



A framework for incorporating evolutionary genomics into biodiversity conservation and management

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REVIEW

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A framework for incorporating evolutionary genomics into biodiversity conservation and management

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Abstract

Evolutionary adaptation drives biodiversity. So far, however, evolutionary thinking has had limited impact on plans to counter the effects of climate change on biodiversity and associated ecosystem services. This is despite habitat fragmentation diminishing the ability of populations to mount evolutionary responses, via reductions in population size, reductions in gene flow and reductions in the heterogeneity of environments that populations occupy. Research on evolutionary adaptation to other challenges has benefitted enormously in recent years from genomic tools, but these have so far only been applied to the climate change issue in a piecemeal manner. Here, we explore how new genomic knowledge might be combined with evolutionary thinking in a decision framework aimed at reducing the long-term impacts of climate change on biodiversity and ecosystem services. This framework highlights the need to rethink local conservation and management efforts in biodiversity conservation. We take a dynamic view of biodiversity based on the recognition of continuously evolving lineages, and we highlight when and where new genomic approaches are justified. In general, and despite challenges in developing genomic tools for non-model organisms, genomics can help management decide when resources should be redirected to increasing gene flow and hybridisation across climate zones and facilitating *in situ* evolutionary change in large heterogeneous areas. It can also help inform when conservation priorities need to shift from maintaining genetically distinct populations and species to supporting processes of evolutionary change. We illustrate our argument with particular reference to Australia's biodiversity.

Keywords: Evolutionary adaptation, Plasticity, Decision framework, Genomics, Management

Climate change threats to biodiversity

The latest IPCC report [1] provides a very clear picture about current and accelerating climate change. Even if CO₂ emissions can be completely curtailed by 2050, it is likely that there will be a further rise in temperature of at least 2°C above the current level of almost 1°C. Given the political challenges associated with emission reductions, it is doubtful whether such a timeframe for emission reduction will be realised. Therefore, the world is more likely facing an increase in mean temperature of 3°C–6°C, approaching the extent of change experienced in the last glacial maximum, coupled with an increase in

temperature extremes. In addition, there will be a gamut of associated changes including ocean acidification, increases in fire incidence and severity, storm activity, the length and intensity of drought and flood conditions, as well as changes in the salinity of coastal areas [1].

The distributions of many species are expected to shift markedly during this period. Climate niche modelling predicts that many areas currently occupied by species and communities will no longer be suitable for them [2]. Similarly, areas suitable for alpine and sub-alpine vegetation and fauna in Europe are expected to decline by more than 90%, e.g. [3,4]. At the same time, changes can be idiosyncratic [5] and some species are expected to benefit from the effects of climate change; groups of invasive species and even some native species are expected to benefit in this way [6,7].

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Predictions based on species distribution models are relatively crude because they assume that current distributions are limited by climatic factors, whereas the climatic space a species can tolerate may be substantially greater than the area where it actually persists [8,9]. To resolve this issue, investigators have explored the limits of tolerance or growth of species [10–12] but controversy remains about the best approach and interpretations of patterns across different species. Tolerance limits often depend not only on the immediate conditions being experienced but also on those encountered during development and the rate at which stresses act, as well as a variety of other factors [13–15]. Species may respond via plasticity, altering growth rates, triggering phenological changes and increasing resistance to extremes, all in an adaptive direction [16]. Behavioural adaptation might also allow species to find areas with suitable microclimates within their current distributions [17] or to track their niches as they move across space [18–20].

Biotic factors introduce another level of uncertainty into predictions, particularly when they generate an additional source of environmental stress, such as the widespread impact of mountain pine beetles on pine tree mortality in North America [21]. Perhaps the most important biotic factor is human population growth and the impacts of more than 7 billion people on a natural environment that is increasingly under stress [22]. The negative impacts of human activities on biodiversity are well documented and range from direct effects triggering species extinctions through overexploitation to indirect effects through removal of habitat for agriculture or resource extraction [23].

It is already clear that large-scale changes to natural communities are occurring and will accelerate over the coming decades [24]. Many (perhaps the majority of) species face local extinction in at least part of their native range. A few species will track climate successfully, others will benefit from vacant space created by departing species, tropical and subtropical species may invade higher latitudes, but many communities will be lost, e.g. [3,5,25]. All of these changes will take place within the context of increased landscape fragmentation due to ongoing vegetation clearing and increasing calls to manipulate the environment to safeguard agriculture and property such as through fire suppression. There will also be flow-on effects of these changes on ecosystem services provided by the natural environment, directly impacting on the ability of species, including our own, to access the resources needed for persistence [26,27].

Opportunities and constraints for evolutionary adaptation

Given the scale and timeframe of climate change effects, what is the likelihood that species and communities can respond through evolutionary changes? Major life forms

have persisted and adapted across geological epochs despite temperature changes that exceed those predicted under anthropogenic climate change. Whilst speciation and evolutionary divergence have occurred over millions of years, current species and populations have persisted through the more recent climatic oscillations of the Pleistocene [28,29]. In addition to these past evolutionary changes over geological time frames, there is also a growing (albeit still small) number of cases of rapid and contemporary evolutionary changes in natural animal and plant populations [30,31] that allow us to track the direct and indirect effects of climate change. Examples include genetic changes in the body colour of owls in response to predation linked to changing snow covers [32], changes in allozyme frequencies and inversions that preserve functional sets of genes in *Drosophila* known to be sensitive to temperature changes [33], and adaptive changes in the flowering time of Brassicas in response to drought [34]. However, not all populations are expected to successfully adapt through evolutionary change. This may reflect a lack of genetic variation in base populations [35], interactions among traits that constrain evolutionary responses in one direction [32,36], and other factors such as the reduced effectiveness of selection in the presence of the plastic responses of individual genotypes (i.e. the extent to which they can be modified by the environment). These types of factors may help account for cases where adaptive evolutionary changes have not occurred, but were expected, as in the case of breeding time in birds [37].

Three interacting demographic factors are widely recognised to have major effects on the likelihood of successful adaptation to rapid climate change—generation time, population size and population structure (Figure 1). Selection responses are typically slower in long lived organisms, although such species can still evolve effectively if able to exploit existing variation within or among populations [38]. The variation available within populations in turn depends on population size; *in situ* evolution will be maximised at larger population sizes [39], and this becomes a major challenge for threatened species living in fragmented landscapes [40]. Population structure has more variable effects on evolutionary potential because gene flow across the landscape can assist evolution or retard it depending on selection gradients and rates of gene flow [41]. Whilst often providing potentially useful new variation for the population in question, it can also swamp processes of local adaptation with an influx of genes that are poorly adapted for the local climate, which might be particularly important for marginal populations [42,43]. On the other hand, gene flow often seems to be related to environmental conditions: processes like flowering, propagule dispersal and mating time can mean that gene flow is higher among

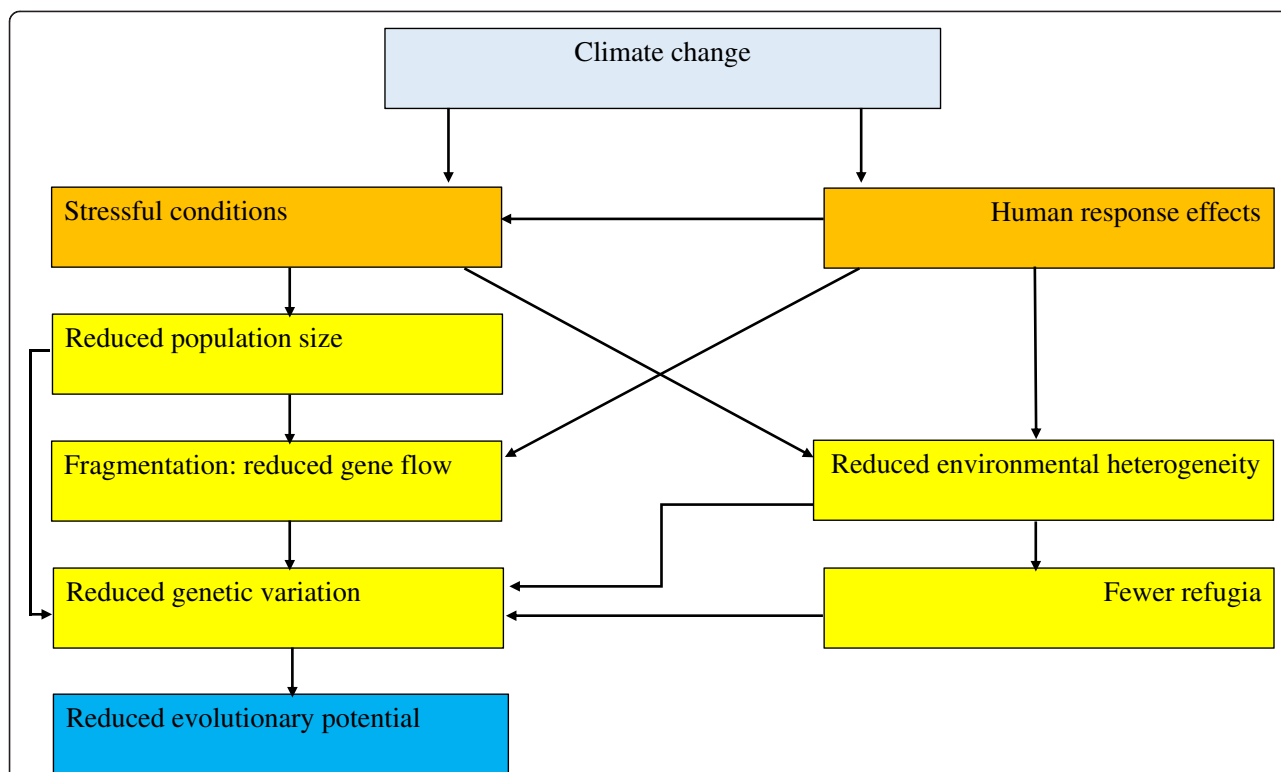


Figure 1 Impact of climate change on evolutionary potential of populations. In many cases, there will be a loss of evolutionary potential derived from a decrease in population size, gene flow and refuge areas.

populations from similar environments, and this might increase rates of adaptive evolution [44].

Both population size and patterns of gene flow have been dramatically affected by human activities. Environments have become increasingly fragmented, leading to increasing levels of genetic distinctness and a loss of accessible genetic variation, e.g. [45,46]. Threatened environments may represent a series of islands surrounded by hostile conditions for the species. At its most extreme, species might be confined to zoos or botanical collections, living in a highly defined set of environmental conditions, at a small effective population size, with only limited scope to recruit new genes even with coordinated programmes to exchange material; see [47].

From an evolutionary perspective, natural populations are therefore threatened by three forces that interact to produce a downward spiral of evolutionary potential (Figure 1): (i) a reduction in genetic variation as a consequence of decreases in population size affecting *in situ* evolution, (ii) a reduction in gene flow preventing an influx of genetic variants from other populations and (iii) a reduction in environmental heterogeneity that can lead to a decrease in adaptive capacity of the species as a whole. The likelihood that evolutionary rescue

(involving forced introgression from other populations or (sub)species) might mitigate some of the threats imposed by environmental change remains unknown [48], although it will likely depend on the availability of genetic variation within the populations/species concerned.

Against this backdrop of gloomy projections, the current revolution in genomics and other -omics technologies is providing unprecedented insights into evolutionary processes and offers an opportunity to significantly improve conservation planning and management decisions. Researchers can now identify parts of the genome that have been or could be involved in adaptive shifts, via new or existing variants *in situ*, or through hybridisation. At a functional level, genomics approaches can also identify the networks of genes/proteins and their expression profiles required for key adaptations. Whilst once limited in application to model organisms, the technology is now increasingly applicable to non-model species despite ongoing challenges around annotation [49,50]. Below, we briefly outline the various methods for generating and analysing genomic data bearing on biodiversity conservation, their strengths and weaknesses, and then describe how genomic information can explicitly be incorporated into a decision-making framework for biodiversity conservation in the face of climate change.

Methods for generating genomic data

The advent of next-generation sequencing has enabled population genetic and microevolutionary studies on a genome-wide scale. From hundreds of millions of dollars for the first draft of the human genome in 2001, the sequencing required to assemble a reference genome for a species now costs just a few thousand dollars. Economical sample preparation strategies reviewed in [51-53] now enable high-throughput genomics studies even without a reference genome [54]. “Home-brew” methods for sequencing library preparation [55,56] have reduced per-sample cost and prices of commercial kits have followed a similar downward trend. It is now realistic to carry out whole genome sequencing (WGS) of 30 individuals of an insect species with a small (250 Mb) genome, for less than US\$4000.

Although multi-individual WGS provides the highest accuracy and power in population genomics, it can still present a significant financial challenge when multiple populations are under investigation. There are several economical alternatives to WGS. First, the recently developed reduced-representation sequencing (RRS), including genotyping-by-sequencing (GBS) and RADSeq [57-59] technologies, can overcome this problem by targeting a subset (approximately 1%) of the genome. These approaches typically involve restriction enzyme digestion of genomic DNA, sample barcoding by attaching unique oligo-nucleotide sequences identifying individuals and selection of a subset of genomic fragments, followed by sequencing of multiple samples in the same lane on an Illumina sequencing platform. They provide data on hundreds to tens of thousands of nucleotide polymorphisms. In some cases, they tag the majority of the genes in the genome and, importantly, reduce per-sample costs substantially. These approaches can be used to answer a wide variety of questions on population structure and phylogeography; see [51,53,57]. RRS strategies typically aim for up to 10× coverage per site, which generally allows for accurate identification of heterozygous sites. Dual-end barcode sets of 384 or more now exist for the identification of individuals, which makes large sample sizes economical. One limitation of RRS approaches is that loci can suffer from “allelic dropout” due to polymorphisms in restriction sites [60,61], which may lead to an overestimate of divergence.

A second alternative to individual-based WGS is combining individuals into a pool, as in Poolseq; see [51,62] (PPS). The Poolseq approach does not allow the data for different individuals to be separated post-sequencing, but it is highly cost-effective for assessing population structure [63], genetic distance [64-66] and genome-wide patterns of heterozygosity. As little as 1× coverage of each diploid individual’s genome is needed, further reducing cost. However, a lower level of coverage will

not adequately represent the pool of individuals, especially when the pool is small to begin with, and can therefore produce misleading population parameter estimates; see [60,61,67]. Guidelines [51,60,61,67] and software packages such as ngsTools [68] and npstat [69], which carry out likelihood-based estimation of allele frequencies, are now available to help tackle these challenges.

Another affordable strategy for population genomic studies is transcriptome sequencing (TS). This approach yields data on genes which are expressed at reasonable levels, representing perhaps 1%–10% of the genome. Variants can then be identified in the sequenced transcriptome. If coverage is deep enough, biases are addressed and appropriate experimental replicates are included; differences in gene expression can also be detected between samples [70]. Transcriptome sequencing is usually performed at the individual level, but it is also possible to estimate allele frequencies from sequencing of pooled samples [71] and to compare different lines and populations [72]. Variant identification from transcriptome data can suffer the same biases from low coverage sequences as Poolseq experiments and can also suffer from allelic dropout when only certain alleles are expressed in individual samples.

Whatever the sequencing platform used, most population genomics studies to date have based their analyses on single nucleotide polymorphisms (SNPs)—single-base variants in either functional or neutral regions of the genome. Whilst SNPs are informative and relatively easy to identify, studies on model species have shown that insertion-deletion polymorphisms (indels) also play an important role in genome evolution and adaptation [73-76]. Identifying indels from high-throughput sequencing data remains a difficult bioinformatic problem [77], and identifying large indels in RRS data is especially difficult because only a small percentage of the genome is sequenced. Indels are not just important for their own sake: if small indels are misaligned, then SNPs may be misidentified in the region [74]. A few programs like the GATK2 best-practice pipeline attempt to resolve this by a local refinement of read alignments, but this can still suffer from discordance [77]. Chromosomal rearrangements and other forms of structural variation are also involved in adaptation (reviewed in [78]). Such variation is still difficult to identify using short-read technology, but improved methodologies are under development [79,80].

RRS, Poolseq and transcriptome sequencing strategies can provide genomic insights into the majority of management questions described in Table 1 below. Some of the common population genetic parameters can be estimated reliably from samples of 30 non-related individuals per population, but if low-frequency alleles are of particular interest, 30 individuals may not be sufficient. Taking spatial structure and landscape features into account may

Table 1 Applications of genomics data to relevant steps in the decision framework

Decisions	Biological issue	How genomics can help inform decisions	Data type	Analysis method	Limitations
Can species tolerate change <i>in situ</i> ?	Determining if a species is currently experiencing stress which suggests it is approaching the limit of physiological tolerance	Screen biomarkers indicative of stress. See [81]	[TS] with [IG]	Gene expression analyses to identify abundance of key gene transcripts	For many species, further research is required to identify biomarkers; however genomics could facilitate this process. A challenge is that biomarkers need to be diagnostic of stress and reproducible—particularly for gene expression markers
	Testing whether a species has sufficient phenotypic plasticity to tolerate projected change	Understand the limits to plasticity under environmental change. See [82,83]	[TS] with [IG]	Gene expression analyses. Gene transcript abundance can be used as a surrogate for overarching phenotypic responses	Observing phenotypes will be more appropriate/cost-effective in some cases, but in other cases, gene expression could screen many phenotypes simultaneously at lower cost per sample. For the latter, links to phenotypic data are required
	Assessing a species' historical demography to see how it responded to past climate change	Greater numbers of loci provide the opportunity to reconstruct demographic history deeper in time. See [84-86]	[WGS], [RRS], [DE] with [IG] or [WGS], [DE] with [PPS]	Bayesian skyline plots/coalescent simulations or likelihood-based diffusion modelling from SNP data	Genomics can provide a comprehensive assessment; however, a similar outcome might be achieved using non-genomic tools (e.g. SSRs), particularly where data sets are already available
Do populations have enough genetic diversity for an evolutionary response?	Determining whether the species or population is currently experiencing inbreeding, which can lead to loss of genetic diversity essential for evolution	Genome-wide sequencing allows accurate estimation of heterozygosity in individuals and populations. See [87]	[WGS], [RRS], [DE], [TS] with [IG] or [WGS],[DE] with [PPS]	Estimate F-statistics and heterozygosity from SNP data	Non-genomic tools (e.g. SSRs) can be applied to estimate diversity, particularly where data sets are already available. However genomics offers better resolution and diversity estimation. The effects of different levels of diversity on adaptability needs to be established through phenotypic comparisons
	Assess whether there is enough standing genetic diversity to provide opportunities to adapt	Accurately estimate the levels of genetic diversity in populations. See [88]		Estimate heterozygosity, DNA sequence diversity estimates (π , θ) from SNP or sequence data	
	Determining whether selection has acted on genetic variation in the species	Test whether major events have resulted in selection on genetic diversity. See [89,90]		Allele frequency spectrum tests (e.g. Tajima's D), linkage disequilibrium, non-synonymous to synonymous polymorphism ratios (e.g. K_n/K_s) from sequence data	
Is genetic diversity strongly distributed across populations?	Identification of centres of genetic diversity, or genetically distinct regions, for prioritised conservation	Examine patterns of population genetic structure to identify outlier populations. See [91]	[WGS], [RRS], [DE], [TS] with [IG] or [WGS],[DE] with [PPS]	Estimation of population differentiation based on SNP data using classical F-statistics, PCA or MCMC and Bayesian derived estimates of admixture (e.g. STRUCTURE)	Non-genomic tools (e.g. SSRs) could be applied to assess population differentiation. However genomics offers better resolution and accuracy of diversity patterns, which may be important for detecting fine scale structure

Table 1 Applications of genomics data to relevant steps in the decision framework (Continued)

Are some populations adapted to local climate?	Identifying whether populations show adaptation to local climate (or other environmental variables)	Identify loci that have been under selection in populations conditioned on local environment. See [92-95]	[WGS], [RRS], [DE], [TS] with [IG] or [WGS], [DE] with [PPS]	Population level or landscape genomics methods based on SNP data: outlier tests, relative rate tests, allelic association with environment and allelic association with adaptive traits. Computational modelling of genomic diversity evolution under environmental change	How to confidently link climate variables to local adaptation, and how to infer adaptive capacity from genomic data are currently pressing questions in population genomics. However these questions also apply to other genetic methods. Characterising the local climate experienced by a species is also a challenge, but microclimate modelling is improving rapidly. Phenotypic data is still essential to determine the extent of adaptation
	How quickly can genetic adaptation occur?	Identify rates of genetic adaptation to environment by screening adaptive variation in natural populations experiencing environmental change, or through simulated or experimental evolution. See [96-98]			
Is gene flow high enough? (or too high?)	Determining the extent of gene flow between existing populations to inform on dispersal capability and also potential for adaptive alleles to spread or be swamped	Provide estimates of ongoing gene-flow and admixture among populations. See [99,100]	[WGS], [RRS], [DE], [TS] with [IG] or [WGS],[DE] with [PPS]	Coalescent genealogy sampling to generate Bayesian and maximum likelihood estimates of migration and gene flow (e.g. Lamarc, Migrate), or MCMC and Bayesian-derived estimates of admixture (e.g. STRUCTURE) based on SNP data. Genomics also has the power to identify recent migrants and so test the efficacy of movement pathways	Genomics can provide a more comprehensive assessment of gene flow compared to non-genomic tools (e.g. SSRs), particularly where rates of gene flow are low
Is a positive evolutionary response possible through natural hybridisation with sympatric species?	Determining whether hybridisation occurs in nature	Estimate rates and genomic extent of hybridisation/gene flow between species <i>in situ</i> . See [101-103]	[WGS], [RRS], [DE], [TS] with [IG] or [WGS],[DE] with [PPS]	Identify hybrid ancestry via comparison to known non-hybrids. Estimate migration and gene flow (e.g. Lamarc, Migrate) and admixture (e.g. STRUCTURE) between species. Admixture quantification also confirms F1 hybrid fecundity	Non-genomic tools are available for identifying hybrids; however genomics gives unprecedented power to detect even low levels of introgression, and to understand how patterns of introgression vary across the genome. Phenotypic data are essential to determine whether hybridisation is adaptive
	Assess how quickly beneficial alleles can move into a population or species	Track introgression of genomic regions under selection following documented hybridisation events. Identify potential for hybrid incompatibilities or swamping. See [104,105]	[WGS], [RRS], [DE], [TS] with [IG] or [WGS],[DE] with [PPS]	Track distribution of species specific alleles in population with regard to null selection models. Transmission distortion in artificial F2 hybrids can indicate genetic incompatibilities	
Can species migrate quickly enough?	Assess potential for migration into climatic refugia given ecological constraints and known rates of gene flow	Provide accurate estimates of gene flow (as described above). See [106]	[WGS], [RRS], [DE], [TS] with [IG] or [WGS],[DE] with [PPS]	Genomic estimates of gene flow can be coupled with data on rates of dispersal or movement and habitat analysis (path analyses, resistance models) to predict viability of dispersal pathways	Genomics can provide a more comprehensive assessment of gene flow compared to non-genomic tools (e.g. SSRs), particularly where rates of gene flow are low

[WGS] whole-genome sequencing. See [107,108].

[RRS] reduced representation sequencing (e.g. RADseq, GBS, DArTseq). See [109-111].

[DE] DNA enrichment (e.g. exon capture, SureSelect, anchored hybrid enrichment). See [112,113].

[TS] genotypes called from transcriptome sequencing or gene expression data. See [114,115].

[IG] sequencing and analyses performed on individual genotypes. See [61].

[PPS] sequencing and analyses performed on pooled population samples. See [69].

also require additional sampling along transects and environmental clines. On the other hand, some other experimental questions, including identification of subspecies and long-range migrants, may require fewer samples.

Significantly, none of these applications absolutely requires individual-based whole-genome sequencing. However, high-quality whole-genome sequencing may be a viable option for species with small genomes and will always provide the most complete data set. Another reason to consider whole-genome sequencing is to assemble a reference genome from one individual or line of the species in question. This can greatly aid in SNP calling, mapping the variants that are identified and associating phenotypes to particular regions, either in genetic crosses or population surveys, involving quantitative trait loci (QTL) mapping and genome-wide association studies (GWAS), respectively [116]. A typical reference genome sequencing project aims for >30× coverage, which is now relatively affordable. Currently, the limiting factor in *de novo* genome sequencing is the bioinformatic expertise required to assemble and annotate genomes to a high quality. At a minimum, annotation involves predicting the location and structure of a gene [117,118]; functional annotation then involves predicting the function of an identified gene, generally by comparison to related annotated genomes [119]. In the latter case, annotations remain challenging for non-model organisms [120], particularly when genome assemblies are of a low quality [118].

Sequencing costs may well continue to decrease in the near future with third-generation sequencing (single molecule sequencing), and innovations such as nanopore-enabled nucleic acid sequencing could further improve quality and reduce costs [121]. With read lengths of 49 kb + projected by companies like Oxford Nanopore [122], it may soon become possible to sequence entire genomes of non-model organisms for less than a thousand (US) dollars. This, combined with re-usable sequencing chips and mini USB-powered sequencers, ensures an increasingly important role for sequencing technologies in population genetic and microevolutionary studies related to climate change adaptation.

A decision framework

A framework for management decisions and subsequent actions for biodiversity conservation under climate change is presented in Figure 2 and Table 1; the framework is modified from that of Shoo et al. [123] to consider the potential for adaptation and possible roles for genomic data. The aim of the framework is to guide thorough but practicable assessments of whether a species can adapt to climatic change through migration, physiological tolerance or adaptive evolution and to recommend appropriate management actions that will help

it avoid extinction and retain genetic variation for long-term survival. Although the framework is designed to consider threatened species, we have interpreted it broadly to include an assessment of adaptation in species that might not be threatened but nevertheless perform a critical function within ecosystems. Each step in the framework requires answering a question relevant to climate change tolerance/adaptation, and those where genomic approaches are particularly relevant are shaded purple in Figure 2 (those best answered with non-genomic information like climatic or ecological data are shaded grey). Questions where genomics are relevant are broken down further in Table 1 into specific experimental approaches that may or may not be appropriate for the species of interest. The limitations of genomic approaches are also noted. The text below considers each step in the framework in turn, expanding particularly on those to which the genomics applies.

Assessing environment suitability and persistence

Assessing the likelihood that environmental suitability will decline

Species distribution models, often also referred to as ecological niche models or bioclimatic envelope models, and methods of modelling community-level turnover such as generalised dissimilarity modelling are currently the main tools used to obtain spatially explicit predictions of habitat (environmental) suitability for species under climate change [25,124,125] (D1 in Figure 2). These approaches use associations between climate and species' distributions to enable projections of future potential distributions under climate change scenarios. Whilst uses of such models have been criticised in the past, models that thoroughly account for algorithmic uncertainties, followed by careful interpretation of results, remain useful and widely used tools for forecasting impacts of climate change on large numbers of species [126].

Assessing whether species can tolerate change *in situ*

If a substantive risk that environmental suitability will decline under climate change has been identified, then the next step is to determine which species and communities should become the focus of ongoing management (D2 in Figure 2). Whilst many species are expected to be at risk from climate change, others may not be threatened because the projected change will fall within their tolerance limits. This section briefly discusses how genomic approaches might be used to determine the extent to which species will be able to tolerate climatic changes *in situ*, without the need for evolutionary responses and management intervention.

The first approach is to screen biomarkers that are consistently linked to levels of physiological stress to determine whether physiological limits are being approached

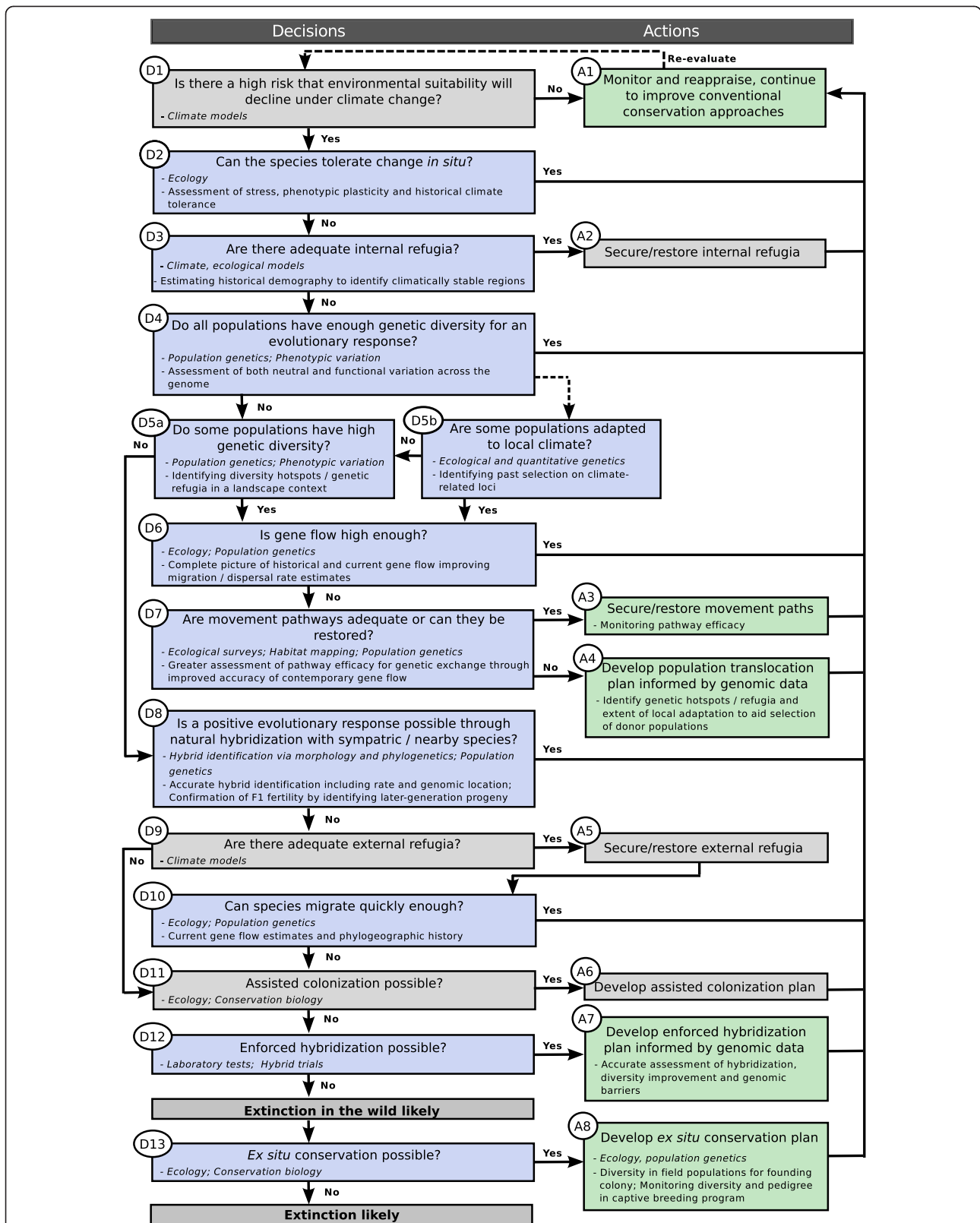


Figure 2 Management framework adapted from [123], highlighting (blue for decisions, green for action) where genomics can benefit decision-making. Decisions and actions are discussed in detail in the text. Italicised points indicate past/current approaches to addressing management questions. Plain-text points indicate the additional information genomics can provide.

or exceeded. Transcriptome sequencing can provide a signal of physiological stress in natural populations and wild-caught individuals [49,127], indicating a population that may not be functioning at its peak; for instance, transcriptomic stress profiling on several fish species has demonstrated a link between changes in the expression of particular genes and the physical condition of the fish [81,128]. One current challenge with this approach is that key biomarker genes have not yet been identified for many groups of organisms, although transcriptomic data for a range of species across various stresses are rapidly accumulating and generalities about useful markers may emerge. A subsequent challenge is to interpret quantitative transcript changes in terms of the critical physiological limits for the species in question [129].

A related approach is to use transcriptomic profiling to determine whether there is a capacity to mitigate the detrimental effects of environmental change via phenotypic plasticity. Just as some aspects of a transcriptomic profile may indicate a species approaching a physiological limit, so can other changes in the profile highlight an underlying capacity to tolerate change through the physiological plasticity of individual genotypes, even when phenotypic responses are not outwardly evident [82,130]. Given the importance of phenotypic plasticity as an adaptive mechanism for organisms facing climate change, such transcriptomic approaches could be used to investigate their capacity to respond physiologically without necessarily involving any evolutionary change. As above, such an approach is currently constrained by the very limited understanding of how gene expression changes link to fitness/performance under stressful conditions, but the situation is expected to improve given the current proliferation of transcriptomic studies. The approach is illustrated by a transcriptomic comparison of populations of the sparrow *Zonotrichia capensis* from altitudinal extremes of its range, carried out on both individuals sampled directly from the field and on those then transferred to a low altitude “common garden” environment [131]. There was no difference between the transcriptomes of the two populations under the latter conditions whereas samples obtained directly from the field differed in their expression of nearly 200 genes, pointing to the involvement of plastic changes in gene expression profiles rather than evolved differences among the populations.

Genomics can provide insights into the way populations of a species may have responded to climate change in the past. Estimates of historical demographic change over recent or long time scales can be obtained from analyses of the scale and structure of sequence variation in extant populations [132,133]. The time course of changes in population size and structure obtained can then be used to link past population expansions and

contractions to historical climate change, giving a clue as to the vulnerability of a species (based on both plastic and evolved responses) to future climate change.

Finally, phylogenetic and phylogenomic studies may provide insights into the capacity of species and lineages to tolerate contemporary climate change (Table 1). The well-supported, well-dated phylogenetic trees that can be produced with genomic data provide an opportunity to assess whether certain taxonomic groups are more vulnerable to climate change than others. The potential insights that might emerge from such studies are illustrated by traditional multi-locus phylogenetic (rather than phylogenomic) studies carried out to date. Thus a continent-wide avian phylogeny showed that European birds whose niches evolved more slowly in the past exhibited greater levels of demographic decline in the twentieth century, both at the individual species and the overall family level [134]. Similarly, Crisp et al. [135] used a phylogenetic framework to show that relatively few groups of southern hemisphere plants have speciated from the alpine biome to the sclerophyll biome, but many have speciated across sclerophyll/arid boundaries, suggesting that groups of alpine species are more at risk of extinction than sclerophyll species given an equivalent amount of climate change.

If a species is predicted to tolerate climatic changes and persist *in situ*, then no further action is required other than ongoing monitoring and assessment (action 1 in Figure 2). If, on the other hand, it is predicted that the species may not be able to persist *in situ*, then the next step in the decision framework is to identify whether there are any climatic refugia, internal to the species range, that might buffer it from change and facilitate persistence.

Identifying climate refugia within a species' current range

Refugia are defined as habitats that species retreat to, persist in and potentially expand from under changing environmental conditions, and are usually places providing environmental heterogeneity and climatic stability as regional environments change [136]. Genomic data can be used in combination with ecological data and species distribution models to identify places where populations of a species have persisted through periods of climatic instability and maintained genetic diversity (D3 in Figure 2). Such places become candidate refugia for the species under future climate change. Genetic signatures of refugia have often been detected using organelle markers [137,138], but as noted above, lineages that have undergone bottlenecks over relatively recent geological timescales (e.g. glacial cycles) can also be identified using high numbers of neutral loci, which can accurately reconstruct temporal changes in effective population size skyline plots [139]. For example, in

antbirds in the Brazilian Atlantic Forest, genetic studies showed that populations in areas with high last glacial maximum (LGM) stability exhibited long-term population growth, whilst populations in less climatically stable regions showed strong demographic fluctuations, supporting previously hypothesised refugial areas [140]. Ongoing work incorporating these genomically estimated demographic effects of climatic changes with spatial modelling is likely to improve future estimations of extinction risk [141]. Climate change in the last glacial maximum (approximately 21 kya) can also be used to project future refugia utilising spatial modelling approaches (see below). Once refugia have been identified, they should be secured from further threat (Action 2 in Figure 2).

Measuring genetic diversity across landscapes: landscape genomics and beyond

Do populations have enough genetic diversity for an evolutionary response?

Conservation geneticists working on threatened species and other key species maintaining ecosystem function have largely focussed on selectively neutral variation to this point, in part because of its ability to provide unbiased estimates of demographic factors like population size, random drift, mutation and migration. The level of neutral variation in threatened and non-threatened species can also provide an indirect but reasonable indication of adaptive diversity when this is dependent on factors like population size [142,143]. Adaptive variation is much more difficult to measure directly because it requires either linking variation in specific genes to adaptive responses or assessing the extent to which variation in traits under selection is genetically determined (heritability and evolvability). Therefore, overall genetic diversity has generally been taken as a reasonable proxy for the small fraction of the diversity that is functionally associated with higher adaptive potential under climate change. However, population and quantitative genomics also now offer some powerful new ways to probe for adaptive variation (D4 in Figure 2).

Population genomics can distinguish particular loci showing signatures of selection from the genomic background, identifying whether adaptive genetic variation is present in the organism. The data required usually involve genome-wide sequencing of multiple genomes from the species in question, although various sampling designs are appropriate depending on the precise nature of the organism and the question. For example, some sampling designs are better able to measure linkage disequilibrium than others, and some designs also allow timeframes for selection to be estimated. The bioinformatics approaches are well established and have been used successfully in many cases, e.g. [144-149]. A key finding of early studies has been that genomic landscapes appear as

mosaics, with some regions providing signatures diagnostic of various forms of positive and balancing selection, and others comprised of apparently neutral or near-neutral diversity [150-152].

This population genomic approach does not itself elucidate the precise targets of selection (which may be in large tracts of the genome spanning coding or regulatory regions) or the specific nature of fitness differences. However, two major quantitative genomics techniques are available that enable genomic data to be linked to the phenotype. In both cases, data are required jointly on genomic and phenotypic variation, either from population samples (GWAS) or crossing experiments (for QTL mapping). A variety of experimental designs are used, mostly based on samples of individuals but in some cases on pooled samples, and either WGS or various RRS, DNA enrichment (DE) or other sequencing strategies can be deployed (Table 1). Issues including the level of linkage disequilibrium and population structure in field populations and ease of breeding and productivity in laboratory crosses will determine which approach is taken and the specifics of the design, but there is a large body of literature to guide such studies; see discussion and references in [153]. Whilst laborious, these experiments are being used more frequently and becoming cheaper. Examples of climate-related phenotypes that have been mapped to particular genetic variants by these means include life history adaptations in various populations of *Arabidopsis* [154] and *Parus major* [155] and tolerance to desiccation resistance [156] and thermal stress [157] in *Drosophila*.

The welter of transcriptomic and other functional genomic studies now being conducted on a wide range of organisms is rapidly expanding our understanding of both the potential functions of particular sorts of genes and their networks of functional relationships [158]. Comparative genomics is helping to identify syntenic blocks and gene families which have expanded or contracted in association with particular ecological niches or adaptations such as frugivory in bats [159] and sensitivity of honeybees to insecticides [160]. A rapidly increasing number of studies are using such approaches to provide functional links between components of the genome and climate-related phenotypes, e.g. [131,161]. We anticipate a time when the results of genome-wide scans of sequence variation will be interpretable in these specific ways.

When assessing changes in genetic variation, museum and herbarium specimens can provide access to temporal series of collections or other material that for various reasons cannot be obtained otherwise. The technology for retrieving data of usable quality from such specimens has improved substantially, e.g. [162], and it has already enabled several studies showing progressive changes in gene frequencies in various organisms over time frames out to about 100 years, e.g. [112,163]. This may provide unique

clues about recent genetic changes, be they losses of genetic variation or of positive selective processes already underway. In either case, they will be important inputs into decisions about interventions such as conservation translocations, both within (reinforcement translocation) and external to (assisted colonisation) species' current ranges ("Defining translocations" section, actions 4 and 6 in Figure 2) and enforced hybridisation (action 7 in Figure 2) discussed further below.

If a species is assessed as harbouring adequate levels of genetic variation, then no direct management actions should be automatically triggered, although ongoing monitoring of genetic diversity can ensure the levels remain sufficient for adaptive responses (action 1 in Figure 2). If some populations harbour more genetic diversity than others, then understanding how that diversity is distributed across the species' range, and the extent to which some populations have adapted to local climatic conditions, may lead to specific management actions (action 3 in Figure 2).

Although genomics provides powerful ways of assessing adaptive and neutral genetic variation, links between the different types of genetic diversity and adaptive capacity can only ultimately be established through phenotypic association studies. Whilst the presence of genetic variation in loci generally, and in those likely to be involved in adaptive changes, can highlight the potential for evolution, it does not necessarily indicate the extent to which phenotypes in populations can be changed by selection.

Do some populations have high genetic diversity?

Understanding how overall genetic diversity is partitioned among populations across a species' range is critical in predicting the adaptive capacity of the species (D5a and D5b in Figure 2, Table 1). Additionally, it informs about the potential for migration to facilitate persistence under climate change [149]. The approaches described in the sections below allow the identification of species that harbour low diversity in some populations and "hotspots" of genetic diversity in others. The latter are obvious targets for conservation and useful source populations for reinforcement translocations ("Defining translocations" section). Low-diversity populations, however, may have low adaptive potential under climate change and may be targets for improving connectivity (see "Gene flow" below, Figure 2 D6) or reinforcement translocations (action 4 in Figure 2, "Defining translocations" section) to increase diversity. The various sequencing strategies outlined above for population and quantitative genomics within populations are also applicable to samples from different populations and, as illustrated below, have often revealed significant differences in divergence levels across the genome.

Are some populations already adapted to local climate?

Where populations differ in their allelic composition, tests for genetic divergence deviating from theoretical neutral expectations can be applied to detect local adaptation (D5b in Figure 2). Quantifying local adaptation is important because this can indicate whether populations already possess genetic variation that could allow persistence under climate change. Measures of population differentiation such as Wright's F_{ST} are commonly used as a metric for local adaptation in methods for detecting adaptive divergence that can include explicit assumptions about demographic history [164-166], although this approach can indicate an excessive number of apparently adaptive loci if assumptions about demography are incorrect [167]. Similarly, tests for consistent differences in the frequencies of alleles between replicated pairs of populations such as the Cochran-Mantel-Haenszel (CMH) test can be used to identify locally adapted loci where population pairs are compared for a common selective constraint [96]. Relative rate tests such as the McDonald-Kreitman (MK) test permit comparisons of diversity within populations to divergences between them (or from related species), where departure from theoretical ratios for neutral loci can imply local adaptation [168,169]. Software is becoming available to allow many of these well-known tests to be carried out on genome-scale data [170-172].

For widespread species whose geographic ranges encompass environmental gradients, the association of allelic variation among populations (or individuals) with environmental factors can also be an indicator of local adaptation, as long as neutral patterns of genetic variation are taken into account [147,173-175]. Several statistical approaches have been developed to test for such associations, many of which incorporate information on demographic history utilising general linear models [176], logistic regression [177], generalised estimating equations [178] or other types of models [179-181]. Central to these analyses is that demographic history is explicitly accounted for, in order to avoid erroneous conclusions of adaptive divergence in allele frequencies [167]. These approaches have identified genomic regions differentiated across climatic gradients, such as four regions repeatedly associated with minimum temperature in the alpine plant *Arabis alpina* [178] and five regions associated with precipitation in the alpine plant *Campanula barbata* [182]. However, isolating the specific environmental factor responsible for spatial genetic variation can still be challenging because different factors will often be spatially correlated.

The above approaches highlight ways to identify genomic regions that are involved either in historic adaptation to longstanding ecological gradients [92] or adaptation to recent environmental change including from anthropogenic

sources [93,183]. This can help distinguish populations that may be at risk due to a lack of adaptive diversity from those which already possess genetic variants that could allow persistence under climate change. However, unless the contribution of specific genes to the size of adaptive shifts is known and the nature of environmental variation linked to the genes has been clearly identified, these types of approaches cannot indicate the rate and extent of an adaptive response possible across a species' range. As with the intra-population variation considered above, quantitative genomics is still needed, both to narrow down the genomic region specifically responsible for the adaptive phenotypic differences and to assess the size of phenotypic effects associated with particular regions.

Is gene flow high enough (or too high)?

If genetic variation has been identified in certain populations of a species that could help other populations adapt to climate change, then it is important to determine whether there is an appropriate level of gene flow between populations (D6 in Figure 2). Gene flow can aid adaptation by increasing genetic variation and/or by introducing better adapted genotypes. Interbreeding with divergent individuals migrating into a population can also generate entirely new genotypes that may be better suited to tolerating the novel conditions expected under climate change [184-186]. Whilst gene flow usually does improve adaptive capacity, high levels of gene flow can also result in a loss of local adaptation and reduction in population fitness [187], although empirical evidence for deleterious gene flow is still limited [44].

Natural or anthropogenic barriers as well as habitat fragmentation can disrupt gene flow by preventing the migration and dispersal of individuals. Topographically complex landscapes with sharp environmental gradients may drive local adaptation and produce regions containing genotypes adapted to different conditions. This in turn may lead to isolation by adaptation—i.e. the exclusion of immigrating individuals from the breeding pool due to higher fitness of local genotypes [188,189]. These landscape-scale processes can also lead to reproductive isolation—such as through mating or flowering phenology—resulting in little or no effective gene flow between geographically close populations [190-192].

Historical and contemporary gene flow between populations can be accurately estimated using genomic data. For example, high contemporary gene flow as well as local adaptation in red abalone has been identified utilising SNPs discovered through transcriptome sequencing [193], whilst historical gene flow between closely related species of *Heliconius* butterflies was identified using targeted enrichment sequencing [99]. RAD sequencing has identified genetic isolation among populations of herring [63] and speciation in cichlid fishes [100]. Gene

flow estimates utilising RAD-seq-derived SNPs detected inbreeding in wild harbour seals, suggesting isolation between natural seal populations [194]. Some of these genomic studies on gene flow, e.g. [99,193] are pointing to highly heterogeneous rates of gene flow across the genome; intra-population and quantitative analyses as outlined in previous sections are then invaluable in ascertaining the adaptive significance of such heterogeneity.

If key populations have been shown to be isolated from adaptive variation, or from high overall variation located elsewhere in the species' range, then the next decision is to determine whether migration pathways can be restored (D7 in Figure 2).

Genetic opportunities—managing for diversity and adaptive capacity

The previous part of the framework deals with the importance of genetic diversity to evolutionary responses to environmental change, how to infer adaptive capacity from measures of genetic diversity and the potential importance of gene flow. The next part considers the potential of more active interventions for species for which the actions outlined to this point are unlikely to be sufficient.

Can degraded landscapes be restored to enhance gene flow and adaptive shifts?

Landscape revegetation is a major programme of activity to address climate change worldwide [195,196]. The aim is generally to restore fragmented and degraded landscapes, thus enhancing the scope, quality and accessibility of key refuge areas for both key species and whole communities [197]. However, little effort is currently invested in assessing the adaptive potential of the trees and shrubs that have been planted and hence the likelihood that they will persist under climate change. At present, most revegetation efforts revolve around the notion of local provenancing, where germplasm is collected from neighbouring areas on the assumption that it is adapted to local conditions. If there is strong local adaptation, then this approach will facilitate short-term establishment, but it may not be the best approach in the longer term, given changing environmental conditions. Compounding the issue, local provenancing often results in seed collections from small local populations that are genetically depauperate [198,199], leading to low genetic variation with inadequate potential for adaptive response to future changed conditions [200,201].

Genomics, in conjunction with functional trait analysis, can play a major role in addressing these issues, as it helps characterise climatic adaptation potential (D7 in Figure 2). Whilst most landscape-scale revegetation programmes do not undertake breeding or selection for specific traits, assessment of genomic sequence variation in

natural populations or provenances under consideration as seed sources can provide important information on standing genetic diversity and, in due course, on adaptive variation in particular regions [202]. In revegetation programmes using foundation species where some selection may be feasible, identification of gene variants that have been targets of environmental selection may be used to guide selections for alleles that may be best suited to projected environments. Such genomic approaches are being undertaken in an increasing number of key species in revegetation programmes in Australia (Table 2).

Several provenancing strategies involving assisted gene flow have been suggested in the context of climate change. Predictive provenancing requires identification of the predicted climate at a certain point in the future and sourcing seed from sites where that climate currently occurs [197]. Composite provenancing involves mixing seed collected from increasing distances away from the site to maximise genetic diversity and mimic natural gene flow [198]. Admixture provenancing suggests collecting seed from a range of environments without regard to the local site conditions [200]. Climate-adjusted

Table 2 Examples of application of genomic approaches to conservation and revegetation of some Australian trees and shrubs

Species	Who	Current status	Genetic/genomics studies	Actions
Grey box (<i>Eucalyptus microcarpa</i>)	The University of Melbourne, CSIRO	Part of threatened vegetation community	Landscape genomics, including effects of fragmentation, and gene trait association	1) Identification of adaptive variation can be applied to guide selection of climatically adapted material based on projected changes to niche envelopes
Yellow box (<i>Eucalyptus melliodora</i>)	Australian National University, CSIRO, Department of Parks and Wildlife	Part of threatened vegetation community	Landscape genomics and gene trait association	
River red gum (<i>Eucalyptus camaldulensis</i>)	CSIRO	Not currently threatened, but at risk due to river regulation	Landscape genomics, landscape transcriptomics and gene trait association [203]	2) Monitor standing diversity in selections to maintain adaptive potential
Ironbox (<i>Eucalyptus tricarpa</i>)	Department of Parks and Wildlife, CSIRO, Edith Cowan University, University of Tasmania	Important species in fragmented communities of south-eastern Australia	Landscape genomics and functional trait assessment across climate gradient [202,204]	3) Guide selections with respect to neutral population structure and gene flow
York gum (<i>Eucalyptus loxophleba</i>)	Department of Parks and Wildlife, CSIRO, Edith Cowan University, Australian National University	Important species in fragmented communities of south-western WA (SWWA)	Landscape genomics and functional trait assessment across climate gradient	4) Screen existing plantings—locally sourced—to assess their resilience based on the above criteria
Gimlet (<i>Eucalyptus salubris</i>)	Department of Parks and Wildlife, CSIRO, Edith Cowan University, Australian National University	Dominant species in Great Western Woodlands of SWWA	Landscape genomics and functional trait assessment across climate gradient [205]	
Jarrah (<i>Eucalyptus marginata</i>)	Department of Parks and Wildlife, Australian National University, CSIRO	Dominant species in jarrah forest system in SWWA	Landscape genomics and functional trait assessment across climate gradient	
Marri (<i>Corymbia calophylla</i>)	Department of Parks and Wildlife, University of Western Sydney	Major co-dominant species in forest and woodlands in SWWA	Landscape genomics and functional trait assessment across climate gradient	
Swamp peppermint (<i>Taxandria linearifolia</i>)	Department of Parks and Wildlife, Warren Catchment Council	Patchy distribution in riparian vegetation along river systems in SWWA	Landscape genomics across river catchment and climate gradient	
Native willow (<i>Callistachus lanceolata</i>)	Department of Parks and Wildlife, Warren Catchment Council	Patchy distribution in riparian vegetation along river systems in SWWA	Landscape genomics across river catchment and climate gradient	
River-bank Astartea (<i>Astartea leptophylla</i>)	Department of Parks and Wildlife, Warren Catchment Council	Patchy distribution in riparian vegetation along river systems in SWWA	Landscape genomics across river catchment and climate gradient	
Waratah (<i>Telopea speciosissima</i>)	University of Western Sydney, The Royal Botanic Gardens, Sydney	Patchy distribution along an environmental gradient from coastal area to inland areas	Common garden experiments, landscape genomics across climate gradients [206]	

provenancing involves sourcing seed from sites along the projected direction of climate change [202,207]. Climate-adjusted provenancing has the advantage of simultaneously mixing seed sources to increase genetic variation and recruiting from populations likely to be adapted to future climates without needing to target any particular population specifically. This approach is particularly suitable for species with long generation times, where the impacts of climate change will be felt within a generation, and maximising the adaptive diversity in the gene pool is essential to future population persistence.

Assisted migration approaches, such as the various provenancing strategies outlined above, may be critical to maintain ecosystems under climate change [207]. However, it should also be noted that the genetic potential of seed sources is just one of the many issues that require management in revegetation programmes (e.g. soil symbionts, disease, weed risk), and there are decision frameworks available for managing these issues [199,208,209] that can be applied in a wider climatic context.

Potential for naturally occurring hybridisation and introgression

If no populations within a species harbour adequate genetic diversity, the next step is to consider whether ongoing evolutionary responses to climate change might be enhanced by naturally occurring hybridisation with closely related species (D8 in Figure 2). Hybridisation has been shown to play a role in moving adaptive gene sets between closely related species, and in such cases, it will alter predictions for future phylogenetic diversity as well as the adaptive capacity of species, e.g. [210-213]. If hybridisation is as common and evolutionarily significant in natural systems as many researchers now consider, it could help species modify their phenotypes rapidly enough to accommodate current rates of environmental change. In the past, statistical evidence for determining the extent of hybridisation in nature has been difficult to obtain because patterns of genetic variation caused by hybridisation look similar to the patterns of genetic variation caused by the incomplete sorting of alleles that can accompany species divergence [212,214,215]. However, analytical approaches that use genome sequence data for robust inferences of hybridisation have recently been developed [215-219], which should help to better understand the extent and adaptive significance of hybridisation in nature.

Importantly, next-generation sequencing (NGS) sequencing of species complexes is showing that adaptive differentiation and introgression do not necessarily involve much of the genome. With our own species, researchers estimate that whilst less than 7% of our genome is introgressed with the DNA of extinct hominid species, the captured DNA sequences have helped humans adapt to a

variety of climates and resist pathogens [211,212,218]. Although “genomic extinction” resulting from hybridisation in nature has been suggested by some researchers to occur where endemic species are replaced by invasive species that acquire endemic adaptations [220], genetic rescue by interspecific hybridisation need not abolish local adaptations. The genetic basis for many of these is likely to be concentrated in islands of adaptive divergence, such as seen in Eurasian *Ficedula* flycatchers [151] and *Heliconius* butterflies [221]. In humans, recent evidence has emerged that there is strong selection against regions of introgressed genomes that are not advantageous, including selection against genes that reduce the fertility of hybrids [218].

Several studies are also now capitalising on inexpensive NGS-based transcriptome analyses to dissect hybridisation and the impact that introgression events have on ecological diversification and reproductive compatibility of plant species (e.g. *Helianthus* sunflowers [222], tomatoes [223], alpine cress [127] and *Senecio* [224]). Biotic and abiotic stress response genes are commonly implicated in ecological diversification and adaptation. Whilst gene expression differences for such genes occur between parent species and hybrids, there is little evidence at this point for regulatory incompatibility between the respective genomes of closely related hybridising species. In an informative study, Moran and Fontdevilla [225] followed up full genome sequencing of two hybridising *Drosophila* species with a QTL analysis of the (incomplete) post-zygotic reproductive barriers between them. They successfully mapped several loci contributing to those barriers and showed they acted cumulatively according to a polygenic threshold model. That is, sterility was more a function of the extent of genetic divergence of the parent species’ genomes than the action of major hybrid sterility genes. Such findings could have important implications for genetic rescue efforts that consider breeding between genetically isolated populations and species, e.g. [226-228], but many more studies will be needed before generalisations will emerge.

Do climate refugia outside species’ current ranges exist and can species reach them?

From an ecological perspective, climatic refugia are often defined as those areas where the projected future environment is most similar to the current environment of a species or community [229] or where environmental and spatial heterogeneity maintains microclimatic variation as regional environments change [136] (D9 and D10 in Figure 2). The premise is that such sites are likely to serve as important refugia for species that are unable to adapt to the novel conditions projected under climate change. Identification of refugia is modelled for individual species using species distribution models as discussed

previously or continent-wide for functional groups of organisms using community modelling [2]. Such refugia can be identified using a range of pattern- and process-based characteristics, including climate projection models, combined with information about current environmental attributes, to estimate the scale of change expected across the landscape and the overall similarity between areas of current and future landscape. For instance, Dunlop et al. [2] modelled the scale of novel environments expected under climate change across the Australian continent, in order to estimate the areas likely to have the least amount of change from their current climate. These projections were then used to estimate how well current environments are represented in the National Reserve System of Australia under future environments and thus how well the reserve system protects biodiversity over the longer term. These methods estimate refugial areas that are inside as well as outside the current range of species, which are important to persistence as long as the species are able to disperse to them and there is vacant ecological space [230].

Once such refugia have been identified, the next management step is to ensure that they are secured against threatening processes (action 5 in Figure 2), such as by inclusion in protected areas, e.g. [2,229]. The key question then is whether the species of concern will be able to reach them and be able to establish there. Modelling approaches estimating the velocity of climate change [231] provide an estimate of the scale of effort required for a species to reach a refugium. Information about current levels of gene flow combined with information about movement pathways, and the extent of landscape

fragmentation/revegetation, is likely to assist in understanding whether species are able to reach refugial areas. If it seems unlikely that the species in question will be able to colonise such refugia because of barriers to gene flow, low rates of migration or the absence of vacant space, assisted colonisation might be considered (Table 3, action 6 in Figure 2).

Last ditch efforts for critical species

Can assisted colonisation, enforced hybridisation and ex situ conservation help?

This section considers interventions for threatened species or populations that have failed or are likely to fail to persist with the management options above and are at, or approaching, endangered or critically endangered status (D11, D12 and D13 in Figure 2). It deals first with translocations aimed at restoring levels of genetic diversity and adaptive capacity within a species' range (reinforcement translocations) ("Defining translocations" section, action 4 in Figure 2). Weeks et al. [199] define these types of translocations as genetic rescue (where the aim is to rescue populations from the genetic effects of inbreeding and associated loss of genetic diversity and inbreeding depression) or genetic restoration (where the aim is to restore levels of adaptive genetic diversity via ongoing translocations from the source population). Note that some of the provenancing strategies considered in the section on revegetation above also have elements of genetic rescue/restoration, the key difference being that the species in question for revegetation are not themselves endangered and the focus for conservation. This section then considers translocations aimed at

Table 3 Definition of terms used in translocations

Term	Definition
Translocation	Human-mediated movement of living organisms or their genes from one area, with release in another. Translocation is an overarching term
Conservation translocation	Intentional movement and release of a living organism or its genes where the primary objective is a conservation benefit: this will usually comprise improving the conservation status of the focal species locally or globally and/or restoring natural ecosystem functions or processes. It can cover translocations either within or outside the species indigenous range
Population restoration	Any conservation translocation within indigenous range and comprises reinforcement and reintroduction
Reinforcement	Part of population restoration. The intentional movement and release of an organism into an existing population of conspecifics. Reinforcement aims to enhance population viability, for instance by increasing population size, by increasing genetic diversity or by increasing the representation of specific demographic groups or stages
Reintroduction	Part of population restoration. The intentional movement and release of an organism inside its indigenous range from which it has disappeared
Conservation introduction	Intentional movement and release of an organism outside its indigenous range. Consists of assisted colonisation and ecological replacement
Assisted colonisation	Part of conservation introduction, involves intentional movement and release of an organism outside its indigenous range to avoid extinction of populations of the focal species
Ecological replacement	Part of conservation introduction involves intentional movement and release of an organism outside its indigenous range to perform a specific ecological function

Definitions (but with addition of genes) according to IUCN (2013) Guidelines for reintroductions and other conservation translocations, version 1.0., IUCN Species Survival Commission, Gland, Switzerland.

hybridising evolutionary significant units [232,233] and sub- or sibling species (assisted colonisation) (action 7 in Figure 2). Finally it discusses the last resort option of *ex situ* conservation (e.g. captive breeding or seed nurseries), which Weeks et al. [199] term genetic capture (action 8 in Figure 2).

Genetic rescue and genetic restoration are appropriate where a key population of a species or subspecies has fallen to such low numbers <1,000 [199,234] that the exposure of genetic load through inbreeding becomes a significant fitness issue (inbreeding depression) compounding the challenges of adapting to a changing environment. Both genetic rescue and genetic restoration involve the translocation of individuals from another, larger population of the species, usually aiming for up to 20% gene flow from the source population [199,235] and, in the case of genetic restoration, also aiming to continue gene flow through ongoing translocation at a rate of at least one effective migrant per generation, which is thought to be enough to reduce the disruptive effects of genetic drift [236]. The goal is to reduce genetic load, inbreeding depression and the detrimental effects of genetic drift whilst also, as with genetic adaptation above, enhancing the prospects for successful adaptation to the changing environment by boosting genetic variation and the opportunities it provides for generating novel recombinants. Hedrick [235] has shown that gene flow of up to 20% into a recipient population is not likely to swamp locally adapted alleles, particularly those under strong selection. Such genetic rescue/restoration has been successful in several recent cases, such as the Florida panthers, greater prairie chickens in North America, adders in Sweden, South Island robins in New Zealand and mountain pygmy possums in Australia [227,237,238]. However, as with translocations for genetic adaptation above, it is still contentious and has been underutilised as a tool in the conservation of endangered species.

Genetic rescue and restoration translocations have partly been underutilised due to concerns around preserving “unique” genetically distinct populations and avoiding outbreeding depression. But uniqueness in endangered populations and species is more likely to be a result of drift processes than mutation alone [239] and the risk of outbreeding depression has clearly been overstated [240]. At any rate, the markers generated using NGS technologies will be more informative than neutral markers (e.g. microsatellites) for differentiating between populations that are adaptively unique, compared with those populations that have lost variation through drift processes by identifying loci under selection [146]. Similarly, NGS might give greater insight into the likelihood of inbreeding and outbreeding depression by assessing the number of genomic regions that are adaptively unique within source and recipient populations, and that decrease fitness, e.g. [241].

Concerns about conserving genetic integrity, and problems with outbreeding depression, become more pronounced when the only option available for genetic rescue involves translocating individuals from a different subspecies or species. However, the increasing pressure from climate change and other drivers of widespread environmental change mean that the potential risks of such genetic rescue are increasingly outweighed by the opportunity to rescue species or subspecies that would otherwise disappear altogether. Whilst it is generally only enacted when population sizes have fallen to a few individuals, there have been some significant successes with this strategy. The classic case of genetic rescue involved the Florida panther (*Puma concolor coryi*) where the introduction of eight female pumas from a different subspecies (*Puma concolor stanleyana*) from Texas restored depleted genetic diversity, reversed inbreeding depression and increased population size [242]. Similarly, the Norfolk Island boobook owl, *Ninox novaeseelandiae undulata*, was reduced to a single female in 1986, and the deliberate introduction of two males of its nearest relative (the New Zealand boobook, *N. n. novaeseelandiae*) saved this subspecies from extinction, albeit in hybrid form [243]. Clearly, there are instances when such radical translocations can save endangered species (or at least some of their genetic history), and more thought needs to be given as to how NGS technologies might better inform about when these instances will lead to success (e.g. by examining patterns of adaptive diversity, developing better estimates of divergence at adaptive loci, etc.). NGS monitoring in the first few generations after the initial hybridisation might also suggest possible further interventions to maximise adaptation.

Another last resort option for endangered species involves *ex situ* conservation. Genetic issues are critical in this option but genetic input into the management of captive breeding/seed nursery programmes has generally been based on relatively few neutral markers [244]. As already noted, genomics can now provide much more comprehensive coverage of neutral markers and give new insights into important adaptive processes. The data quality can also be improved by having access to a reference genome from the target species (or related species) and, as already noted, the costs of this continue to decrease, making it a viable option for conservation programmes.

More specifically, NGS resequencing will permit genetic relatedness among individuals to be accurately estimated across the genome. This in turn will enable better decisions, both in captive breeding strategies and in the selection of individuals for release back into particular field populations [146]. This increases the chances of avoiding inbreeding depression and perhaps even increasing adaptive variation both in captive and natural populations. The prospects of avoiding some of the

specific deleterious fitness effects that have plagued *ex situ* captive breeding programmes [245,246] are also improved, for example, by ensuring maximum diversity is retained in key genomic regions related to disease resistance and by reducing the frequencies of alleles that have been associated with mating incompatibility or specific recessive conditions, either in the species under study or in others [244]. Changes in gene frequencies over generations of captive breeding can also alert managers to avoid alleles that may be associated with adaptation to captivity and to compare the genetic composition to natural populations, either overall or in specific localities targeted for reintroductions [244]. As knowledge of gene function improves, it may be possible to identify and select for alleles associated with particular environments (e.g. desiccation resistance, drought tolerance or phenology that may be required under future climate scenarios [146]).

However, it should be emphasised that decisions around assisted colonisation and *ex situ* conservation will involve many considerations unrelated to genetic variation and evolutionary capacity. These include factors like evaluating the impact of removing individuals from source populations as well as the impact of introductions on existing biota in target sites, assessing the likely costs of such translocations within the context of other demands on conservation budgets, and social or cultural aspects such as the value placed by the public on a threatened species.

Concluding remarks

Our framework highlights the potential of genomic studies to contribute to strategies for conserving biodiversity. Both population and quantitative genomics are crucial, aided by, but not dependent on, a good reference genome sequence. However, these genomic approaches do not provide a panacea for the problems in biodiversity conservation under climate change. Their value will be easier to realise in some decision areas than others.

Population genomic data can now be used in relatively straightforward experiments to assess genetic diversity within and between species, to map levels of genetic diversity across landscapes, and to understand the relative importance of neutral evolutionary processes like genetic drift and migration in driving population dynamics. They can also now be used to understand the extent to which genetic changes have occurred as a consequence of natural selection driving local adaptation and to make inferences about the relative importance of evolutionary adaptation versus neutral process in driving patterns of biodiversity across landscapes. As such, population genomic data can provide unprecedented insights into the extent and evolutionary consequences of naturally occurring hybridisation in nature, and to assess and monitor

the outcomes of management decisions that involve translocations, and efforts to restore degraded landscapes and communities through revegetation programmes.

However, the key limitations with population genomic approaches are that they do not of themselves identify the precise genetic variants that causally underpin adaptive responses to climate change, nor do they tell us about the size of the adaptive differences mediated by variation in particular genomic regions. Quantitative genomics, combined with appropriate ecological and quantitative evolutionary work, can address both these issues, although it is challenging in such studies to define complex physiological traits that are relevant to the ecology of species. One major issue with QTL mapping (but not GWAS) is its absolute dependence on managed breeding programmes, which may be not be feasible or affordable in many cases. Where population and quantitative genomics approaches can be undertaken, it may be possible to identify and implement substantively more effective and efficient management strategies for biodiversity under climate change.

Zoos and other breeding establishments will be important resources for the genomics work required for threatened fauna, as they have unique capabilities in rearing and breeding animals and are increasingly concerned with conservation issues. One of their major contributions to date has been in restoration programmes, breeding captive populations of animals for eventual release into the wild. This exercise has often suffered from a low success rate, due to factors such as ongoing inbreeding, genetic adaptation to captivity at the expense of adaptation to wild conditions and so on [247,248]. Whilst avoiding inbreeding and the exposure of deleterious recessive conditions is already a major goal in their breeding programmes, genomic approaches together with evolutionary thinking could provide data which are both more comprehensive and more precise on this point. Zoos could also play a larger role in the future in the quantitative and population genetics needed for evaluating other key management options, such as translocations and hybridisation by, for example, testing the viability and various adaptively important phenotypes of F1 and F2 offspring generated from crosses between populations, subspecies and other taxa.

Herbarium collections, seed banks and botanic gardens could fulfil the same sorts of functions for plants. In addition, where seed material can be maintained across years, there is an opportunity to capture the genetic variation present at a particular point in time and preserve it for later re-establishment of populations [249,250]. Such a resource could provide a valuable source of genetic variation and capture novel genotypes across regions as plant populations adapt to changing environmental conditions [251].

Finally, once genomic approaches become routine components of conservation programmes and restoration efforts, novel ways of thinking about the role of evolution in management programmes to maintain biodiversity and ecosystem functions are likely to emerge. New examples of phenomena like incomplete allele sorting and islands of adaptive divergence during speciation, and various introgression scenarios following hybridisation, have already become evident from the application of genomics to a range of non-model species. New levels of understanding of climate change adaptation and the role of hybridisation in adaptive processes are likely to emerge from this work. This understanding in turn will suggest novel approaches to biodiversity conservation and the maintenance of ecosystem function under a rapidly changing climate.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors contributed to the development of the ideas presented in this review through discussions, as well as providing references and contributing written material. All authors read and approved the final manuscript.

Acknowledgements

This paper arose out of a workshop funded through the Office of the Chief Executive Science Team at CSIRO and the Science Industry Endowment Fund.

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Received: 19 September 2014 Accepted: 17 December 2014

Published online: 28 January 2015

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