REVIEWS

Detecting genetic responses to environmental change

Ary A. Hoffmann and Yvonne Willi‡*

Abstract | Changes in environmental conditions can rapidly shift allele frequencies in populations of species with relatively short generation times. Frequency shifts might be detectable in neutral genetic markers when stressful conditions cause a population decline. However, frequency shifts that are diagnostic of specific conditions depend on isolating sets of genes that are involved in adaptive responses. Shifts at candidate loci underlying adaptive responses and DNA regions that control their expression have now been linked to evolutionary responses to pollution, global warming and other changes. Conversely, adaptive constraints, particularly in physiological traits, are recognized through DNA decay in candidate genes. These approaches help researchers and conservation managers understand the power and constraints of evolution.

Plastic change
A change in phenotypic expression, but not in the genotype, because of environmental change.

Inbreeding depression
A decline in fitness owing to inbreeding that is caused by the expression of (deleterious) alleles in the homozygote state.

Neutral genetic marker
A sequence of DNA that is polymorphic within a population or a species and that is not under selection (for example, a microsatellite).

**Centre for Environmental Stress and Adaptation Research, Bio21 Institute, Department of Genetics, The University of Melbourne, Parkville 3010, Australia.*
‡Institute of Integrative Biology, Eidgenössische Technische Hochschule Zürich, 8092 Zürich, Switzerland.
Correspondence to A.A.H.
e-mail: ary@unimelb.edu.au
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Environments are changing rapidly. There is now abundant evidence for shifts in species distributions and abundances in response to the localized effects of climate change[1]. As the human population continues to increase and the ecological footprint of individuals continues to expand[2], massive local environmental changes are imposed by deforestation, pollution, runoff and erosion, with further potential effects on local climate[3].

The persistence of a species depends on its vulnerability to these environmental changes, which is determined by its genetic constitution and physiological tolerance[4]. Rates of phenotypic change, involving either genetic or plastic changes, are particularly high within anthropogenic contexts[5]. There is a growing appreciation that genetic changes can occur rapidly, as illustrated by recent evolution in response to climate change[6–8]. However, population persistence might not be a universal pattern as there are often low levels of genetic variation in traits that limit the distribution and abundance of species[9,10], and there are potentially large costs involved in plastic changes[11]. Moreover, environmental changes are predicted to decrease population size[12]; as a result, overall levels of genetic variation might decrease, and this — in combination with inbreeding depression — can limit further adaptive responses[13].

Genetic markers could be used to monitor whether environmental changes influence species at the level of DNA sequences, to assess the nature of selection imposed by environmental changes and to assess the potential of populations to respond by evolutionary adaptation. Within a conservation context, this information could be used to identify populations most threatened by environmental changes and even the nature of the environmental threats as experienced by organisms. Neutral genetic markers currently predominate in conservation and management applications of population genetics. These markers inform about population demographic processes and have the potential to measure shifts in population size arising from environmental change[14] and adaptation[15]. But neutral markers have limitations for monitoring the effects of environmental changes on populations because variation in these markers only decreases when population size drops sharply and gene flow is interrupted.

An alternative to neutral markers is to assess the adaptive genes that are directly involved in responding to environmental changes. This has in the past been considered too difficult because we know so little about the candidate loci that underlie responses, even though these markers potentially provide information on the nature of selection on populations, and on the potential of populations to adapt. It is now known that genetic changes at candidate loci might have a profound influence on populations, allowing them to persist under changing conditions. This has been reinforced recently by new clinal analyses and reciprocal transplant experiments showing large survival and reproduction differences among genotypes[16], and by studies showing that single gene polymorphisms (for example, the phosphoglucose isomerase polymorphism in butterflies)[17] can influence population growth rate. The impact of adaptive changes on population processes are also apparent from the

dramatic increases in survival and abundance of pest plants, insects, mites and rodents after evolution of resistance to pesticides and herbicides.

Because of advances in genomic tools to identify genes under selection and biochemical tools to link these genes to phenotypes, there is renewed interest in genetic markers associated with environmental changes for monitoring population responses[18]. Our aim in this Review is to consider recent literature that indicates what might be achieved by studying neutral and adaptive genetic markers. We first update early work on the effects of pollutants and other stresses on genetic variation in populations as measured by neutral markers[14]. Can this approach still be useful in monitoring stressful conditions? We then consider literature on abiotic stress resistance and insights gained into adaptive responses. What approaches are available to generate candidate sets of genes for adaptive responses? To what extent are genetic changes predictable across different populations and species? Can marker changes indicate the types of environmental stressors acting on populations? The final question we consider is whether the potential of organisms to undergo adaptive change might be predicted. Are there signatures of the adaptive potential of populations and species? We will show that genetic imprints of environmental changes are indeed becoming apparent in an increasing number of populations as candidate gene sets emerge. These sets are being identified in plants, insects and mammals, and they have indicated adaptive responses to toxicants, thermal stresses and drought responses, whereas decay in genes is also being linked to a lack of response to such stressors.

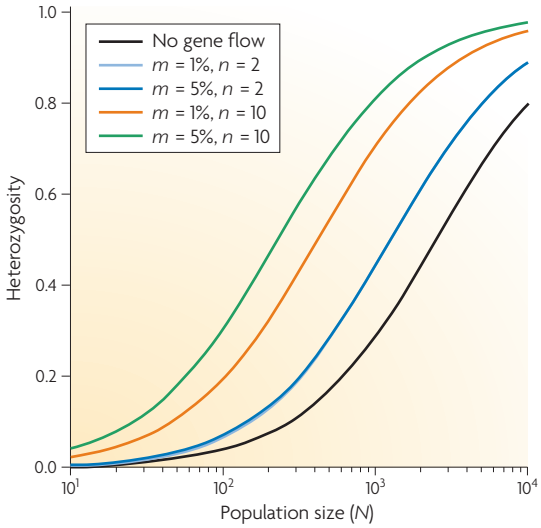

Figure 1 | **Relation between population size and genetic variation.** Equilibrium single-locus heterozygosity in relation to local population size (*N*), migration rate (*m*) and the number of connected populations (*n*) according to the infinite alleles model with a mutation rate of 10^{-4} (REF. 121). The green and blue lines illustrate that heterozygosity rises with increasing gene flow into the population, assuming 10 and 2 connected populations of equal size, respectively[122]. The two curves for $n = 2$ are so similar as to be nearly indistinguishable.

Monitoring neutral variation

If environmental stresses cause a persistent decline in population size and/or a reduction in gene flow, this might result in a general decrease in genome-wide within-population genetic variation, and an increase in between-population genetic variation. Heterozygosity at neutral loci decreases at a rate of $1/[2N_e]$ per generation, where N_e is the effective population size[19], although gene flow can prevent this change under a range of scenarios[13] (FIG. 1). There is good evidence that neutral-marker variation closely follows expectations in laboratory populations[20] and also that this variation decreases in natural populations that are experiencing reduction in size[21–23]. This has led to the hypothesis that neutral markers could be used to monitor pollution effects in populations[14,24]. The use of this approach to detect ecologically relevant effects is illustrated by a recent study in which selection for cadmium resistance in the least killifish (*Heterandria formosa*) led to increased levels of resistance, but also a decrease in genetic variation as measured by microsatellite markers[25].

However, comparisons of natural populations that are exposed to pollution stresses have produced equivocal results[26,27], probably for a number of reasons. First, environmental monitoring by neutral markers only works if the decline in population size is sufficient to affect genetic variation (FIG. 1). Second, populations must be relatively isolated because a small amount of gene flow is enough to maintain high heterozygosity (FIG. 1). Third, when contaminants are introduced into the environment, mutation rates might increase following exposure, increasing rather than decreasing the level of genetic variation at some markers[28]. Finally, the success of detecting population decreases using genetic markers relies on the availability of multiple unaffected control populations. In light of these drawbacks, neutral markers seem most usefully applied to isolated populations of threatened organisms, because such populations often exhibit a reduction in genetic variation before extinction[29]. An example is the reduction in microsatellite heterozygosity detected in the mountain pygmy possum (*Burramys parvus*); a sharp reduction from 0.65 to 0.20 occurred in response to a loss of habitat following ski area development, whereas extensive fires in other populations did not decrease heterozygosity[22].

Using neutral markers to track population declines can be improved by more intensive data collection and new statistical tools. Higher precision might be achieved by serial sampling across a known number of generations and genotyping at several tens of loci with many alleles[30]. On the analysis side, more sensitive methods include tracing changes in the frequency of rare alleles or in ratios of the number of alleles to allele size range[31,32]. These methods are more sensitive because genetic drift reduces the number of rare alleles more strongly than it affects heterozygosity that is dominated by alleles of intermediate frequency. Coalescent methods, likelihood methods[30] and the approximate Bayesian computation method[33] also estimate population declines with more accuracy than classical moment-based methods.

Gene flow
The exchange of genes between populations that is caused by the dispersal of propagules or individuals.

Clinal
Referring to a gradual change along a geographic axis in allele frequencies or phenotypes.

Effective population size
The size of the population that contributes to the next generation — it determines the importance of genetic drift and the amount of inbreeding.

Microsatellite marker
A non-coding section of DNA consisting of short repeats of 1–4 nucleotides.

Coalescent method
A method of reconstructing population history by simulating the genealogy of genes back to the most recent common ancestor of all alleles currently in the population.

Likelihood method
The estimation of an unknown parameter based on its likelihood of producing the observed data (for example, population size is estimated based on a change in heterozygosity).

Approximate Bayesian computation method
A method that compares summary statistics calculated from the data with those produced by a simulation model in which parameters are drawn at random.

Moment-based method
An approach in which the 'true' population parameters are equated to parameters obtained from observed samples (mean, variance and so on).

Box 1 | **Quantitative traits or neutral markers?**

An alternative to measuring the effects of environmental changes through neutral genetic markers is to consider genetic variation in quantitative traits that are easy to measure on individuals. Genetic variation in morphological traits could be readily monitored in populations if animals from populations are routinely trapped and if relatedness among individuals is known. If a quantitative trait has an intermediate heritability and if variation in the trait is controlled by additive effects, this approach can be quite powerful. For example, Carvajal-Rodríguez *et al.*[109] estimate that a single quantitative trait provides the same information as 10–20 neutral genetic markers when assessing within-population changes in genetic variance that is due to pollution or other types of stresses that decrease population size. Estimates of quantitative genetic parameters can be prone to large errors and can vary with environmental conditions. However, accurate estimates can often be obtained in wild populations when relatedness among individuals in a population is known across multiple generations[35]. Quantitative genetic variation in traits can also be assessed in combination with variation at neutral loci to establish if selection is acting on the traits. Populations are compared for the extent to which they differ genetically in quantitative traits (Q_{ST}) and at neutral markers (F_{ST}). Typically, Q_{ST} values are larger than F_{ST} values[36], and this can be taken as evidence for natural selection causing divergence among populations.

The coalescent framework simulates genealogies based on frequency data while accounting for mutation, recombination and migration[34].

Changes in the genetic variance of quantitative traits might also be a sensitive approach for monitoring changes in the genetic diversity of a population exposed to stressful conditions (BOX 1). The advantage of a quantitative approach is that each quantitative trait represents the contribution of multiple loci; therefore, scoring changes in variation in a single quantitative trait is equivalent to scoring variation at multiple loci. However, the technique has not been applied widely because quantitative genetic variation can only be scored when the relatedness among a substantial number of individuals in a population is known. Even so, the technique could be used in situations where pedigree information is available[35]. Patterns of variation in quantitative traits among populations can also be compared with those for neutral markers to determine if selection is acting on populations; selection is implicated when differentiation in quantitative traits is stronger than for neutral markers[36].

Monitoring adaptive markers

In light of limitations in the types of information that neutral genetic markers can provide about adaptive changes, attention has turned to markers that are affected by selection. Initially, the focus was on identifying adaptive markers from model organisms, but adaptive markers are now also being actively sought for many non-model species.

A useful adaptive marker set needs to include loci that contribute to a substantial part of the genetic variance in a trait within and between populations and that undergo a substantial change in allele frequency with environmental change (FIG. 2). If such loci can be identified, there is the potential to monitor allelic changes to understand how selection acts on a population. Each locus should contribute to a substantial proportion of the variance (>5%) to be useful in this context; otherwise, the number of loci needed to characterize a selection response might become prohibitively large and the power to link genetic changes to environmental effects would be low (FIG. 2). Furthermore, candidate alleles that are initially rare might only be recognized after several generations of environmental selection because there is a lag phase in the selection response.

Recent theoretical models of adaptation have highlighted that a mixture of alleles of small and large effects are expected to contribute to adaptive shifts in quantitative traits[37]. The types of alleles favoured seem to depend on conditions such as mutation rate, selection intensity and rate of environmental change. Kopp and Hermisson[38] showed that if the environment changes quickly and imposes strong selection, alleles of large effect and with the highest selection coefficients will be most important. However, if the environmental change is slow relative to the appearance of new mutations, alleles of small effect become fixed first because they are already favoured during the initial stages of change. Because genes of large effect are easier to detect, candidate genes are more likely to be revealed if the environmental change is rapid.

Which alleles will be favoured under selection depends on a number of other conditions. When an environmental change favours alleles that influence a particular trait, these alleles might not increase in frequency because of pleiotropic effects (or close linkage) leading to selection in the opposite direction[39] or because of other constraints influencing directional evolution[40]. Therefore, genes that contribute to an adaptive shift are only a subset of those that contribute to variation in traits in populations. The impact of pleiotropic effects on selection responses has been particularly well documented for the evolution of resistance to pesticides. This might involve alleles that substantially decrease fitness owing to pleiotropic effects being replaced by alleles with few pleiotropic effects, as happened during the evolution of organophosphate resistance in *Culex* mosquitoes[41].

Identifying candidate gene sets

Sets of genes that are candidates for adaptive shifts in response to environmental change can be identified through various genetic tools, including microarrays that measure expression changes, selection experiments that test for correlated responses in candidates, QTL mapping, and comparisons of strains that test for specific genetic associations. Most techniques lead to the identification of candidate sets of loci rather than specific alleles. Nevertheless, the specific alleles that are involved might eventually be identified both in the non-coding and transcribed regions of genes[41–43]. It seems that both regulatory and structural changes in genes, as well as gene duplication and gene loss, can have a role in adaptive shifts in response to environmental changes (BOX 2). Each of the methods used for identifying potential marker genes is discussed below, highlighting advantages and disadvantages (see TABLE 1 for a summary); TABLE 2 provides a detailed example of an adaptive gene set for heat resistance in *Drosophila melanogaster*, generated using a range of approaches.

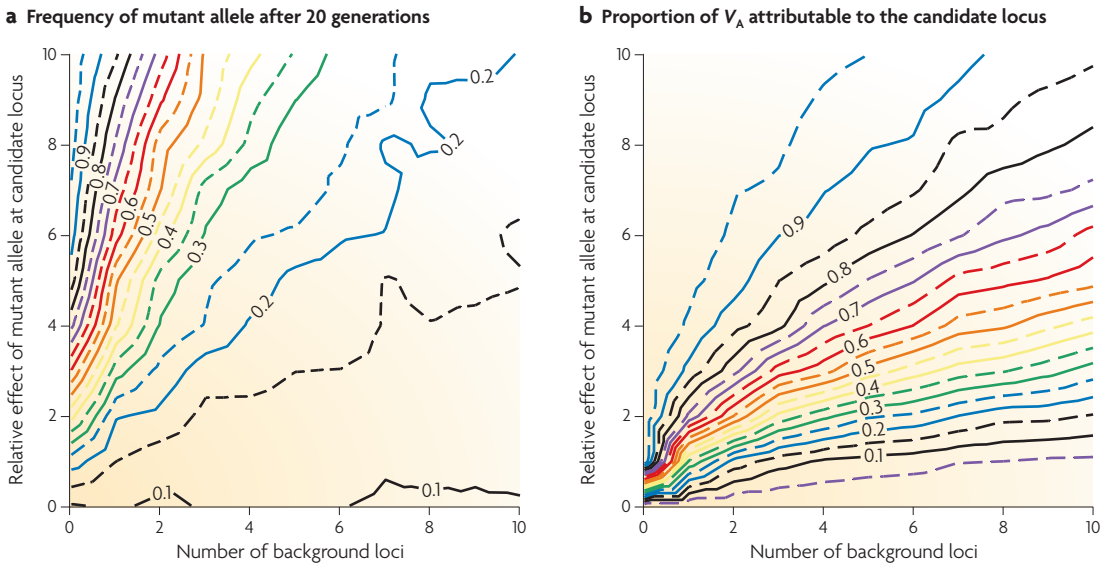

Figure 2 | **Evolution at a candidate locus depending on its effect and genetic background.** The evolution of a beneficial mutant allele at a candidate locus (**a**) and the proportion of total genetic variance (V_A) of a polygenic trait explained by it (**b**) depend on the number of background loci influencing the trait and the effect of the mutant allele on the phenotype. The contour plots are drawn from individual-based simulations in populations of 10,000 individuals with the outcome assessed after 20 generations. Assumptions for background loci were: two alleles per locus with an initial frequency of 0.5 each and gene effect of 0.05, which reflects the fitness advantage of the favoured homozygote relative to the heterozygote. Assumptions for the candidate locus were: two alleles per locus with an initial frequency of the beneficial mutant of 0.1, and its gene effect expressed relative to the effect of the advantageous allele of background loci. The population was diploid, loci were unlinked and alleles acted additively.

A priori *candidates*. Candidate genes are sometimes obvious from biochemical studies and/or previous associations of genetic markers with environmental gradients. In *Drosophila melanogaster*, the alcohol dehydrogenase gene (*Adh*) is involved in ethanol metabolism, and enzyme forms that are associated with common genotypes differ in thermal sensitivity. The frequency of the Adh^S allele increases in natural populations of *D. melanogaster* as temperature rises[44], making *Adh* a candidate gene for monitoring thermal responses. Consistent with this notion, the Adh^S allele that is most frequent in tropical populations has increased in frequency with climate warming over the last 20 years along a cline in eastern Australia[8]. Other allozyme polymorphisms that indicate thermal selection include phosphoglucose isomerase[17,45].

Several allozyme polymorphisms in trees are geographically associated with climatic factors and provide candidates for climatic adaptation[46]. An example is the polymorphism at the glycerate dehydrogenase (*Gly*) locus of *Pinus edulis* from southwestern United States, which is associated with moisture conditions within forests and among mountain ranges[47]. One of the three segregating alleles, *Gly-3*, is more common on drier sites, and its frequency is tightly and negatively correlated with summer precipitation. *Gly-3* homozygotes have longer and narrower stomata than other genotypes[47], and they have higher viability and growth rates on dry volcanic soils[48]. Clearly, *Gly* is a promising candidate locus for monitoring adaptation to changing moisture levels in this species.

Knowledge of physiological pathways can suggest genes that are involved in adaptation to environmental changes. Potential candidate loci for drought tolerance in plants include genes that are involved in the synthesis of abscisic acid (ABA), transcriptional regulators of drought-inducible pathways, or late embryogenesis abundant (LEA) proteins and other stress-related gene products. In *Arabidopsis thaliana*, four independent gene regulatory systems operate in response to water stress, of which two are ABA dependent[49]. LEA proteins are expressed in vegetative organs under water-deficit stress and are involved in the maintenance of protein and membrane structure, sequestration of ions and chaperone-like functions[50]. LEA proteins are generally upregulated under stress[51], and there is now evidence for fixed differences in the level of upregulation among cultivars varying in drought tolerance, suggesting that heritable adaptive changes have taken place in response to past droughts[52].

As knowledge about relevant pathways and gene functions in model species increases, functional roles of candidate genes in relation to specific traits can be tested for. As an example, Pool and Aquadro[53] applied knowledge of pigmentation pathways to identify putative candidate loci for pigmentation variation in natural populations of *D. melanogaster*, whereas Steiner *et al.*[42] followed the same approach in identifying candidate genes controlling cryptic colouration in oldfield mice (*Peromyscus polionotus*). In another example, Rako *et al.*[54] tested candidate genes suggested from a variety of sources for contributions to variation in traits that diverged along a climatic gradient. In fact, recently

Box 2 | **Regulatory or structural changes?**

Comparisons of gene expression patterns between populations emphasize the adaptive changes in the control of gene expression that are due to changes in promoter regions or other regulatory elements. Conversely, traditional allozyme studies have emphasized the importance of allelic changes in genes coding for proteins in adaptation, whereas some of the pesticide resistance literature has emphasized the potential role of duplications in resistance development.

In practice, all of these changes might be involved in adaptive responses involving the same sets of genes. This can be illustrated with respect to *Mt* genes, which encode metallothioneins — proteins involved in detoxifying heavy metals as well as other processes. Recent data suggest that changes in *Mt* gene expression, structural changes in *Mt* genes and the evolution of novel *Mt* genes are all potentially involved in adaptation to heavy metals. Populations of *Orchesella cincta* springtails differ in heavy-metal resistance and this has been linked to the expression of metallothioneins[110]. Differences in expression seem to be due, at least in part, to variation in the promoter region of the *Mt* gene in *O. cincta*[111]. By contrast, in *Drosophila* populations, metal resistance seems to be associated with a duplication of the metallothionein gene[112], and, in populations of other species from metal-contaminated areas, novel *Mt* genes have been isolated[113]. Finally, allelic forms of *Mt* genes are associated with heavy metal responses — in the oyster, *Crassostrea gigas*, field and experimental populations exposed to metals have a high frequency of two alleles for one of the *Mt* genes[114].

The evolution of thermal responses associated with heat shock protein (*Hsp*) genes is also likely to involve changes in expression, structural changes involving *Hsp* alleles and, potentially, gene duplication. Heat shock proteins are chaperone molecules that help to protect other macromolecules from degradation, although some of these proteins have other functions. Expression patterns in heat shock protein genes have been linked with adaptation to thermal environments across a range of organisms, including insects, fish and bacteria[88,115,116]. Variation in the expression of *Hsp* genes might be related to the presence of transposons in promoter regions of the genome, at least in *Drosophila* species[117]. Alleles of *Hsp* genes in *Drosophila melanogaster* might vary in frequency geographically and also change in frequency in response to laboratory selection[118,119]. Duplications in *Hsp70* vary in copy number within and among species of the *Drosophila virilis* group, and copy number relates to thermotolerance[120].

invaded and established latitudinal clines that vary along climatic gradients[55], or newly introduced plants that have become locally adapted to novel conditions[56], are ideal experimental systems for finding candidates genes. By contrast, long-standing spatial patterns of differential adaptation might be less informative because evolution is likely to have happened over long periods of time, during which *de novo* mutations and genetic rearrangements might have been important[57], and multiple drift events might have occurred[58].

Gene expression studies. Microarrays and other techniques for monitoring changes in transcript levels can be used to compare gene expression patterns between environments and between strains of the same species. Microarrays can be applied to non-model organisms[59,60] and they can be used on material sourced from the field as well as that obtained under laboratory conditions[61]. Although their application to non-model organisms has so far depended on the availability of existing arrays for model organisms, breakthroughs in genomics now allow the development of customized, species-specific cDNA arrays[62]. Microarrays are usually applied to monitor plastic responses, but also provide a way of comparing expression patterns in lines selected for altered environmental stress resistance[63] and population comparisons[64].

Gene expression studies can help discover stress response genes when the plastic responses of populations exposed to different conditions are compared. For example, Knight *et al.*[59] compared the responses to water stress of populations of the *A. thaliana* relative *Boechera holboellii* from dry and moist sites. Based on cDNA-amplified fragment length polymorphism (cDNA-AFLP) expression profiling, they identified genes that showed plastic responses to water stress as well as transcripts that were differentially expressed between the two populations and are therefore likely to be involved in adaptive responses to stress. Similarly, Larsen *et al.*[60] used cDNA microarrays in flounders (*Platichthys flesus*) to identify several genes that showed different expression patterns in transplanted fish populations originating from different salinity environments. Around 5% of genes showed different expression patterns between the populations, compared with only 1.4% of genes that responded plastically to salinity. Several of the genes that were expressed differently between populations were expected to influence physiological traits involved in salinity responses.

A limitation of microarray studies is that plastic changes in expression patterns are often not particularly consistent across studies (TABLE 1). For example, Bray[65] compared the results of three microarray studies performed with *A. thaliana* populations under water-deficit stress. About 800 genes were upregulated, summed over the three studies, but only 27 of these were consistent in all studies. For this reason, expression studies aimed at identifying candidate gene sets should focus not only on plastic responses to a stress but also on comparisons of populations that have adapted to that stress[64].

Genetic mapping. QTL mapping can identify genome regions in which adaptive change has taken place, including genetic changes that result in altered protein structure and gene expression. In *D. melanogaster*, regions of the genome controlling resistance to a range of stresses have been identified by crossing lines that have diverged through artificial selection. A large part of the difference between selected and control lines for thermal resistance maps to a region without any changes in expression in genes located within the region[66]. Line differences for starvation resistance map to two genomic regions each of approximately 100 genes[67].

In *A. thaliana*, flowering time — a drought-escape trait with a relatively simple genetic basis and linked to climate-change adaptation[7] — has been the focus of the most intensive QTL studies performed in relation to environmental adaptation[68]. This effort has revealed approximately 28 loci that contribute to natural variation in flowering time under different conditions. Commonly, one to four loci show a moderate to large effect, some loci interact epistatically, and other loci have a detectable effect only under specific environmental conditions[68,69]. Flowering time QTLs have been linked to specific genes by fine mapping and positional cloning, and by linkage disequilibrium mapping[68]. Just recently, selection on some of these genes has been shown in a field experiment[70].

cDNA-amplified fragment length polymorphism (cDNA-AFLP) expression profiling
A fingerprinting method that allows the genome-wide analysis of expression in any species without the need for prior sequence information. The mRNA of an organism is reverse transcribed to cDNA, then cut and fused with small adaptor sequences. The amount of adaptor represents the expression levels of the gene attached to it.

Finding candidate genes by traditional QTL approaches has several shortcomings (TABLE 1), particularly the low resolution of the mapping. Much better resolution for localizing candidate loci is emerging from new techniques that allow a dense map of genetic markers to be generated for model as well as non-model organisms. Such maps can then be used in association studies to link markers to quantitative traits. Dense maps can be generated in model organisms through microarray hybridization or hybridization to a tiling array to reveal numerous sequence polymorphisms, which are then used to generate a dense genetic map[71,72]. Markers that are identified as being associated with traits can be assumed to be either within the gene affecting the trait or in a neighbouring sequence at linkage disequilibrium with this gene, in which case further fine-scale mapping is needed to identify the gene. If a very dense genetic map with markers over the whole genome is available, traits can be directly linked to causative genes in outbred natural populations[73]. In humans and mice, this requires markers tagging genomic regions every 100 kb or so. Within this distance, strong linkage disequilibrium occurs among markers. This means that hundreds of thousands of markers are required for establishing causal associations in many species[74]. When genome sequences and tiling arrays are unavailable in non-model organisms, a high density of markers can

Table 1 | **Approaches available for developing candidate gene sets**

Approach	Description	Strengths	Weaknesses	Refs
A priori *candidates*				
Associations between traits and known candidate gene polymorphisms	Associations between environments, traits and polymorphisms are tested in sets of lines or as correlated responses to selection	Potentially fast when information is available about genetic polymorphism and adaptive function	Gives a biased understanding of environmental change if other, less obvious, genes and traits are overlooked	54
Microarrays, transcriptomics and expression profiling				
Environment-induced changes	Changes in gene expression are scored following exposure to an environmental change	Comparison reveals plastic responses to environmental change. If divergent genotypes are included, it can also trace non-plastic genetic differences. Can be applied to non-model organisms and field-collected material. Sensitive to changes in non-structural genes	Hundreds of genes typically show altered expression, making candidate genes difficult to identify. Microarray results are environment sensitive. Resistance can involve changes in target sites that are not detectable by altered gene expression	61
Selected line or geographic comparisons	Differences in expression patterns are compared across diverged lines			59
QTL analysis				
Mapping based on defined crosses	This approach usually involves crosses among inbred lines	Comparison reveals genetic differences underlying divergence. Gene interactions (epistasis and pleiotropy) can be detected	Gives a biased understanding of environmental change if other important traits are overlooked. The association between QTL and phenotypic trait is environment and genotypic-background sensitive. Leads to underestimation of QTLs of minor effect as well as multiplicity of epistatic QTL effects	66
Mapping based on hitch-hiking	Follows association between markers and traits during divergence			67
Screening for selective sweeps				
Genome scans for population divergence among multiple loci	Outlier markers are identified from increased population divergence	No crosses have to be performed. Can be executed on field-collected material, provided a high-density map is available. Genes with low positive-selection coefficients can be detected if selection has been acting for some time	To reveal the exact position of the gene, the scan needs dense coverage of the genome with markers. The function of the candidate gene remains unknown. The method works best when combined with other methods, such as ESTs	90
Genome scans for decreased population variability	Outlier markers are identified from decreased variability in allele number or allele size within populations			78
Comparisons of nucleotide variability in candidate loci	The spread of the favoured allele in the population decreases the level of nucleotide variability, this is detectable from direct sequencing or microarray DNA typing			79
Screening for mutants				
Screening of mutant and deletion sets	This technique is available in model species in which large sets of mutant or deletion lines are available for screening	A powerful tool to reveal physiological pathways and gene functions	Restricted to model organisms	83
Trait-directed mutagenesis	This approach usually involves ethyl methanesulphonate mutagenesis followed by mapping to identify candidate genes, and has occasionally been applied to quantitative traits			80

Selective sweep
A reduction in genetic variation in DNA that surrounds a locus that is under strong directional selection.

Directional selection
Natural selection that favours phenotypes or genotypes on one side of a distribution.

also be developed. One approach is to cut DNA with a restriction endonuclease to identify polymorphisms in restriction sites, and then further shear the DNA to produce numerous small fragments that act as markers; these can then be scored on custom microarrays[71]. However, using high-density maps in association studies comes at a price; typically, several thousand individuals need to be typed to detect associations between SNPs and species traits, particularly if traits have a low heritability and SNP alleles are at a low frequency[74].

Screening for selective sweeps. Candidate loci can be identified by searching for signatures of selective sweeps in different populations of the same species across an environmental gradient. Methods for identifying genes under selection based on patterns of divergence fall into two categories: those that test whether divergence among populations at specific loci is greater than expected, and those that test whether genetic variation within populations is less than expected[75,76]. Both patterns imply that the loci are under directional selection, which produces genetic divergence among populations and a reduction in genetic variation within populations. Tests for selective sweeps do not require crosses and can be executed on field-collected material, provided a high-density map is available[77]. However, they are only a first step in identifying genes under selection because connections need to be established between the markers and phenotypes that influence field fitness along gradients.

The efficiency of this approach for locating candidate genes can be improved by focusing on genes that are expressed as opposed to markers that are randomly scattered throughout the genome. In sunflowers (*Helianthus annuus*), Kane and Rieseberg[78] examined allelic diversity and variance in allele size (number of short repeat sequences) at 128 microsatellite markers located close to expressed genes, for eight populations originating from four habitats (desert, plains and two types of salt marsh). They identified 17 loci with low diversity and/or variance that are potentially linked to candidate genes under selection. Many of these genes seemed to be under selection in the salt-marsh environment, providing a list of candidates for further testing. The approach can also become more efficient if focused on selective sweeps in sets of candidate genes — such as the *cyp* detoxification genes of *D. melanogaster* that are likely to be associated with selection for resistance to pesticides[79].

Mutant studies. Mutants can be isolated either through screening existing sets of lines (such as lines containing small deletions) for traits of interest, or by generating new mutants *de novo* — typically through chemical mutagenesis. Because hundreds or thousands of lines need to be screened, these approaches are limited to model organisms. They have been applied to traits like insecticide resistance that can be easily scored, although mutant screens have also been completed for physiological traits[80].

Table 2 | **List of candidate genes for heat resistance in *Drosophila melanogaster****

Gene	Name	Evidence	Function	Refs
DMU14395	–	Association	Microsatellite marker, no known function	54
Est-6	Esterase 6	Microarray, geographic	Carboxylesterase activity, involved in reproductive and metabolic processes	123
Gpdh	Glycerol 3 phosphate dehydrogenase	Microarray, geographic	Catalyzes the oxidation of *sn*-glycerol 3-phosphate to dihydroxyacetone phosphate, involved in metabolism and in protein folding and assembly	124
Hsf	Heat shock factor	Association	Regulates the expression of heat shock protein genes	125
Hsp22	Heat shock protein 22	Microarray, QTL	Function is unknown, expression is linked to stress, lifespan and development	126
Hsp23	Heat shock protein 23	Association, microarray, RT-PCR , QTL, geographic	Function is unknown, expression is linked to stress, lifespan and development	123
Hsp26	Heat shock protein 26	Microarray, mutation, QTL, geographic	Function is unknown, expression is linked to stress, lifespan and development	117
Hsp27	Heat shock protein 27	Microarray, QTL	Function is unknown, expression is linked to stress, lifespan and development	125
Hsp68	Heat shock protein 68	Association, microarray, QTL	Binds unfolded proteins, linked to stress and defence responses	127
Hsp70‡	Heat shock protein 70	Microarray, mutation, RT-PCR, association, QTL	Binds incomplete and unfolded proteins, assists protein transport, induced to high levels by stress, linked to stress and defence responses	123
Hsp83	Heat shock protein 83	Association, QTL	Binds unfolded proteins, involved in development, stress and defence responses, macromolecule and organelle assembly	128
Hsrω	Heat shock RNA ω	Association, correlated response, QTL	Non-coding gene that is thought to regulate the synthesis of other heat shock proteins and metabolism following heat stress	127
mth	methuselah	Microarray, mutation, correlated response, selection, QTL	Has G-protein coupled receptor activity, linked to stress responses and longevity	128
Tot‡	Turandot	RT-PCR, QTL	Peptides of unknown function, involved in response to heat and other stresses	129

* The list excludes cases where gene expression was altered in comparisons of selected lines or following heat stress. ‡This includes multiple variants of *Hsp70* and *Tot*.

Transferring markers

To what extent do sets of markers have to be developed for different situations and groups of organisms? Will each evolutionary response be highly species or population specific, or is there some potential to extend markers across different groups? Although gene loss and duplication occurs during evolution, the vast majority of genes are conserved across related organisms; the recent comparison of 12 *Drosophila* genomes shows that 97.5% of protein-coding genes are present ancestrally, despite several million years of independent evolution[81]. Sets of candidate genes therefore have the potential to be applied across related species. However, genomic comparisons also indicate that genes associated with environmental responses, such as chemoreception, detoxification and defence, tend to evolve more quickly and undergo a more rapid rate of loss and duplication[81] and that comparative studies need to test the extent to which such changes have occurred. In microorganisms like *Prochlorococcus* marine cyanobacteria, there are core sets of genes that are conserved, but there are also other genes that are involved in light and other responses that evolve more quickly[64].

Even if there is conservation at the gene level across lineages, responses to selection might be highly unpredictable when different options are available for selection, such that alterations in different genes affect the same trait. This caveat has been highlighted in laboratory populations of model organisms that are adapting to uniform environmental conditions. For example, Matos and co-workers have found consistent genetic responses underlying changes in fecundity in populations of *Drosophila subobscura* adapting to laboratory conditions, but different genetic responses for adaptation leading to starvation resistance and adaptation to altered larval conditions[82].

However, four lines of evidence suggest room for optimism. First, in the case of insecticide- and fungicide-resistance genes that have evolved in response to the presence of chemicals in the environment, there is often a high degree of predictability in terms of the adaptive genetic changes — even across species. Changes in target-site genes that encode resistance to organophosphate and other insecticides have been shown to involve a highly specific set of mutations[75]. In the case of resistance to organophosphates, which occurs through the evolution of insensitive forms of acetylcholinesterase, the same mutation is involved in the mosquito vectors *Anopheles gambiae* and *Culex pipiens*[76]. Sequences of allelic changes, duplications and transposition events can be involved in the development of resistance, but these all occur around the same target gene or detoxification gene, even if they do not involve the same mutations[83]. Similarly, resistance against Qo inhibitor fungicides, which impede mitochondrial respiration, has evolved in many fungal species, and the most common and potent resistance type is caused by a point mutation at the same codon in cytochrome *b*, which causes a glycine to be replaced by an alanine[84]. Interestingly, those species for which such resistance has been sought but not found have an intron just after that codon; splicing of the intron might be hindered by alanine, preventing resistance evolution[84]. This emphasizes the limited genetic options available for different organisms when evolving resistance.

The second line of evidence is that there are universal mechanisms present across organisms for dealing with environmental stresses. Perhaps the best example involves the heat shock proteins, in particular Hsp70, which are linked to heat responses in a variety of organisms ranging from microorganisms to plants, invertebrates and vertebrates (BOX 2). Heat shock proteins are also upregulated in insects following the onset of cold conditions, and this upregulation seems to be essential for increasing cold resistance during diapause[85].

Third, genetic changes in microorganisms appear to have some degree of predictability. In independent lineages of the bacteriophage ϕX174 that were adapting to high temperature stress, a third of the nucleotide substitutions were the same across lineages[86]. Different lines of *E. coli* adapting for 2,000 generations to high temperature stress often showed the same genomic changes leading to an increase in fitness[87]. Changes in expression patterns of genes also show a degree of conservation in these lines of *E. coli*[88], as they do in *Drosophila*[63] and to some extent in *Fundulus* fish evolving resistance to toxic chemicals in the field[89].

Finally, genome scans and mapping experiments often indicate that the same markers or genomic regions exhibit adaptive patterns of divergence in different situations. In the snail *Littorina saxatilis*, the same 15 AFLP markers showed high F_{ST} values (the extent to which populations differ genetically at neutral markers) differentiating two snail morphs along three different clines[90], whereas in whitefish, 6 loci showed parallel patterns of divergence between different ecotypes sampled from different lakes[91]. QTL mapping of thermal responses in *Drosophila* essentially led to the same regions being identified both when crosses involved selected lines from a single population and in crosses between continents[66]. These findings suggest some consistency in responses across conspecific populations, although this has not yet been tested across species as it has been for the first two lines of evidence.

Importantly, predictability — especially in the context of complex environmental change — can be improved by keeping in mind the ecological context. For example, in the face of stressful environmental change caused by drought, adaptation in plants can take two forms: escape or tolerance. An escape strategy is early flowering before the dry season[92], whereas strategies of tolerance include growing bigger roots relative to shoots, improving water-use efficiency and developing different leaf morphology[59]. Whether selection favours these mechanisms might depend on the timing of drought. Heschel and Riginos[93] raised inbred lines of the plant *Impatiens capensis* in a field study. In a year with a late-season drought, they found selection for increased water-use efficiency. In another year with an early-season drought, selection favoured lower water-use efficiency and early flowering. Favoured traits and genes might depend on the nature of selection and pleiotropic effects, such as when flowering time alleles have pleiotropic effects on water use[94].

Once there is information on candidate gene sets and underlying allelic variation, there is the potential to use genetic markers to elucidate the nature of environmental stresses acting on populations. Changes in frequencies of resistance alleles are already being used to argue for the impact of chemicals on non-target organisms[95]. Frequency changes at other sets of loci could indicate the presence and impact of other types of stressors on populations.

Markers for limitations of adaptive potential

There are an increasing number of cases in which evolutionary responses to selection seem to be limited[40], including cases associated with anthropogenic stresses related to pollution[96] and climate change[97]. Many species seem to be at physiological limits in a part of their distribution, with little evidence of evolutionary change to extend these limits[98]. In predicting the evolutionary potential of populations and species to adapt to environmental changes, it should be possible to derive an indication of the likelihood of an adaptive response from genetic data. This might consist of some measure of quantitative genetic variation, such as narrow-sense heritability or the additive genetic variance — low genetic variance or low heritability can translate into a lack of selection response[9,10].

Recent comparative genomic studies provide evidence for gradual DNA decay, pseudogenization and gene loss in classes of genes that are no longer under strong purifying selection. Such genetic variation can no longer contribute to future adaptation, at least in the short term. In microorganisms, a link between ecological niche and pseudogenization and/or gene loss was suggested several years ago[99]. Closely related microorganisms differ markedly in pseudogene number; for instance, the causative agent of leprosy, *Mycobacterium leprae*, has hundreds of extra pseudogenes when compared with its relative *Mycobacterium tuberculosis*, which has only six[100]. Decay in genes that are associated with anaerobic respiration is likely to account for the inability of *M. leprae* to colonize warmer parts of the body.

Links between gene loss or pseudogenization and ecological niche are also emerging from comparative genomic studies of animals. In honeybees, there are decreases in the size of gene families associated with detoxification, which is likely to reflect the managed environment in which bees develop[101]. Compared with both *Anopheles* mosquitoes and *Drosophila*, there are fewer genes encoding classes of detoxification enzymes such as cytochrome P450s and glutathione S-transferases, making bees highly susceptible to many pesticides[101]. In mammals, the receptors that sense bitter taste are encoded by the TAS2R family of receptor genes, which help animals in dealing with poisonous foods. Mice have more functional TAS2R genes and fewer pseudogenes than humans, and in human populations there are segregating null alleles in the remaining functional genes[102]. Bitter-taste genes are now unlikely to be under selection in human populations, owing to low levels of toxic-plant food in human diets. There has also been gene loss and decay in the olfactory receptor genes and detoxification genes of *Drosophila* species that are adapted to highly specialized habitats when compared with more widespread species[81,103].

DNA decay might occur both when a gene is no longer under selection and accumulates mutations, and also when there is direct selection for loss of function. Early flowering in *A. thaliana* is associated with non-functional alleles on the *FRIGIDA* locus[104]. Loss of function of these alleles is presumably due to absent portions in the coding and promoter region[105], and there is evidence that the non-functional alleles have been under positive selection[106]. Once decay occurs, organisms become restricted to particular environments, because too many changes are required to reactivate genes that allow adaptation. The ability to detoxify some compounds is difficult to reacquire once the detoxification genes contain numerous point mutations and indels. Rather than being driven solely by selection and adaptation, ecological specialization might therefore be at least partly driven by DNA decay, which limits the evolutionary potential of species. Genes with environment-specific effects, like those involved in chemoreceptors and cytochrome P450s, are more likely to evolve into pseudogenes[99]or undergo loss from the genome[81], and thereby limit niche breadth. The presence of decay in these genes is a slow process but it provides rapid signatures for the potential of species to adapt to environmental changes, such as a lack of genetic variation. As candidate gene sets develop for traits, these might in turn be used to indicate the potential for adaptation in a range of species from different environments.

Conclusions and future perspectives

We now have a much better understanding of the ways in which variation in genes influence traits under selection in natural populations, and there are an increasing number of cases where changes in candidate markers have been linked to adaptive shifts. Alleles in structural or regulatory sequences of genes, or sometimes duplicated sequences, are favoured in new environments and lead to rapid adaptation. Rapid genetic changes have been detected in a few traits in response to environmental shifts, and these are likely to provide additional material for testing and isolating new genetic markers for monitoring adaptive changes. These include rapid changes in photoperiod responses in pitcher-plant mosquito species[6], rapid changes in flowering time in an annual *Brassica* species[7] and adaptive changes in acid tolerance of copepods in response to acid levels in lakes[107]. As sets of candidate genes emerge in model organisms, they can be used to design primers for identifying similar genes in non-model but related organisms. Comparative analyses of genomes, which are becoming possible in relevant species as more sequences become available, make it easier to extend candidates across organisms, including non-model species. Recent advances in resequencing technologies, in which short reads of 50–250 kb can be scored cheaply on populations of individuals[108], assist in linking variation in candidate genes to traits in natural polymorphic populations of species, even if these species are not amenable to traditional genetic mapping approaches.

Recent technological and analytical advances promise to accelerate the development of genetic methods for monitoring adaptation to environmental change in several ways. After a high density of polymorphic markers has been developed across the genome, association studies can be used to isolate narrow regions where candidate genes are located, as in the identification of genes affecting human diseases[74] and plant traits[72]. High-density marker scans are also being increasingly used across environmental gradients to identify genomic regions carrying candidate genes. However, the cost of typing thousands of individuals for hundreds of thousands of markers remains high.

The routine use of genetic markers to study and monitor adaptive changes in natural populations, and to predict the potential for adaptive changes in populations, is still some way off. Nevertheless, there is room for optimism given the rapid progress in recent years, and given the increasing number of cases where the genetic basis of adaptive shifts has been identified from a combination of candidate gene studies, genome scans and expression studies[17,42,53,76].

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