UNDERSTANDING QUANTITATIVE GENETIC VARIATION

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Until recently, it was impracticable to identify the genes that are responsible for variation in continuous traits, or to directly observe the effects of their different alleles. Now, the abundance of genetic markers has made it possible to identify quantitative trait loci (QTL) — the regions of a chromosome or, ideally, individual sequence variants that are responsible for trait variation. What kind of QTL do we expect to find and what can our observations of QTL tell us about how organisms evolve? The key to understanding the evolutionary significance of QTL is to understand the nature of inherited variation, not in the immediate mechanistic sense of how genes influence phenotype, but, rather, to know what evolutionary forces maintain genetic variability.

MULTIFACTORIAL GENETICS

QUANTITATIVE TRAIT LOCI (QTL). Genetic loci identified through the statistical analysis of complex traits (such as plant height or body weight). These traits are typically affected by more than one gene and also by the environment.

HERITABILITY
The fraction of the phenotypic variance due to additive genetic variance (V_A/V_P).

ENVIRONMENTAL VARIANCE
The variance in the trait among genetically identical individuals. This variation might be due to the different environmental conditions experienced by different individuals, or to essentially random factors.

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The study of evolution and inheritance in natural populations began at the end of the nineteenth century, with the analyses of Francis Galton, Karl Pearson and W. F. R. Weldon[1]. The statistical tools of correlation and regression that they developed were the foundation of a sophisticated body of theory, with wide applications in plant and animal breeding. However, the emphasis that this 'biometric' school placed on natural selection acting on minor variants was opposed by the newly founded Mendelian genetics, and it took more than a decade for it to be recognized that minor Mendelian variants could account for inherited variation in continuous traits. Considerable efforts were made to identify the genetic basis of trait differences within and between species, using essentially the same methods as in modern studies of QUANTITATIVE TRAIT LOCI (QTL). However, such work was severely limited by a dependence on visible markers.

During the 1980s, quantitative genetics was increasingly applied to evolution in natural populations, and, at the same time, quantitative genetic theory began to be related more directly to the underlying variation in genes[2]. However, the key issue of what causes variation in continuous traits received relatively little attention, and was overshadowed by the more-prominent controversy over whether protein and DNA sequence variation is explained by selection or by random drift (see REF. 3, Ch. 20). The genetic basis and evolutionary causes of quantitative variation are now receiving renewed attention, both within evolutionary biology, and in applications to human genetics and agricultural genetics.

Most traits, in most populations, show substantial HERITABILITIES. There are a few patterns; for example, components of fitness, such as longevity or survival, show lower heritability than morphological traits, such as skeletal shape[4]. However, their heritability is lower because fitness components tend to have much higher ENVIRONMENTAL VARIANCE; when scaled appropriately, their GENETIC VARIANCE is actually higher[5]. The most important and general observation is that much trait variation is inherited. This is surprising, for the same reason that high levels of molecular variation were a surprise when discovered during the 1960s: the simplest forms of selection would be expected to eliminate variation, and the simplest mechanisms for maintaining variation seem unlikely to apply across very different traits and species[6].

More specifically, it is generally believed that many quantitative traits are under STABILIZING SELECTION, which would tend to deplete genetic variation. Admittedly, this belief is based more on intuition than on direct evidence, as the strength of stabilizing selection acting on a trait is hard to measure accurately. Although there are clear examples in which extreme phenotypes have lower

GENETIC VARIANCE
The variance of trait values that can be ascribed to genetic differences between individuals.

STABILIZING SELECTION
Intermediate phenotypes have greater fitness than extreme phenotypes.

reproductive success (for example, the reduced survival of babies with high or low birth weights[7]), it is usually hard to know whether the fitness differences are actually caused by the observed trait[8]. A plausible alternative is that deleterious alleles have pleiotropic effects on quantitative traits, so that unfit individuals tend to have extreme phenotypes. Moreover, a recent survey[9] shows that although there is evidence for DIRECTIONAL SELECTION in nature, there is, overall, no more evidence for stabilizing selection than for DISRUPTIVE SELECTION. Nevertheless, the slow rates of change seen in the fossil record, together with pervasive genetic variation, require that traits with even a slight effect on fitness must, in the long run, be under stabilizing selection towards a constant optimum.

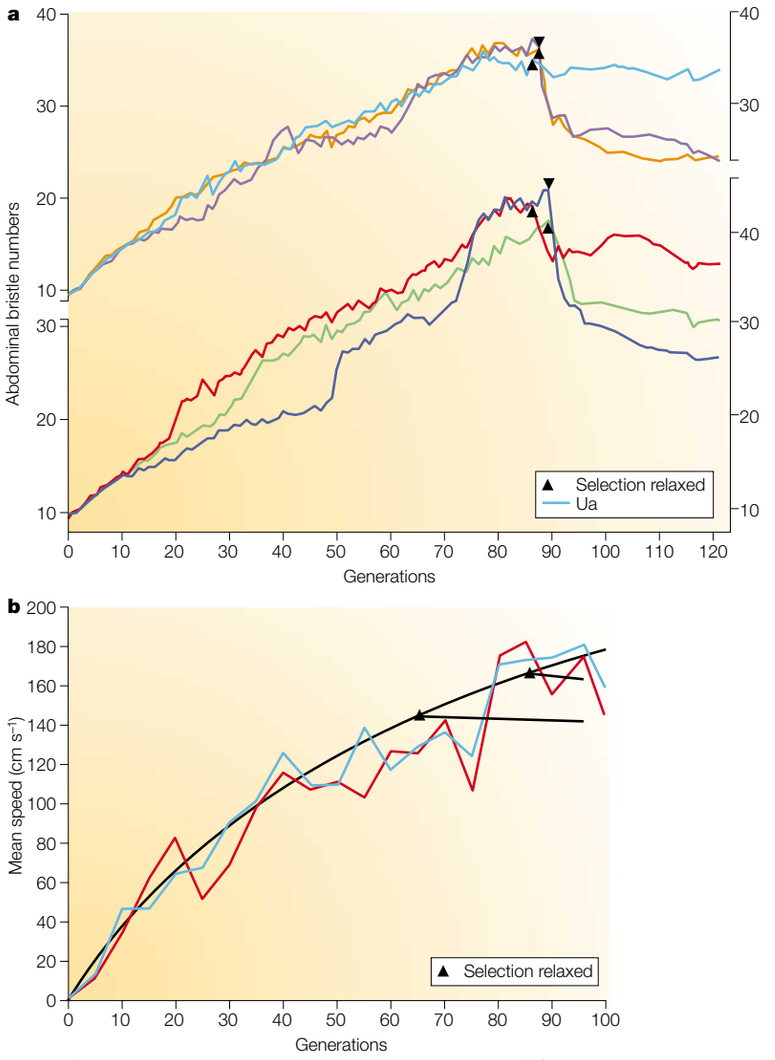


Figure 1 | **Examples of long-term selection response. a** | Yoo[116] took a large outbred population of fruitflies, and selected six replicate lines for increased numbers of abdominal bristles: the top 20% of the population (50 pairs) was selected every generation. All six lines responded; by generation 85, the average number of bristles had increased by 16 phenotypic standard deviations. Sharp jumps were attributed to selection of recessive lethals; these added up to 11 bristles when heterozygous. At the end, when selection was relaxed (black triangles), mean bristle score fell rapidly in those lines that carry lethals[13]. However, not all of the response was due to lethals: one line (Ua) carried none, but responded. Moreover, genetic variance did not decrease appreciably over the experiment, even after discounting the contribution from high-frequency lethals[117]. **b** | Weber[14] selected on ability to fly upwind in a wind tunnel. The apparatus allowed very large numbers to be selected with high intensity: the 4.5% strongest fliers were selected, and EFFECTIVE POPULATION SIZE was kept at 500–1,000. Mean flying speed increased from 2 cm s^{-1} to 170 cm s^{-1} over 100 generations, at about the same rate in two replicates. There was no detectable loss of fitness, and when selection was relaxed (black triangles), the mean did not decline significantly. The smooth curve is fitted to the mean of the two replicate lines.

The issue at stake, then, is the nature of the evolutionary forces that maintain genetic variation. This issue is important in itself, but also has practical relevance. For example, the feasibility of using markers to assist artificial selection, or to assess the risk of disease, depends on the nature of the underlying genetic variants. The purpose of this article is, therefore, to consider the genetics of quantitative traits from an evolutionary perspective. In the next section, we review some of the evidence from artificial selection experiments that shows how much genetic variation is present, and then go on to consider the nature of that variation. We also consider how these differences are generated and, finally, turn to the more difficult issue of how selection shapes the genetic architecture of quantitative traits.

Sustained responses to artificial selection

High heritabilities allow rapid responses to artificial selection and, with few exceptions, such responses are seen[3] (FIG. 1). Moreover, change can be sustained for 100 or more generations, which leads to remarkable changes in phenotype. The most obvious explanation is that trait variation is based on very many genes of very small effect, an assumption known as the INFINITESIMAL MODEL[10]. So, selection causes no appreciable change in the frequency of any particular allele, and does not erode heritable variation, which allows the response to selection to continue. However, QTL of large effect are frequently identified in mapping experiments, and this makes steady and sustained selection responses puzzling: alleles of large effect should be fixed rapidly, after which no further response would be seen. Two factors might help to explain this apparent paradox. First, QTL-mapping experiments underestimate the numbers of QTL and overestimate their effects (BOX 1). Second, mutation generates alleles of large effect, which can be picked up quickly enough by selection to sustain a continuing selection response[11] — a possibility that we examine in more detail below.

In explaining sustained responses to artificial selection, of the type seen in FIG. 1, the key genetic questions are whether the response is due to alleles of large or of small effect, and whether these alleles were present at the start or arose by mutation during the course of selection. Any alleles of large effect in the base population will quickly be either fixed or lost, and their contribution dissipated. They will contribute longest if they are initially rare and are recessive, but even if they increase from just one copy, such alleles will be fixed in relatively few generations. Specifically, a response to strong selection based on additive alleles that increase the trait by around half a phenotypic standard deviation can last no longer than ~20 generations; more generally, the time span is inversely proportional to the size of the effect (FIG. 2a).

Box 1 | Biases in quantitative trait loci analysis

Typically, quantitative trait loci (QTL) are located by measuring associations between Mendelian markers and the trait of interest in a 'mapping population' (for example, an F_2 from a cross between selected lines)[12,18]. However, several factors make it difficult to estimate the 'true' numbers and effects of loci that influence a quantitative trait.

- **Closely linked QTL with opposite effects tend to be missed, as there are few recombinants that could reveal their presence.**
- **There is a lower limit for the size of a QTL that can be detected, which will vary according to the size of the experiment and the properties of the trait; real QTL with effects below this limit are nearly always undetected.**
- **Closely linked QTL with effects in the same direction tend to give the appearance of a single QTL of larger effect. Indeed, simulation studies have shown that under the infinitesimal model, the chance coupling of linked factors can lead to the appearance of large-effect QTL[100,101]. The effect can be exacerbated if recombination rates vary[102], or if the actual loci tend to be clustered (for example, in a multi-gene family). Hyne and Kearsey[103] have pointed out that in a typical experiment (heritability ~40%, ~300 F_2 individuals), no more than ~12 QTL are ever likely to be detected. Empirical data on the numbers of QTL detected in plants seem to support this[17].**
- **Unless samples are large (>500, for example), the effects of statistically significant QTL are substantially overestimated[104]. This 'Beavis effect' comes about because only QTL with a significance that exceeds some genome-wide threshold are reported, and their estimated effects tend to be inflated owing to a contribution from environmental variance.**

Alleles must have extremely small effects if they are to remain in the population for maybe 50 generations, and must, therefore, be present at very many loci to contribute significant heritability (FIG. 2b).

Therefore, sustained responses must be based either on many extremely minor variants present in the base population, or on new mutations. It is now clear that mutation rates for quantitative traits are high enough to make a substantial contribution[12]. Mutations of small effect take a very long time to be established, whereas mutations with large effects on heterozygotes quickly contribute to the selection response. For example, in the study by Yoo[13] (FIG. 1a), much of the response can be explained as the immediate consequence of new mutations of large effect — including several recessive lethals. Mutations of large effect are expected to contribute even more in bigger populations, because the total number of mutations available to selection is proportional to the population size. This is not inconsistent with the smooth and replicable response seen in large-scale experiments, such as Weber's[14] experiment on flight ability in *Drosophila* (FIG. 1b), because, in a large population, mutations arise often enough for their individual effects to be smoothed out (FIG. 2c). Although new mutations are expected to have deleterious effects, fitness might have remained high in this example because intense competition in Weber's dense cages eliminated deleterious alleles, or because more benign alternative alleles were more likely to be picked up in larger populations[15].

The nature of quantitative trait loci

There have been recent reviews of the genetic basis of between-species differences[16], and QTL studies in plant species[17]. Both surveys point to extreme variation between experiments in the numbers and effects of QTL. In several instances, most of the difference between important phenotypes is explained by a few genes (maybe less than five) of large effect, whereas others point to a strongly polygenic basis. Furthermore, there seem to be no obvious rules, based, for example, on the kind of quantitative trait that would allow us to make a prediction *a priori* about the genetic basis of a given trait in a given cross. In BOXES 2 and 3, we show two QTL mapping studies that span the extreme diversity of results that can arise from line-cross experiments.

Analysis of standing variation within natural populations is much more challenging. The magnitude of genetic variation is much lower than that of non-genetic fluctuations and, therefore, almost all successful studies have relied on candidate genes, which were already known to be likely to affect the trait. Following a long tradition, Mackay, Langley and colleagues have been searching for associations between bristle number and molecular markers in or near several candidate genes in *Drosophila*[18]. The basis of the test is that allelic variants that are associated with a change in trait value must be in LINKAGE DISEQUILIBRIUM with the causal genetic factor(s); as linkage disequilibrium is generally strong over only short genomic regions in wild *Drosophila*, such associations imply tight linkage. The first result was surprising: large DNA insertions (probably transposable elements) at or near the *Achaete-scute complex* are associated with fewer bristles[19]. The effect was strong and replicable[20]. This systematic association with a certain type of variant is good evidence for a causal effect, as opposed to mere correlation. In *Drosophila*, at least, much quantitative variation could therefore be caused by transposable element insertions, which remain rare in a mutation–selection balance[21].

Variation in bristle number was also significantly associated with single-nucleotide or small insertion/deletion variants in *scabrous*[22], *achaete-scute*[20] and *Delta*[23], and the variance associated with statistically significant molecular variants was quite large. However, the true amount of variation due to the causal factors is uncertain, for the various reasons discussed in BOX 1. A further caveat is that linkage disequilibrium can be generated by various forms of demographic structure, in which case the inference that the variation associated with a marker allele is caused by tightly linked alleles in the candidate gene might be spurious. In much of the *Drosophila* genome, linkage disequilibrium decays rapidly over short distances (that is, a few tens of kilobases), but there is evidence of longer-range weak disequilibrium, even between unlinked sites[24], and for patterns of molecular variation at neutral sites that could signal the effects of natural selection or demographic structure on linked sites[25–27]. A possible control is to test for associations with other traits, for which the genes tested are not strong candidates; the results should generally be negative.

Even apparently 'straightforward' cases, such as variation in enzyme activity, have proven surprisingly complex. Stam and Laurie[28] conducted a landmark study on the contribution of natural molecular variation to quantitative trait variation by dissecting the

DIRECTIONAL SELECTION
Natural selection that acts to promote the fixation of a particular allele.

DISRUPTIVE SELECTION
Intermediate phenotypes have lower fitness than extreme phenotypes; the opposite of stabilizing selection.

INFINITESIMAL MODEL
A simple model of the inheritance of quantitative traits, which assumes an infinite number of unlinked loci, each with an infinitesimal effect.

EFFECTIVE POPULATION SIZE
The size of the ideal population in which the effects of random drift would be the same as observed in the actual population.

LINKAGE DISEQUILIBRIUM
The condition in which the frequency of a particular haplotype is significantly greater than that expected from the product of the observed allelic frequencies at each locus.

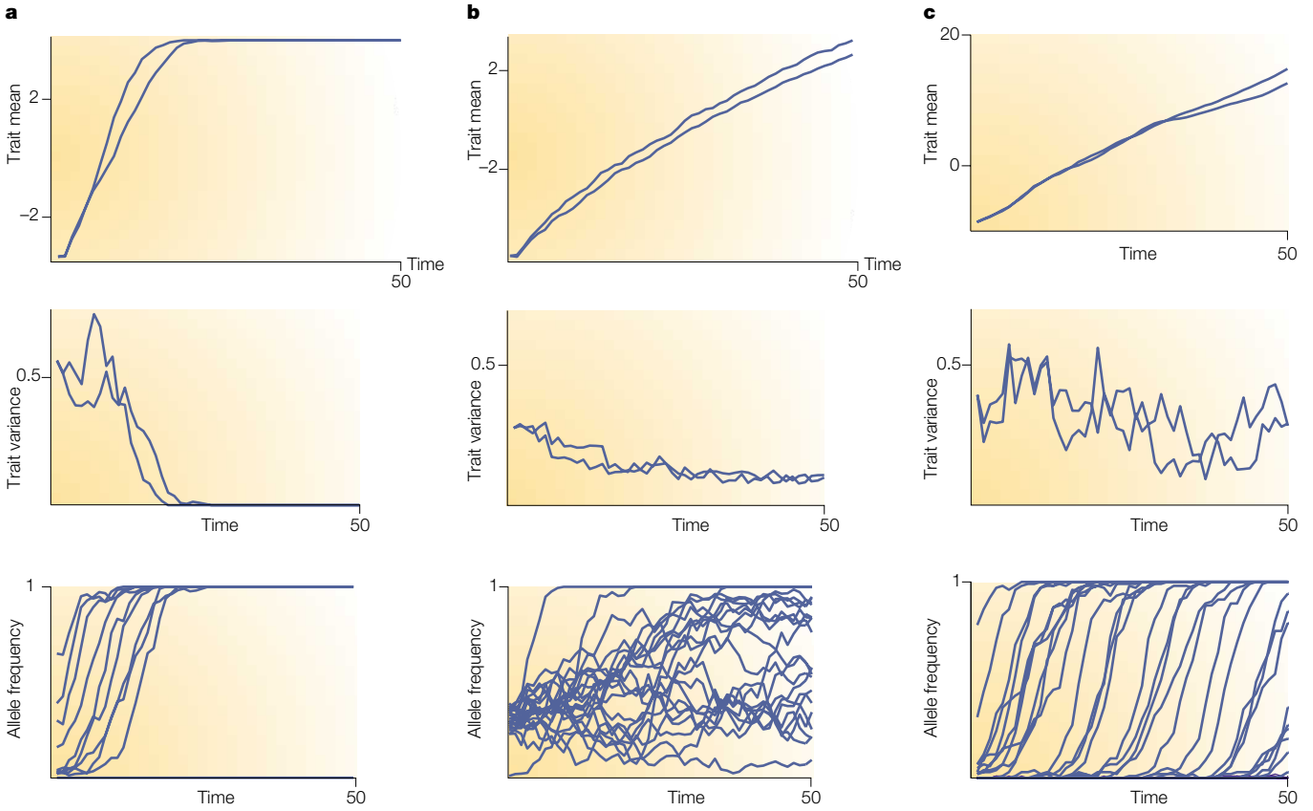


Figure 2 | **Alternative genetic models for long-term selection response.** Simulations were made of artificial selection on an additive trait with environmental variance, $V_e = 1$. The 50 highest-scoring individuals were chosen from 250 in each generation; initial heritability ~30%, and effects of major alleles $\alpha = 0.5\sqrt{V_e}$. Upper row: trait mean over time (two replicates). Middle row: genetic variance, V_g. Bottom row: allele frequencies (one replicate shown). **a** | Ten unlinked loci with major alleles, **b** | 400 unlinked loci with minor effects ($\alpha = 0.03\sqrt{V_e}$) apart from two major loci, which fix first (top left of bottom panel), **c** | 100 unlinked loci; initial allele frequencies as in **a** at ten loci; the remaining loci start fixed, but with mutation rate such that mutational variance, $V_m = 0.0023V_e$.

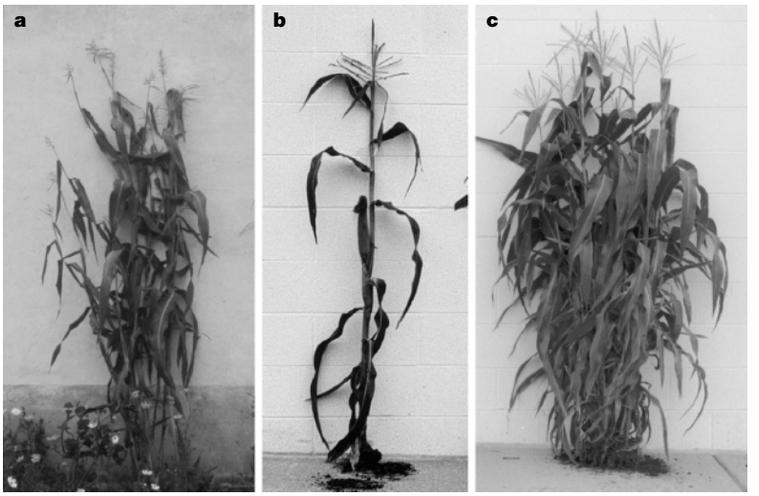
contribution of allelic variation at the *Adh* locus to the level of alcohol dehydrogenase activity in *Drosophila melanogaster*. There are genetic factors at sites linked to *Adh*, as well as unlinked loci, that contribute to the *Adh* enzyme activity variation that is observed in populations[29]. At *Adh* itself, the famous Fast/Slow amino-acid polymorphism contributes an ~2.5-fold catalytic activity difference, but variation in activity is also contributed by regulatory sites elsewhere at the locus, which influence *Adh* protein concentration. Stam and Laurie[28] constructed all eight possible allelic combinations of three different segments of the *Adh* gene *in vitro*, and transformed these into flies that lack the *Adh* gene. Analysis of the transgenic lines showed that natural polymorphisms in all three segments contribute to enzyme activity, and that there was a significant interaction between sites. Other studies had also revealed substantial linkage disequilibrium between sites in the *Adh* region that contribute to the activity differences. So, Stam and Laurie[28] argued that the combined effects of several linked polymorphisms could generate a 'superallele', which would be interpreted as a major gene in a QTL-mapping experiment.

We do not attempt to review the extensive literature on association studies between molecular variants and quantitative traits (mainly disease states) in humans, as there have been several recent reviews on this topic (for example, see REFS 30,31). The caveats that apply to the *Drosophila* studies mentioned above also apply to humans, and have led to pessimism about the prospect of identifying QTL that are associated with disease in population-wide surveys[32].

For practical purposes, it might suffice to locate a small region of the genome that is associated with trait variation. However, more fundamental questions require precise identification of the sequence variation involved. The prospects for cloning specific QTL will vary from case to case, because many QTL 'break up' into several linked factors when subjected to fine-scale mapping (for example, see REFS 33–39). But, there have been several recent reports of the cloning of QTL (notably in maize[40] and tomato[41,42]), which convincingly show that at least some QTL involve individual genes with large effects.

Even if the trait is simple, the molecular basis of variation might be complex. Stam and Laurie's[28] transgene

Box 2 | **The domestication of maize**



The ancestor of modern maize (*Zea mays mays*) is believed to be a wild Mexican grass, teosinte (*Z. mays parviglumis*)[43]. The two subspecies are interfertile, but show many morphological differences[105]: teosinte has many long, lateral branches that terminate in male inflorescences (tassels) (panel **a**), whereas the branches of maize are short and terminate with female inflorescences (ears) (panel **b**). Doebley and Stec[106] carried out a QTL analysis in an F_2 population derived from a cross between teosinte and a primitive variety of maize. All traits seemed to be under polygenic control, but, in most cases, one or two QTL accounted for a greater proportion of the phenotypic variance than all the remaining QTL put together. For example, a major QTL on maize chromosome 1 accounts for ~30% of the phenotypic variance for lateral-branching structure traits, and a QTL on chromosome 4 accounts for 44% of the phenotypic variance in a key fruit-case architecture measure. Whether defined by the amount of variance explained, or the change in phenotypic means, these are QTL of 'large effect'[16]. The fruit-case architecture QTL behaves as a Mendelian locus when backcrossed into a pure maize or teosinte background, and has been named *teosinte glume architecture1* (*tga1*) (REF. 107). The chromosome 1 QTL that affects lateral branching mapped to within 0.5 cM (averaged over traits) of a previously known major mutation, *teosinte branched1* (*tb1*)[108] (panel **c** shows the mutant homozygote) and it fails to complement a reference *tb1* allele — strong evidence that it is an allele of *tb1*. This locus is the first case of a QTL that has been cloned on the basis of its map position[40].

A small number of loci seem to have been key players in the genetic changes that occurred during the domestication of teosinte. We do not yet know whether substitutions at the (probably) five key loci were made in many small increments, or whether there were major-effect mutations, fixed during domestication. It is also possible that the fixation of major-effect alleles required many substitutions elsewhere in the genome to ameliorate undesirable pleiotropic effects. It would be interesting to know how teosinte containing short chromosome segments that carry the five major maize QTL alleles would function as a crop. Figure reprinted with permission from REF. 40 © (1997) Macmillan Magazines Ltd.

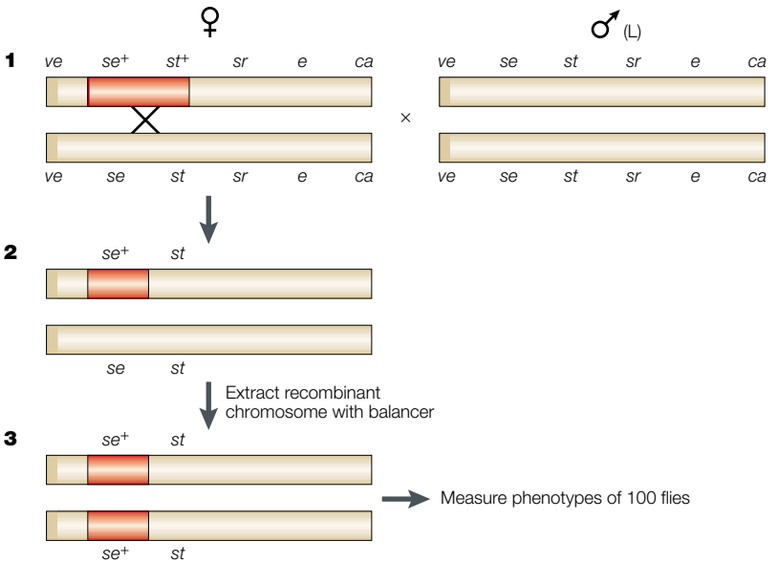
analysis of the *Drosophila Adh* locus showed that interacting allelic variants in coding, 3′ untranslated and intronic DNA affect enzyme activity. Non-translated regions are also implicated at several other QTL (see also REF. 18). In the case of *teosinte branched1* (*tb1*), Wang *et al.*[43] examined the nature of the allelic differences within and between populations of maize and teosinte to look for the molecular signature of selection under domestication. This could take the form of reduced molecular diversity due to a recent SELECTIVE SWEEP relative to a neutral sequence in a part of the gene within a maize population. Moreover, if the genetic changes at the locus involved several adaptive substitutions, there could be an excess of fixed differences between maize and teosinte, relative to a segment of neutral DNA. Wang *et al.* found evidence for the former in the 5′ non-transcribed region of the gene, but no evidence of fixed differences. This might imply that the target(s) for selection under domestication lie outside the chromosome region analysed. This is surprising, because reduced diversity due to selection seemed to be fairly localized. In tomato, the fruit weight QTL identified by fine mapping and transgenesis[41], and the soluble solids QTL identified by ultra-resolution mapping[42] could both be due to substitutions in non-coding DNA. Until the functions of non-coding DNA are better understood, it might turn out to be difficult to pinpoint precisely the causal factors that are responsible for QTL.

SELECTIVE SWEEP
After the fixation of a new favourable mutation, the surrounding region of the genome is also fixed; neutral diversity is therefore 'swept' out of the population.

Maintenance of variation by mutation

As already discussed, the response to artificial selection, beyond the first 20 or so generations, is increasingly caused by selection of new mutations; eventually, of course, all evolutionary change depends on an input of mutational variability. The standing variation might also be due to a balance between mutation and some kind of selection. Explanations for the maintenance of variation based on a balance between mutation and selection are attractive, because they are simple, and depend on just a few parameters that are (in principle) measurable. Moreover, the rate of increase in heritability due to mutation is substantial and surprisingly uniform: most estimates are in the range 0.1–1% per generation, so that in the absence of selection or random drift, heritability would be replenished over 100–1,000 generations[12]. A mutational explanation is, therefore,

Box 3 | **Bristle number selection lines in *Drosophila***



Shrimpton and Robertson's[77,109] ambitious study of sternopleural bristle number in *Drosophila melanogaster* marked the end of the era in which QTL were mapped with visible genetic markers or isozymes; these were soon replaced with DNA-based markers (for example, see REF. 110). Their analysis started with five recombinant sublines that carry different sections of chromosome from a high bristle line (H; indicated in red) on a multiply marked low-bristle-line background (L)[109]. H and L were derived by artificial selection for bristle score. These marker-assisted introgression lines are now known as 'nearly isogenic lines' (NILs), a common starting point for high-resolution QTL mapping[111], although the idea originated much earlier[112]. Comparison of these studies showed that effects on bristles were unequally distributed among the sections, and there was some evidence of interaction between them. The five sections identified in this first analysis together accounted for a phenotypic difference of ~24 bristles (~13 phenotypic standard deviations). Further analysis[109] increased this minimum estimate to 17 genes; extrapolating to the whole genome[113], the minimum becomes 39 genes.

Shrimpton and Robertson's strategy to map the factors within sections was to search for recombinants within sections, and then accurately estimate their homozygous effects — a powerful technique for high-resolution QTL mapping[112,114,115] (see figure). The low-line chromosome (L) was multiply marked with *ve*, *se*, *st*, *sr*, *e* and *ca*. A NIL that was heterozygous for the high-line genomic segment that contains the genetic markers *se* and *st* was allowed to recombine with L (1), and recombinants identified on the basis of the genotype of the flanking markers (2). Recombinants were made homozygous with the aid of BALANCER CHROMOSOMES (3), and their trait values accurately measured. Trait values (each estimated from 100 flies) could be resolved into a minimum of five groupings, indicating the presence of at least five QTL. There was broad agreement between the sizes and locations of the QTL inferred from recombinants coming from each side (that is, se^+st or $se\,st^+$), which indicates little overall genetic interaction. The largest chromosome 3 effect mapped very close to the *hairy* locus — a major Mendelian bristle number locus — and Shrimpton and Robertson[77] proposed that the factor could be allelic with it. However, a higher degree of resolution with a denser marker map would be necessary before this hypothesis could be accepted. It is possible that candidate genes of this sort make a substantial contribution to quantitative genetic variation[18], but the mere fact that a candidate gene maps close to a peak in LOD (logarithm of the odds) score in a genome-wide QTL scan is not in itself strong evidence of allelism[17]. A random scatter of small-effect QTL can generate large peaks in LOD score[101], and these could coincidentally map close to one or more candidate genes.

an attractive candidate for high heritabilities across diverse traits and organisms. However, although its simplicity makes mutation–selection balance the obvious working hypothesis, we should not exaggerate its plausibility as an explanation of a complex world.

If mutation is the immediate source of quantitative genetic variance, we can make two robust predictions. First, if an input of mutational heritability at a rate of 0.1–1% per generation is to be balanced at high heritability, then selection must eliminate variation at a similar rate: the alleles that are responsible for trait variation must reduce fitness by 0.1–1%[44]. Second, as mutation rates per locus are low (maybe 10^{-5} per gene), the deleterious alleles held in mutation–selection balance must be rare[45].

The key issue is to know what kind of selection is involved. Most attention has focused on stabilizing selection on the trait in question[45]. In this case, the equilibrium genetic variance equals the ratio between the total genomic mutation rate to alleles that affect the trait, *U*, and the strength of stabilizing selection. The size of the mutational effect is irrelevant: alleles with large effect reduce fitness more, and so are held at lower frequency, which makes the variance contributed independent of the effect. Although this prediction is remarkably simple, it is hard to test: the total mutation rate is much harder to measure than the mutational heritability, because the effects of individual mutations must be discerned, rather than just their aggregate effect on the net genetic variance. On the basis of an often-quoted estimate[12,46] for the

BALANCER CHROMOSOME
Chromosome with recessive lethal mutations and inverted segments that suppress recombination.

strength of stabilizing selection, high heritability of a particular trait would require a total mutation rate to alleles that affect the trait of $U = \sim 0.025$. If the mutation rate at each locus is small (for example, 10^{-5}), this would imply contributions from a very large number of loci.

It is possible that stabilizing selection is much weaker than has generally been assumed[9]. Moreover, individuals with extreme phenotypes might tend to have lower fitness, not because of the direct causal effects of the measured trait, but because both extreme phenotypes and low fitness are associated with poor condition, owing to unrelated environmental or genetic factors[8,44,47]. If so, a lower net mutation rate, U, would be required to maintain high heritability, and so fewer loci might be involved.

Although genome-wide mutation rates might be high enough to balance stabilizing selection directly[48], each mutation can potentially affect many traits: it is not possible to consider each trait in isolation. A direct mutation–selection balance then becomes much harder to accept, because selection on all the traits acts to reduce variation at each locus[44,49,50]. Once it is accepted that each gene might influence many traits, which in turn influence fitness, the simplest approach is to consider just the net effect of quantitative variation on the trait of interest and on net fitness[44,51]. In this view, mutation maintains extensive genetic variation, and some of this variation happens to influence the trait of interest. All that is required to maintain high heritability is that some alleles have appreciable effects on the trait, but small effects on fitness. The joint distribution of the effects of P-element insertions on *Drosophila* bristle number and viability has been estimated, and indicates that such alleles might exist[52]. However, it is not feasible to measure the extremely small fitness effects that are likely to be involved.

Maintenance of variation by selection

For bristle number in *Drosophila*, rare deleterious mutations with large effects are known to contribute both to selection response[13] (FIG. 1a) and to standing variation[20]. However, the proportion of variation that can be explained in this way is unknown, and for other traits and organisms, the issue is unresolved. BALANCING SELECTION might be at least as important as mutation in maintaining heritable variation. As with mutation, selection might act either directly on the trait, or indirectly, by maintaining variation at loci that have pleiotropic effects on the trait that is observed[44].

Direct selection can maintain variation by inducing heterozygote advantage — for example, heterozygotes might be closer to the trait optimum[53,54], or might be less sensitive to the environmental fluctuations that deviate from the optimum[55–57]. However, OVERDOMINANCE cannot be a complete explanation, because self-fertilizing organisms maintain heritable variation, despite the lack of heterozygotes (for example, see REFS 58,59). The alternative is that rare phenotypes are somehow favoured, so that a stable equilibrium is maintained. Such frequency-dependent selection can occur when different genotypes exploit different limiting resources. For example, specialization to eat different types of seed might maintain the striking dimorphism in beak size in *Pyrenestes* finches[60]. Diverse mechanisms are possible[61–63], but all act in essentially the same way: alleles exploit different limiting resources, giving them an advantage when they are rare relative to their preferred resource. A different mechanism applies when the trait optimum fluctuates in the absence of any spatial or ecological subdivision: if fluctuations are sufficiently strong, a very low level of mutation can sustain considerable variation[64–66]. Finally, migration between local populations in which selection favours different trait values or alleles might maintain substantial variation[2].

These mechanisms — overdominance, frequency-dependent selection, migration or temporal fluctuation — can maintain heritability as a side effect of polymorphism, even if selection does not act directly on the trait[44,67,68]. Indeed, as argued above, when we realize that each allele is likely to affect several traits, it becomes more plausible to suppose that variation in any one trait is a pleiotropic side effect of polymorphisms that are maintained for other reasons. All that is needed is that balanced polymorphisms should be abundant and influence quantitative traits. The relative plausibility of mutation versus balancing selection as factors that maintain heritability then depends largely on their relative importance in maintaining sequence variation. Despite much effort, this is still an unresolved issue.

There is good evidence that balancing selection maintains substantial heritability in specific cases. When selection is known to maintain polymorphism, quantitative traits are often influenced — for example, flight activity in *Colias* butterflies varies, in part, because of the balanced polymorphism at phosphoglucose-isomerase[69]. However, such examples are few, and so more-general predictions are needed. One such example is that traits should regress after strong artificial selection is relaxed. However, artificial selection can easily be strong enough to eliminate variation at some loci, which impedes return and, in any case, regression is also expected if variation is due to deleterious alleles (for example, see FIG. 1a).

The strongest prediction is that, in almost all models of balancing selection, alleles are maintained at high frequency. Observation of common alleles that are associated with heritable trait variation has, therefore, been seen as evidence for balancing selection[20]. The pattern might be obscured by neutral divergence in allelic classes. However, even if many haplotypes segregate, balancing selection might still be detected by an excess within-population variability near the selected site[70], and by the presence of ancient lineages, maintained by selection for longer than would be plausible under random drift[71]. This approach requires, however, that the QTL responsible for within-population variation be mapped and sequenced.

To summarize, we know that both mutation and balancing selection are responsible for some heritable variation, but we do not know their relative importance. Any one mechanism of balancing selection can be criticized, as it is unlikely to apply across a wide range of traits and organisms; in addition, most mechanisms that invoke direct selection on the trait

BALANCING SELECTION
Selection that acts to maintain two or more alleles in a population.

OVERDOMINANCE
The phenotype of the heterozygote is greater than that of either homozygote. Overdominance for fitness can lead to the maintenance of both alleles in the population.

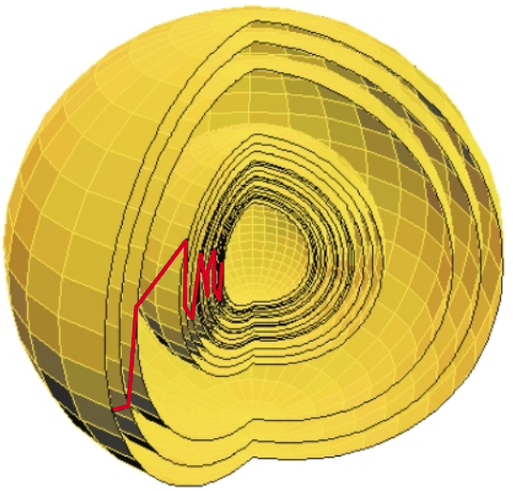


Figure 3 | **Adaptation in the Fisher/Orr model.** The population begins at a point on the outer sphere, at a distance $d/2$ from the central optimum (d is the diameter of the sphere); the simulation is of ten dimensions, although only three can be shown. The first successful mutation has magnitude $r = 0.137d$, and takes the population 8.7% of the way to the optimum (first part of line, leading to the second sphere). The third successful mutation has the largest magnitude, $0.271d$; it is followed by smaller steps that, on average, follow a geometric series. Reproduced with permission from REF. 118 ©1998 Macmillan Magazines Ltd.

maintain variation at only a few loci (for example, see REFS 54,61; also see REF. 72). However, various mechanisms might together explain widespread heritability. The clearest path to distinguishing the alternatives requires that we identify the frequency and sequence structure of alleles at QTL.

What type of QTL do we expect to see?

Classical population genetics does not, by itself, predict what type of genetic variation we expect to be responsible for phenotypic variation, although it can show what kinds of allele will be sieved out by selection and other processes. For example, the evolution of the genetic system has been understood by assuming abundant genetic variability for recombination rate, degree of selfing, ploidy cycle, and so on, and then asking what types of system will emerge. However, there has been little discussion of the issue that concerns us here: what types of allele do we expect to underlie variation in continuous traits? Progress here requires some model of the relationship between genotype and phenotype.

One such model was first proposed by R. A. Fisher[73] to support his argument that adaptation is based on mutations of small effect. Fisher imagined that the phenotype consists of a large number, n, of continuous traits, and that fitness increases smoothly towards some optimum. The model can be visualized as a space of n dimensions, with the optimum at the centre, surrounded by contours of decreasing fitness (FIG. 3). A mutation takes the phenotype in some random direction, and increases fitness if it brings the genotype closer to the optimum — that is, inside the n-dimensional shell on which the current genotype lies. Fisher's argument was that mutations of small effect, r, relative to the distance from the optimum, d, are as likely to increase fitness as to decrease it. However, mutations of sufficiently large effect ($r > d$) must decrease fitness. Moreover, the chance that a mutation is favourable depends on $r/\sqrt{n}$: changes of a given magnitude are more likely to disrupt a complex organism than a simple one.

Kimura[74] introduced the first evolutionary analysis of Fisher's model, by pointing out that although small mutations are more likely to increase fitness, they are less likely to be fixed. Overall, then, mutations of intermediate magnitude are most likely to contribute to adaptation. Orr[75] considers a sequence of substitutions, which take a population towards the optimum in steps of (on average) decreasing size. He shows that the largest step in the whole sequence is likely to be much larger than Fisher or Kimura's calculations suggest. However, Fisher's argument is still qualitatively correct, in that the typical magnitude of a successful mutation decreases with $\sqrt{n}$.

Two results from Orr's analysis are relevant to understanding the nature of QTL. First, for plausible distributions of mutational effects, those that are fixed have effects that are approximately exponentially distributed — roughly speaking, we expect a few QTL of large effect, plus many more with small effects. So, if populations adapt by fixing a sequence of additive mutations, we expect an exponential distribution of QTL effects. Observed distributions[76,77] are consistent with this prediction, although there is little statistical power to detect deviations, and the distributions are distorted by the various biases discussed in BOX 1.

The second result[78] is that the rate of adaptation decreases inversely with the number of dimensions in which the organisms is adapting. This is essentially because the chance that a random mutation is favourable decreases with the number of constraints that it must satisfy. Orr proposes that, in principle, the effective number of dimensions could be measured by examining the distribution of fitness effects of random mutations. Given a microbial strain that is adapted to some well-defined environment, the 'size' of various mutants could be measured by the decrease in fitness that they cause. If one then takes a suboptimal strain, the distribution of fitness effects caused by mutations of known 'size' leads to an estimate of the effective number of dimensions: with more dimensions, the chance that a random mutation will increase fitness becomes smaller, as does the typical size of those mutations that do turn out to be favourable. The difficulty of adapting under many constraints — the essence of the Fisher and Orr argument — implies that genetic manipulations of large effect will usually have deleterious side effects, which can best be ameliorated by the selection of minor alleles.

We can hardly imagine that organisms live in the abstract geometrical world proposed by Fisher. However, his model does lead to robust predictions (see above), and indicates research that would be valuable regardless of its interpretation within the Fisher and Orr framework. For example, True and

Lindquist[79] found that yeast defective in translation termination had a wide range of growth rates, which depended strongly on the environment and genetic background; in many cases, the defect actually increased growth rates. However, this finding is difficult to assess without knowing the distribution of fitness effects of other types of genetic perturbation. Such systematic work is starting to be carried out in large-scale studies of gene function (for example, see REFS 80,81). Orr's model indicates possible interesting interpretations for such selective screens, in terms of an "effective number of dimensions". Alternative models are needed, and a few exist: for example, Kauffman's[82] NK model for gene interaction and models of metabolic networks[83] (see below). More specific models of development and gene regulation are also being constructed[84], but the difficulties of obtaining accurate measurements of many parameters, and the need for a broader understanding applicable to diverse systems, at present favours a more abstract and general approach.

Epistasis

Quantitative genetics focuses on the additive effects of individual alleles. This approach is valid even if there are pervasive interactions between genes — that is, DOMINANCE and EPISTASIS. It is important to distinguish here between the technical definitions of gene interaction in quantitative genetics, as deviations from an additive statistical model, and the wider definition in terms of genetic mechanisms. The rate of change of a trait, and the genotypic variance, depend primarily on additive effects, so that interaction terms can often be neglected. Conversely, a good fit to an additive model (for example, see REF. 85) does not imply that the underlying genes do not interact at a mechanistic level.

The form and amount of epistasis that we expect for quantitative traits depends crucially on the specific details of gene action. For example, consider a linear chain of enzymes that converts X to Y; the trait is the flux to Y. Null mutations clearly generate large epistatic effects, but minor alleles (say, doubling or halving enzyme activity) are expected to generate little epistasis[86]. In such metabolic models, epistasis is 'directional', so that selection for increased or decreased flux has different consequences. As alleles that raise enzyme activity increase in frequency, metabolic-control theory predicts that their effects on flux will increase[87]; conversely, there are diminishing returns for downwards selection. This might be one explanation for the widespread asymmetrical responses to artificial selection[3], although it is possible that DIRECTIONAL DOMINANCE is a more common cause of asymmetry than directional epistasis[88].

Perhaps surprisingly, given the complexity of metabolism and development, there is little evidence for widespread interactions between QTL that differentiate distantly related taxa[16]. However, genome-wide surveys of populations that are derived from crosses between artificially selected lines often find evidence for substantial epistasis between QTL (for example, see REFS 89–91). The interpretation of these pairwise interactions is difficult, however, because so many epistatic terms need to be evaluated. By crossing together near-isogenic lines that contain different QTL, interactions can be studied more directly. Experiments of this type in crosses between wild and cultivated tomatoes indicate possible diminishing returns epistasis between QTL for fruit weight[92], in which each additional favourable allele adds a smaller increment to the trait. This would be consistent with the metabolic model mentioned above. In maize, crosses that involve *tb1* and a second, large-effect QTL revealed epistatic effects for morphology of magnitude that are comparable with the main additive effects; the interaction of the two QTL might, therefore, have been crucial in domestication[93]. It should be noted, however, that these QTL might have extreme additive effects, which might make strong interactions between them more likely.

Gene interactions are important because they cause the additive effects of alleles to change as the genetic composition of the population changes. For example, there are strong theoretical arguments that a high mutation rate can only be sustained if each deleterious allele significantly reduces fitness, and yet the cumulative load due to many deleterious alleles is less severe than the sum of their individual effects. This type of negative epistasis can also favour sex and recombination[94]. Gene interaction might also slow down selection response, because alleles might only become favourable as the genetic background changes during the course of selection. *In vitro* selection experiments on ribozymes have shown how individual variants rise and then fall as their selective value changes with the genetic background[95]. Such patterns might be widespread, and yet go undetected.

Conclusions

The alleles that are responsible for standing variation in quantitative traits might be different from those that contribute to natural adaptations, or to the long-term success of plant and animal breeding. In particular, if most variation and selection responses are a side effect of deleterious mutations, then we might have explained less than it seems. Although transposable elements in *Drosophila* are associated with trait variation at candidate loci, they are rarely fixed in evolution[21], and are likely to disrupt gene regulation, rather than be involved in building new complex functions. Similarly, recessive lethals can hardly be responsible for much long-term evolution. Alleles with severe deleterious side effects might eventually be replaced by more benign alleles, and modifiers might also evolve to ameliorate fitness costs (for example, see REFS 96,97). In this view, short-term selection response would tend to be based on alleles of larger effect than those responsible for species differences. There is no sign of such a pattern, but it might well be obscured by accumulation of multiple substitutions at the same locus to eventually give a compound allele of large effect[16,98]. The same kind of comments apply if balancing selection maintains variation; again, there might be a conflict between selection on the trait and on the underlying genes.

DOMINANCE
A genetic interaction between the two alleles at a locus, such that the phenotype of heterozygotes deviates from the average of the two homozygotes.

EPISTASIS
In the context of quantitative genetics, epistasis refers to any genetic interaction in which the combined phenotypic effect of two or more loci is less than (negative epistasis) or greater than (positive epistasis) the sum of effects at individual loci.

DIRECTIONAL DOMINANCE
The phenotype of individuals that are heterozygous for the multiple loci that affect a trait deviates from the average of the phenotypes of homozygous individuals.

Extrapolation from within-population variation to long-term evolution is further complicated by chance variation. Rare, favourable alleles will tend to be missed, so that selection response based on standing variation will vary between replicates; the same applies to the contribution from new mutations. In some cases (for example, in insecticide resistance), only a few alleles at a locus might contribute; even for a complex trait, such as bristle number in *Drosophila*, the common involvement of candidate genes indicates a surprisingly consistent response to selection. However, we still expect that if selection response is based primarily on rare alleles or new mutations, and epistasis is common, the QTL that are responsible for adaptation will not be predictable from variation in the population at large. This diversity is seen most strikingly in the recent adaptations of human populations to resist malaria, which has involved a wide variety of alleles in α- and β-globin, and in many other genes[99].

Progress towards understanding the basis of quantitative genetic variation is likely to come from studying allelic variation at specific QTL. Cloning of QTL that cause phenotypic differences between selected populations should become more common, aided by the revolution in genomic technology, and should lead to the elucidation of the nature of such QTL. We can hope to have much more information on whether multiple allelic substitutions are typically involved, whether regulatory or structural changes predominate, and the extent of dominance and epistasis. Achieving a satisfactory understanding of variation within populations is likely to be much more challenging. The information that we are seeking is the distribution of sizes of allelic effects at individual QTL and their frequencies within the population in question, as well as information on their effects on fitness. Highly replicated experiments that isolate QTL by fine-scale mapping could give us information on the allelic variants that segregate at specific regions of the genome or even specific loci, along with their frequencies within a population. Association studies should also reveal whether allelic variants repeatedly correlate with phenotypic variation for specific traits. Gaining such information will require very large experiments, larger perhaps than have previously been contemplated, but are essential if we are to know the causes of variation among organisms — including our own species.

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