# Ecological genomics of local adaptation

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Abstract | It is increasingly important to improve our understanding of the genetic basis of local adaptation because of its relevance to climate change, crop and animal production, and conservation of genetic resources. Phenotypic patterns that are generated by spatially varying selection have long been observed, and both genetic mapping and field experiments provided initial insights into the genetic architecture of adaptive traits. Genomic tools are now allowing genome-wide studies, and recent theoretical advances can help to design research strategies that combine genomics and field experiments to examine the genetics of local adaptation. These advances are also allowing research in non-model species, the adaptation patterns of which may differ from those of traditional model species.

Fitness
The contribution of the genes of an individual to the next generation, usually approximated through measuring survival and reproductive success.

Phenology
The timing of life history events, such as the start of growth or flowering in plants or the arrival to breeding grounds by birds.

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doi:10.1038/nrg3522

Spatial environmental variation is ubiquitous, and populations of a wide range of species are adapted to local abiotic or biotic conditions. Formally, the strict criterion for local adaptation is that a population must have higher fitness at its native site than any other population introduced to that site[1] (FIG. 1). Phenotypic and genetic differentiation along environmental gradients, or across contrasting habitat types, can also be indicative of local adaptation[2,55]. For example, there is genetically based variation in phenology along latitudinal gradients in many plants[3] and animals[4]. Ecological specialization through local adaptation can eventually lead to speciation[5–7], and local adaptation is also an important component of responses to changing environments[8]. During climate change, remaining locally adapted can allow population persistence[9,10], although phenotypic plasticity[11,12] and migration[13] have received more attention so far. Furthermore, breeding for local adaptation has become an important goal for both crop and animal breeding in the face of climate change[14,15].

Although there is evidence of widespread local adaptation in plants[16] and animals[17,18], the genetic basis of local adaptation remains poorly understood in general. However, the genetics of several adaptations with simple modes of inheritance have been characterized. For example, genes or genomic regions have been mapped for the classic colour polymorphism in the peppered moth *Biston betularia*[19,20], for the reduction of armour plate numbers in three-spined stickleback populations that have colonized freshwater environments[21,22], for coat colour variation in mouse species in response to changing environment background colour[23,24], for the response to salinity in *Arabidopsis thaliana*[25] and for trichome variation that results in resistance to herbivory in *Arabidopsis lyrata*[26].

In most cases, the traits that confer local adaptations are polygenic quantitative traits, and the identification of the loci that govern variation in such traits is a challenging task, especially with limited genomic resources[27]. Studies using full genome data have been possible only in species with well-characterized genomes, as exemplified by studies on human climatic adaptation through variation in regulatory regions[28], human height (which is possibly related to climatic adaptation)[29], latitudinal differentiation in *Drosophila melanogaster* that is probably due to climatic selection[30], climatic adaptation in *A. thaliana*[31] and freshwater adaptation in three-spined sticklebacks[32]. However, improving genomic tools (BOX 1) now also allow genome-wide studies of non-model species that may show different patterns of adaptation compared with traditional model species, which are often short-lived, weedy or commensal.

In this Review, we focus on the evolutionary genomics of adaptation to contrasting environments, rather than on species-wide adaptations[33] that have been the focus of several recent reviews[33–35]. We first examine the conditions under which local adaptation can be expected to evolve, as these conditions have major implications for the design and interpretation of experiments. We then review alternative, often complementary, approaches to studying the genomic underpinnings of local adaptation. Our focus is on population genomics and quantitative

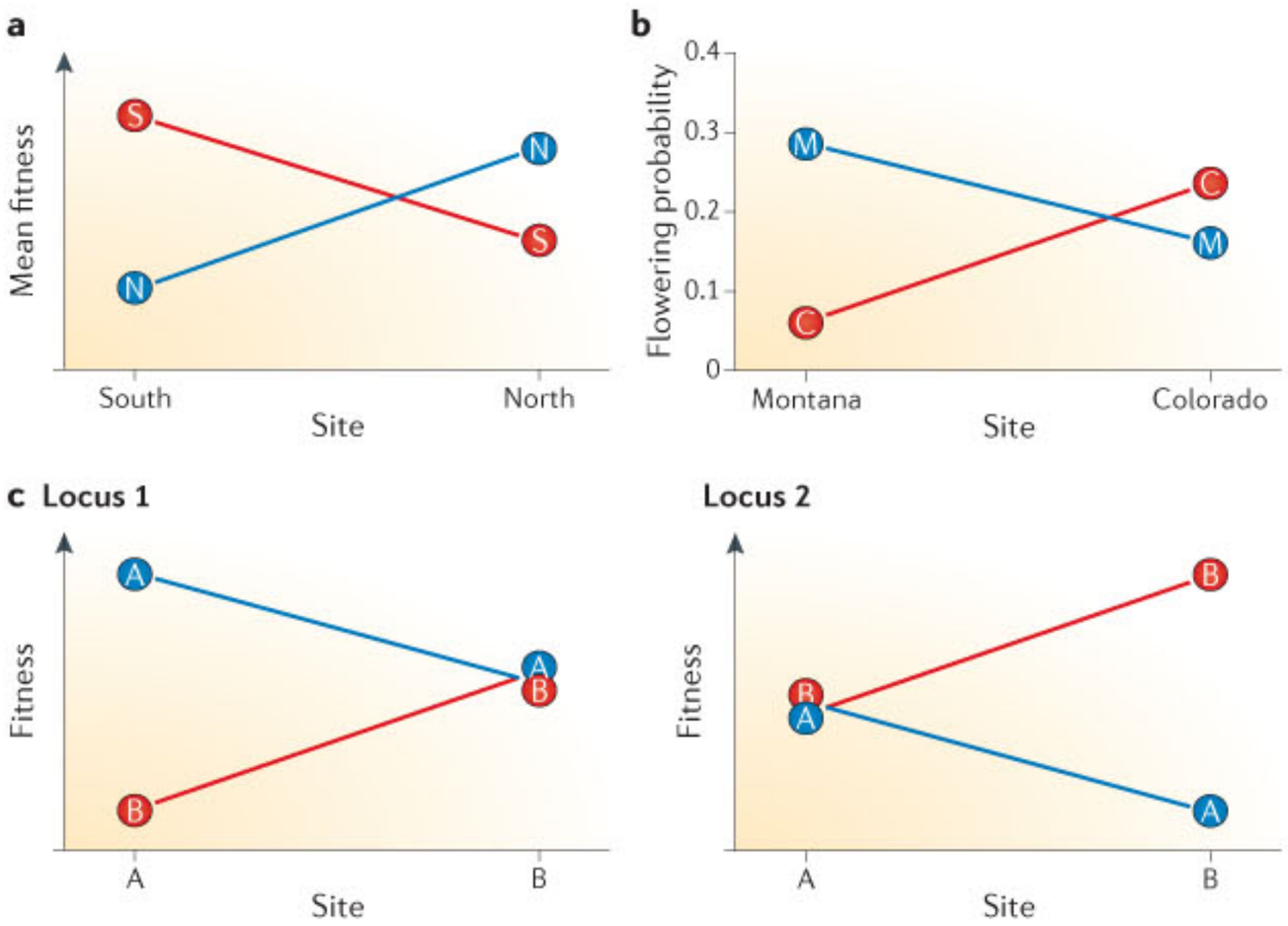



Figure 1 | **Defining local adaptation.** Fitness comparisons between populations are shown. In reciprocal transplant experiments there is a pattern in which each locally adapted population in its native site has higher fitness than any other population in the same site[1,3] (part **a**). Fitness comparisons at individual genetic loci are shown in parts **b** and **c**. Antagonistic pleiotropy at the single-locus level: each allele at the shown quantitative trait locus (QTL) has the highest flowering probability at its home site in a *Boechera stricta* reciprocal transplant experiment that was carried out in Montana (M) and Colorado (C), USA[80] (part **b**). Conditional neutrality is illustrated for two QTLs affecting the same fitness trait (part **c**). The local allele at one QTL (locus 1; shown on the left) shows fitness advantages at site A relative to the non-native allele, whereas the alleles do not differ in their fitness at the other site (site B). At the other QTL (locus 2; shown on the right), the native allele shows fitness advantage relative to the non-native allele at site B, whereas at site A the alleles do not differ in their fitness effects[174]. N, north; S, south. Part **b** is modified, with permission, from REF. 80 © (2013) John Wiley & Sons, Inc. Part **c** is modified, with permission, from REF. 174 © (2012) New Phytologist Trust.

genetics approaches, as functional genomic approaches have been covered in many recent reviews[35–37].

## Predictions about local adaptation

The questions that can be addressed in local adaptation studies crucially depend on experimental design, and especially on the choice of populations. A crucial factor is whether the populations are, or at least were recently, connected by migration (BOX 2).

***Populations with gene flow.*** When populations exchange migrants, local adaptation (or the absence of it) is the result of the balance between selection and migration. Spatially varying selection can lead to local adaptation and, by definition, to genetic differentiation, but not under all conditions. For example, temporally varying selection or frequent extinctions or recolonizations of populations will hamper the evolution of local adaptation[1]. The evolution of adaptive plasticity[11,12] can also prevent local adaptation that is based on genetic differentiation. With these caveats in mind, the conditions for the evolution of genetic differentiation are best illustrated by a simple single-locus–two-allele deterministic model[38] (BOX 2). For local adaptation to occur, high fitness in one environment must have a cost in the other environment, and migration should not overwhelm the effect of local selection[1]. Importantly, in contrast to species-wide adaptations, local adaptation must be due to ongoing or very recent spatially varying selection[1].

The evolution of local adaptation for quantitative traits, which are typically controlled by multiple loci, is not yet as well understood theoretically as for single-locus–two-allele systems[1], even though local adaptation is often based on quantitative traits[3,17]. Polygenic models with many loci often make strong assumptions, such as the assumption that the alleles at all loci have equal effects on the phenotype and that these effects are additive within and across loci. Drift, migration and selection have more complex effects on mean fitness and local adaptation in polygenic models than in single-locus–two-allele models[39]. Nevertheless, the polygenic models allow predictions about the expected genetic architecture. When selection for local adaptation takes place with ongoing gene flow, evolution towards fewer loci with larger-effect-size alleles is expected[40,41] (FIG. 2a). These large-effect loci are more likely than small-effect loci to undergo strong local selection and thus remain polymorphic despite migration and drift (BOX 2). Furthermore, when there is migration, the evolution of local adaptation requires alleles that confer high fitness in one environment to confer lower fitness in the other environment[1]. Thus, fitness trade-offs at the phenotypic level are expected to be accompanied by trade-offs at the level of individual loci[1] (FIG. 1b). If this were not the case, the allele with the highest overall fitness would be expected to invade the other population, and the locus would become fixed (that is, monomorphic)[42]. Additionally, selection for local adaptation is expected to result in clusters of adaptive loci through multiple mechanisms[41,43,44]. It will be challenging to test this prediction experimentally, as distinguishing several close, but distinct, loci from one locus is difficult. This also complicates effect size estimation.

Therefore, although there has been much recent progress in developing theory in this area, many issues remain open (BOX 2), and there is a need to test predictions of theoretical models experimentally by using populations that are exchanging genes through migration.

***Clinal variation.*** Selection along environmental gradients in continuous populations (as opposed to that in populations in discrete environments) often results in clines (BOX 3). The development of the genetic differentiation that gives rise to phenotypic clines depends on the balance between selection and the average dispersal distance of the organism in question[45–47] (BOX 3).

Alternative models of clinal variation lead to different predictions of the underlying genetics. For example, one can assume that a set of populations is distributed along an environmental continuum, in which neighbouring populations are most likely to exchange genes and have different fitness optima. Alternatively, one can assume an island model, in which all populations can exchange genes. The continuum model predicts that, along the environmental gradient, one can observe many sequential steep clines of allele frequencies at genetic loci

**Adaptive plasticity**
The phenomenon by which a genotype can result in alternative phenotypes in different environments so that the overall fitness of the genotype is increased.

**Deterministic model**
A model in which the same starting conditions result in the same outcome, as opposed to stochastic models in which chance effects influence the results.

**Effect size**
The contribution of a locus or an allele to phenotypic variance in a trait.

**Clines**
The gradual phenotypic or allele frequency changes along a geographical or environmental gradient.

Box 1 | **Molecular tools for ecological genomics**

Various molecular tools can be used to detect the genomic basis of local adaptations, but the different study designs have different requirements.

**QTL-mapping approaches**

Genotyping for quantitative trait locus (QTL) mapping approaches does not require a dense set of molecular markers. The most economical ways to obtain such markers are various adaptations of restriction-site-associated DNA sequencing (RAD–seq)[144], such as genotyping by sequencing[145] or double-digest RAD–seq[146]. These approaches allow the simultaneous recovery and genotyping of many markers, depending on the restriction enzyme, without needing a reference genome. For species in which single-nucleotide polymorphisms (SNPs) have been mapped, efficient genotyping methods can rapidly produce high-quality data, but this approach might be more costly.

**Association-mapping approaches**

The genotyping strategy for association-mapping approaches and, in particular, the density of markers required, depends on the range of linkage disequilibrium (LD) in study populations. In cases of low LD, genotyping the specific SNPs of interest or other SNPs that are close to these specific SNPs of interest may require a high number of markers. For higher LD, sparser genotyping is sufficient. In the self-fertilizing *Arabidopsis thaliana*, 250,000 SNPs have been used successfully[31]. If the SNPs were discovered in a small sample of individuals of a population , rare SNPs were likely to have been missed. Such bias can be corrected for if the source of the SNPs is known[147]. Recently, RAD–seq-based techniques that use limited sequencing have been used to provide higher genome coverage[148], but even a high number of anonymous markers may be insufficient if LD is low and genome size is large. Another solution is to focus efforts on the coding part of large genomes by using exome capture and sequencing[149], a method that is now also used in studies of wild populations. As sequencing costs decrease, genotyping through sequencing will become the most economical option. However, if there is much LD, such as in population isolates, a lower density of markers (as provided by SNP-genotyping microarrays) may suffice.

**Population genetics**

Population genetics analyses are currently carried out using combinations of microsatellites, SNPs and DNA sequencing. The best data input for population genetics analyses is high-quality resequencing data without ascertainment bias. Next-generation sequencing data require careful filtering that ensures high-quality data but that does not introduce biases[150,151]. Genomic analyses of non-model species are typically hindered by the lack of a high-quality reference genome, although methods to overcome this challenge are rapidly developing[152].

that govern the variation of the trait[46,47] (BOX 3), whereas the island model predicts that allele frequency differentiation is limited among populations and that most phenotypic differences are due to covariances of alleles between loci[48] (BOX 2). More realistic evolutionary models that allow for the evolution of effect sizes are available for two-population systems[41] and might give a better fit to the data when applied to clines.

***Isolated populations.*** Isolated sets of populations can also provide important insights into local adaptation. For example, replicate studies of adaptation in isolated populations allow issues of parallel evolution to be addressed, as demonstrated by the case of repeated colonization of freshwater environments by three-spined sticklebacks[49]. In addition, experimental-evolution studies compare ancestral and derived populations over time and analyse mutational changes and their effects[50–52]; these studies can be highly informative about adaptation to specific environmental conditions without gene flow (BOX 4). However, the study of isolated populations is not directly related to migration–selection balance and informs us about the end result of local adaptation, rather than about the process of local adaptation. In the absence of gene flow, each population is expected to adapt independently to its environment, as described by Orr's[53] models of directional selection. Hence, we expect an exponential distribution of specific allelic effect sizes in which variation at a few large-effect loci and many small-effect loci influences the trait (FIG. 2) (see REF. 27 for a discussion of assumptions). With a changing environment, models that incorporate a moving optimum also need to be considered. Additionally, with isolated populations, trade-offs at the fitness level (FIG. 1a) do not necessarily involve trade-offs between individual alleles (FIG. 1b) but can also be due to different loci that influence the trait in the two populations. Consider the following example in which only two loci influence fitness (FIG. 1c): at locus 1, the native allele confers high fitness in its native environment (A) but it has no effect on fitness in the other environment (B), whereas at locus 2, the native allele confers high fitness in environment B but it has no influence on fitness in environment A (which is known as conditional neutrality). As there is no gene flow, the 'high-fitness' alleles do not spread from one environment to the other[54].

## Field experiments

***Reciprocal transplant experiments.*** Common garden experiments, in which all experimental plants or animals share the same environment, are classic tools for studying local adaptation. Among these, reciprocal transplant experiments are particularly relevant because they allow a direct test of local adaptation, whereby the fitness of the native population is compared with that of the introduced population (or populations). Early reciprocal transplant experiments with North American *Potentilla* spp.[56] uncovered evidence of local adaptation. Reciprocal transplant experiments have mostly been carried out using plants, but studies of wood frogs (*Rana sylvatica*)[57,58], pea aphids[59] and salmonids[18] are classic examples in animals. Meta-analyses of reciprocal transplant studies have revealed that ~50–70% of

Resequencing
A method to obtain population data by sequencing multiple individuals of a species that has already had a reference genome sequenced.

Parallel evolution
The repeated and independent evolution of similar adaptations (phenotypic or molecular) in multiple populations.

Migration–selection balance
The phenomenon in which the relative strengths of migration and selection determine the level of polymorphism.

Conditional neutrality
A situation in which some alleles may be advantageous in one environment but neutral in other environments.

Reciprocal transplant experiments
Field experiments in which individuals from at least two populations are reared in their respective native and non-native environments.

population pairs show local adaptation by the native versus non-native criterion[16,17]. In general, these studies show that larger environmental differences and larger populations are associated with more frequent local adaptation[16,17]. This is consistent with theoretical predictions that strong selection and limited drift favour the evolution of local adaptation[60,61].

Reciprocal transplant experiments have important limitations. If the strength of selection varies over time, as was recently found in the first large-scale experiment with *A. thaliana*[62], then it is important to evaluate fitness over multiple episodes of selection. However, a recent meta-analysis suggested more constant selection over several years[63]. Additionally, some fitness components,

Box 2 | **Theoretical aspects of local adaptation: some promising avenues**

**Spatially varying selection and drift**

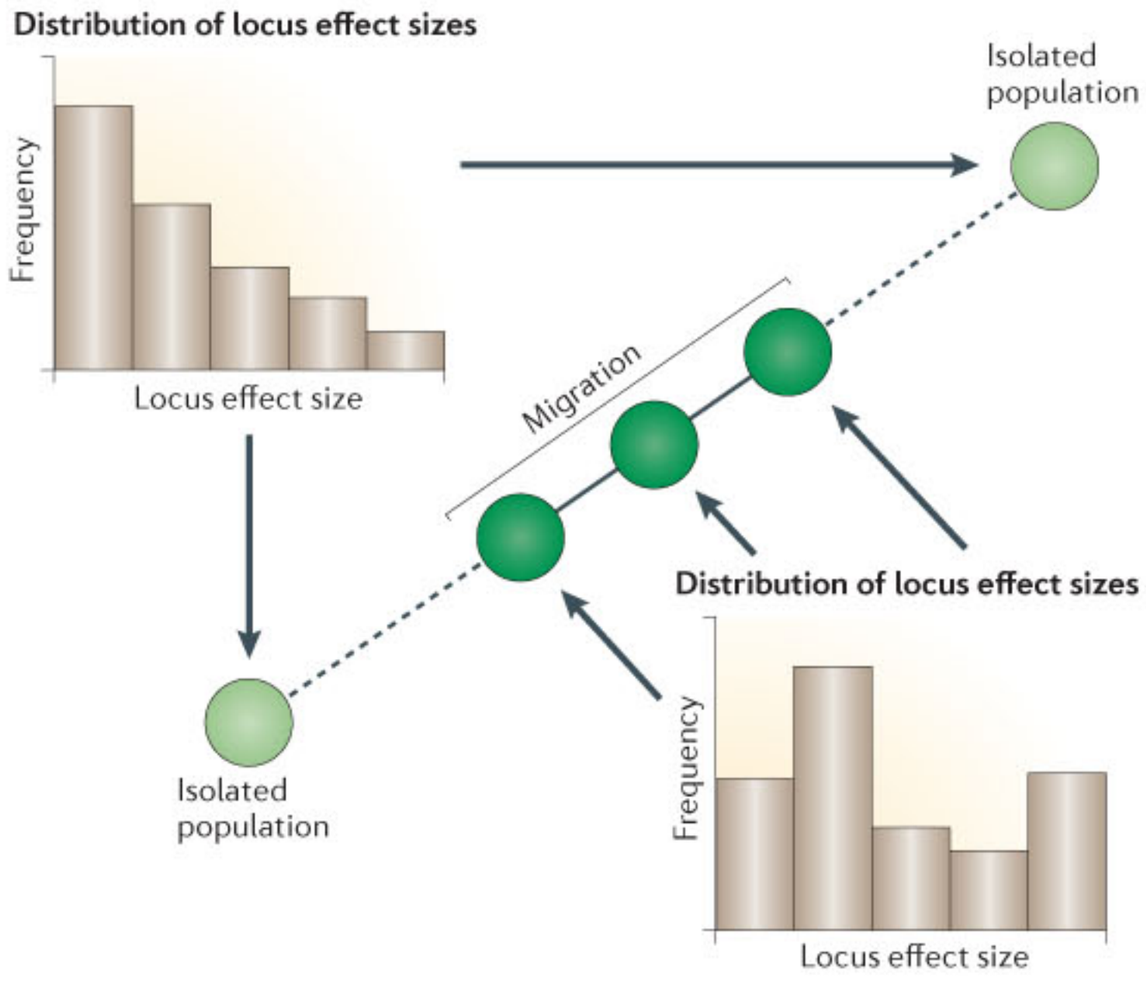
There is a long tradition of theoretical studies that aim to understand the relative importance of migration, selection and other evolutionary forces in creating and maintaining local adaptation. Here, we highlight some recent studies that nicely illustrate the interplay between the various forces involved and indicate new research directions. A recent advance[61] has been the addition of genetic drift to Bulmer's[153] deterministic two-population–two-allele model with migration and divergent selection. This work showed that the joint effect of migration and selection can be effectively summarized by a single diversification coefficient and that drift can be added by replacing the strength of selection with this diversification coefficient in Kimura's[154] equation for the probability of fixation. These results illustrate some salient features of migration–selection balance models. First, the system has threshold behaviour: above a certain level of migration, polymorphism will not be maintained. Second, the addition of drift dramatically changes the conditions for the maintenance of polymorphisms. Importantly, this might occur even with reasonably strong selection. The logic is that selection and migration can cancel each other out, and that their joint effect can be overwhelmed by drift.

**Selection for local adaptation and genetic architecture**

Another study[41] used simulations to consider two populations that are connected by migration and that are undergoing stabilizing selection towards different optima; it characterized the architecture of a quantitative trait that results from such a system. Adaptation with migration typically leads to a genetic architecture with fewer, larger and more tightly linked quantitative trait loci (QTLs) than in isolated populations (see the figure). Each circle represents a population; populations at both extremities are no longer exchanging migrants, unlike the populations in the middle of the figure. The distributions of locus effect sizes are schematic and illustrate the fact that more theoretical and empirical work is needed to acquire a comprehensive understanding of these distributions.

In a second series of promising studies[48,155] on the evolution of quantitative traits under migration–selection balance, the starting point was the observation that forest trees could show a low level of differentiation at random markers (as estimated through the Wright fixation index ($F_{ST}$)), while showing high genetic differentiation at quantitative traits (as measured by $Q_{ST}$). This is, in essence, a consequence of the Bulmer effect[156]. Namely, under the infinitesimal model of quantitative genetics, changes in the variance of genotypic values of a trait under selection primarily result from changes in covariances in genotypic values among loci[157]. LeCorre and Kremer[48,155] extended this idea to a set of structured populations under migration and selection. In a structured population, covariances in genotypic values among loci will have two sources: intra-population linkage disequilibrium (LD) and migration, which will depend on the differences in allele frequencies among populations. $Q_{ST}$ can be expressed as a simple function of these two covariance terms and $F_{STQ}$, which is the $F_{ST}$ value at loci underlying the quantitative trait. This relationship provides interesting insights. For example, the largest discrepancy between differentiation at the phenotypic and molecular levels is obtained under strong divergent local selection and under moderate to high levels of gene flow[45,155].

These studies indicate that there is a complex interplay between the genetic architecture of quantitative traits and the selection–migration balance. Thus, this interplay has important consequences for the search of QTL or of the actual underlying quantitative trait nucleotide (QTN), which has generally been carried out under the expectation that the distribution of effect size of adaptive substitution is approximately exponential[27]. Finally, an added strength of the approach used by LeCorre and Kremer is that their results are directly expressed in terms of quantities such as LD and $F_{ST}$ values that can be measured and are familiar to experimentalists.




Stabilizing selection
A situation in which phenotypes that are close to an optimum have highest fitness.

Infinitesimal model
A model in which variation in a quantitative trait is assumed to be due to small effects at many loci.

such as male reproductive fitness or germination under natural conditions, are hard to assess[64]. Similarly, given the ubiquity of transgenerational environmental effects[65,66] and the long generation times of many organisms, rigorous field- and laboratory-based experiments are time consuming, especially as several populations should be included to increase statistical power. Nevertheless, recent conceptual advances in interpreting experimental results have provided fresh ideas for designing studies of local adaptation[67].

***Mapping local adaptation genes in reciprocal transplant experiments.*** To investigate the genetic basis of local adaptation, and to identify the traits that confer fitness advantages, the populations should show evidence of local adaptation in fitness comparisons[68,69]. Mapping is possible when the progeny of crosses between populations are included in the experiments. Quantitative trait locus (QTL) mapping is based on the joint analyses of phenotypes and genotypes, preferably with a large number of markers, in a segregating progeny[70]. The $F_2$ progeny of crosses between a pair of parental lines are often studied, but other designs are also possible. Genetically identical individuals of the study population can be generated through vegetative propagation[71] or by producing recombinant inbred lines (RILs) of self-fertilizing species. These lines can be genotyped (or even fully sequenced) and studied in multiple environments[42]. For outcrossing species, three-generation pedigrees will avoid complications that are due to inbreeding depression or self-incompatibility[72]. For species with long generation times without suitable crossing progeny, such as forest trees, maternal families can also be used.

QTL mapping is a powerful tool, but many crosses need to be carried out to uncover more variation than that found between one parental pair[73]. Similarly, because of the extended linkage disequilibrium (LD) in the progeny after only one generation of recombination, the location of the QTL cannot be accurately estimated. Multiple-generation pedigrees of wild populations, which capture more recombination events and allow more detailed mapping, are increasingly being used[74,75] but have not yet been applied to studies of local adaptation. Finally, the accurate estimation of the effect size of individual alleles requires large sample sizes[75] (see below).

***Emerging genetic results from reciprocal transplant experiments.*** So far, the limited mapping has focused either on examining the size of the effect or on identifying whether the QTLs that underlie adaptive traits show antagonistic pleiotropy or conditional neutrality. The first study to map local adaptation genes, in self-fertilizing wild barley, showed large QTL effects; a few of these QTLs explained large proportions of the observed variance, although the study may have had little power to detect small effects. Most of the QTLs detected had effects of the same direction in both environments, thus showing no evidence of antagonistic pleiotropy[76,77]. These polymorphisms would not be maintained if the populations were not isolated. In many other studies, most findings have indicated

Quantitative trait locus
(QTL). A genomic area that is found to be associated with variation in a quantitative trait in the progeny of a genetic cross.

Recombinant inbred lines
(RILs). Lines generated by first crossing differentiated parents to produce heterozygous offspring that are self-fertilized. After a few generations, the self-fertilized lines contain different mixtures of parental genomes in a homozygous state.

Inbreeding depression
The decreased fitness of progeny owing to mating between relatives.

Linkage disequilibrium
(LD). The nonrandom association of alleles at two or more different loci.

Antagonistic pleiotropy
The phenomenon in which alternative alleles at a given locus are favoured in different environments.

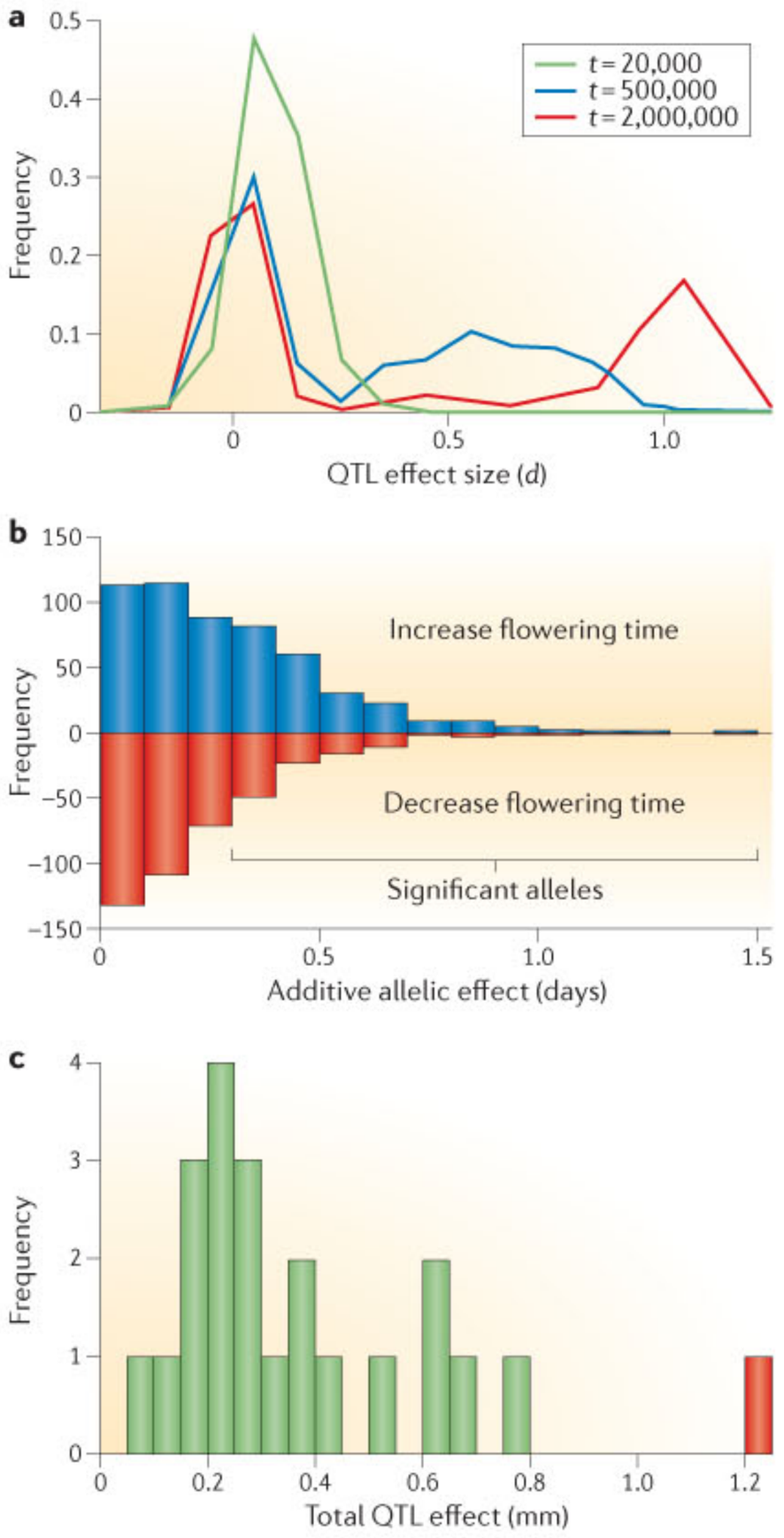
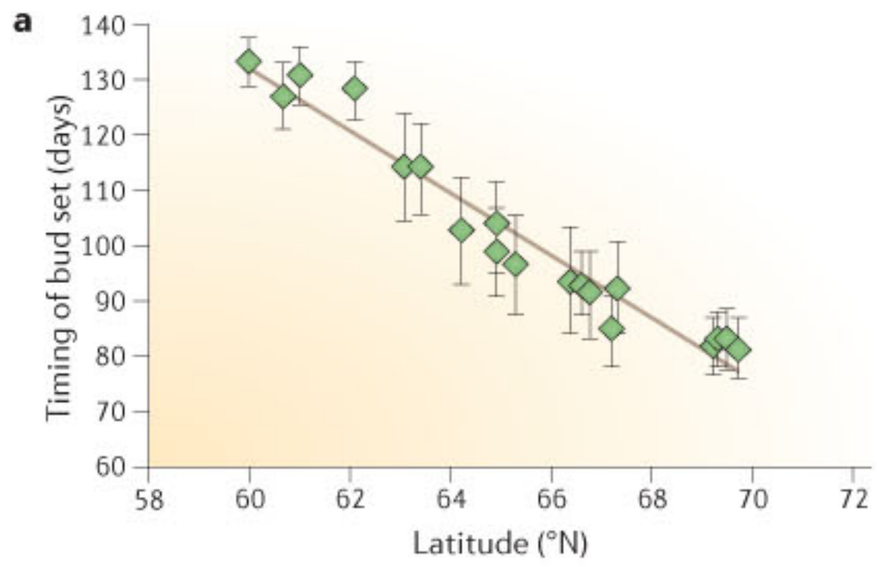


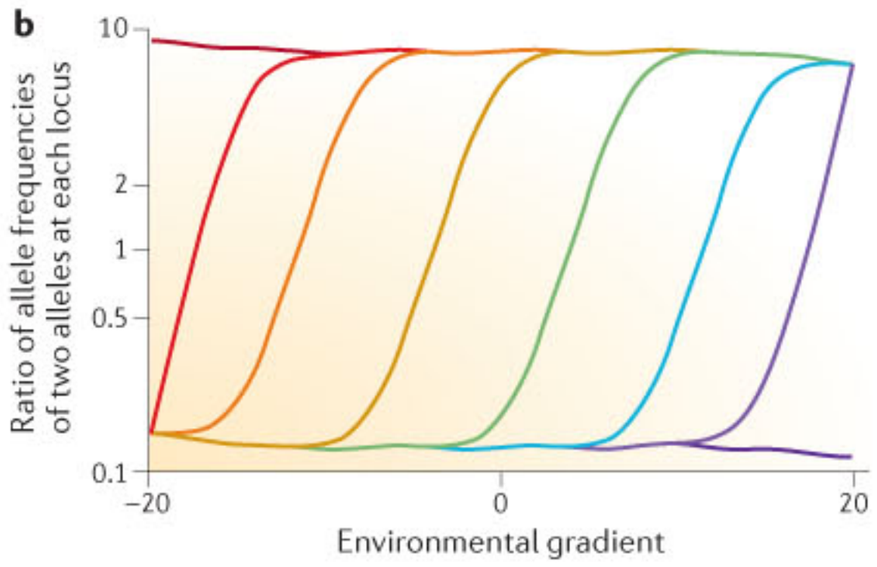

Figure 2 | **Effect sizes of alleles from association or QTL mapping studies.** **a** | The distribution of the quantitative trait locus (QTL) effect size (*d*; the difference between effects of the alleles in the two populations that are adapting to different environments) is expected to evolve with time under migration–selection balance. Three different numbers of elapsed generations (*t*) are shown[41]. Evolution towards fewer loci with larger-effect-size alleles is expected under divergence[41]. **b** | Effect sizes for flowering time alleles of maize in a large experiment that involves the QTL analysis of many different crosses are shown. These alleles can either decrease or increase the time of female flowering, but nearly all of them affect flowering time by less than 1.0 day[105]. **c** | The gamma-shaped effect-size distribution of three-spined stickleback body-size QTLs from an interpopulation cross shows moderate effects between Japanese and North American populations. Effect size was measured as the difference between two homozygotes[175]. Effect sizes for QTLs are shown in green and the effect size of the sex-determining region is shown in red for comparison. Part **a** is modified, with permission, from REF. 41 © (2011) John Wiley & Sons, Inc. Part **b** is modified, with permission, from REF. 105 © (2009) American Association for the Advancement of Science. Part **c** is modified, with permission, from REF. 175 © (2008) John Wiley & Sons, Inc.

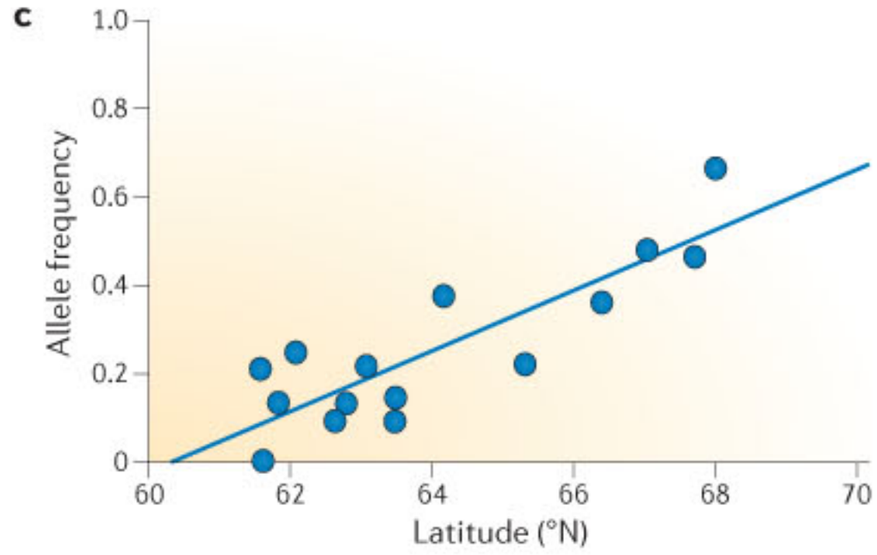
Box 3 | **Local adaptation and studies of clinal variation**

It is important to demonstrate that correlations between traits and abiotic or biotic environmental factors are due to spatially varying natural selection that leads to local adaptation, rather than due to demographic processes. Clinal patterns of variation are often ascribed to selection. Examples of this include the latitudinal clines for body size in *Drosophila subobscura*[158], the circadian rhythm in *Drosophila littoralis*[159] and the phenology (flowering time, bud set and bud flush) of many plant species[84,160] that has been detected in common garden experiments. However, in complex environments it may be difficult to disentangle the roles of demographic history and selection. For example, a recent re-analysis[98] of a classic case of local adaptation in grasses, and the mesic and xeric ecotypes of wild oats (*Avena barbata*) in California, USA[161], indicated that there was no evidence of local adaptation, but that one of the genotypes may be spreading throughout the northern parts of the state. Disentangling the roles of demographic history and selection may be easier in some species than in others. For example, *Arabidopsis thaliana* displays patterns of phenotypic clinal variation[162]. However, clines are generally stronger in randomly mating species with large population sizes, in which selection is expected to be more efficient[101,163].

The analysis of phenotypic clinal variation due to selection can be, as a first step, a fruitful approach to uncover local adaptations and their genetic underpinnings. Phenotypic clines along environmental gradients are common in forest trees, such as for variation in the mean timing of bud set in *Pinus sylvestris*[84,164] along a latitudinal gradient (see the figure, part **a**). This is probably due to differential selection because background neutral markers have low estimates of differentiation ($F_{ST}$)[3]. Theoretical models and simulations[46,47] predict that at the underlying loci, the frequency of one allele increases from a low frequency to a high frequency within a short geographical distance along the gradient (see the figure, part **b**), and that this this would occur sequentially at many loci. By contrast, island model-based simulations predict little allele frequency change at individual loci[48,155] (BOX 2). Empirical findings, however, show gradual changes of allele frequencies at candidate loci[131] along a large geographical distance, such as for a locus in Norway spruce (see the figure, part **c**). More realistic theoretical studies and more extensive empirical data are needed to resolve this discrepancy. Part **a** is modified, with permission, from REF. 84 © (2013) John Wiley & Sons, Inc. Part **b** is modified, with permission, from REF. 46 © (1999) Cambridge University Press. Part **c** is modified, with permission, from REF. 131 © (2012) Genetics Society of America.




conditional neutrality, such as in the self-fertilizing *Avena barbata*[78], in the RILs of *Mimulus guttatus*[42], in the $F_2$ generation of a cross between northern European and North American populations of the outcrossing *A. lyrata*[72], and in the RILs of *Boechera stricta*[79]. In *B. stricta*, allele frequency changed at individual loci in the mapping population during one growing season and resulted in increases of the native-allele frequency at 8% of the loci at one site (which is consistent with conditional neutrality), whereas at 2.8% of the loci, the native-allele frequency increased at each site (which is consistent with antagonistic pleiotropy)[80]. Strong evidence of antagonistic pleiotropy was also observed for an inversion polymorphism in *M. guttatus* by multiple reciprocal transplant experiments between inland and coastal populations[73]. This study provided evidence for the important role of chromosomal inversions in local adaptation[44].

***Future directions for reciprocal transplant studies.*** So far, in most studies, including genomics, differentiated and geographically distant population pairs have been chosen to increase the power to detect phenotypic differences, and self-fertilizing species have been favoured owing to reduced gene flow to facilitate the genetic analyses. To address predictions on the genetic architecture under migration–selection balance it will be important to examine populations that are subjected to

### Box 4 | Evolutionary insights from local adaptation studies

**Parallel evolution**

There have been many studies on traits with simple inheritance in animals. For example, in mammals, colour polymorphisms that allow animals to camouflage in their different coloured backgrounds have been repeatedly found to be due to the same loci (often melanocortin 1 receptor (*MC1R*)) and even due to the same mutation[165] in different species. Repeated adaptation of three-spined sticklebacks to freshwater has been found to involve changes at the same set of loci (ectodysplasin (*EDA*) and paired-like homeodomain transcription factor 1 (*pitx1*))[21,32,136,166]. Similarly, the evolution of shared adaptive traits in natural populations is frequently caused by parallel genetic changes[167,168]. In plants, a comparison of two *Arabidopsis lyrata* populations that were adapted to serpentine soils (which contain heavy metals) with two populations that were found on non-serpentine soils identified several polymorphisms that are strongly associated with soil type and that are enriched at genes encoding heavy-metal detoxification enzymes, and calcium and magnesium transporters[81]. Finally, recent experimental-evolution studies in *Escherichia coli* have shown that the evolutionary responses to higher temperatures or to the availability of two different carbon sources in independent lineages were repeatedly due to the same genes or at least due to the same functional groups of genes[50,52].

**Standing variation or new mutations**

Both biogeography and studies on contemporary adaptation[169,170] tell us that local adaptations may arise rapidly. For example, plants have colonized the previously glaciated areas of northern Europe in the past 10,000 years, yet many tree species have developed clines in phenology[131,164,171]. There is evidence for even faster local adaptation in recent range expansions, such as the evolution of body size cline in *Drosophila subobscura* over a 2,000 km-long latitudinal cline in North America within two decades[158]. There is now plenty of evidence for adaptive genetic responses to environmental changes that have occurred in the time span of a few years to hundreds of years[170]. These findings suggest that much of local adaptation stems from standing variation, rather than from new mutations. This has some important implications to studies of local adaptation. For example, the signals of selective sweeps on standing variation are expected to be weaker than those signals that are based on new mutations[172].

**Partial selective sweeps as evidence of local adaptation**

If sampling is appropriately designed, it is possible to detect selective sweeps that have influenced only some populations, probably owing to local adaptation. Such regionally restricted sweeps have been detected, for example, in *Arabidopsis thaliana*[31] and in humans[173], although species-wide sweeps were rarer.

ongoing gene flow. Cases of short-range adaptation — such as plants on different soils (for example, serpentine or non-serpentine soil)[81,82] or populations growing on mines and adjacent areas[7,83] — might provide interesting opportunities for such studies. Forest trees also show evidence of adaptation despite extensive gene flow. Studies of allele frequency changes in existing long-term transplant experiments of forest trees[84] may also provide opportunities to identify the genetic basis of adaptive differentiation in the presence of gene flow.

Studies with only two populations and their progeny at two sites are demanding, as large sample sizes (>500 $F_2$ individuals from a cross between inbred lines) are required to obtain unbiased estimates of effect sizes, but many populations are needed to generalize the findings[75]. These large sample sizes are in contrast to the low number of individuals per population that was recently suggested for the phenotypic analysis of transplant experiments[67].

Improving genomic and statistical tools now offer powerful ways to estimate historical[85] and/or current[86] gene-flow rates, which could help to better understand the migration–selection balance. We emphasize that, when there is an overlap between the genomic locations of QTLs (or known candidate genes) and the loci underlying fitness, further evidence is needed to verify that variation in both fitness and focal traits is governed by the same genetic variants[87]. A first step towards this is provided by biological replication, such as in the case of the study in *M. guttatus*[73], and by adding evidence from association mapping or population genetics methods (see below).

***Other common garden experiments in the field.*** Much useful information on the genetics of adaptation can also be obtained from common garden experiments other than reciprocal transplant experiments, for example, by planting a high number of populations together at a few sites[67]. Such approaches have been extensively used in forest trees to identify the best seed sources[84] or to test for local adaptation[3,88], and many more such analyses seem to be feasible in additional species[84]. Estimating fitness, measuring related traits in the field and correlating this variation to environmental variation in the sites of origin can be informative about the genetics of local adaptation[67]. Association mapping[89] — that is, examining the correlation between phenotypes and genotypes — can then be used to identify the genomic loci that influence these traits (discussed in more detail below).

The first genome-wide study on *A. thaliana* characterized fitness and trait variation of hundreds of accessions at several field sites and genotyped ~250,000 single-nucleotide polymorphisms (SNPs)[31]. Alleles that confer higher fitness in northern or southern Europe were more common in the north or in the south, respectively. Antagonistic pleiotropy was rare, as few SNPs had fitness effects at multiple experimental sites[31]. The same set of common garden experiments also allowed modelling of the influence of different flowering time loci in field conditions[90]. These and other experiments on *A. thaliana* have emphasized the importance of field studies, the conclusions of which can substantially differ from those of studies under laboratory conditions[91].

Animal studies that are based on common garden approaches in the field have rarely been able to combine information on fitness and genomics. In one well-studied case, differential predation by owls is likely to maintain coat colour differences of beach mice between environments despite extensive migration[92]. In this migration–selection balance situation, large-effect mutations in the *cis*-regulatory region of the *Agouti* locus and in the coding region of the *Mcr1* locus account for much of the coat colour variation[23]. The rapid development of genomic resources should allow the extension of this type of study to other non-model organisms.

### Mapping adaptation without fitness estimates

In many cases, reciprocal transplant experiments or common garden experiments in field sites are not feasible for biological, practical, or even legal and ethical reasons. In such cases, even if fitness measurements are not possible, studies of the genetic basis of differentiated traits that are associated with local adaptation can still provide useful insights into the genomic basis of adaptation.

The genetics of population differences in traits is related to adaptation. The genetic basis of natural variation has been extensively characterized for many traits in both animals[93] and plants[84], such as in sticklebacks[22] and in recent studies of flowering time in *A. thaliana*[94,95]. In order to focus on local adaptation, we concentrate on the studies that have explicitly sampled populations from relevant contrasting environments. Genetic differences between populations from contrasting environments, or that are associated with traits which vary along environmental gradients, can be due to selection for local adaptation, especially if these patterns are replicated. However, historical demographic events can generate similar patterns[96,97], as has been recently suggested for *A. barbata*[98]. In the absence of fitness data from reciprocal transplant experiments, comparing the differentiation of quantitative traits and molecular markers between environments may help to identify adaptive quantitative trait variation[99] (BOX 3). Related studies can also be carried out in the laboratory by varying relevant environmental factors, such as temperature, day length or soil type. Of note, if the development of the trait is not sensitive to the environment, such as armour plates in sticklebacks, the control over environmental conditions may not be crucial.

***Association mapping.*** Similar to QTL studies, association mapping is based on correlating phenotypes with genotypes but it considers populations rather than pedigrees[89]. Individuals of the populations that were sampled have therefore undergone more generations of recombination events than those in pedigree-based studies. The LD value is thus expected to be much lower in association mapping than that in pedigree-based studies, which allows more accurate QTL mapping than that in pedigree-based QTL studies. For genome-wide association studies, a dense set of markers is needed, and the number of markers depends on both the extent of LD and the size of the genome[100]. An alternative approach has been to examine associations based on a limited set of candidate genes; that is, loci that are thought to potentially influence the trait. The identification of the genetic basis of quantitative traits through association mapping is still challenging, partly owing to the difficulty of identifying small-effect loci[27].

Association studies are most powerful when carried out in a population in which it can be assumed that, at each locus, the variation is due to only one mutation, as in population isolates. When samples from differentiated populations are included, phenotypic variation in the population may be due to variation either at different loci or at different alleles at an individual locus. For example, an association study of *A. thaliana* that encompassed Europe-wide sampling showed that different non-functional deletion alleles at the *FRIGIDA* (*FRI*) locus give rise to early flowering[101]. Patterns of adaptation that are confined to particular loci or alleles could remain undetected if many populations are pooled in the analysis. A more regional sampling may help to uncover patterns that are related to local adaptation, such as the patterns seen along altitudinal clines in Spanish populations of *A. thaliana*[102].

However, to map genes that are related to local adaptation, by definition, genetically differentiated populations need to be studied. This differentiation at loci that are related to local adaptation is due to selection, whereas the patterns of differentiation in the rest of the genome are probably due to demographic effects (that is, population history) and will have a different distribution[103]. As an example of such problems, an early study on maize reported that variation in the *DWARF8* gene (also known as *D8*)[104] had a strong effect on the time of pollen shedding, but later studies that corrected for differentiation of the genetic background found that *DWARF8* actually had a small effect[105,106]. The existing methods of association studies address such problems by correcting for relatedness and population structure[107–110], which is appropriate when the trait of interest is expected to have a similar distribution across populations. However, if the underlying genes of the trait of interest are differentiated among populations, as one would expect when considering local adaptation, such statistical correction may also eliminate the signal in loci that are responsible for local adaptation. Combining the examination of association between and within populations could help to resolve this problem.

In summary, although association studies still carry a high risk of either spurious associations (false positives) or unidentified association (false negatives), they allow the accurate mapping of effects. Combining association studies with QTL mapping[105] or artificial multi-accession crosses[105,111] allows the consideration of many parents and seems to be promising for increasing mapping accuracy and resolving confounding problems, as demonstrated by a detailed analysis of flowering time in maize[105]. Combinations of association mapping with other methods that are less sensitive to the population structure will also help to identify causative genes. Finally, there have recently been exciting attempts to incorporate molecular biology knowledge, for example, gene regulatory networks, a priori in association studies rather than a posteriori as

is generally done. This could lead to a gain in statistical power and new biological insights[100].

***Insights into effect sizes from QTL and association mapping.*** The choice of the traits under study is seldom random: in many plant studies, traits that are likely to be influenced by local adaptation are studied, whereas in many animal studies, striking visible polymorphisms (for example, colour variation) have often been favoured. This choice of traits influences the distribution of effect sizes, as the predictions of the theoretical models for polygenic traits differ from the traits that are influenced by individual loci.

Heterogeneous patterns of effect-size distributions have been uncovered across species. In *A. thaliana*, laboratory-based studies have detected large effects due to fairly common alleles in Europe-wide collections. For 40 of the 100 traits that were detected, one frequent SNP accounted for at least 20% of variation[101]. In contrast to *A. thaliana*, in forest trees, many QTL mapping and association studies have identified small to moderate effects[84,112–114]. A study of *Silene vulgaris* populations connected by gene flow showed that adaptation to serpentine soils was due to large-effect loci that influence differences in nickel tolerance[82]. Overall, a common output of these studies is the identification of a few large-effect loci and many small-effect loci[115] (FIG. 2).

In natural animal populations, mapping experiments have uncovered several examples of loci with large effects on discrete traits. Such examples include coat colour in beach mice[23], as well as armour plating[116], pelvis reduction[117], and other morphological and colour variation traits in three-spined sticklebacks[118,119] (FIG. 3). Mapping of continuously distributed traits, such as shape in sticklebacks[120] or body size in a cross between mice[121], has uncovered much higher numbers of small-effect QTLs. A recent study suggested that selection coefficients on the 1,400 loci that were found to be associated with human height were small, with alleles that increase height being favoured in northern versus southern Europeans[29,41]. In sticklebacks, adaptation to a new environment that was different from the ancestral one resulted in selection for larger-effect alleles, compared with situations in which the difference between the old and new optimum was smaller[120].

Detecting QTLs and estimating the size distribution of QTL or SNP effects are demanding tasks. Even medium-sized studies suffer from a bias towards larger effects, as small effects are difficult to detect[27,75]. Clusters of several small QTLs or quantitative trait nucleotides (QTNs) can have the appearance of one large-effect QTL[24]. As mentioned above, such clustered loci are expected for local adaptation[41]. Nonetheless, one trend to emerge is that a self-fertilizing species (such as *A. thaliana*) shows larger-effect loci than the outcrossing forest trees (or maize[105]) for similar traits. Careful comparisons between closely related outcrossing and self-fertilizing species that control for other factors are warranted. The heterogeneity of the results[27] should also encourage a more in-depth examination of the factors that are expected to influence the distributions, such as the level of gene flow (BOX 2), the duration of selection, the geographical distance separating the populations or the distance between the adaptive peaks[41,60,120].

## Population genetics approaches

Although the previous approaches started with the phenotypes, the starting point for population genetics approaches is variation at the DNA level. Most of the tests that were developed to detect the presence of natural selection from sequence data look for departures from the standard neutral model (such as Tajima's D test[122] and related tests) or are based on the comparison between polymorphism and divergence (such as the MacDonald–Kreitman test[123] or the Hudson–Kreitman–Aguadé tests (HKA tests))[124]; they are therefore not directed towards testing for local adaptation. The effects of directional selection are seen as selective sweeps (BOX 4) and can influence the whole species, such as in *Drosophila simulans*[125], but similar methods can be used to search for traces of selection for local adaptation[31].

There are currently three main groups of methods for detecting the footprints of local adaptation at the molecular level: population differentiation through scans of the Wright fixation index ($F_{ST}$), correlation between allele frequencies and environmental variables, and $F_{ST}$–$Q_{ST}$ comparisons. As the $F_{ST}$–$Q_{ST}$ comparison approach has recently been reviewed[99], it is not considered here.

***Population differentiation.*** The first group of methods is based on genetic differentiation between populations; these include both $F_{ST}$-based methods that extend the Lewontin–Krakauer approach and methods that are based on LD between populations[126]. There are many methods that aim to detect $F_{ST}$ outliers[130]. These methods differ in the underlying demographic model that they assume (for example, the island model or hierarchical model), in the statistical approach that they adopt (for example, the frequentist or Bayesian approach) and whether selection is explicitly included in the method. As a consequence, the methods vary in how conservative they are (see below). In particular, even those that account for a hierarchical population structure can still lead to many false positives[127]. Also, it is important to keep in mind that processes other than local adaptation can also lead to $F_{ST}$ outliers; such processes include selection owing to deleterious alleles, species-wide selective sweeps, cryptic hybrid zones and stochastic effects in expanding populations[128]. Therefore, $F_{ST}$ outliers are only candidate loci of local adaptation, and further investigation is required to determine whether they are truly involved in local adaptation.

***Correlation with environmental variables.*** The second group of approaches has been developed more recently and provides tools to analyse associations between SNP frequency and environmental variables. The major implementation of these methods is the program Bayenv[129]. To control for population structure, Bayenv estimates a covariance matrix of allele frequencies that is used as a null model and is tested against an alternative

**Polygenic traits**
Traits that are influenced by variation at many loci.

**Quantitative trait nucleotides**
(QTNs). The causative nucleotides that govern the expression of variation in given traits.

**Hudson–Kreitman–Aguadé tests**
(HKA tests). Tests of selection versus neutrality, based on the comparison of divergence between species with diversity within species between different genomic areas.

**Selective sweeps**
When a mutation with a beneficial fitness effect arises in a population, natural selection will rapidly increase the frequency of the mutation to a high frequency (partial sweep) or to fixation (complete sweep), which results in a reduction of diversity at and around the selected locus.

**Wright fixation index**
($F_{ST}$). The proportion of the total genetic variability that occurs among populations. It is typically used as a measure of the level of population genetic differentiation.

**$F_{ST}$–$Q_{ST}$ comparisons**
Tests for selection that compare the degree of differentiation in quantitative traits ($Q_{ST}$) with the population genetic differentiation at the marker loci ($F_{ST}$).

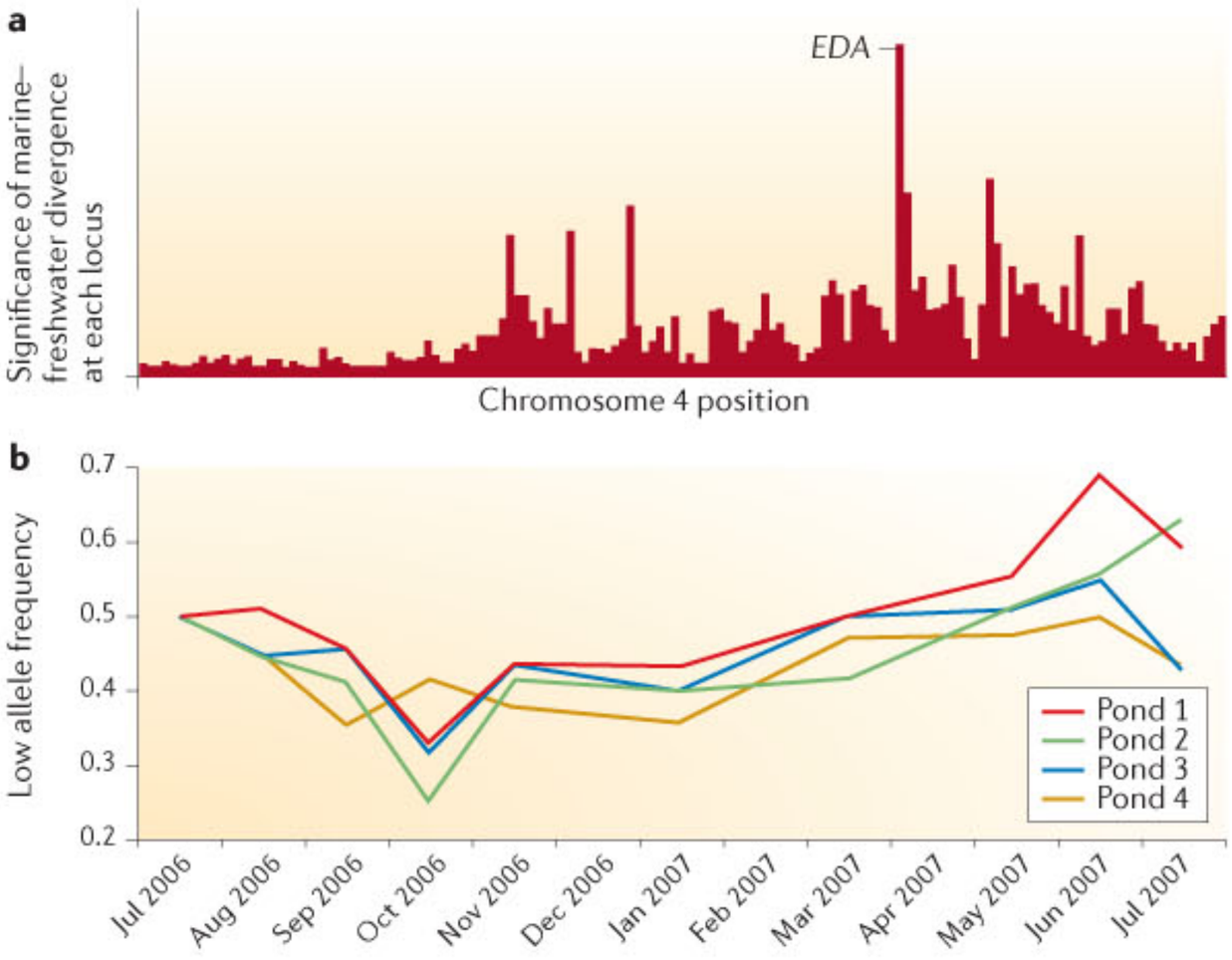



Figure 3 | **Evidence for local adaptation in lateral plate numbers in three-spined sticklebacks using different approaches.** **a** | Increased divergence is seen at the ectodysplasin (*EDA*) locus among marine and freshwater populations of sticklebacks from a resequencing approach[32]. **b** | Experimental evidence shows allele frequency changes at the *EDA* locus in replicate freshwater colonizations of sticklebacks in different ponds[176]. **c** | Evidence shows adaptive differentiation both in lateral plate numbers and in underlying alleles at the *EDA* locus in a high gene-flow marine environment, the Gulf of Finland in the Baltic Sea[177]. The inner pie chart shows the frequencies of *EDA* alleles that are associated with low numbers of lateral plates (light blue sectors) and high numbers of lateral plates (dark blue sectors) in the samples. The dark red area in the outer ring (where applicable) indicates the actual average number of lateral plates (expressed as the proportion of 25 lateral plates). **d** | The evidence for repeated selective sweeps in the *EDA* locus in global pairwise populations is obtained from data in REF. 136. *EDA* allele frequencies (shown in pie charts) and dominant lateral plate phenotypes (represented by the fish symbols) are given for each pair of freshwater and marine populations. The frequencies of the *EDA* allele for full-plate morph (dark blue sectors) and low-plate morph (light blue sectors) are shown. The asterisks (*) indicate cases with evidence for selection on *EDA* markers. Part **a** is modified, with permission, from REF. 32 © (2012) Macmillan Publishers Ltd. All rights reserved. Part **b** is modified, with permission, from REF. 176 © (2008) American Association for the Advancement of Science. Part **c** is modified, with permission, from REF. 177 © (2013) John Wiley & Sons, Inc. Part **d** is modified, with permission, from REF. 136 © (2011) John Wiley & Sons, Inc.

model, which includes a linear relationship between allele frequencies and an environmental variable. As for $F_{ST}$-based methods, correlation with environmental variables is not necessarily causal, and further investigations or evidence are required to determine whether the loci are truly involved in local adaptation (see below).

***Relative merit of the different methods.*** A recent study based on forward simulations of different demographic models and sampling schemes tested five $F_{ST}$-based methods and three methods that are based on the correlation between genotypes and environmental variables[130]. This revealed that, for methods that are based on the correlation between genotypes and environmental variables, those that account for the underlying correlation structure of allele frequencies, such as Bayenv[129], are more powerful and yield fewer false positives than those that do not, and hence have been successfully used[31,131–134]. Given that the population genetic model assumed by Bayenv[129] is a simple ancestral population that split into independent populations through drift alone, one can foresee further improvements if more complex demographic models can be accommodated. The $F_{ST}$-based methods are less powerful than correlation-based methods, but they (especially the BayeScan program[135]) lead to fewer false positives. Finally, under the models simulated[130], sampling many populations led to more reliable results than sampling many individuals per population.

***Caveats and a general suggestion.*** As emphasized above, $F_{ST}$-based methods and correlation-based methods can lead to false positives. What we advocate here is that a careful study design can substantially decrease the uncertainty that is inherent to these methods. For example, in a study designed to detect molecular footprints of adaptation to freshwater environments in the three-spined stickleback[136], paired sets of globally distributed freshwater–marine populations were used to look for footprints of natural selection in markers at many physiologically important genes (FIG. 3d). The detection of signatures of selection in such comparisons can be interpreted in two ways: they could be true signatures of selection, or they could be caused by demographic effects that are associated with the colonization of small freshwater bodies from large ancestral marine populations. In this study[136], negative and positive controls were used to exclude the demographic explanations. First, signatures of selection were detected more frequently in candidate genes than in negative-control extragenic regions, whereas demographic factors should influence both classes of markers in a similar way. Second, selection was always detected in the positive-control locus, which in this case was a marker that is associated with the ectodysplasin (*EDA*) gene[21] (FIG. 3a); after colonizing freshwater habitats, three-spined sticklebacks usually lose most of their lateral plates as an adaptation to life in freshwater, and this loss is attained by an allelic substitution at the *EDA* locus[21,22,137]. Hence, this example illustrates that through appropriate study design more confidence can be based on the results from $F_{ST}$ outlier studies. Notably, the repeated freshwater adaptation at the *EDA* locus occurs from standing genetic variation[137]; hence, the use of outlier tests is not limited to detecting adaptation that is based on novel mutations.

Standing genetic variation
Existing variation in a population as opposed to variation that is emerging owing to mutation.

## Conclusions

Despite the powerful molecular tools and analysis methods that are currently available, it is still a daunting task to obtain a full picture of the effect of local adaptation at the genomic level. We summarize below some areas in which advances can be made.

***Experimental design and sampling.*** Given that local adaptation is the outcome of a dynamic balance between selection and migration, planning an experiment that investigates local adaptation always involves difficult choices and trade-offs, especially if it combines phenotypes with genomic data[67]. These relate to issues such as the number of populations to study, their ecological and evolutionary divergence, the parts of the genome to be sampled, and the statistical methods to associate genotypic and phenotypic variation. We believe that rapid progress will be made in these areas, especially when guided by advances in modelling local adaptation. Recent theoretical and methodological developments are promising first steps in this direction[39,67,138].

***Gaps between theory and data.*** This Review also highlights gaps in our knowledge and interesting discrepancies between theoretical predictions and empirical findings. These suggest possible future directions for research. For example, despite recent advances[43], the task of understanding and measuring the distribution of effect sizes is and will remain challenging. The incorporation of more biological information, such as information on gene regulatory networks, is likely to be more fruitful than simply trying to increase the size of the experiments, which is beyond the reach of most laboratories that work on non-model species[100]. Combining information from genomic and gene expression[18] studies could also yield a better biological understanding of the traits underlying local adaptation. Moreover, scans for local adaptation that are based on $F_{ST}$ generally detect a few outlier loci, whereas models predict small changes in covariances in allele frequencies at underlying loci across populations rather than detectable changes at individual loci (BOX 2). Can more complex models of local adaptation account for this apparent paradox? Do QTLs with high $F_{ST}$ values have specific biological properties, such as having key roles within the set of loci controlling the trait? In these cases too, more functional data would be useful. We also lack polygenic selection models that predict gradual change at the causative loci of adaptive traits, which is a pattern that has been detected in many empirical studies[139].

***Genomics and local adaptation.*** Genome-wide data offer many advantages over sparser sets of genetic markers. The large number of markers allows a highly accurate estimation of relatedness, such as in the context of association studies. Many tests of selection are based on

Restriction-site-associated DNA sequencing
(RAD–seq). A technique for partial DNA sequencing, in which DNA is first cut with restriction enzymes and the DNA next to these sites is then sequenced.

examining the spatial patterns of variation, for example, detecting selective sweeps. It is now possible to detect the patterns and to examine them against genome-wide variation[32,140], even with limited genome-wide data, such as those obtained with restriction-site-associated DNA sequencing (RAD–seq) (BOX 1). Furthermore, sequencing the coding part of the genome allows genome-wide tests for selection based on the comparison of synonymous and non-synonymous sites[133]. Until recently, research on the model organisms with genomic resources, such as *A. thaliana* or sticklebacks, have provided the first views of adaptation at the whole-genome level[31,32]. Extending these studies to carefully chosen additional species will allow the examination of different factors that influence adaptation genomics.

Finally, genomic studies of local adaptation can also be expected to have a central role in improving our understanding of responses to climate change. Currently, the challenge faced by evolutionary studies of adaptation to climate change is in the difficulty of recovering genetic evidence to differentiate between local adaptation and phenotypic plasticity as causes of observed phenotypic changes in various animal and plant populations[141,142]. It will be important to identify and study both kinds of situations. When long-term field experiments can be combined with genomic analyses, it will be possible to examine what genetic changes have occurred and even to estimate strengths of selection[29]. If there is no evidence of local adaptation, then the mechanisms of phenotypic plasticity (for example, epigenetic variation) can be studied[12]. As our understanding of the genomic basis of both fitness and trait variation increases, prediction of longer term climate change responses are also likely to improve[84,143].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### Acknowledgements
The authors thank P. Leinonen for comments on the manuscript. O.S. is supported by the Academy of Finland (Grant number 132611) and the ProCoGen FP7 EU project; M.L. by the Swedish Research Council, Erik Philip-Sörensen Fund and BioDiversa (Linktree and Tiptree projects); and J.M. by the Academy of Finland (Grant number 252597).


### Competing interests statement
The authors declare no competing financial interests.