Journal of FISH BIOLOGY

Journal of Fish Biology (2016) **89**, 2519–2556
doi:10.1111/jfb.13145, available online at wileyonlinelibrary.com

On the maintenance of genetic variation and adaptation to environmental change: considerations from population genomics in fishes[a]

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The first goal of this paper was to overview modern approaches to local adaptation, with a focus on the use of population genomics data to detect signals of natural selection in fishes. Several mechanisms are discussed that may enhance the maintenance of genetic variation and evolutionary potential, which have been overlooked and should be considered in future theoretical development and predictive models: the prevalence of soft sweeps, polygenic basis of adaptation, balancing selection and transient polymorphisms, parallel evolution, as well as epigenetic variation. Research on fish population genomics has provided ample evidence for local adaptation at the genome level. Pervasive adaptive evolution, however, seems to almost never involve the fixation of beneficial alleles. Instead, adaptation apparently proceeds most commonly by soft sweeps entailing shifts in frequencies of alleles being shared between differentially adapted populations. One obvious factor contributing to the maintenance of standing genetic variation in the face of selective pressures is that adaptive phenotypic traits are most often highly polygenic, and consequently the response to selection should derive mostly from allelic co-variances among causative loci rather than pronounced allele frequency changes. Balancing selection in its various forms may also play an important role in maintaining adaptive genetic variation and the evolutionary potential of species to cope with environmental change. A large body of literature on fishes also shows that repeated evolution of adaptive phenotypes is a ubiquitous evolutionary phenomenon that seems to occur most often *via* different genetic solutions, further adding to the potential options of species to cope with a changing environment. Moreover, a paradox is emerging from recent fish studies whereby populations of highly reduced effective population sizes and impoverished genetic diversity can apparently retain their adaptive potential in some circumstances. Although more empirical support is needed, several recent studies suggest that epigenetic variation could account for this apparent paradox. Therefore, epigenetic variation should be fully integrated with considerations pertaining to role of soft sweeps, polygenic and balancing selection, as well as repeated adaptation involving different genetic basis towards improving models predicting the evolutionary potential of species to cope with a changing world.

Key words: adaptation; conservation; environmental change; parallel evolution; population genomics; RADseq.

INTRODUCTION

Ample empirical evidence has accumulated that species across the globe are experiencing drastic changes in environmental conditions as a result of human activities (Smith

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[a]This paper was presented at the FSBI Symposium, Bangor, in July 2016. Its content may not follow the usual style and format of the *Journal of Fish Biology*.

& Bernatchez, 2008; Carroll *et al.*, 2014). Among these, change in temperature induced by increased greenhouse gas emissions is of greatest concern (Hoffmann *et al.*, 2015.). In particular, aquatic species must also face other associated environmental challenges including ocean acidification, extremes in length and intensity of drought and flood conditions, changes in the salinity of coastal areas and novel pathogens. Moreover, anthropogenic activities also impose many additional stressors on species, including physical habitat alterations (*e.g.* dams and reservoirs), pollution and the introduction of new species that are altering predation and competition dynamics. Selective harvesting of wild populations (*e.g.* fishing) has the potential to radically modify population dynamics and selection regimes (Allendorf *et al.*, 2008). These environmental changes are happening rapidly, almost instantaneously, they incur substantial economic costs and threats to biodiversity as well as diminish a species' potential to adapt to future environments. As a consequence of these interactions, a global, yet unplanned, evolutionary experiment is being witnessed with direct effects on the biotic diversity of the planet (Smith & Bernatchez, 2008).

Understanding and predicting how organisms respond to human-driven environmental change is therefore of major concern for scientists. For instance, this was identified among the top five priorities in a recent perspective outlining the molecular ecology strategy for the next 20 years (Andrew *et al.*, 2013). Despite the scale of the problems at stake, however, there remains much to be done to understand how species can adapt and under what conditions this may occur (Hansen *et al.*, 2012). Three (non-exclusive) options exist by which organisms can cope with a changing environment. In the shorter term, animals and plants may acclimate to shifting environmental conditions *via* phenotypic plasticity, *i.e.* by expressing particular adaptive phenotypes in response to local environmental conditions. In the longer term and depending on an organisms' life history, species distributions may shift to sites that are more favourable to their physiology and ecology. Lastly, the ultimate response to global change is to adapt to the prevailing environmental conditions, which implies evolutionary (genetic) change in response to the new selective pressures. Arguably, in order to assess the potential of species to cope with environmental change, it is necessary to differentiate among these aforementioned responses and to document their respective roles in different contexts and under different conditions.

The application of principles from evolutionary biology to these challenges may improve the ability to respond to human-driven environmental change (Carroll, 2011). For instance, by applying an evolutionary framework the three most important demographic factors that facilitate successful adaptation to rapid environmental change, such as generation time, population size and population structure can be identified (Carroll *et al.*, 2014). A plethora of studies have provided evidence that species differ largely in their potential to adapt to rapid changes, probably owing to variation in these three factors. On one hand, numerous studies have documented human-driven 'contemporary (adaptive) evolution' (Stockwell *et al.*, 2003). Evidence for rapid evolutionary adaptations, as seen in the peppered moth *Biston betularia* (Kettlewell, 1973), or changes in resistance levels to heavy metals in plants inhabiting contaminated sites (Wu & Kruckeberg, 1985), once considered atypical, is now commonplace. Indeed, many studies showed that over recent decades, environmental change has led to heritable, genetic changes in both plants and animals (Bradshaw & Holzapfel, 2006; Hoffmann *et al.*, 2015). Studies on aquatic species and in fishes in particular have provided evidence that species can rapidly adapt to newly invaded environments

(Lee & Gelembiuk, 2008; Vera *et al.*, 2016), to increased pollution levels (Williams & Oleksiak, 2008; Bélanger-Deschênes *et al.*, 2013), captive environments (Makinen *et al.*, 2015; Christie *et al.*, 2016) or hybridize following environmental perturbations (Seehausen *et al.*, 2008*a*). Yet other studies have documented changes in the genetic basis of growth in exploited species as a result of size-selective fishing (Conover & Munch, 2002; Dunlop *et al.*, 2009). More dramatic, however, are the challenges faced from cancer, pests and pathogens that can evolve rapidly over short time frames (Palumbi, 2001; Thomas *et al.*, 2013; Carroll *et al.*, 2014). On the other hand, not all populations are expected to be capable to adapt to anthropogenic changes *via* evolutionary change. Indeed, species that are characterized by long generation times (*e.g.* several years), or by fragmented population structures make them less able to adapt quickly enough to keep pace with the rate of human alterations to the environment. These biological characteristics contribute, together with non-evolutionary factors, to what has been termed the imminent prospect of Earth's sixth mass extinction (Carroll *et al.*, 2014).

While many studies have attributed phenotypic changes in natural populations to adaptive evolution, there is still significant controversy and uncertainty surrounding these inferences (Merilä & Hendry, 2014). Genetic evidence is needed to demonstrate that adaptive evolution has occurred, yet it has proven very challenging to link this evidence to phenotypic changes and thus, to distinguish between the proportions of phenotypic changes that are genetically based *v.* changes resulting from phenotypic plasticity. Indeed, the genetic underpinnings of most fitness-related phenotypic traits are still poorly documented (Ellegren & Sheldon, 2008; Savolainen *et al.*, 2013). There are several explanations for this. First, the vast majority of studies have focused on measuring phenotypic responses potentially associated with increased individual fitness in new environments (*e.g.* growth, fecundity, physiology and morphology), whereas the molecular pathways underlying rapid adaptive phenotypic responses and the change in genetic variation have been less studied (Laikre *et al.*, 2010). Second, progress towards addressing these questions has been methodologically hampered until recently because the technical and analytical resources necessary to investigate the genetic basis of adaptation lacked power (Pardo-Diaz *et al.*, 2015). This is particularly true for non-model species, where genomic resources have been limited to the genotyping information of a few dozen (*e.g.* microsatellites) or at best a few hundred (*e.g.* amplified fragment length polymorphism, AFLP) mainly neutral and anonymous markers.

Recent development in genomics and other omic technologies are now providing unprecedented insights into the evolutionary processes and molecular basis of adaptation, equipping on how to now address the challenge. In particular, the genomic revolution associated with the development of various next-generation sequencing methods has transformed the field of population and functional genomics over the past 5 years by allowing to perform, for relatively modest costs, genome-wide single nucleotide polymorphism (SNP) genotyping for any species (Andrews *et al.*, 2016) and for species with small and simple genomes, whole genome (re)sequencing (Jones *et al.*, 2012; Ellegren, 2014). In turn, this makes it possible to document in much more detail the genome-wide architecture underlying adaptive traits (Star *et al.*, 2011; Barson *et al.*, 2015), as well as the genome-wide response to selection induced either by natural (Hohenlohe *et al.*, 2010) or anthropogenic factors (Bélanger-Deschênes *et al.*, 2013; Laporte *et al.*, 2016).

Although this explosion of genomic data is very promising, this only represents a first step, as genomic data in itself are not sufficient for predicting the evolutionary potential of species and their future. This requires building models that would integrate parameters that allow predicting the potential for adaptive responses of a given species in a given environmental context. For instance, theory and simulation studies have demonstrated that the number, effect size, geographical distribution and starting allele frequencies of beneficial variants can drastically alter the probability of adaptation (Ralph & Coop, 2015; Yeaman, 2015). While empirical genome-wide data are now available for many species, these are rarely integrated into theoretical frameworks that would, for instance, allow a better evaluation of how the adaptive capacity may vary across taxonomic groups with different life histories or that are occupying distinct ecological niches. In sum, progress towards forming predictive hypotheses has been relatively modest because of the existing gap between these two main lines of inquiries: (1) what is learnt from population genomic studies about the potential evolutionary change in existing populations and (2) do these findings match existing theory?

Understanding and predicting a species' capacity to retain its evolutionary potential in the face of new selection pressures is further hampered by the fact that existing theory and models pertaining to environmental adaptation are quite recent and commonly assume that phenotypic traits are controlled by single genes driven to fixation (Uecker *et al.*, 2014). For instance, such models predict that an environmental tolerance allele, that provides a sufficient selective benefit in the novel environment and is present at a high enough frequency in the contemporary population, will be driven to fixation (Orr & Unckless, 2014). In turn, this assumption also implies the erosion of genetic diversity and therefore the reduction of a species' evolutionary potential. The prevailing and gloomy perspective stemming from these approaches is that natural populations are threatened by interacting forces that together create a downward spiral that reduces the evolutionary potential as a result of strong directional selection and reductions in population size, leading to impoverished genetic variation and an increased extinction risk.

In sharp contrast to this view, however, is the notion that the sole search for quantitative trait nucleotides of large effects, or the QTN programme (Rockman, 2012), could lead to an underestimate of the potential of species to retain their genetic diversity and evolutionary potential. After all, organisms can sometimes adapt surprisingly quickly to new environmental conditions (Messer & Petrov, 2013), even when census sizes are very small, as commonly observed in captive and domesticated populations (Andersson, 2013). Moreover, empirical studies using population genomics techniques are increasingly revealing a prevailing role for standing genetic variation in environmental tolerance within populations (Barrett & Schulter, 2008). Clearly, there exist mechanisms by which genetic variation and evolutionary potential can be maintained by natural populations and it appears that this can happen in a more prevailing way than has previously been assumed.

In the following sections, the first goal is to briefly introduce what Whitlock (2015) coined the 'modern approaches to local adaptation', with a focus on the use of population genomics data to detect signals of natural selection. Several mechanisms are discussed that may enhance the maintenance of genetic variation and evolutionary potential that have been overlooked and should be considered in future theoretical development and predictive models. These are the prevalence of soft sweeps, polygenic basis of adaptation, balancing selection and transient polymorphisms, recurrent parallel evolution and epigenetic variation. Given the focus of this volume on 'Fish, Genes and Genomes: Contributions to Ecology, Evolution and Management', exemplary population genomic studies from the recent fish literature that illustrate the usefulness of applying modern approaches to local adaptation for management and conservation purposes is then presented. This review also provides empirical fish examples showing that species may be able to retain more genetic variation and evolutionary potential than currently assumed. Fishes are particularly relevant in this context since both marine and freshwater environments belong to the types of ecosystems that are thought to be suffering the most from the various human-induced stressors. They further represent the group of vertebrates with the highest number of threatened species and possibly also one of the best studied groups of organisms for documenting evolutionary change in human-altered habitats. Finally, while trying to represent most of the relevant literature to the topic, there is a bias towards highlighting some of my own research, given that this paper was delivered as an invited keynote lecture.

MODERN APPROACHES TO STUDY GENOMICS OF LOCAL ADAPTATION

Many new theoretical and analytical approaches have been developed in recent years to study local adaptation (Savolainen *et al.*, 2013; Haasl & Payseur, 2016). With the ever decreasing costs and increasing analytical power to dissect DNA, it has now become possible to identify some of the loci responsible for local adaptation using genome-wide, population-level data. Such studies are ideally performed using whole genome resequencing or high density SNP chip data, but the costs of developing and applying these tools have so far limited their use to either model species or species characterized by small and notionally simple genomes. Arguably, the most common genotyping approach currently applied to fishes is restriction site-associated DNA sequencing (RADseq), which enables the low-cost discovery and genotyping of thousands of genetic markers (Andrews *et al.*, 2016). Regardless of the exact analytical approach, the hope of using genome-wide data is to interrogate variation across the genome to discover genes responsible for local adaptation without the need to rely on *a priori* expectations. If the loci can subsequently be annotated and thus associated with a biological function, then the phenotypes upon which selection has acted on can potentially be inferred on the basis of gene function (Primmer *et al.*, 2013). The analytical and statistical methods currently applied to identify the targets of selection can be divided into two main groups.

GENOME SCANS

The first group of methods is based on variation in genetic differentiation between populations. Broadly speaking, these comprise F_{ST} based approaches that extend the Lewontin & Krakauer (1973) method, whereby a genome-wide average of differentiation is taken to provide a baseline approximation of neutral processes, including demographic and genetic processes. Then, outliers, which exhibit greater differences in allele frequencies between populations than expected under neutral assumptions, are considered as the most likely loci under divergent selection, or at least, physically linked to genomic regions involved in local adaptation. There are many methods to detect F_{ST} outliers, which differ in the underlying demographic models, the statistical approach and whether selection is explicitly included in the method (Narum & Hess, 2011; Lotterhos & Whitlock, 2015; Haasl & Payseur, 2016). These methods also vary in their relative biological realism.

LANDSCAPE GENOMICS

The second, newer group of methods is based on detecting selection by finding statistical associations between local allele frequencies and environmental variables that could be selective agents of local adaptation (De Mita *et al.*, 2013; Joost *et al.*, 2013; Frichot & Francois, 2015). The assumption of these analyses is that the distribution of alleles across a landscape (or seascape or riverscape) is a function of adaptation to contemporary environments, with each population being adapted to local conditions. Again, the different methods differ in their underlying neutral assumptions, statistical approaches or the way they control for population structure, which in turn influences their relative performance in different contexts of population structure and demography (De Mita *et al.*, 2013; de Villemereuil *et al.*, 2014; Rellstab *et al.*, 2015). Admittedly, a simple correlation with environmental variables does not readily imply a causal relationship, as this would require additional evidence to conclude whether these loci are truly involved in local adaptation. Ideally, landscape genomic analyses should be combined with experimental and functional approaches in order to demonstrate more firmly that fitness trade-offs of genetic variants are truly associated with contrasting environments (Soria-Carrasco *et al.*, 2014; Egan *et al.*, 2015; Storz, 2016).

When applying any of the aforementioned methods, it is always important to keep in mind that confounding factors, other than local adaptation, can cause F_{ST} outliers, or incidental associations with environmental variables (Bierne *et al.*, 2013; Gagnaire *et al.*, 2015). In particular, any factor (other than divergent selection) that causes genetic variation to differ among loci may affect the success of these methods, with the main determinants of genomic heterogeneity being mutation, recombination, background selection and the genetic architecture of adaptive traits (Haasl & Payseur, 2016). Methodological failures to properly account for different genetic structures, demographic history or non-equilibrium conditions, further add bias to these methods and can cause high false-positive rates. On the contrary, limited sampling of the genome, reduced power caused by the combination of small sample sizes and the necessity for multiple comparisons, can all lead to an increase false-negative rates. Consequently, a common problem encountered in genomic studies of local adaptation is that the different methods often only detect partially overlapping sets of loci (Gagnaire *et al.*, 2015). The ongoing refinement of methods towards fixing these caveats is underway, while trying to minimize loss of power (de Villemereuil & Gaggiotti, 2015; Whitlock & Lotterhos, 2015). Meanwhile, a common practice to partially circumvent these problems is to combine genome scans with landscape genomic methods as a way to identify more reliably the most likely targets of selection (de Villemereuil *et al.*, 2014).

In fish-related research, the combined use of RADseq data in genome scans with the aim of identifying targets of selection in freshwater, anadromous and marine species has been increasingly popular, such that it is nearly impossible to provide an exhaustive list of all published studies. References to less recent studies can be found in several review papers (Hemmer-Hansen *et al.*, 2014; Pujolar *et al.*, 2014, 2015; Ulrik *et al.*, 2014; Willette *et al.*, 2014; Gaither *et al.*, 2015; Guo *et al.*, 2015; Elmer, 2016; Picq *et al.*, 2016, as well as references in the next section as examples of the most recent studies). In contrast, fewer fish studies have used a landscape genomic framework to detecting selection by finding statistical associations between local allele frequencies and environmental variables and those have mainly been performed on salmonids (Zueva *et al.*, 2014; Hecht *et al.*, 2015; Hand *et al.*, 2016). For instance, Hecht *et al.* (2015) used RADseq to genotype nearly 2000 Chinook salmon *Oncorhynchus tshawytscha* (Walbaum 1792) from 46 localities at about 20 000 SNPs in order to document environmental adaptation throughout the North American range of the species, from California to Alaska. They applied a multivariate landscape genomic technique (redundancy analysis, RDA), to identify correlations between genetic markers and 24 environmental and climatic traits. They identified 566 putative adaptive loci as targets of environmental adaptation. Depending on the genetic lineages, their results revealed that between 5·8 and 21·8% of genomic variation could be accounted for by environmental and climate features, the most influential being precipitation, variation in mean diurnal range in temperature, as well as migration distance. Thus, this study allowed identifying environmental features that are potential strong drivers of adaptive genomic divergence in *O. tshawytscha*, which provided a basis to investigate in greater detail how this species might respond to global environmental change.

A similar study was performed by our group a few years ago on Atlantic salmon *Salmo salar* L. 1758 (Fig. 1). In one of the first large-scale landscape genomic studies in fishes, Bourret *et al.* (2013) documented adaptive and neutral differentiation across 54 North American populations of *S. salar*. Using an SNP array, about 4000 SNPs were genotyped in 640 individuals and 49 environmental variables were characterized. The authors combined genome scans (hierarchical F_{dist}), linkage mapping and a multivariate RDA to associate population genetic structure and environmental variables. The results confirmed the regional structure of the species in North America, consistent with previous data that *S. salar* form seven regional groups in the study area [Fig. 1(a)]. Hierarchical F_{dist} detected 179 outliers distributed throughout the *S. salar* genome, that are potentially under divergent selection among these regional groups and for which genetic variation was four times the observed percentage for neutral SNPs (28·04 and 6·42%). The RDA revealed that up to 81% of the variation at these markers could be accounted for by environmental features, with RDA axes 1 and 2 representing 62% of the variation [Fig. 1(b)]. Climate (temperature and precipitation) and geological characteristics were the main variables associated with this potentially adaptive genetic divergence [Fig. 1(c)]. Annotation of the 179 outliers identified 12 gene ontology (GO) terms that were over-represented and associated with growth functions. Taken together, this integrative landscape genomic approach identified both putative environmental selective agents and candidate genes potentially involved in the process of local adaptation in *S. salar*. Although these results call for experimental confirmation of the adaptive hypotheses, this study provided one of the first illustrations how landscape population genomics may improve the understanding of evolutionary processes affecting fish populations. Insights from such studies may help to develop conservation tools that integrate both genetic and environmental variables and their interactions.

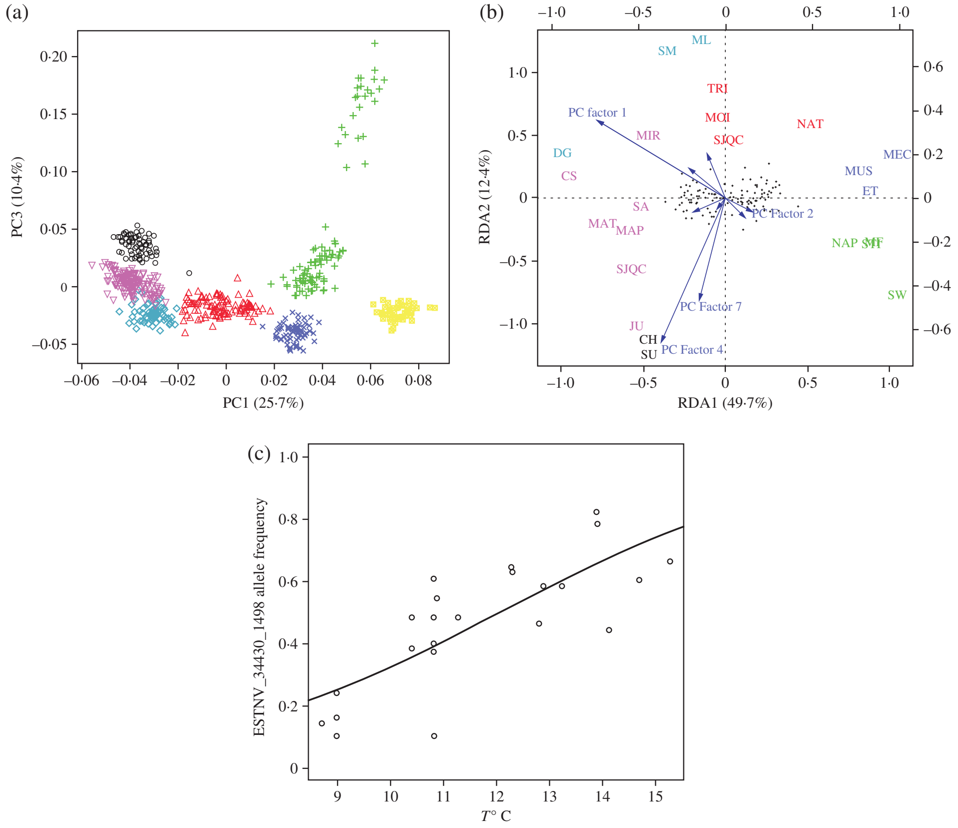

FIG. 1. Landscape genomics in *Salmo salar*: searching for gene–environment interactions driving local adaptation. (a) Principal component analysis clustering *S. salar* into seven regional genetic groups in Canada: ○, Anticosti; ▽, Southern Québec; +, Labrador; ×, Lower North Shore; ◇, Québec City; △, Higher North Shore; ⊠, Ungava. (b) Redundancy analysis (RDA) showing the position of allele frequency vectors for the 179 outlier single nucleotide polymorphism (SNP) and significant environmental principal component factors (PCF): PCF 1, temperature; PCF 2, precipitation, PCF 4 and 7, geological features. Markers identified by + and colours reflect regional groups identified in (a) and abbreviated population names are those identified by Bourret *et al*. (2013). (c) Example of relationship between allele frequency at an SNP correlated with average summer temperature (T ° C).

ALLELIC FIXATION DOES NOT UNDERLY LOCAL ADAPTATION IN FISHES

Numerous studies provided evidence for adaptation at the molecular level, either from the identification of outlier genomic regions or from the association between genetic and environmental factors (Jensen *et al.*, 2016). A striking observation stemming from these studies is that pervasive adaptive evolution seems to almost never involve the fixation of beneficial alleles, in the form that is classically inferred from hard sweeps. Without performing a rigorous survey, this seems particularly clear from the fish literature. Even though actual heterozygosity values for individual loci are not readily available for most population genomics studies, evidence for the rarity of allelic fixation can be interpreted from the maximum values of F_{ST} outliers. The rationale here is that the alternate fixation of SNPs is associated with differential adaptation as a result of hard sweeps (values should be 1 or at least close to this value). Of course, F_{ST} values < 1 does not rule out the possibility that some of the populations are fixed for locally beneficial alleles. Yet, F_{ST} of 1 (or close to it) should be relatively common if the prevailing mode of adaptation implies hard sweeps. Clearly, this is not what has been reported in almost all studies. For instance, in a study on *S. salar* populations occupying distinct freshwater habitats that differ in climate, substratum and precipitation, Bourret *et al.* (2013) reported maximum F_{ST} values for outliers varying between 0·30 and 0·58. A similar study on *O. tshawytscha* reported F_{ST} values for outliers in the range of 0·40–0·80 (Brieuc *et al.*, 2015). In another Pacific anadromous species, the eulachon *Thaleichthys pacificus* (Richardson 1836), Candy *et al.* (2015) observed maximal outlier values ranging between 0·15 and 0·30, whereas outlier values reported by Hess *et al.* (2013) between anadromous Pacific lamprey *Entosphenus tridentatus* (Richardson 1836) populations performing different lengths of reproductive migration were in the range of 0·10–0·50, with only one exceeding value of 0·83. In marine fishes, Corander *et al.* (2013) found 117 outliers among 4756 divergent SNPs between Atlantic herring *Clupea harnegus* L. 1758 populations across a salinity gradient in the Baltic Sea, corresponding to a mean outlier $F_{ST} = 0{\cdot}128$ (0·125–0·131). In Atlantic cod *Gadus morhua* L. 1758, Hemmer-Hansen *et al.* (2013) observed maximum outlier F_{ST} values of 0·73 between stationary and migratory stocks and maximal outliers values in the range of 0·5–0·7 were reported by Karlsen *et al.* (2013). In *G. morhua* also, Bradbury *et al.* (2010) observed F_{ST} values for outliers ranging from 0·15 to 0·45 between populations across a strong thermal gradient in both North America and Europe. In freshwater fishes, Fraser *et al.* (2015) reported a mean maximum F_{ST} of 0·68, which varied from 0·50 to 0·92 depending on rivers between Trinidad guppy *Poecilia reticulata* Peters 1859 populations adapted to different predator densities. Higher values and evidence for alternate fixation were reported in the three-spined stickleback *Gasterosteus aculeatus* L. 1758. Hohenlohe *et al.* (2010) observed several differentially fixed SNPs between marine and freshwater populations, including for the classic ectodysplasin (*eda*) gene region on chromosome 4, which is strongly associated with variation in armour plate numbers. Very high values were also reported between lake and stream parapatric pairs where fixation was observed for few loci in one of the pairs in a study by Roesti *et al.* (2012), whereas maximum F_{ST} values in the other three pairs were in the range of 0·2–0·6. A very similar pattern was observed in lake whitefish *Coregonus clupeaformis* (Mitchill 1818) sympatric dwarf-normal pairs with values ranging between 0·8 and 0·9 and a few alternate fixations in two lakes, whereas outliers values were generally in the range of 0·2–0·6 in the other lakes (Gagnaire *et al.*, 2013). Recently also, Le Moan *et al.* (2016) reported a few cases of alternate fixations between European anchovy *Engraulis encrasicolus* (L. 1758) ecotypes. Here, however, local adaptation is not the only factor at play since the speciation process is quite advanced and involved a phase of long geographic isolation during which genomic incompatibilities probably evolved. Finally, most outliers detected between young species in the radiation of Nicaraguan crater-lake cichlids involved shifts in frequencies of the same alleles, although near fixation was observed for several of those (Kautt *et al.*, 2012). In addition to the fact that this review is not exhaustive, a caveat that could apply to these examples is that failure to detect locus-specific F_{ST} values close to 1 could be a resolution issue, *i.e.* if the causative sequence polymorphism lies some distance from the SNPs that are actually assayed. This could result in lower F_{ST} values through incomplete linkage. Yet, the general emerging pattern is that adaptation in freshwater, anadromous and marine fishes in the form of either trophic or migratory ecotypes, or populations adapted to distinct predatory, salinity or thermal regimes rarely (if ever) involves the fixation of alternate alleles. It is only in extreme cases of population differentiation, for instance where the processes of ecological speciation is well underway and genomic incompatibilities causing postmating reproductive isolation has developed (such as in some Coregoninae, Gasterosteidae, Engraulidae and young Cichlidae), that fixation has been mainly observed. In general, therefore, adaptation seems to proceed most commonly by shifts in frequencies of alleles being shared between differentially adapted populations.

HOW IS STANDING GENETIC VARIATION BEING MAINTAINED?

THE PREVAILING ROLE OF SOFT SWEEPS IN ADAPTATION

Perhaps because of computational complexity, most available models to predict the potential for adaptation to environmental change have been developed on the basis that a single locus affects a single trait (Orr & Unckless, 2014; Uecker *et al.*, 2014). Similarly, the prevailing approaches applied in empirical population genomics studies have been to search for signals of selection of large effects on a locus-by-locus basis (or linked genomic regions), for instance by using one of the many available methods described in the previous section, but also Haasl & Payseur (2016) and Jensen *et al.* (2016). Hard sweeps and selection driving major adaptive changes (and ultimately fixation) in allele frequencies certainly occur in nature and are informative about genetics changes that can cause evolution (Savolainen *et al.*, 2013). For example, genes or genomic regions have been identified and located for colour of the polymorphic peppered moth (van't Hof *et al.*, 2011), the reduction of armour plates in *G. aculeatus* populations that have colonized freshwater environments (Colosimo *et al.*, 2005) and coat colour variation in mouse species adapted to different environmental background colours (Steiner *et al.*, 2007). Despite this, there are many theoretical and empirical reasons to suspect that QTNs of large effect associated with hard sweeps and allelic fixation may not be the most informative in terms of addressing the molecular basis of phenotypic evolution or for estimating the roles of evolutionary forces in shaping adaptation (or maladaptation).

Based on both empirical evidence and modelling approaches, Pritchard & Di Rienzo (2010) and Messer & Petrov (2013) argued that adaptation may predominantly occur without dramatic allele frequency changes and adaptive fixation. Instead, adaptation should commonly produce soft selective sweeps, whereby multiple adaptive alleles sweep through the population at the same time, as opposed to hard sweeps that would rapidly lead to fixation. They also argued that most well-known examples of rapid molecular adaptation indeed show signatures of such soft selective sweeps and that fixed differences among human populations are exceptionally rare, despite sufficient time. Instead, much local adaptation appears to result from more modest changes in frequencies of several to many genes, something that is also consistent with studies highlighting the importance of standing genetic variation. Thus, the footprint of adaptation observed in fishes and summarized above is in line with these other studies.

THINK POLYGENIC

Rockman (2012) argued that large-effect QTNs are insufficient to explain general evolutionary phenomena, including those pertaining to the maintenance of genetic variation. Indeed, such loci may be unrepresentative of the alleles that matter most for rapid adaptation, given that models and experimental findings suggest that evolution often acts *via* large numbers of small-effect polygenes, which are hard to detect individually (Pritchard & Di Rienzo, 2010; Le Corre & Kremer, 2012). Thus, as the number of causative loci increases, the intensity of selection experienced by each of them decreases and consequently, selection on a highly polygenic trait generates only small changes in allele frequencies at causative loci. Instead, the response to selection derives mostly from allelic co-variances among causative loci (McKay & Latta, 2002; Le Corre & Kremer, 2012). Polygenic selection is therefore not expected to lead to the fixation of loci under selection, which means that allelic diversity, and therefore evolutionary potential, should be retained when exposed to new selective pressures, for instance, by novel environments (Yeaman, 2015). Given the evidence for a ubiquitous polygenic basis of adaptation, it is somewhat of a paradox that the vast majority of studies investigating the genomic basis of adaptive traits or signatures of selection have been based on genome-wide association studies (GWAS) performed on a locus-by-locus basis or genome scans that are biased towards the detection of large effects at single loci (Jensen *et al.*, 2016). Similarly, studies that experimentally document the effect of selection at the genetic level in natural populations have mainly focused on methods best designed to quantify selection coefficients on a locus by locus basis (Thurman & Barrett, 2016). Accordingly, it is expected that these methods will often fail to identify selective events involving polygenic selection (Haasl & Payseur, 2016). This is well-exemplified in studies on humans. For instance, classical GWAS have found significant associations with phenotypic variation, but these explained only a minute proportion of heritable trait variation. This so-called missing heritability is best illustrated in studies on variation of human height, a trait with a heritability of 80% (Visscher *et al.*, 2008). Yet, GWAS performed on 90 000 individuals with thousands of SNPs identified only around 50 genes that together accounted for 5% of the heritable height variation (McEvoy & Visscher, 2009). Taking together, evidence for the occurrence of adaptation involving both allelic change of large effects (sometimes involving fixation), as well as co-varying changes of small effects both indicate that studies aiming at detecting the effect of selection in natural populations should combine methods that are best designed to detect either types of signals. This is also theoretically inferred, for instance under Orr's (1998) exponential effect-size model, which predicts that the effect-size distribution of adaptive substitutions is approximately exponential, with a few large-effect and numerous small-effect mutations.

Wellenreuther & Hansson (2016) reviewed recent analytical advancements to investigate the polygenic basis of phenotypic variance as well as signals of polygenic selection in wild populations. Their review shows that the development and application of mixed models that incorporate confounding factors, such as environmental noise and population structure, has led to substantial improvements in the accuracy of polygenetic modelling. Different variants of the mixed-model family comprise a univariate linear mixed model (LMM) for estimating the proportion of variance explained (PVE) of genotypes, a multivariate linear mixed model (mvLMM) for testing marker associations with multiple phenotypes while simultaneously controlling for population stratification and a Bayesian sparse linear mixed model (BSLMM), which allows estimating

PVE of genotypes and identifying associated markers by jointly modelling all markers while controlling for population structure. The power of such multivariate approaches is nicely shown by the study of Yang *et al.* (2010) who revisited the genetic basis of human height variation. The authors quantified the proportion of variance for human height explained by nearly 300 000 SNPs across 4000 individuals using a linear model analysis combined with a restricted maximum likelihood analysis (REML) to estimate the variance explained by SNPs. They found that 45% of variance could be explained when considering all SNPs simultaneously, a proportion nine times higher than the 5% estimated by classical GWAS (McEvoy & Visscher, 2009). Moreover, they showed that the remaining heritability was due to incomplete linkage disequilibrium between causal variants and genotyped SNPs. This suggests that most of the heritability for human height is not missing, but that the previously detected individual effects were too small to pass stringent significance tests.

Pathway analyses present another family of methods to investigate the polygenic control of phenotypic trait expression, as well as signals of polygenic selection. One of the most promising methods is the random-forest (RF) algorithm, which is a tree-based ensemble-machine learning tool that is particularly well suited for studies with a 'large number of indicators and small sample sizes' (Chen & Ishwaran, 2012). The RF approach is particularly well suited for genomic applications, because this kind of data contains typically a large number of loci relative to the number of individuals. RF also accounts for correlation and interactions among loci, making them particularly suitable to search for signals of polygenic selection. While this approach is currently popular in the fields of medicine and agriculture, it is still infrequently used in studies of natural populations. One pioneer example is the study by Holliday *et al.* (2012) who used RF to predict adaptive phenotypes related to climate from multi-locus genotypes in Sitka spruce *Picea sitchensis*, an important tree for the forestry industry. The authors were able to explain 37 and 30% of the phenotypic variation, respectively, when retaining the 20 most important SNPs as predictors for two locally adaptive traits: autumn bud-set timing and cold hardiness. Their results illustrate the power of RF to identify subsets of markers that are most important to climatic adaptation and suggest that co-variation among these loci may be widespread.

Good examples of RF applications also come from recent fish studies. In an elegant study on anadromous *O. tshawyscha* populations, Brieuc *et al.* (2015) integrated multiple approaches to examine the genetic basis of local adaptation in a key life-history trait (migration-run timing) sampled from contrasting environments. First, they used RF, to detect markers linked to run timing across 14 populations representing four distinct evolutionary lineages within *O. tshawyscha* in order to detect loci of small phenotypic effect. Second, they performed a discriminant analysis of principal components (DAPC) of the identified predictor loci to determine whether structuring at these loci was best explained by run timing or by lineage. Third, they performed a genome scan using BAYESCAN (http://cmpg.unibe.ch/software/BayeScan/) to detect outliers of pronounced divergence between these populations. All loci identified were also positioned on a high-resolution genetic map. Sequencing of over 9000 RAD markers identified 33 predictor loci which explained nearly 80% of variance in run timing. Analysis of the spatial relationship between populations based on DAPC analysis on the predictor loci defined groups based on run timing, rather than on lineage. This suggested some parallel genetic changes underlying evolution of the run-timing phenotype across lineages, although there was also evidence of lineage-specific affinity. While differences between populations were largely explained by changes in minor allele frequency, BAYESCAN provided some evidence of soft selective sweeps on specific loci, with five of the 33 predictor markers mapped to a small region on specific outlier chromosomal regions. Taken together, the data suggest that the evolution in run timing in *O. tshawyscha* has occurred through both shared and novel evolutionary pathways, although the authors could not rule out other processes that have acted on undetected markers. These results are also consistent with theory implying that genetic variation underlying life-history traits is explained mainly by loci of small phenotypic effect without excluding a role for loci of major effect. This study of Brieuc *et al.* (2015) provides a clear illustration of the limitations of a strictly single-locus approach in searching for the genomic basis of adaptation.

Laporte *et al.* (2016) also combined RF with multivariate analyses (distance-based redundancy analyses, db-RDAs) and genome scan (BAYESCAN) to test for the occurrence of polygenic selection within-generation in response to anthropogenic organic and metal contamination in both American *Anguilla rostrata* (leSeuer 1817) and European *Anguilla anguilla* (L. 1758) eels. The goal was to identify loci that would discriminate eels from control *v.* polluted environments and be associated with specific contaminants acting as putative selective agents. RADseq resolved 23 659 and 14 755 filtered loci for the *A. anguilla* and *A. rostrata*, 141 and 142 co-varying predictor markers discriminating from control *v.* polluted sampling localities were found using RF in *A. anguilla* and *A. rostrata*, respectively. The db-RDA was then used to assess the relationships between these co-varying markers and concentration of 34 contaminants measured for each individual eel. Three contaminants (PCB153, 4040DDE and selenium) were consistently associated with co-varying markers for both species, thus identifying these contaminants as important selective agents in contaminated sites. About 30 out of the 141 or 142 predictor markers for each species could be annotated and gene enrichment analyses suggested that sterol regulation plays an important role in the differential survival of anguillids in polluted environments. In contrast to the polygenic analyses, a genome scan provided little evidence for selection with no loci potentially under selection identified in *A. anguilla* and only two divergent ones in *A. rostrata*, which were also identified by RF. As for Brieuc *et al.* (2015), this study illustrates the power of combining methods for detecting signals of polygenic selection and for associating variation of markers with putative selective agents in studies aiming at documenting the dynamics of selection at the genomic level and particularly so in human-altered environments.

The RF approach was also recently applied to detect signals of polygenic selection imposed by different natural habitats used in *A. anguilla* (Fig. 2; pers. obs.). This species presents a paradox; despite inhabiting drastically different environments, the species is panmictic and as such cannot develop local adaptation (Côté *et al.*, 2013). Yet, depending on rearing habitat, individuals exhibit drastically different ecotypes, with individuals reared in fresh water tending to grow slowly and maturing older and being more likely to be female in comparison with individuals reared in brackish–saltwater habitats. Pavey *et al.* (2015) used RF to perform a genome-wide association study to investigate a possible polygenic basis of these habitat-specific anguillid ecotypes. They found 331 co-varying predictor loci out of 42 424 RADseq loci that consistently were associated with ecotypes, allowing a reclassification of 90%. Of these 331 associated markers, 55% ($n = 182$) allelic diversity was highly reduced in one ecotype: with 137 markers being associated with freshwater and 45 with brackish–saltwater locations, referred to as freshwater and brackish–saltwater

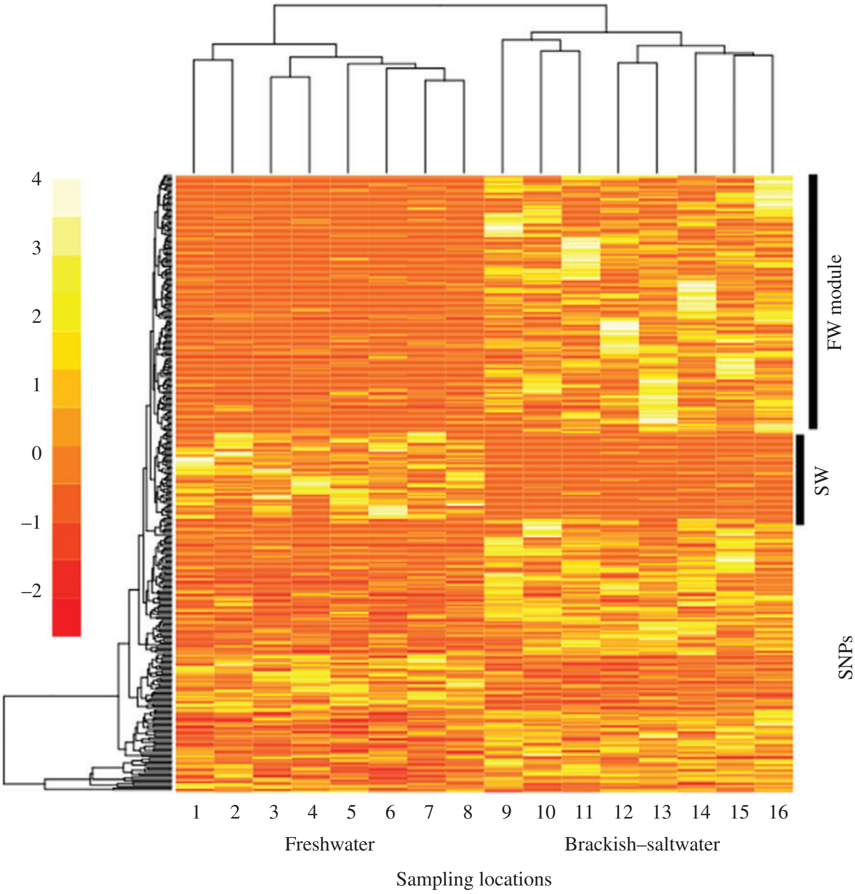

FIG. 2. RAD sequencing highlights polygenic discrimination of habitat ecotypes in the panmictic *Anguilla rostrata*. Heat map (−2 to +4 arbitrary units) illustrating the allele frequencies at the 331 predictor single nucleotide polymorphisms (SNP) for 16 study sampling locations. Rows represent specific SNP, and each column, a sampling site. Colours represent normalized (by row) allele frequencies. Half of the markers are nearly fixed in one ecotype and comparatively variable in the other. SNPs exhibiting this pattern are designated as either fresh water (FW: 137 SNPs) or brackish–saltwater (SW: 45 SNPs) modules.

modules, respectively (Fig. 2). The finding that the freshwater module is three times larger than the brackish–saltwater module suggests that more genes are influenced by intra-generational directional selection in the freshwater habitat. The 331 predictor SNPs were associated with 101 genes that represent various functions, including vascular and morphological development, calcium ion regulation, growth and transcription factors and olfactory receptors. These results are consistent with divergent natural selection acting on phenotypes causing genetic differences between habitats, occurring every generation anew in this panmictic species. They thus support the hypothesis that ecotype differences between anguillids occupying different habitats is not the solely caused by plasticity, as traditionally assumed, but that functional genetic differences stemming from intra-generational spatially varying selection is also of importance.

THE ROLE OF BALANCING SELECTION IN THE MAINTENANCE OF GENETIC VARIATION

Population genomic studies searching for signatures of adaptive evolution have so far paid little attention to the finding that selection does not always act in a directional manner but may instead be balancing, thereby contributing to the maintenance of standing genetic variation (Fijarczyk & Babik, 2015). Indeed, Whitlock (2015) argued that ‘although we still do not know, even after almost a century of research, the relative importance of different mechanisms for the maintenance of genetic variation, it is reasonable to believe that whatever genetic variation is preserved by spatial balancing selection is likely to be more useful in allowing the population to respond to future challenges than other mechanisms that maintain genetic variation’. Balancing selection can take different forms, such as: frequency-dependent selection; heterozygote advantage (overdominance); selection that varies in direction and intensity across space or time; antagonistic evolution within species, including sexual conflicts, as well as disassortative mating (Rice, 1992; Castric & Vekemans, 2004; Charlesworth, 2006; Hedrick, 2006). Any of these mechanisms would allow alleles to persist at intermediate frequencies in a population, potentially for long periods (but see below). Consequently, selection imposed by environmental stressors (*e.g.* climate change) may not necessarily cause the loss of adaptive genetic diversity. For instance, Orozco-Terwengel *et al.* (2012) combined laboratory imposed natural selection in fruit flies *Drosophila melanogaster* with genome-wide sequencing of DNA pools (Pool-Seq; https://omictools.com/pool-seq-category/) to identify alleles selected in a fluctuating thermal regime and traced their trajectories during 15 generations. They observed a genomic selection response in 5000 SNPs, consistent with a model of polygenic selection. Furthermore, they observed that numerous alleles at first increased rapidly, but then eventually reached a plateau of intermediate frequencies. While the authors could not identify the exact causal factors, their results suggest that overdominance or antagonistic pleiotropy, two inferred mechanisms of balancing selection, could be the cause. Also in fruit flies, Bergland *et al.* (2014) tested the hypothesis that seasonal environmental fluctuations can impose temporally variable selection that could drive repeatable adaptive oscillations at balanced polymorphisms. Using whole genome resequencing data, the authors identified hundreds of polymorphisms whose frequency oscillated among seasons, most likely due to strong and temporally variable selection. Their results also suggested that adaptively oscillating polymorphisms can be very old. These polymorphisms were also associated in predictable ways with seasonally variable phenotypes. Therefore, their results are consistent with a model of balancing selection whereby rapid temporal climatic fluctuations over generational time maintains adaptive genetic diversity at loci underlying polygenic fitness variation. In fact, Bergland *et al.*’s (2014) results nicely corroborate predictions of a recent realistic evolutionary model of temporally varying environments (Gulisija & Kim, 2015). In particular, this model predicts that cyclic selection can vary in magnitude, either spatially or temporally and can promote the maintenance of elevated levels of non-neutral genetic polymorphisms in finite populations.

The maintenance of adaptive polymorphisms by balancing selection may be particularly prevalent in species facing few physical barriers to dispersal during their early life-history stages, or with weak philopatry at the adult stage and that must cope with heterogeneous environments varying in time and space (Lenormand, 2002). This is characteristic of many marine fishes and invertebrates for which random mating and dispersal exist over large geographic areas. Here, theory predicts that genetic polymorphism at genes differentially affecting fitness in contrasting environments will be maintained by a balance between selection and dispersal across the heterogeneous seascape (Hedrick, 2006). In extreme situations where both dispersal across habitats and mating are random, local adaptation will be impossible, yet adaptive polymorphism may be protected by selection (Yeaman & Otto, 2011). In such a case, and for the many marine species characterized by a time lag between recruitment and reproduction, selection could be particularly efficient at filtering beneficial allelic variants in a given environment, leading to local genetic adaptation within each generation (Hedrick, 1986), due to a form of balancing selection called spatially varying selection.

In practice, the discovery of locally adaptive polymorphisms subject to balancing selection is hampered by a lack of suitable methods, resulting in false positives, even when the selection coefficients are large relative to the migration rate (Beaumont & Balding, 2004; but see Fijarczyk & Babik, 2015 for new developments). In addition and particularly in situations of quasi or complete panmixia, there might be a negative trade-off between the number of loci affected by spatially varying selection and individual locus effects on fitness, such that the whole adaptive load owing to selection on unlinked loci remains sustainable for the population (Gagnaire *et al.*, 2012). Moreover, recombination is expected to rapidly erase the effects of selection in proximity to the selected sites, such that soft sweeps on new targets of balancing selection is expected to leave only transient genomic footprints, which will diminish the chances of finding them (Charlesworth *et al.*, 1997; Gagnaire *et al.*, 2015). Despite these shortcomings, several recent population genomic studies provide evidence for a role of balancing selection in maintaining adaptive genetic variation in marine species.

In a study that investigated the evolutionary potential of table-top corals to tolerate climate change, Bay & Palumbi (2014) genotyped over 15 000 SNPs within a natural temperature mosaic. Despite panmixia, they identified 114 highly divergent SNPs candidates for thermal selection. Their data were best explained by mild selection for alternate alleles at many loci, in accordance with a polygenic model of selection, accompanied by the maintenance of adaptive polymorphism through multilocus balancing selection in this heterogeneous environment. Bay & Palumbi (2014) suggested that natural populations of corals harbour a reservoir of alleles preadapted to high temperatures, suggesting evolutionary potential to respond to future climate change. In another study, Pespeni *et al.* (2013) aimed at documenting the adaptive capacity of the purple sea urchin *Strongylocentrotus purpuratus* to ocean acidification. By genotyping nearly 20 000 loci in larvae cultured under realistic future CO_2 levels, they found significant allelic change in 40 functional proteins classes involving hundreds of loci. Their results revealed the capacity for rapid evolutionary response to ocean acidification, probably due to standing genetic variation representing a reservoir of resilience to climate change in coastal upwelling ecosystems. Pespeni *et al.* (2013) proposed spatio-temporal variation in CO_2, pH and carbonate ion concentrations in the California Current System as the causal environmental agents of polygenic balancing selection maintaining this adaptive genetic variation.

There are still few population genomic studies that investigated the role of balancing selection in maintaining genetic variation in wild fish populations. Clearly, the best dissected case to date is the study by Barson *et al.* (2015) that documented sex-dependent dominance at a single gene of large phenotypic effect (vestigial-like family member 3 gene or *vgll3*), which explains 39% of the variance in age at maturity of *S. salar*.

Adaptive polymorphism at this gene is being maintained due to sex-dependent dominance that promotes earlier and later maturation in males and females, respectively. This is the first empirical example of dominance reversal allowing greater optimization of phenotypes within each sex, contributing to the maintenance of adaptive genetic variation resulting from sexually antagonistic selection (a form of balancing selection) and the resolution of sexual conflict in a major and widespread evolutionary trade-off between age and size at maturity.

In a study predating the genomic era, Pogson (1995) compared allozyme patterns to anonymous nuclear DNA loci in *G. morhua*. Highly significant DNA marker differences were observed among all populations consistent with an isolation-by-distance population structure. In contrast, they observed very limited spatial variation in allele frequencies at allozyme loci. Pogson (1995) proposed that the discrepancy between both types of markers was caused by balancing selection maintaining uniform protein polymorphism among populations. In another study on *G. morhua*, Therkildsen *et al.* (2013) performed genome scans in four overfished populations studied over an 80 year period to evaluate how quickly natural populations can adapt to changes in their environment and how temporal and spatial variation in selection pressures interact to shape patterns of genetic diversity. Screening about 1000 gene-associated SNPs, they identified 77 loci that showed elevated levels of differentiation. Their analysis suggested that temporal allele frequency shifts at certain loci may correlate with local temperature variation. More importantly, they observed pronounced sequential shifts in spatial outliers, with no locus maintaining elevated spatial differentiation throughout the study period. The authors proposed that the observed genetic changes are likely to be at least partly driven by highly dynamic temporally and spatially varying selection that contributed to maintain genetic variation and evolutionary potential in this high gene flow species. Finally, Hohenlohe *et al.* (2010) conducted a genome scan of nucleotide diversity and differentiation in marine and freshwater populations of *G. aculeatus*. Genotyping of over 45 000 SNPs identified genomic regions exhibiting signatures of divergent selection consistent across multiple, independently derived populations. They also identified several genomic regions with high levels of diversity and heterozygosity, which suggested the effect of balancing selection maintaining a common pool of genetic variation within and among populations. Interestingly, the authors identified several candidate targets of balancing selection in those genomic regions, namely genes implicated in the first line of defence against pathogens and orthologues of several inflammation pathway genes, as well as genes potentially implicated in innate immune responses.

In sum, the above theoretical considerations and the few available empirical examples document that balancing selection of various forms may play an important role in maintaining adaptive genetic variation and contributes significantly to the evolutionary potential of species to cope with environmental change and particularly so in marine ecosystems.

THE PARADOX OF POLYGENIC ADAPTATION AND THE TRANSIENT NATURE OF POLYMORPHISM MAINTENANCE

The above sections suggest that molecular adaptation occurs most commonly by means of soft selective sweeps that very rarely lead to fixation and instead, that allelic polymorphisms are maintained in the form of standing genetic variation. The sections also suggest that the prevailing occurrence of soft sweeps may relate to the polygenic nature of most adaptive traits, whereby the intensity of selection experienced by the underlying locus may be small, thus generating modest changes in allele frequencies. Moreover, the maintenance of adaptive genetic variation at such polygenes may in some cases be further enhanced by balancing selection of various forms. All of these observations seem to contradict theoretical predictions of population genetic models inferring that alleles with small selection coefficients will be swamped by migration or lost by drift and therefore will rarely contribute to local adaptation (Yeaman & Otto, 2011). Moreover, there are controversies regarding the importance of balancing selection in adaptive evolution. Recent theoretical studies indicate that balancing selection may be only a short-term process that cannot efficiently promote the long-term maintenance of adaptive polymorphism (Fijarczyk & Babik, 2015). Consequently, the traditional view to date is that balancing selection mainly affects a few classes of genes (*e.g.* related to immune responses) and that its overall role in maintaining variation is relatively minor (Asthana *et al.*, 2005). One argument against the evolutionary significance of balancing selection is that balanced polymorphisms may be difficult to maintain for a long time because of the genetic load that they create. Also, environmental changes may cause shifts in allele frequencies that could result in the loss of formerly stable polymorphisms. Indeed, there are an accumulating number of studies from a wide array of genes and organisms reporting the transient nature of balancing selection (Fijarczyk & Babik, 2015). These observations then raise a fundamental question: if most alleles contributing to standing variation are of small effect and are often temporally transient, how then does local adaptation proceed? Yeaman (2015) recently simulated this paradox using a modified version of Nemo (Guillaume & Rougemont, 2006) and showed that local adaptation occurs much more readily with alleles of large effect, but also that adaptation can involve polygenic response. Thus, even when population genetic models predict the replacement of individual alleles at loci of small effect, Yeaman (2015) showed that considerable local adaptation can evolve at the phenotypic level, as long as there is sufficient standing genetic variation. More importantly, Yeaman's (2015) simulations suggest that in such a case, the underlying architecture of adaptive divergence is transient, whereby no locus makes an important contribution for long periods, thus causing the genes responsible for adaptive divergence to vary over time. These results also suggest that genetic drift can play a critical role in shaping the architecture of local adaptation, both through randomly eroding standing genetic variation, as well as affecting the rate of polymorphism turnover with redundant phenotypic effects. Since most quantitative traits have substantial levels of standing genetic variation (Houle, 1992), local adaptation *via* the temporal turnover of a polygenic architecture may be particularly common when selection acts on metatraits (*e.g.* complex life-history traits and growth) that may also involve many biochemical pathways (Yeaman, 2015). Because this kind of local adaptation may happen mainly due to transient frequency changes and allelic co-variance, these architectures will be difficult to detect using the most currently used approaches to study the genomic basis of adaptation, which may partly explain why empirical evidence for such allelic transient state is still scarce. Yet, the finding that adaptive evolution mainly involves soft sweeps, that balancing and polygenic selection may be quite common and that these processes seem to promote the maintenance of ample standing genetic variation, suggest altogether that adaptation by temporal turnover of a polygenic architecture underlying adaptive traits is plausible.

As far as is known, no study has directly addressed this question. In fishes, however, at least two studies support this possibility. Gagnaire *et al.* (2012) evaluated the extent to which spatially varying selection on panmictic *A. rostrata* can explain the retention of adaptive genetic variation. By combining a genome scan and candidate SNP approach to genotype nearly 1000 individuals from 16 sampling sites at different life stages of the same cohort, clear evidence for spatially varying selection was found at 13 genes. These genes showed correlations between allele frequencies and temperature across the entire sampling range. Subsequent simulations using a multiple-niche Levene's model estimated the relative fitness values among genotypes. This model rarely predicted a stable polymorphic equilibrium at these loci, but predicted the fixation of globally advantageous alleles with spatially variable effects on fitness. This in turn suggested a transient turnover of loci involved in within-generation adaptation to the different environments encountered by anguillids throughout their extensive range, in line with Yeaman's (2015) individual-based simulations. The study of *G. morhua* by Therkildsen *et al.* (2013) provides a second example suggesting that the molecular basis of local adaptation may be transient, given their observation of pronounced sequential shifts in spatial outliers, with no locus maintaining elevated spatial differentiation over time. Clearly, much remains to be learned about the possible role of transient polymorphisms in adaptation. If more evidence is found, this would provide yet another way by which species may manage to cope with environmental change. Moreover, this could also suggest that polygenic traits may be less susceptible to share the same adaptive architecture across populations compared with simple traits, or in other words, that multiple genetic solutions can underlie a given environmental adaptation in polygenic adaptive traits (Yeaman, 2015). Therefore, this process could partly explain why parallel evolution often does not involve the same genetic architecture, as presented below.

REPEATED ADAPTATION, WITH OR WITHOUT SIMILAR GENETIC ARCHITECTURE

A vast number of empirical studies have provided evidence that populations can evolve in parallel to the same environmental problem either *via* the same or different genomic architectures (Elmer & Meyer, 2011). Yet, predictive models of organismal potential to adapt to environmental change have largely neglected the fact that adaptive phenotypes are not irreplaceable, that is, populations of a given species can in parallel evolve the same phenotypes independently. Classic examples of parallel adaptive phenotypic evolution in vertebrates include adaptive radiations of the Caribbean Anolis lizards and cichlids in African rift lakes (Schluter, 2000). These cases of parallel phenotypic evolution provide a unique framework to infer the genetic basis of adaptation. Indeed, progress in sequencing technologies and other genomic approaches makes it now feasible to address these fundamental questions. In particular, there has been a major focus to assess whether parallel phenotypic evolution can be achieved through the same molecular basis, *i.e.* whether parallel evolution involves homologous nucleotide change in the same genes, different mutations in the same gene or non-homologous mutations in different genes (Elmer & Meyer, 2011). A recurrent question has also been whether these changes are occurring *de novo* or are the product of standing genetic variation (Schluter & Conte, 2009). Clearly, work towards this goal will contribute to a better understanding of genetic (*e.g.* mutational or pleiotropic) constraints to adaptive evolution (Losos, 2011; Ralph & Coop, 2015). More generally, this may improve our ability to predict adaptive responses to human imposed selection. To this end, examples to explore in fishes include the adaptive evolution during the process of domestication (Roberge *et al.*, 2006; Sauvage *et al.*, 2010), in response to pollution (Williams & Oleksiak, 2011) or to thermal gradients (Bradbury *et al.*, 2010).

According to Storz (2016), the number of possible mutations capable of producing an adaptive change depends on the particular proteins under consideration and the nature of the selected phenotype. For instance, if adaptive modifications of protein function require fine-tuned changes in catalytic activity or substrate specificity, then the number of potentially adaptive mutations may be limited to a fairly restricted set of active sites. Alternatively, if adaptive changes involve a more generalized property, then numerous possible mutations at multiple sites may be capable of producing the required change. Consequently, empirical evidence to date has shown that parallel phenotypic change in different populations can occur both through similar or different genetic trait architectures (Conte *et al.*, 2012; Laporte *et al.*, 2015). Comparative studies of naturally evolved proteins have documented a number of striking cases of convergence and parallelism at the amino acid level (Storz, 2016). For example, studies on humans reported independent adaptations to the same nucleotide change (Jeong & Di Rienzo, 2014). In other mammals, parallel evolution of colour polymorphisms important in camouflage are due to changes in the same loci (*e.g.* melanocortin 1 receptor, MC1R) and in some cases to the same mutation, even across different species (Manceau *et al.*, 2010). There are also examples of contemporary molecular parallelism driven by human activities; for instance, in proteins that mediate insecticide resistance, herbicide resistance or antibiotic resistance (Storz, 2016). In fishes, the iconic example of parallel evolution involving a similar genetic basis is *G. aculeatus*, which repeatedly evolved a reduction in defensive structures (*e.g.* armour plates and pelvic spines) from the same set of genes (*e.g.* ectodysplasin, *eda*) and the paired-like homeodomain transcription factor 1 (Pitx1) following the independent colonization of new freshwater habitats (Colosimo *et al.*, 2005; Chan *et al.*, 2010).

The above cases illustrate that the same adaptive traits have evolved in parallel in a variety of organisms by means of a shared genetic basis. Is it also possible, however, that adaptations evolve *via* alternative genetic routes? The answer is yes, as an increasing number of examples now document that different populations of a species have adapted to similar environments by means of different genetic changes, sometimes involving very distinct genes and pathways (Ralph & Coop, 2015). Theoretical considerations support this finding and indicate that pronounced spatial population structure, for instance caused by geographically limited dispersal, increases the chance of parallel evolution involving a different genetic architecture (Ralph & Coop, 2014). This is because in spatially structured populations, the spread of adaptive mutations can be impeded by the time it takes until individuals with mutations can migrate to more distant populations. If migration takes much longer than a new adaptive mutation to arise, then novel adaptive mutations of independent origin will instead arise and spread. Also, highly structured populations make it more likely that the local pool of alleles responding to selection may be different due to genetic drift (Barton, 1989). In humans, a classic example of parallel adaptation involving a different genetic basis is lactase persistence that evolved in parallel in Eurasia and Africa through independent mutations (Enattah *et al.*, 2008). Other examples include the evolution of pesticide resistance in the fruit fly (Menozzi *et al.*, 2004), drug resistance in malaria parasites (Nair *et al.*, 2007), adaptive coat colour variation in mice populations (Hoekstra *et al.*, 2006) or species-specific changes in skeletal form (Indjeian *et al.*, 2016).

Among fishes, although *G. aculeatus* is the most cited example involving the same genetic basis underlying parallel evolution, recent studies have shown that parallel evolution can also proceed *via* different genetic routes (Elmer & Meyer, 2011). For instance, pelvic reduction is generally associated with a cis-regulatory deletion upstream of the *pitx1* gene, but this deletion can be of variable size among populations, indicating that the genetic basis is not the same. Moreover, the *pitx1* deletion is not always the origin of pelvic reduction (Chan *et al.*, 2010). Instead, multiple genetic mechanisms have been shown to account for pelvic reduction in the nine-spined stickleback *Pungitius pungitius* (L. 1758) (Shapiro *et al.*, 2004). Similarly, while worldwide loss of armour plates in freshwater populations is associated with *eda* alleles, there is at least one exception to this rule (Colosimo *et al.*, 2005). Finally, whole genome resequencing has shown that most of the genetic differentiation between freshwater and marine populations of Gasterostidae is not parallel (Jones *et al.*, 2012). These results suggest that a combination of standing genetic variation, different mutations at homologous genes and changes to different genes have been involved in the repeated parallel phenotypic adaptation of Gasterostidae to freshwater habitats. Cases of parallel phenotypic evolution have also been documented in cichlids. For instance, studies of genotype–phenotype associations of Lake Victoria cichlids in vision genes (opsins) revealed parallel adaptation to divergent water depth and clarity (Seehausen *et al.*, 2008*b*). In contrast, species flocks in Lakes Tanganyika and Malawi showed parallel opsin gene expression profiles that were not accompanied by parallelism in coding sequence variation (O'Quin *et al.*, 2010). Machado-Schiaffino *et al.* (2015) showed that cichlids of different origins colonized Ugandan crater lakes and independently evolved a more slender (limnetic-like) body shape relative to the ancestral populations. Another classic example of parallel phenotypic evolution is the *P. reticulata*, for which repeated adaptation to low and high predation environments has been documented. Fraser *et al.* (2015) used genome scans to examine whether phenotypic convergent evolution to high and low predation environments left comparable footprints at the genome level. They found only a small number of regions across the genome with the same selection signatures across populations. Consequently, they proposed that *P. reticulata* populations evolved parallel adaptation to low and high predation regimes through a different genetic basis.

While parallel phenotypic evolution is common, it is often not perfectly repeated, as illustrated by the research programme that has been conducted for many years on sympatric species pairs of *Coregonus* spp. (Figs 3–6). Here, parallel phenotypic evolution can be explained by two distinct views (Losos, 2011): first, natural selection produces optimal solutions to problems repeatedly posed by the environment; second, constraints on evolutionary processes lead to biases in the production of phenotypic variation. The expected outcome under either of these two views would be populations showing perfect evolutionary parallelism. It is increasingly being reported, however, that studies of populations that diverged in response to apparently similar environmental gradient show no or imperfect parallelism, including variable progress towards adaptive divergence (Nosil, 2012). Also, studies show that parallel phenotypic evolution can involve parallel genetic evolution or not, depending on the nature of the genetic basis of underlying phenotypes and historical contingency (Gompel & Prud'homme, 2009).

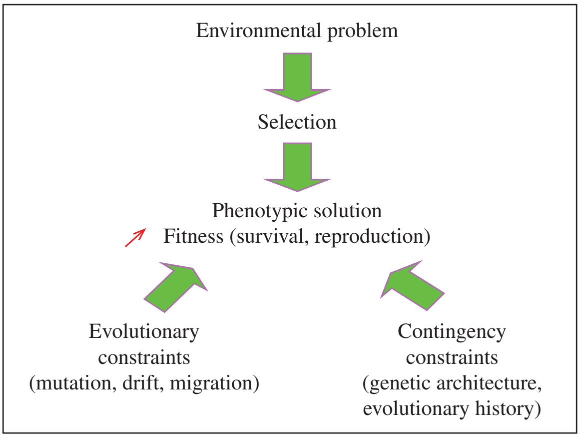

FIG. 3. Schematic framework illustrating how the comparative analysis of populations may help to elucidate the fine tuning of adaptive evolution. In a case of perfect parallelism, a same phenotypic solution optimizing fitness in the face of a same environmental problem will evolve as a consequence of identical selective pressure. This may involve the same genetic basis or not, depending on the extent of evolutionary or contingency constraints. In a situation of imperfect parallelism, different phenotypic solution could evolve because: (1) the environmental problem (and therefore selective pressure) is not exactly the same; (2) there are evolutionary constraints that prevent similar phenotypes to evolve in parallel in different populations and (3) contingency constraints (*e.g.* different evolutionary origin) could also limit the extent of parallelism.

Elucidating the mechanisms responsible for parallelism *v.* non-parallelism is a fruitful, yet underexploited research area towards elucidating the fine tuning of adaptive evolution (Fig. 3).

This conceptual framework has been used to investigate the nature of adaptive evolution in dwarf (limnetic) and normal (benthic) sympatric species pairs of *Coregonus* spp. that have evolved rapidly, independently and in parallel in different North American lakes (pers. obs.). There are multiple life history, morphological, physiological and transcriptomic traits differing between the two species type (Bernatchez, 2004; Bernatchez *et al.*, 2010; Evans & Bernatchez, 2012; Dalziel *et al.*, 2015; Laporte *et al.*, 2015). The framework allowed elucidation of some important aspects of the pattern of adaptive evolution between and across sympatric coregonid species: a similar phenotypic solution (dwarf–normal species) evolved at a different pace in each lake (Fig. 4) and this apparently happened as a function of the local environment (*e.g.* biotic and abiotic factors favouring competitive interactions). In turn, these differences in biotic and abiotic variables among lakes imposed different intensity of divergent selection in each lake which also resulted in variable genome-wide divergence (Fig. 5). Also, different contingency constraints (evolutionary and demographic histories) also probably affected the continuous pattern of phenotypic and genetic divergence between dwarf and normal coregonids observed among lakes (Fig. 6).

In summary, the above examples show that repeated evolution of adaptive phenotypes is a ubiquitous evolutionary phenomenon. More importantly, these studies demonstrate that this phenomenon can occur through various genetic solutions that may either involve the repeated use of the same genetic variants or very different genetic architectures, the latter particularly so in highly structured populations.

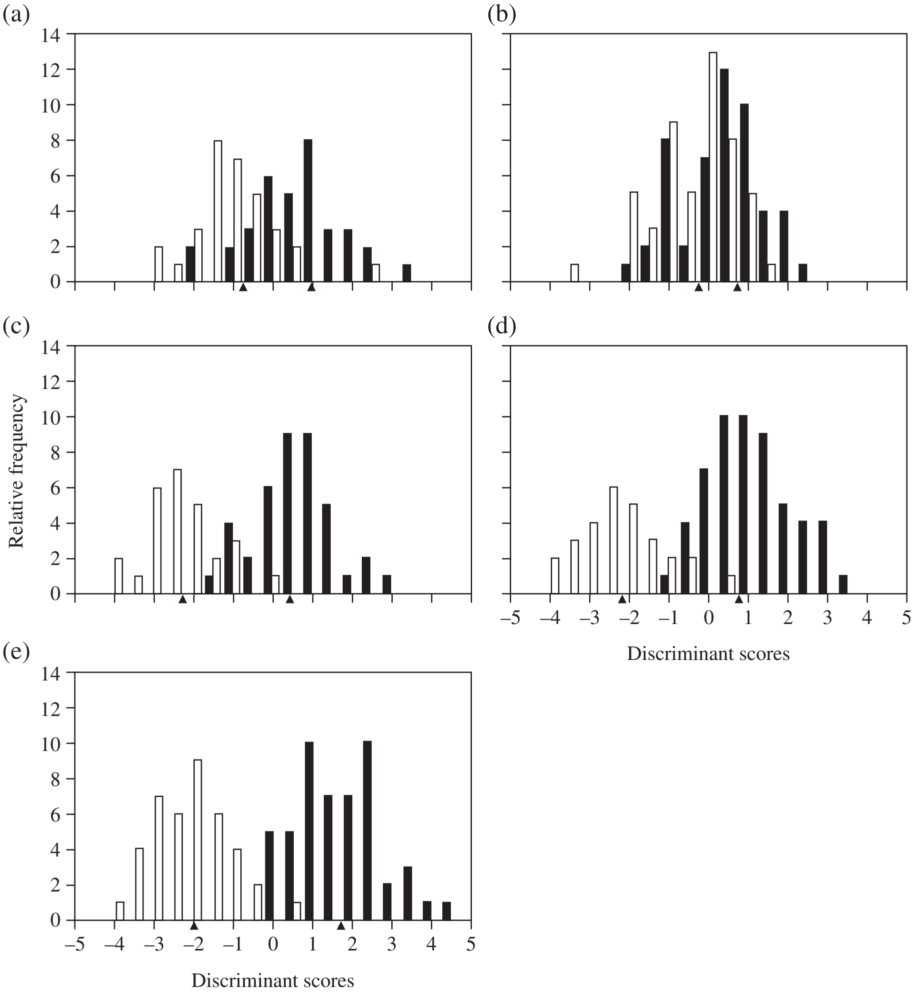

FIG. 4. Multivariate morphological analyses across (a) Témiscouata Lake, (b) East Lake, (c) Webster Lake, (d) Indian Pond and (e) Cliff Lake revealed a continuum of phenotypic divergence among five sympatric pairs a dwarf (■) and normal (☐) *Coregonus clupeaformis*. This continuum of phenotypic divergence was accompanied by lake-to-lake differences in potential for competitive interactions (Landry *et al.*, 2007).

ADAPTIVE POTENTIAL IN SMALL POPULATIONS: IS THERE A ROLE FOR EPIGENETIC VARIATION?

Despite the occurrence of various mechanisms explaining why and how standing genetic variation may be maintained, impoverished genetic diversity has been commonly documented, particularly so in isolated populations with highly reduced effective population sizes. While theory predicts that the adaptive potential of such populations should be severely compromised, recent studies in fishes suggest that it is not necessarily the case. For instance, in a study on brown trout *Salmo trutta* L. 1758 populations founded by a small number of parents in Kergulen, Indo-Southern Ocean, Labonne *et al.* (2016) showed that contrary to theoretical predictions, minimal genetic

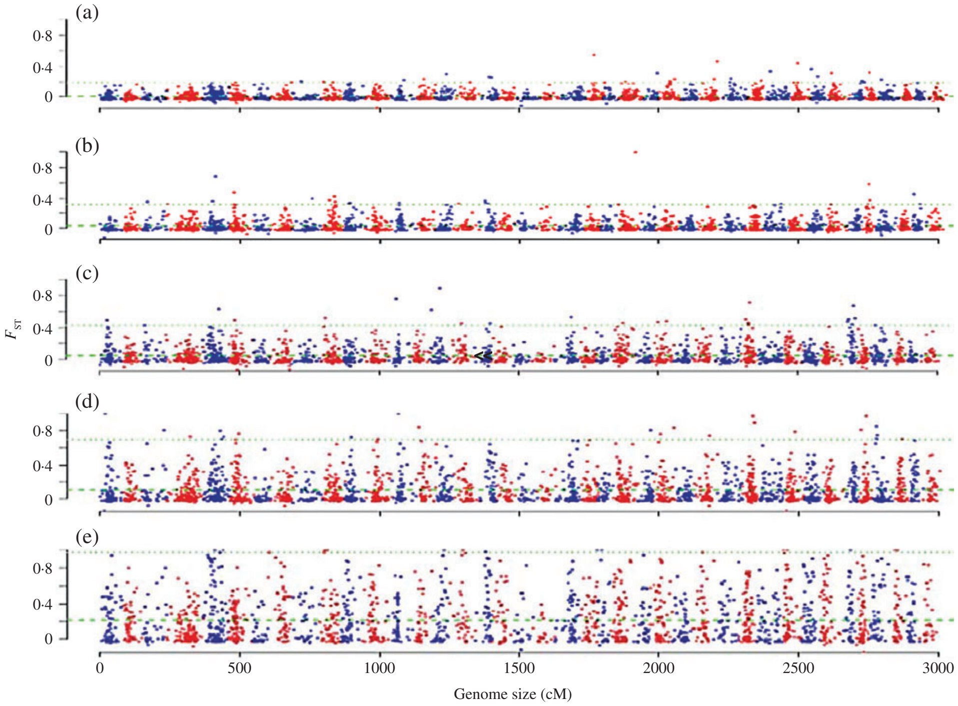

FIG. 5. A genome scan performed using RADseq among these same *Coregonus clupeaformis* sympatric pairs as in Fig. 4 revealed a continuum of genome-wide divergence that strongly correlated ($r^2 = 0{\cdot}849$) with the extent of phenotypic divergence (Gagnaire *et al*., 2013): (a) Témiscouata Lake - - - - - $F_{ST} = 0{\cdot}008$; (b) East Lake $F_{ST} = 0{\cdot}029$; (c) Webster Lake $F_{ST} = 0{\cdot}049$; (d) Indian Pond $F_{ST} = 0{\cdot}105$; (e) Cliff Lake $F_{ST} = 0{\cdot}216$ (Gagnaire *et al*., 2013). The alternating groups of ● and ● data points indicate different linkage groups.

variation and very strong founder effects did not preclude the species to colonize a new environment never occupied by *S. trutta* and apparently to adapt to it. Wood & Fraser (2015) investigated plasticity response of brook trout *Salvelinus fontinalis* (Mitchill 1814) to increasing temperature in a common-garden experiment using populations varying 50 fold in census size. They observed similar responses of small and large populations that also differed strikingly both in their effective population size and genetic diversity. These results suggest that phenotypic plasticity is not necessarily reduced as population size and genetic diversity decreases and that even very small populations might have the ability to respond to climate change by means of an adaptive plastic response. Finally, Wood *et al.* (2016) performed a meta-analysis to investigate the links between natural selection, quantitative genetic variation and population size across a large number of populations and species in nature. They found that heritability does not decrease with population size unless it is extremely small ($N_e < 10$), which suggests that potential for response to selection at small population size might be more extensive than previously assumed in evolutionary and conservation biology. Admittedly, the number of empirical studies supporting the view that wild populations of very small effective sizes can retain adaptive potential is too small to draw generalities about the ubiquity of this phenomenon. Indeed, Wood *et al.* (2016) admitted that their meta-analysis had some important limitations, partly imposed by the current lack

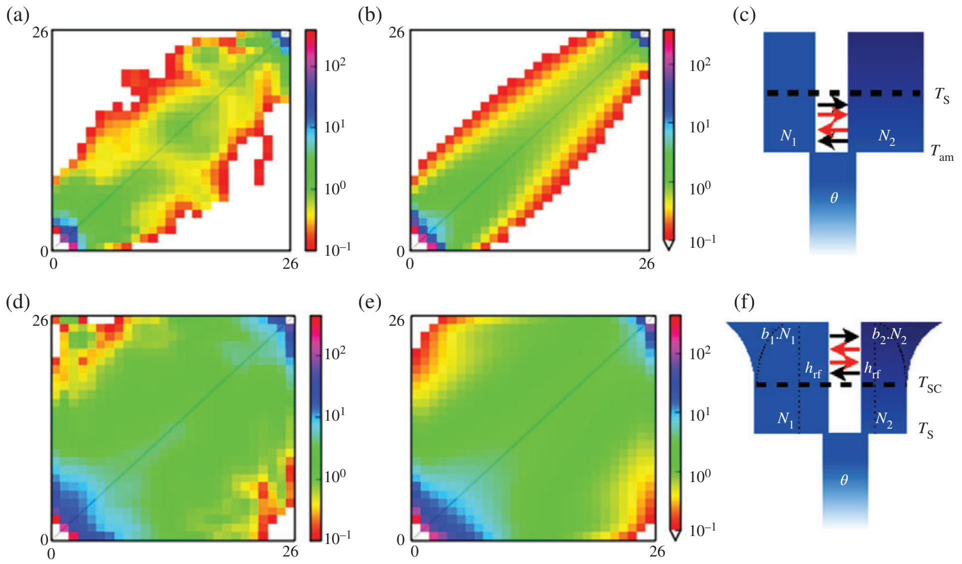

FIG. 6. Demographic inferences (∂a∂i software; Gutenkunst *et al.*, 2009) based on likelihood calculation of RAD-seq joint–allelic–frequency spectrum (JAFS) and model comparison (AIC) revealed different evolutionary and demographic history among *Coregonus clupeaformis* populations (C. Rougeux, L. Bernatchez & P.-A., unpubl. data). Examples of two lakes with distinct demographic histories: (a–c) Webster Lake, the (a) data best fits a history of sympatric origin with (b) an ancient migration [AM; (c) model] in the early stage of divergence. (d–f) The best fit for Indian Pond (d) data is a history of (e) secondary contact [SC; (f) model] of two glacial lineages after an allopatric phase, both with heterogeneous gene flow. The demographic history of the two lakes also differs in the values of and the different model parameters: (c) AM2m, the Ancient migration (AM) model with 2 m chromosomal variations in migration rate; θ, factor that allows scaling the inferred demographic parameter values; N_1 and N_2, the effective population size after split for dwarf (N_1) and normal (N_2) populations; T_S, time parameter for split of the ancestral population and end of migration; T_{am}, time parameter for start of migration between diverging populations before complete isolation and (f) SC2N2mG, the Secondary contact (SC) model with chromosomal variations of effective population size (2 N); G, temporal variation in effective populations size; b, the growth coefficient for dwarf (b_1) and normal (b_2) populations; b_iN_i, corresponds to the contemporary effective population size; h_{rf}, the Hill–Robertson factor corresponding to the degree to which the effective population size is locally reduced due to the effect of background selection and selective sweep effects; T_{SC}, time parameter for start of migration following allopatric phase.

of data for very small and isolated populations. Consequently, until more empirical population genomics studies from different biogeographic contexts (*e.g.* over a range of predictable and unpredictable environmental instability) are obtained, results of the above studies should be interpreted cautiously and as showing how unpredictable evolution (including habitat-specific selection) can be when N_e becomes very small.

That being said, the solution to this apparent paradox, whereby evolutionary potential might sometimes be retained despite small population size and impoverished genetic diversity, could lie in epigenetic variation, a non-genetic source of variation that may increase the potential of species to quickly adapt to environmental change and upon which natural selection can also act (Verhoeven *et al.*, 2016). Indeed, epigenetic variation meets the requirements to be acted on by natural selection: it is ubiquitous; it controls the expression of adaptive traits and may be inherited over several generations (Angers *et al.*, 2010; Klironomos *et al.*, 2013; Rey *et al.*, 2016). The best evidence to date for the association between epigenetic variation and adaptation to local environmental conditions comes largely from plant studies. For instance, using the reduced-representation bisulphite sequencing (RRBS) technique, Gugger *et al.* (2016) assessed whether climate is associated with variation in DNA methylation levels among 58 naturally occurring and species-wide samples of valley oak *Quercus lobata* collected across climate gradients. Environmental association analyses revealed 43 specific single-methylation variants (SMV) that were significantly associated with four climate variables, mainly with mean maximum temperature. This pioneer study provides initial evidence for a role of CG methylation in locally adaptive evolution (including adaptive plasticity) in plant response. Keller *et al.* (2016) recently performed a multivariate association study between genome-wide DNA methylation and climate across the range of mouse-ear cress *Arabidopsis thaliana*. Comparing genomic clusters of methylated and unmethylated cytosines, they found that climate and space variables explain much greater amounts of variation in DNA methylation than those explained by variation at the single-nucleotide level. They also found that methylation polymorphisms showing the strongest associations with climate were enriched in transposable elements. These findings therefore indicate that epigenetic variation across the genome contributes to an adaptive response to climate conditions.

In animals, Norouzitallab *et al.* (2014) found that exposure of brine shrimp *Artemia* spp. to heat-induced heritable changes in DNA methylation and histone acetylation patterns associated with increased *hsp70* production, which enhanced tolerance to heat stress. Other studies revealed that epimutations may occur at higher rates than mutations, or reported higher levels of variation at the epigenome than at the genome level. Liebl *et al.* (2013) reported that epigenetic diversity was negatively correlated with genetic diversity in house sparrow *Passer domesticus* populations that invaded new habitats in the past century. This suggests either that methylation increases phenotypic variation or that plasticity increases in response to new environments and therefore plays an important source of variation related to adaptation. Epimutations may also be triggered by environmental stressors (Feil & Fraga, 2012) and consequently, epigenetic variation could potentially promote rapid and inherited phenotypic diversity in response to global change.

There are few studies on epigenetic variation performed on natural fish populations. In a pioneer study on the clonal finescale dace *Chrosomus eos-neogaeus* (Cope 1857), Massicotte *et al.* (2011) documented ample epigenetic variation in the total absence of genetic variation, demonstrating that this process can be an important source of variation. Baerwald *et al.* (2016) investigated the role of epigenetic regulation of gene expression in linking phenotypic plasticity to variation in migration-related life history tactics between migratory steelhead and non-migratory rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) lines. They found 57 differentially methylated regions (DMR) between migratory and resident juveniles, many of which encode proteins with activity relevant to migration-related transitions. This is the first evidence of a relationship between epigenetic variation and life-history divergence associated with migratory traits in any species. Finally, Smith *et al.* (2016) tested the hypothesis that populations in the earliest stages of divergence should differentiate in their methylome prior to any genetic differentiation. They showed that epigenetic differentiation was indeed greater than genetic divergence among closely related populations. Epigenetic divergence was also a stronger predictor of the strength of behavioural reproductive isolation. This study therefore suggests a role for epigenetic variation in both the initiation of divergence and the maintenance of species boundaries.

Although this remains to be supported by more empirical studies, results to date support the view that the retention of high epigenetic diversity could account for the apparent paradox of the maintenance of evolutionary potential in small populations despite very low genetic diversity. They also suggest that such variation may be adaptive and probably play a role in local adaptation and even possibly in reproductive isolation.

CONCLUSION

The goal of this perspective paper was not to provide arguments for downplaying the importance of environmental challenges that living organisms (and fishes in particular) are currently facing, as well as the importance of genetic monitoring. After all, fishes, in particular freshwater populations, provide examples of rapid extinctions associated with fragmentation, demographic stochasticity, sometimes associated with genetic erosion. Therefore, ongoing efforts of incorporating genetic data into conservation practices and translating genetic findings into management policies remains totally warranted and justified. Rather, the aim was to bring a more balanced view on this issue by surveying theoretical considerations and current empirical facts regarding several important mechanisms by which species may maintain their evolutionary potential in the face of these challenges and adapt to them, perhaps more efficiently than generally assumed or predicted to date.

Research performed over recent years on fish population genomics has contributed greatly at providing evidence for local adaptation at the molecular level. A striking general pattern emerging from these studies is that pervasive adaptive evolution seems almost never to involve the fixation of beneficial alleles, in the form that is classically inferred from hard sweeps and the QTN programme. This confirms that the view whereby adaptation proceeds *via* selective sweeps at key loci of large effect is too limited. Instead, it may be that sweeps and other modes of selection (*e.g.* polygenic selection) are not mutually exclusive. For instance, it could be that sometimes the alleles with largest effect sizes sweep to fixation, although much of the adaptive response is caused by smaller changes in allele frequency (Hermisson & Pennings, 2005; Pritchard & Di Rienzo, 2010). Fish studies also support the view that genetic diversity and associated evolutionary potential is being retained in the long-term mainly *via* standing genetic variation (more than from new mutations), despite selection driving the evolution of pronounced divergent adaptations. They also add to the growing evidence suggesting that adaptive alleles may persist at intermediate frequencies, particularly so when selection pressures are highly variable, such as along a species' range or across time. This has some important implications towards predicting the potential of species to adapt to environmental changes and perturbations. Standing genetic variation has several advantages over new mutations (Messer & Petrov, 2013). In particular, alleles have already avoided stochastic loss immediately after arising and intermediate allele frequencies may underlie important parts of a phenotype's heritable (and therefore selectable) variation, with allelic effects being either large or small.

Perhaps the most obvious factor contributing to the maintenance of standing genetic variation is that adaptive phenotypic traits are most often highly polygenic and as a consequence, evolution may proceed more commonly *via* high numbers of small-effect polygenes. The response to selection should thus derive mostly from allelic co-variances among causative loci. Theory predicts that such polygenic selection will generally not lead to the fixation of loci under selection, meaning that allelic diversity and therefore evolutionary potential, should be retained when exposed to new selective pressures. While such signals of selection are more difficult to detect at the genome level than say, the effect of hard sweeps, the few studies performed on fishes using new analytical approaches, including various multivariate analysis framework as well as tree-based ensemble machine learning tools such as RF, showed their efficiency and relevance for detecting signals of polygenic selection. Clearly, more studies aiming the investigation of polygenic selection must be performed in other species occupying different types of environments and with different demographic and population structure characteristics in order to obtain a more complete view of how natural selection shapes genome architecture and genetic diversity. This should contribute to improve predictions regarding the potential of species to cope with a changing environment. Similarly, future population genomic studies should also consider that selection is not only divergent or directional. Rather, there are many reasons to believe that balancing selection may be at play more commonly than currently assumed and consequently be an important process contributing to the maintenance of genetic variation allowing species to respond to future challenges. Arguably, detecting true signals of balancing selection remains very challenging and therefore, new analytical and empirical development towards this goal appears particularly crucial.

Future modelling efforts towards predicting the evolutionary fate of species in the face of environmental changes should also consider that repeated evolution of adaptive phenotypes is a ubiquitous evolutionary phenomenon. Studies on fishes have made very substantial contributions in empirically supporting this fact. These studies also showed that repeated evolution often involve different genetic architectures, perhaps more commonly than involving the same genetic basis. This implies that while some of the genetic variation underlying the expression of adaptive traits may be lost in some populations, *e.g.* due to genetic drift, such traits might evolve again *via* a distinct genetic architecture. The high prevalence of repeated adaptation with or without the same genetic architecture adds to the potential of species to cope with a changing environment. As illustrated by research on coregonids also, while parallel phenotypic evolution is common, it is often not perfectly repeated, or sometimes unobserved where it was expected to occur. Future studies would benefit by aiming to elucidate the mechanisms responsible for parallelism *v.* non-parallelism, which arguably represents a very fruitful, yet underexploited research area towards elucidating the fine tuning of adaptive evolution.

Finally, although this remains to be supported by many more empirical studies, results to date support the view that the retention of high epigenetic variation could account for the apparent paradox that represents the maintenance of evolutionary potential retention in small populations despite their impoverished diversity. They also suggest that such variation may be adaptive and probably play a role in local adaptation and even possibly in reproductive isolation. Therefore, the aim should be towards fully integrating epigenetic variation with considerations pertaining to role of soft sweeps, polygenic and balancing selection, as well as repeated adaptation involving different genetic basis towards improving models predicting the potential of species to cope with a changing world.

I am thankful to M. Wellenreuther for a thorough revision of a previous version, A. Carrier, B. Sutherland, M. Laporte and P.-A. Gagnaire for their thoughtful inputs, as well as E. Normandeau for helping in screening the literature on genome scans. I am also grateful to G. Carvhalo and three anonymous referees for their constructive comments and suggestions. This research is funded by the Natural Sciences and Engineering Research Council of Canada and the Canada Research Chair in Genomics & Conservation of Aquatic Resources.

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