 Taxonomy of Methods for Population Genomics in Ecology and Evolution

Traditional genetic methods: These methods include Sanger sequencing of particular loci and any non-sequence-based method for genotyping a set of loci. In some cases, some prior genetic knowledge is required to target specific loci, for instance, to develop PCR primers for amplification. Depending on the loci targeted (e.g., mitochondrial or coding versus noncoding nuclear DNA) and the rate at which it evolves, sequence data can provide insights into a range of time scales from ecological population-level processes to long-term phylogenetic relationships among taxa. Non-sequence-based genotyping methods include allozymes, restriction-fragment analyses, and microsatellites. These techniques are used to produce genotypes for a set of loci across individuals, and these techniques are often most useful for ecological and evolutionary insights within species.

Whole-genome sequencing (WGS): One approach in population genomics is simply to sequence the complete genome of every individual in a sample (e.g., Jones et al. 2012; Ellegren et al. 2012; Robinson et al. 2016). Typically, this is done when a reference genome assembly or physical map is available, so that short-sequence reads from sampled individuals can be aligned against the reference. This approach is also called "whole-genome re-sequencing" because a reference genome has already been sequenced for the species. Samples can either be individually sequenced at high enough coverage to provide individual-level genotype data or pooled to provide population-level allele frequency data. An advantage of WGS is that in addition to identifying single-nucleotide variation, larger-scale genetic variants such as insertion/deletion, copy number variants, and inversions can be identified that may play an important role in adaptation (e.g., Chain et al. 2014; Feulner et al. 2015).

Reduced representation sequencing: While whole-genome sequencing costs continue to decline, making it feasible for ecological and evolutionary studies, it often may not be the most efficient allocation of sequencing effort given the goals of a study, and it imposes substantial bioinformatic burdens. An alternative is to focus sequencing on a reduced representation – a subset – of the genome, so that sequencing effort can be spread across many more individual or population samples. There are several ways to focus on a subset of the genome:

Anonymous reduced representation sequencing includes techniques in which sequencing cannot be targeted at prior-defined loci and may not even be known beforehand. The most common family of such techniques is restriction site-associated DNA sequencing (RADseq; Andrews et al. 2016), a group of techniques united by their use of restriction enzymes to focus

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Box 1 (continued)

sequencing effort on DNA fragments adjacent to enzyme recognition sites. Restriction enzymes digest DNA at characteristic short (4–8 bp) nucleotide sequences that may occur anywhere in the genome. While the distribution of recognition sites may be biased to some degree (e.g., by GC content or methylation sensitivity), RADseq loci are essentially a random sample across the genome and occur in both coding and noncoding regions.

Transcriptome sequencing focuses sequencing effort on the subset of the genome that is transcribed, by reverse transcribing RNA to DNA during construction of sequencing libraries (Wang et al. 2009; Ekblom and Galindo 2011). In many organisms, such as vertebrates, the transcriptome is a small fraction of the total genome size. To the extent that adaptive variation exists in coding regions (or in regulatory regions tightly linked to coding regions), this approach can increase the chances of identifying adaptive variants, but it also may provide a biased sample of the genome relative to neutral evolutionary processes such as demography.

Sequence capture methods use a prior designed set of probes to focus sequencing effort on a set of hundreds to tens of thousands of loci (Jones and Good 2016). Probes may target genes of interest, putatively neutral loci, or any combination, but they must be designed ahead of time based on prior sequence information. For large, complex genomes, capture methods may allow researchers to avoid repetitive or non-informative genomic regions (McCartney-Melstad et al. 2016). A recent approach combines RADseq and sequence capture, in a protocol called "rapture," to target a subset of previously identified RADseq loci for efficient genotyping across a large number of individuals (Ali et al. 2016).

Multiplex PCR amplicon sequencing is a set of techniques for efficiently amplifying multiple loci with standard PCR primers and then using next-generation sequencing techniques to sequence these loci across many individuals in a single experiment. Like sequence capture methods, multiplex amplicon sequencing requires some prior work to identify loci and design PCR primers, which may target SNPs or other previously identified polymorphisms useful for population genetic studies. An example is the protocol developed by Campbell et al. (2015), called "Genotyping in Thousands by sequencing" (GT-seq), which can target roughly 50–500 loci. GT-seq uses dual barcoding to allow up to thousands of individuals to be multiplexed in a single lane of Illumina sequencing and later separated bioinformatically. Because it targets a relatively modest number of loci, multiplex amplicon sequencing is not suited for conducting, for instance, an initial genome scan for selection, but rather expanding from an initial list of loci of interest to a wider set of populations or individual samples.

How should researchers choose a population genomic approach in an ecological or evolutionary study? The overriding consideration is the goal of the study; the choice of method should be driven by the particular question(s) being addressed and the type of data that would best answer them, given the biology of the system (Andrews and Luikart 2014; Benestan et al. 2016). Methods differ widely in their power to make statistical inferences in natural populations, as well in the cost associated with each method and the trade-offs inherent in sampling design. While no approach is ideal in all cases, the range of options provides flexibility in addressing particular study goals and biological systems and adjusting to constraints of total cost and laboratory or bioinformatics expertise. Within each of the methods in Box 1, there is also wide latitude to adjust technical details, in addition to sampling and experimental design, to tailor genomic techniques to each scientific question. Optimizing these details depends on a large number of considerations (Box 2); a few are discussed in more detail below and illustrated by case studies later in the chapter.

Box 2 Key Questions in Designing a Population Genomic Study

Before embarking on a population genomic study in ecology and evolution, researchers would be well-advised to answer as many of the questions below as possible. These answers will drive the best molecular and bioinformatic approaches to be used, as well as sampling design.

- What are the goals of the study, and what type of data would provide the best statistical power of inference?
- Are genomic techniques necessary at all? Or would a traditional population genetic tool be sufficient and less expensive in time and resources?
- What are the characteristics of the genome? (e.g., total genome size, proportion made up of genic regions, amount of duplicate sequence from whole-genome duplication or transposable elements, etc.)
- What are the prior genomic resources available? (e.g., Is there a genetic map or transcriptome assembly available? Is there a reference genome sequence from the focal species, and how well assembled and annotated is it? Or is there a reference genome from a related species, and if so how divergent?)
- What proportion of the genome, or number of markers, is necessary to cover?
- What are the budget limitations? Total sequencing cost is allocated across several factors: proportion of the genome interrogated, number of markers, number of individuals or populations, length and type of sequencing reads, and depth of coverage.
- What bioinformatics expertise and computational resources would be required to analyze the data?

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Box 2 (continued)
- How important is reproducibility of the set of loci and compatibility of the data with future studies, for example, applying a similar technique in a related taxon?
- To what extent is the data designed to solely address a particular question or to provide a base of genomic information for multiple future studies?

The first question when designing a study should be: Are genomic approaches appropriate or needed at all? Traditional genetic approaches remain effective tools for addressing a range of questions in ecology and evolution, such as demography, population structure, parentage or sibship, or detection of hybridization. In systems where a technique is established (for instance, where a set of microsatellite loci has been validated), it may be most efficient to avoid the expense and bioinformatic burden of using next-generation sequencing. In addition, this allows newly collected data to be completely compatible with previous studies, for instance, in long-term monitoring studies. However, in the absence of any prior tools or established protocols, genomic techniques like RADseq can be applied to simultaneously identify and genotype a large number of markers across many individuals. For ecological and evolutionary studies of non-model organisms, some genomic techniques are now more cost-efficient than traditional genetic techniques for an initial foray into a new system, even when the focal questions could be addressed with traditional techniques. Furthermore, the use of broad-sense genomics may often improve the accuracy and precision of population genetic estimates and lay the groundwork for further narrow-sense genomic studies.

1.3 How Much of the Genome Should Be Assayed?

Genomic techniques differ widely in what proportion of the genome of each sample is examined. At one extreme, whole-genome sequencing (WGS) provides nearly complete genetic information for each sample, and on the other, reduced representation methods can be dialed down to just a few hundred markers (Andrews et al. 2016; Jones and Good 2016; Ali et al. 2016). As sequencing costs continue to drop, it may seem intuitive to choose the first option – WGS of every sample in a study. However, the costs of WGS still limit most researchers in ecology and evolution to far fewer samples than are optimal to address many research questions, although new techniques may change that in the near future (Therkildsen and Palumbi 2017). There are ways to increase the number of individuals sampled with WGS, for instance, by pooling or low-coverage sequencing. However, a further consideration is that WGS data can impose a substantial computational burden. Researchers in nearly any population genomic study should plan to spend more time on bioinformatics than data generation, and this is certainly true for WGS data. In addition,

growth in computational processing and storage capacity has not kept pace with growth in sequence data-generating capacity. Thus, the bioinformatic costs, beyond the sequencing costs, may outweigh the benefits of WGS data for many ecological and evolutionary studies. Nonetheless, WGS and reduced representation genomic data provide different types of information that are appropriate for addressing different questions in ecology and evolution, as illustrated by case studies below.

As an alternative to WGS, anonymous reduced representation techniques like RADseq can provide a wide range of marker densities across the genome. Some recent discussion has centered around the question of whether the RADseq family of techniques can generate sufficient marker density to address ecological and evolutionary questions (Lowry et al. 2016; McKinney et al. 2017; Catchen et al. 2017). A key consideration is the extent of linkage disequilibrium (LD) across the genome, which effectively scales the density of markers to the proportion of the genome that can be assessed. This is because the signature of evolutionary forces like selection acting at any particular location in the genome will only be measurable if that location is in LD with one or more assayed markers. LD typically decays with distance along a chromosome, although this decay is often far from smooth; in some cases there may be regions of relatively high LD, called "haplotype blocks," punctuated by breakpoints that may reflect locations of elevated recombination rate (Dawson et al. 2002). The extent of LD is not just characteristic of a species but varies among populations due to demographic history, selection, chromosomal structural variation, and other factors (Dunning et al. 2000; Reich et al. 2001). Accordingly, there is vast variation by several orders of magnitude among biological systems in the size of haplotype blocks and thus the density of markers needed to sample a large proportion of them (McKinney et al. 2017).

Under the broad-sense definition of population genomics, many study goals do not require sampling even a majority of haplotype blocks; rather, only a relatively small sampling of the genome is required. Many of these questions could be answered with traditional genetic techniques. However, the increase in markers with genomics can improve accuracy and precision (e.g., below we discuss the relative value of microsatellite loci versus single-nucleotide polymorphism (SNP) loci for statistical inference).

Under the narrower definition of genomics, the proportion of haplotype blocks that are sampled determines the likelihood of detecting functionally important loci (Tiffin and Ross-Ibarra 2014; Catchen et al. 2017). However, even when the goal is to distinguish adaptive variation from the neutral background in a genome scan approach, many scientific questions do not require finding all adaptive loci. Such questions include: Is there a signature of adaptation across the sampled portion of the genome, either within or between populations (Epstein et al. 2016; Funk et al. 2016)? What is the geographic distribution of adaptive variation (White et al. 2013; Ferchaud and Hansen 2016)? Does population structure or phylogeny at adaptive or ecologically relevant loci match that across the rest of the genome (Funk et al. 2012; Wagner et al. 2013)?

In a study addressing these narrow-sense genomic questions in the context of predicted climate change, Bay et al. (2018) identified loci associated with climate

variables across the range of yellow warblers (*Setophaga petechia*) using RADseq. They then assessed genomic vulnerability as the mismatch between current and predicted allele frequencies based on genotype-environment association analyses, in order to predict the species' capacity to adapt to future conditions. They found that populations showing recent declines also tend to be more vulnerable to future selection pressures, potentially informing conservation and monitoring efforts. Studies like Bay et al. (2018), and those addressing the questions above, are narrow-sense genomic ones because they rely on identifying adaptive loci and distinguishing them from the genome-wide background; but they do not require identification of all adaptive loci, let alone functional validation of them. Instead, only a subset of adaptive loci may be detected, but these are still sufficient to address the study goals.

2 Broad-Sense Genomics

2.1 Selectively Neutral Processes

Population genomic approaches can provide more accurate estimates of genetic statistics than traditional techniques. For example, compared to pedigree-based estimates of inbreeding, genomic techniques can provide more accurate estimates of individual and population-level inbreeding. This results from surveying enough markers to determine the actual level of identity by descent within each individual, rather than the expectation based on pedigree relationships (Kardos et al. 2015; Luikart et al. 2018). Population genomic data can also provide greater power to detect inbreeding depression; for example, Hoffman et al. (2014) observed a much higher correlation of fitness and heterozygosity using SNPs compared to microsatellites in harbor seals (*Phoca vitulina*), because their RADseq approach yielded over 14,000 SNP loci.

Several recent studies have directly compared the utility of microsatellites versus genomic SNP data, such as that derived from RADseq or other reduced representation approaches. Because of the number of possible alleles, each microsatellite locus contains potentially much more information than a single SNP locus, which is typically expected to have just two alleles. However, the number of SNP loci commonly available in genomic studies often more than compensates for the lower information content per locus. For example, Malenfant et al. (2015) and Jeffries et al. (2016), studying polar bears (*Ursus maritimus*) and crucian carp (*Carassius carassius*) respectively, found that a RADseq dataset better detected fine-scale population structure than microsatellites. For crucian carp, this was true even when a much smaller sample of individuals per population was used for the RADseq data (Jeffries et al. 2016). Similarly, a study of the Amazonian plant species *Amphirrhox longifolia* using ~4,000 ddRAD loci found that sample sizes of eight were sufficient to estimate diversity when $\geq$1,000 SNPs were used, and sample sizes as low as two provided accurate estimates of F_{ST} when $>$1,500 SNPs were used (Nazareno et al. 2017). These cases illustrate that even when the sample of

individuals is far too small to estimate allele frequency or F_{ST} at any single locus with any accuracy, a large number of loci can still accurately estimate the average F_{ST} across the genome (Nazareno et al. 2017). Similarly, Puckett and Eggert (2016) found that 1,000 SNP loci outperformed 15 microsatellites in assignment of American black bears (*Ursus americanus*) to their natal range. In contrast, Fischer et al. (2017) found that estimates of genetic diversity in *Arabidopsis* populations were not closely aligned between microsatellite and SNP datasets. In fact, heterozygosity at SNP loci was more closely correlated with allelic richness in microsatellite loci than with heterozygosity at microsatellite loci, possibly a result of the different mutation processes in each type of locus.

It is useful to consider the "conversion rate" between microsatellites and SNPs in terms of the information content for different types of analyses. For example, Kaiser et al. (2016) found that a panel of 97 SNPs was equivalent to 6 microsatellite loci in estimating parentage in black-throated blue warblers (*Setophaga caerulescens*). Elbers et al. (2017) found that 100 SNP loci were required to correlate with the results of 10 microsatellite loci in estimating population differentiation (F_{ST}) in the gopher tortoise (*Gopherus polyphemus*), but 800 SNPs were needed to correlate with the same 10 microsatellites in estimating expected and observed heterozygosity. Note that the absolute estimates of F_{ST} and heterozygosity (and other population genetic statistics, like effective number of breeders N_b; Linløkken et al. 2016) may differ between SNPs and microsatellites because of the different mutation rates involved. Overall, these studies put the "conversion rate" between SNPs and microsatellites at anywhere from 10:1 to 80:1, depending on the analysis. However, it is typically feasible to get several orders of magnitude more SNP markers than microsatellites in most cases, in which case the conversion rate no longer matters (Fischer et al. 2017). For example, the study by Elbers et al. (2017) above subsampled their SNP markers from a dataset of nearly 18,000 SNP loci from sequence capture.

2.2 *Neutral Population Genetic Structure and Population Units*

The broad-sense definition of genomics includes the use of genomic tools to improve upon accuracy, precision, and efficiency compared to previous genetic approaches for estimates of, for example, population structure (see below), levels of admixture and inbreeding (Kardos et al. 2015, 2016), or effective population size (N_e). For example, Larson et al. (2014) used RADseq data to estimate N_e in Chinook salmon (*Oncorhynchus tshawytscha*) using the method *N_eEstimator* (Do et al. 2014), which relies on linkage disequilibrium among loci. In this case having a genetic map of the genome can allow the removal of physically linked loci, which can downwardly bias estimates of N_e (Park 2011; Larson et al. 2014). Larson et al. (2014) found that estimates of N_e based on 1,118 RADseq-derived SNPs had far smaller confidence intervals compared to estimates based on 39 previously identified SNP loci.

In the case of identifying genetic structure among populations, the increased precision of genomic tools may identify genetic differentiation that was cryptic to traditional methods. For instance, a phylogeographic study using RADseq for a mosquito (*Wyeomyia smithii*) in eastern North America revealed insights into demographic history that were not identified using traditional markers (Emerson et al. 2010). In this study, the authors used RADseq of pooled population samples to estimate consensus genotypes at a large number of SNP loci for each population and then used these data in a phylogenetic analysis. Because most of the populations are the result of recolonization from refugia following the last Pleistocene glaciation, genetic differentiation among them is relatively recent (beginning 22,000–19,000 years ago). Whereas previous mitochondrial DNA sequence data produced poorly resolved relationships among current populations, the pooled RADseq approach revealed a distinct geographic pattern of recolonization northward and then westward (Fig. 1). One possible factor in this discrepancy is that mitochondrial DNA sequence represents a single locus with different inheritance patterns than nuclear loci, while genomic techniques can sample a large number of loci across the much larger nuclear genome. Particularly in cases like this, with

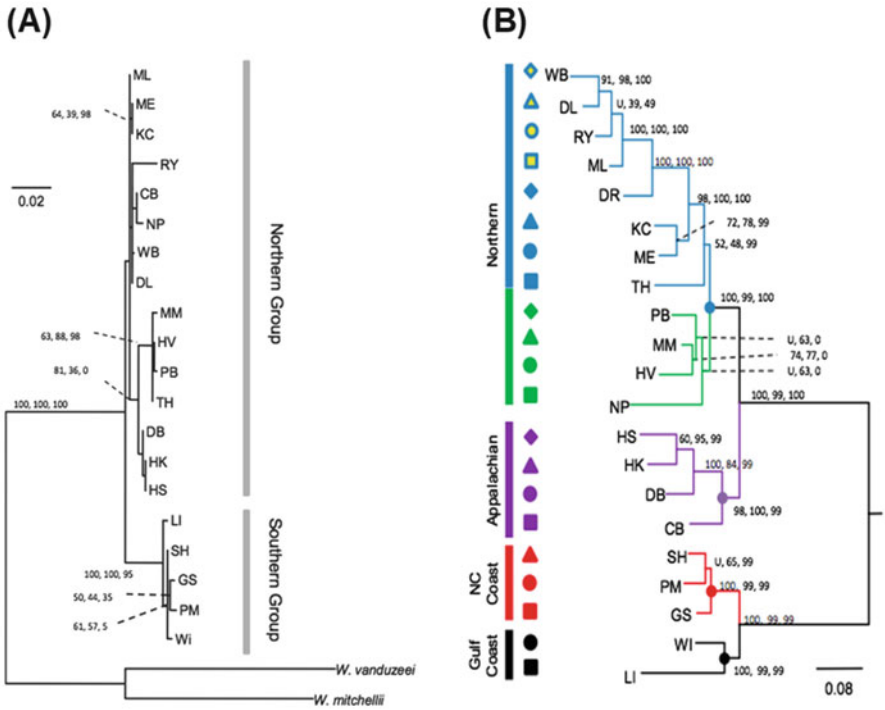

Fig. 1 Improvement of phylogeographic inference in the mosquito *Wyeomyia smithii* using broad-sense population genomic tools. (**a**) Maximum likelihood tree of relationships among populations based on mitochondrial *COI* sequence data. (**b**) Maximum likelihood tree based on 3,741 nuclear SNP loci derived from pooled RADseq data. Modified from Emerson et al. (2010)

recent differentiation among populations within a species, loci across the genome may reflect different phylogenetic histories due to incomplete lineage sorting and migration after formation of the populations. Analytical methods should account for this discrepancy in phylogenetic history among loci.

Genomic approaches also provide great promise for increased power in population assessments and stock identification for managed or harvested species, particularly marine taxa. Stock identification in harvested species is important for conservation and management of populations to avoid overharvest and local population extirpation (Palsbøll et al. 2007). For example, Benestan et al. (2015) used RADseq for American lobster (*Homarus americanus*) to define populations that were previously unresolved using microsatellite markers and to identify a set of loci that could assign individuals to source populations despite the weak genome-wide population structure for this species (mean $F_{ST} = 0.00185$). The authors identified and genotyped 10,000 SNPs using RADseq and then identified a subset of 3,000 high-F_{ST} loci (identified using a training set of samples and validated on an independent set, following Anderson (2010)) that assigned individuals to their source location with 80% success. Low genome-wide values of F_{ST} are expected to be characteristic of wide-ranging taxa with long-distance dispersal and large N_e (Bernatchez 2016). However, functionally important differentiation may occur at a small number of loci, and genomic approaches can identify these loci for ecological and evolutionary inferences. Even if the study goal is not to identify functionally important loci or loci under selection (as it is in "narrow-sense" genomic studies discussed below), the ability of genomic techniques to identify so many markers that a subset of highly differentiated markers can be extracted allows for finer-scale discrimination of population structure.

2.3 Phylogenomics

Genomic tools are increasingly being used for assessing phylogenetic relationships among species and higher taxa (Chan and Ragan 2013; McCormack et al. 2013; Ree and Hipp 2015; Barrett et al. 2016). A major challenge for such phylogenomic studies is that the many parts of the genome sampled by genomic tools may represent different lineage histories, and this has required building on traditional phylogenetic tools that assume a single history. Several different genomic techniques are applied in phylogenomics, including anonymous reduced representation techniques such as RADseq (Ree and Hipp 2015), targeted sequence capture (Bragg et al. 2016), and even whole-genome sequencing (Jarvis 2016). In this latter case, whole-genome data provided a detailed phylogeny and comparative genomic study of an entire vertebrate class, birds (Jarvis 2016). But even for short-sequence techniques such as RADseq, the accessible scale of taxonomic resolution can be quite deep (e.g., over 80-million-year divergence in octocorals, *Paragorgia* spp.; Herrera and Shank 2016). However, Leaché et al. (2015) found conflicting results from sequence capture and RADseq phylogenetic estimates in phrynosomatid lizards. Interestingly,

the best concordance between the sequence capture and RADseq-based SNP trees occurred when less conservative filtering was applied to the RADseq data, providing a large set of SNPs (roughly 16,000) with substantial missing data. This suggests that conservative filtering of genomic SNP data may cause misestimation in some cases.

While conflicting gene trees among loci can be a problem when the goal is to estimate a single species tree, variation among loci may reflect truly different evolutionary histories because of reticulate evolution (Vargas et al. 2017). The power of modern sequencing technology allows for phylogenetic estimation across multiple species or groups on a landscape, so that patterns of reticulate evolution and conflicting gene trees can be examined in a comparative framework (Edwards et al. 2016). While this can challenge the development of new demographic models and phylogenetic analysis tools (Edwards et al. 2016), it can also reveal insights into the adaptive consequences of hybridization and introgression (Keller et al. 2013; Nadeau et al. 2014; further discussion below).

3 Narrow-Sense Genomics

3.1 Detecting Ecologically Relevant and Adaptive Variation

At the heart of many population genomic studies in ecology and evolution is the detection of adaptive or functionally important loci (Luikart et al. 2003, 2018). One way to identify such loci is traditional genetic mapping techniques, made more powerful with the density of loci provided by population genomic approaches. Quantitative trait locus (QTL) mapping is possible for species that can be crossed experimentally (e.g., Miller et al. 2012; Liu et al. 2014) or for which pedigrees are known for natural populations (e.g., Slate et al. 2002; Beraldi et al. 2007; Santure et al. 2013). Genome-wide association studies (GWAS) are also feasible, even in many natural populations of ecological or evolutionary interest, in part because of the "democratization" of genomic techniques to non-model organisms. Some natural systems may be particularly well-suited to this approach; for instance, Nadeau et al. (2014) took advantage of a natural hybrid zone between phenotypically divergent butterfly (*Heliconius* spp.) subspecies to map wing color traits.

A long-standing method to distinguish adaptive loci from the genome-wide background is to identify high-F_{ST} outliers that are suspected to be under divergent natural selection among populations (Lewontin and Krakauer 1973; Beaumont and Nichols 1996). Outlier tests have received some criticism and perhaps been misapplied in some cases (Hermisson 2009; Hohenlohe et al. 2010; Hoban et al. 2016), in part because methods differ in model assumptions. Violations of model assumptions, such as historic demographic fluctuations, can increase variance in F_{ST} among loci and create false positives (Hohenlohe et al. 2010; Whitlock and Lotterhos 2015).

More recently, parallel to the development of landscape genetics and genomic approaches, there is increased interest in directly associating allele frequencies with

environmental variables, through genotype-environment association tests (GEAs; Joost et al. 2007; Coop et al. 2010; Hancock et al. 2011; Fumagalli et al. 2011; Schoville et al. 2012; Frichot et al. 2013; Rellstab et al. 2015; Forester et al. 2016; Hoban et al. 2016). GEAs are conceptually similar and complementary to GWAS approaches in that gene frequencies are associated with environmental factors, whereas in GWAS loci are associated with phenotypic traits. In humans, where very large sample sizes are feasible, several studies have used GEAs to identify important loci linked to environmental factors (Hancock et al. 2011; Fumagalli et al. 2011). In most ecological and evolutionary studies, samples sizes of both individuals and number of markers may be much smaller.

The move toward GEAs has been prompted by greatly increased availability of environmental and genomic data and growing understanding that signatures of adaptive selection can be difficult to distinguish from the selectively neutral genomic background (Schoville et al. 2012). For example, genetic variation underlying polygenic traits may be difficult to detect because the effect size and allele frequency shifts at any single locus may be quite small (Bernatchez 2016). Simulation-based studies have found that, in general, GEAs have more power to detect loci under selection than outlier-based approaches but have higher rates (20–50%) of false positives (De Mita et al. 2013; Frichot et al. 2013; Forester et al. 2016). Recent work has also suggested that multivariate approaches (principal component analysis, redundancy analysis, and population graphs) might help reduce the number of false positives and maintain reasonable power to detect true correlations (Forester et al. 2016; Rajora et al. 2016). Several other methods are also available for detecting loci under selection from population genomic data, and they are appropriate for different population scenarios, data types, types of selection, and time scales (Hohenlohe et al. 2010; Rajora et al. 2016; Luikart et al. 2018).

Bernatchez (2016) outlined a number of factors that can maintain adaptive variation in natural populations and therefore make signatures of adaptation difficult to identify. These include soft selective sweeps, traits with a polygenic basis, epistatic interactions among genes, epigenetics, and various types of balancing selection. Under these conditions, selection does not often drive single beneficial alleles to fixation; rather, the response to selection is relatively slight shifts in allele frequencies. In the potentially large number of cases in which adaptation depends on a large number of loci, detecting selection may be improved by alternative approaches. For instance, a promising recent approach in outlier tests for local adaptation is to focus on allele frequency covariance among loci, rather than allele frequency variation at individual loci (LeCorre and Kremer 2012; Rajora et al. 2016; Lind et al. 2017). Although reliably detecting adaptive loci remains challenging, a large and growing number of studies have detected adaptive variation with population genomic tools and provided insights into multiple aspects of species biology (Luikart et al. 2018). Below we discuss a few case studies.

3.2 Adaptive Population Structure

Advances in the discovery of non-neutral (i.e., candidate adaptive) markers have improved our ability to examine how selection shapes population genetic structure and how and why population structure differs at fitness-related loci compared to the genome-wide background dominated by neutral forces like demography. Recent genomics studies have revealed significant adaptive divergence at outlier loci, even in systems of high gene flow, such as marine organisms and forest trees. This is especially apparent in marine species, presumably because of large effective population sizes and large dispersal differences, which reduce neutral population divergence and allow for selection to act effectively on adaptive loci (Limborg et al. 2012; Corander et al. 2013; Hess et al. 2013; Milano et al. 2014). Genomic patterns of adaptive divergence often vary across spatial scales within a species, and adaptive loci often reveal finer-scale differentiation than neutral loci (Matala et al. 2014; Hand et al. 2016). Simulation-based modeling has further shown that inferences about local adaptation based solely on neutral genetic markers risk incorrectly identifying the underlying mechanisms driving population structure (Landguth and Balkenhol 2012).

In one example, Steane et al. (2015) used genome-wide diversity array technology (DArTseq; Sansaloni et al. 2011) to identify and genotype 16,122 high-quality dominant markers (presence/absence) in gimlet trees (*Eucalyptus salubris*; Steane et al. 2014). *E. salubris* is an obligate seeder that does not survive wildfire; however, it is also a key species for revegetation in a moderate (mesic) to arid region in Southern Australia (Nicolle 2006; Steane et al. 2015). Steane et al. (2015) identified a set of 24 putatively adaptive loci that showed high rates of differentiation ($F_{ST} > 0.7$ and many close to fixation) between two cryptic lineages in *E. salubris*, which appeared to be associated with climate adaptation along a strong aridity gradient. In this case, genome-wide scans were essential in identifying putatively adaptive markers of high differentiation that otherwise would have gone undetected by traditional neutral genetic techniques or phenotypic traits alone.

Killer whales (*Orcinus orca*) provide another example illustrating how neutral and adaptive markers can show different patterns of genetic structure. This species is the most widely distributed marine mammal. Despite the propensity for long-range dispersal and the movement of individual social groups over wide geographic ranges, there appears to be very little ancestral dispersal among sympatric ecotypes that differ in foraging behavior (Moura et al. 2014; Morin et al. 2015). Mitogenomes and 42 independent nuclear loci were found to be in concordance, indicating very limited gene flow among ecotypes (Morin et al. 2015). Moura et al. (2014) further identified a set of putatively adaptive loci (168 of 3,281 variable SNPs). Neutral genetic structure agreed with previous studies in identifying significant differentiation between populations in sympatry. However, adaptive genetic structure differed from neutral patterns and included a reduced set of high-F_{ST} outliers ($F_{ST} > 0.7$) with putative physiologically relevant function related to digestion and reproduction (Moura et al. 2014). The difference in neutral vs. adaptive genetic differentiation offered additional evidence that differentiation among sympatric populations was related to ecological processes more so than genetic drift.

3.3 Adaptive Introgression and Hybridization

Hybridization and introgression have important evolutionary consequences, and understanding these consequences is aided by dense, genome-wide marker coverage. Several powerful tools have been developed for inferring historical patterns of hybridization and introgression from population genomic data (e.g., TreeMix, Pickrell and Pritchard 2012; ALDER, Loh et al. 2013). In systems of hybridizing native and non-native species, introgression can lead to genetic extinction of the native (and often endangered) species (Allendorf et al. 2010).

One of the most well-studied systems involves the Flathead River system in the Northern Rocky Mountains, USA (Boyer et al. 2008; Muhlfeld et al. 2009, 2014; Hohenlohe et al. 2011, 2013; Amish et al. 2012; Hand et al. 2015; Kovach et al. 2015, 2016). Here, native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) is greatly threatened by hybridization with rainbow trout (*O. mykiss*), the world's most widely introduced fish (Halverson 2010). Hohenlohe et al. (2013) showed improved accuracy in measuring individual admixture proportions when using 3,180 diagnostic SNPs vs. 7 microsatellite loci (Boyer et al. 2008). The use of paired-end RADseq in this study allowed for identification of candidate genes by providing longer contiguous sequence around significant SNPs than previous approaches. Subsequent work included the publication of a reference genome (Berthelot et al. 2014) and the identification of more diagnostic markers for identifying parental ancestry, made possible with a larger sample of individuals and reference-based rather than de novo locus identification (Hand et al. 2015). These technical advances further refined the understanding of the system, revealing that selection in hybridized populations acts primarily against genetic variation from the invasive rainbow trout (Kovach et al. 2016).

Two more illustrative examples of adaptive introgression, and its signature on genomic variation, are from cichlid fish and butterflies. Keller et al. (2013) used RADseq in closely related cichlid taxa (*Pundamilia* and *Mbipia* species) from Lake Victoria. Five taxa were identified by several phenotypic traits, including male coloration. Across much of the genome, the taxa are poorly differentiated, but a subset of loci putatively associated with adaptive differentiation suggests two introgression events among lineages within the group that carried genetic variation for male coloration and opsin alleles (Keller et al. 2013). Similarly, Nadeau et al. (2014) examined striking color pattern differentiation among subspecies of the butterfly *Heliconius melpomene*, using RADseq to identify both loci under divergent selection (high-F_{ST} outliers) and loci associated with phenotypic variation in color pattern (GWAS). They found that signatures from both F_{ST} outlier tests and GWAS converged on a small number of major effect loci, providing evidence that narrow hybrid zones are maintained by strong selection on color pattern.

3.4 Demographic History

Genomic data provide a powerful ability to reconstruct the demographic history of populations. Previous genetic markers, such as microsatellites and mitochondrial DNA sequence, allowed some inference of historical fluctuations in population size using bottleneck tests and approximate Bayesian computation (ABC) methods (e.g., Fontaine et al. 2012; Spurgin et al. 2014). However, genomic data can provide much greater statistical power with ABC methods (Cornuet et al. 2014). For instance, large numbers of SNP loci can be used to estimate the allele frequency distribution, which can be used to test alternative models of demographic history across a set of populations using the method ∂a∂i (Gutenkunst et al. 2009). This method can test for changes in population size (expansion, contraction) as well as migration among populations (Fig. 2). While developed originally for human populations, with the advent of genomic techniques for non-model species, ∂a∂i has been applied widely. For instance, this type of demographic inference can be conducted in a comparative framework, as across six taxon pairs of birds that share similar distribution patterns in disjunct South American dry forest habitats (Oswald et al. 2017).

Estimation of demographic history from SNP data can be combined with detection of outlier loci that show greater differentiation between populations and differential patterns of gene flow among populations. For instance, Leroy et al. (2017) used ∂a∂i and ABC to infer the history of four European white oak species (*Quercus* spp.). They found that a long period of isolation generated some reproductive barriers, but that recent secondary contact due to postglacial warming resulted in

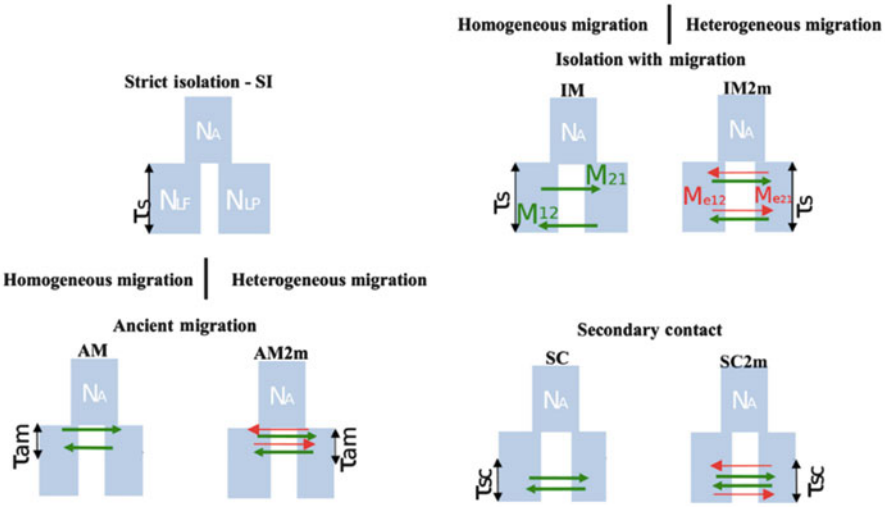

Fig. 2 Demographic scenarios tested in lamprey ecotypes. Four general models are shown for the history of two populations since divergence: strict isolation, isolation with migration, ancient migration, and secondary contact. In each model, parameters are estimated for the population sizes and timing of events. Reproduced with permission from Rougement et al. (2016)

secondary contact and gene flow at some loci, but not uniformly across the genome. Similarly, Rougement et al. (2016) illuminated the effect of migration on divergently selected loci in two lamprey species (*Lampetra* spp.), and Schield et al. (2017) applied a range of tests for migration and selection in western diamondback rattlesnakes (*Crotalus atrox*). All of these studies used reduced representation methods to genotype several thousand SNP loci across dozens to hundreds of individuals, illustrating the ability of this sampling design to be informative about adaptive variation.

Alternatively, the whole-genome sequence of even a single diploid individual can be used to infer the historical course of effective population size using pairwise sequentially Markovian coalescent (PSMC; Li and Durbin 2011). For instance, McManus et al. (2016) reconstructed the demographic history of lowland gorillas (*Gorilla* spp.), identifying a population contraction that appears to correspond with reduction in forest cover at the end of the last glacial maximum. Similarly, Nadachowska-Brzyska et al. (2016) linked climate changes to population fluctuations in *Ficedula* flycatchers (Fig. 3). Both of these studies emphasize that individuals from different populations are likely to exhibit different demographic histories, as might be expected, and a critical assumption in these analyses is that the sequenced individuals are representative of the population unit under study. Additionally, historic population structure can violate assumptions of the model and lead to false signatures of fluctuations in population size (Mazet et al. 2016). A way around these problems may be newer methods that allow analysis of multiple individuals, such as SMC++ (Terhorst et al. 2017). In addition to demographic reconstruction, WGS data can be used in a comparative framework to identify adaptive loci across closely related species, as illustrated in a study of large cats (*Panthera* spp.; Cho et al. 2013).

Demographic fluctuations have important consequences for current levels of genetic diversity and adaptive potential in natural populations. For instance, two studies have addressed this issue in island foxes (*Urocyon littoralis*) using two different genomic methods. Island foxes persist in six populations, each restricted to a separate island off the coast of Southern California, that have historically small population sizes in addition to recent bottlenecks. Robinson et al. (2016) used whole-genome sequencing of a single fox from each island (with the exception of one island represented by two individuals) and found extremely low levels of heterozygosity and the presence of deleterious variants. The approach of using WGS in a very small number of samples is justified here, because populations are likely to be well-mixed within each island and avoid the violation of assumptions mentioned above (Mazet et al. 2016). Funk et al. (2016) addressed the issues of genetic diversity in island foxes using RADseq. This approach assayed far fewer loci but across a total of 188 individuals. This study similarly found low levels of genetic diversity within each population. Because of the larger number of individuals sampled, it was possible to use F_{ST} outlier tests to detect selection, and despite the low overall diversity and differentiation among islands due to drift, there was also

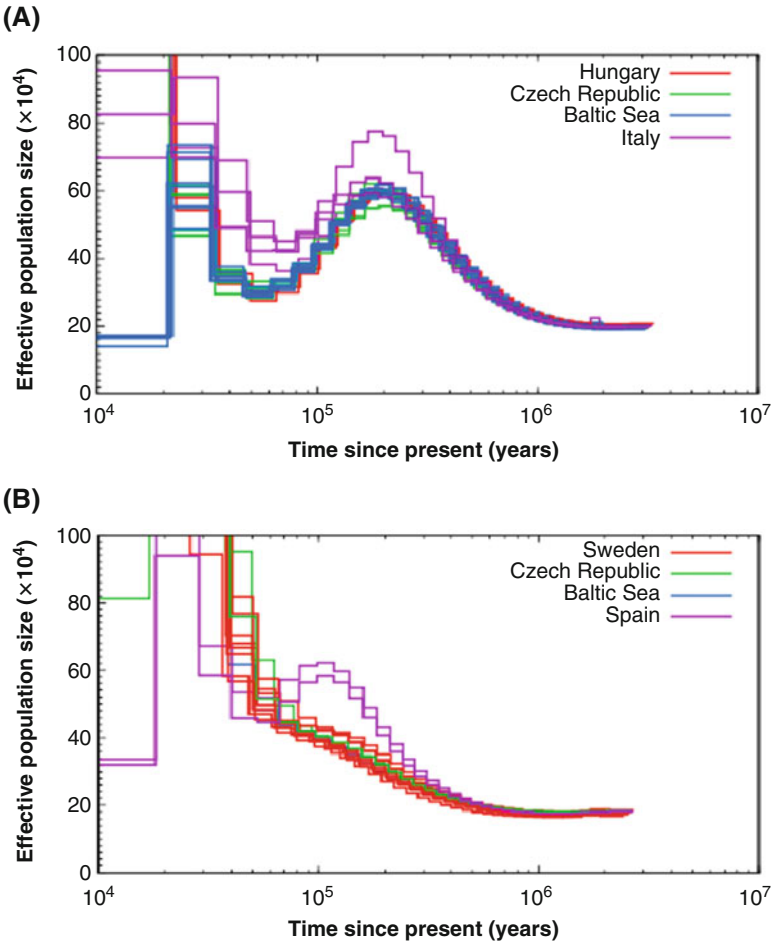

Fig. 3 Reconstruction of the demographic history of populations of (**a**) collared flycatchers (*Ficedula albicollis*) and (**b**) pied flycatchers (*Ficedula hypoleuca*), using the pairwise sequentially Markovian coalescent (PSMC) method (Li and Durbin 2011) on whole-genome sequence data. As illustrated here, PSMC can result in uncertainty at recent time scales, but it allows comparative demographic inference among related taxa occupying the same region. Modified from Nadachowska-Brzyska et al. (2016)

evidence of adaptive divergence among islands. Note that the study by Funk et al. (2016) is a case where not all haplotype blocks, and thus not all potentially adaptive loci, were sampled with the RADseq approach; nonetheless, a narrow-sense genomic question (is there evidence for adaptive differentiation among populations?) was still able to be answered.

4 Conclusions and Future Perspectives

Population genomics has provided numerous insights into ecological and evolutionary processes in natural and managed populations. The wealth of molecular and analytical techniques provides great flexibility in tailoring a population genomic approach to the goals of any particular study and the challenges of any particular biological system. The field is changing rapidly. The cost of acquiring sequence data continues to drop, and novel analytical techniques incorporate improved models of genomic processes and increased statistical power. In particular, whole-genome sequencing may be the best-suited approach to an expanding range of population genomic applications, but nonetheless a variety of reduced representation and targeted sequencing approaches are likely to continue to provide efficient alternatives. It is imperative for researchers in ecology and evolution to educate themselves about the trade-offs involved in designing population genomic studies. With careful consideration of the range of options, population genomics will continue to provide remarkable insights into ecological and evolutionary processes.

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