Epistasis and quantitative traits: using model organisms to study gene–gene interactions

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Abstract | The role of epistasis in the genetic architecture of quantitative traits is controversial, despite the biological plausibility that nonlinear molecular interactions underpin the genotype–phenotype map. This controversy arises because most genetic variation for quantitative traits is additive. However, additive variance is consistent with pervasive epistasis. In this Review, I discuss experimental designs to detect the contribution of epistasis to quantitative trait phenotypes in model organisms. These studies indicate that epistasis is common, and that additivity can be an emergent property of underlying genetic interaction networks. Epistasis causes hidden quantitative genetic variation in natural populations and could be responsible for the small additive effects, missing heritability and the lack of replication that are typically observed for human complex traits.

Main effect
The effect of a variable averaged over all other variables; also known as marginal effect.

Heterosis
The phenomenon whereby the mean value of a quantitative trait in the F_1 progeny of two inbred lines exceeds, in the direction of increased fitness, either the mean value of the parental lines (that is, mid-parent heterosis) or the mean value of the best parent (that is, high parent heterosis); also known as hybrid vigour.

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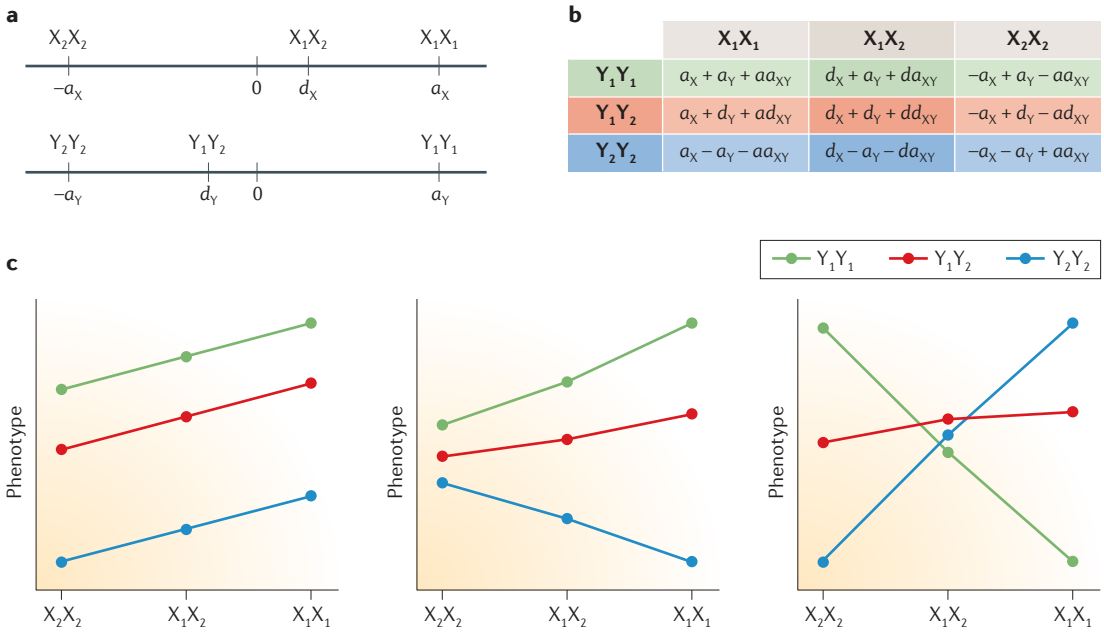
A major challenge of contemporary biology is to understand how naturally occurring variation in DNA sequences causes phenotypic variation in quantitative traits. Efforts to chart the genotype–phenotype map for quantitative traits using both linkage and association study designs have mainly focused on estimating additive effects of single loci (that is, the main effect of the polymorphic locus averaged over all other genotypes). However, quantitative variation in phenotypes must result, in part, from multifactorial genetic perturbation of highly dynamic, interconnected and nonlinear developmental, neural, transcriptional, metabolic and biochemical networks[1]. Thus, epistasis (that is, nonlinear interactions between segregating loci) is a biologically plausible feature of the genetic architecture of quantitative traits. Deriving genetic interaction networks from epistatic interactions between loci will improve our understanding of biological systems that give rise to variation in quantitative traits[2], as well as of mechanisms that underlie genetic homeostasis[3,4] and speciation[5,6]. Knowledge of interacting loci will improve predictions of individual disease risk in humans, response to natural selection in the wild, and artificial selection and inbreeding depression (and its converse, heterosis) in agricultural animal and crop species.

Mapping epistatic interactions is challenging experimentally, statistically and computationally. The experimental challenge is the large sample sizes that are required both to detect significant interactions and to sample the landscape of possible genetic interactions. The statistical challenge is the severe penalty that is incurred for testing multiple hypotheses. The computational challenge is the large number of tests that must be evaluated. Genetically tractable model organisms afford the opportunity to use experimental designs that incorporate both new mutations and segregating variants to detect epistasis, and many recent studies in model organisms have highlighted the importance of epistasis in the genetic architecture of quantitative traits. In this Review, I describe the quantitative genetics of epistasis and the reasons that the role of epistasis has been controversial. I then review experimental methods to detect epistasis in yeast, *Drosophila melanogaster*, mice, *Arabidopsis thaliana* and maize, and summarize empirical results showing that epistasis is pervasive. I discuss the implications of pervasive epistasis in model organisms for evolutionary models of the maintenance of quantitative genetic variation and speciation, and for both animal and plant breeding. Given that epistasis is pervasive in model organisms, it is also likely to be a hallmark of the genetic architecture of human complex traits. I discuss how underlying epistasis can give rise to the small additive effects, missing heritability and the lack of replication that are typically observed in human genome-wide association studies. I do not discuss statistical and computational methods for assessing epistasis, as these have been reviewed previously[7,8].



	X_1X_1	X_1X_2	X_2X_2
Y_1Y_1	$a_X + a_Y + aa_{XY}$	$d_X + a_Y + da_{XY}$	$-a_X + a_Y - aa_{XY}$
Y_1Y_2	$a_X + d_Y + ad_{XY}$	$d_X + d_Y + dd_{XY}$	$-a_X + d_Y - ad_{XY}$
Y_2Y_2	$a_X - a_Y - aa_{XY}$	$d_X - a_Y - da_{XY}$	$-a_X - a_Y + aa_{XY}$

Figure 1 | **Two-locus genotypic effects.** Genotypic values for loci X and Y, each with two alleles (X_1, X_2, Y_1 and Y_2), are shown (part **a**). The additive effect (*a*) of each locus is one half the difference in mean phenotype between the two homozygous genotypes. The dominance effect (*d*) is the difference between the mean phenotype of the heterozygous genotype and the average phenotype of the two homozygous genotypes[10]. $d = 0$ indicates additive gene action; $d \neq 0$ denotes departures from additivity due to dominance. Genotypic values for two-locus genotypes are shown (part **b**). The first two terms for each genotype denote the additive combination of single-locus additive and dominance effects. With epistasis, additional terms that reflect additive-by-additive (aa_{XY}), additive-by-dominance (ad_{XY} and da_{XY}) and dominance-by-dominance (dd_{XY}) epistasis contribute to the genotypic value. Graphical representations of genotypic effects at two biallelic loci are shown (part **c**). The left panel shows additive gene action at locus X, partial dominance at locus Y and no epistasis between X and Y. The middle panel shows epistasis in which the additive effect of locus Y is much greater in the X_1X_1 genetic background than that in the X_2X_2 genetic background. The right panel shows epistasis in which the additive effects of locus X are opposite in the Y_1Y_1 and Y_2Y_2 genetic backgrounds.

Missing heritability
The phenomenon whereby the fraction of total phenotypic variance that is explained by all individually significant loci in human genome-wide association analyses for common diseases and quantitative traits is typically much less than the heritability that is estimated from relationships among relatives.

Di-hybrid cross
A cross between parental lines that are fixed for alternative alleles at two unlinked loci (for example, $A_1A_1B_2B_2$ x $A_2A_2B_1B_1$, where A and B denote the loci and the subscripts represent the alleles) in which nine genotypes segregate in the F_2 generation.

Dominance effects
Differences between the genotypic values of the heterozygous genotypes and the average genotypic values of the homozygous genotypes at loci that affect quantitative traits.

Quantitative genetics of epistasis

In classical Mendelian genetics, epistasis refers to the masking of genotypic effects at one locus by genotypes of another, as reflected by a departure from expected Mendelian segregation ratios in a di-hybrid cross[2]. In quantitative genetics, epistasis refers to any statistical interaction between genotypes at two or more loci[9–11]. Epistasis can refer to a modification of the additive effects and/or dominance effects of the interacting loci (FIG. 1a,b); for two diploid loci, it can be easily visualized by plotting the phenotypes of the nine different genotypes (FIG. 1c). Epistatic interactions for quantitative traits fall into two categories: a change of the magnitude of effects, in which the phenotype of one locus is either enhanced or suppressed by genotypes at the other locus; or a change of the direction of effects. In the absence of epistasis the estimates of additive and dominance effects at each locus are the same, regardless of the genotype of the other locus. With epistasis, the effect of one locus depends on the genotype at its interacting locus.

The role of epistasis in the genetic architecture of quantitative traits has been controversial since early formulations of quantitative genetic theory[12,13], and this controversy continues today[7,14]. Different perspectives regarding the importance of epistasis arise, depending on whether one focuses on epistatic interactions at the level of individual genotypes or at the level of epistatic genetic variance in populations[2,9]. Epistatic interactions at the level of individual genotypic values (known as genetical, biological or physiological epistasis[15]) are independent of allele frequencies at the interacting loci. In populations, the total genetic variance is partitioned into orthogonal components that are attributable to additive, dominance and epistatic variance, which depend on allele frequencies[10,11].

Epistasis (FIG. 2a) can have peculiar effects in populations because the effects of one locus (that is, the target locus) vary depending on the allele frequency of an interacting locus (FIG. 2b). If the allele frequency of the interacting locus varies among populations, the effect of the target locus can be significant in one population but not in another, or can even be of the opposite sign. Epistatically interacting loci generate substantial additive genetic variance over much of the allele frequency spectrum because of non-zero main (that is, additive) effects (FIG. 2c). Epistatic variance is maximal when both interacting loci are at intermediate frequencies and is of much smaller magnitude than the additive genetic variance unless the genotypic values at one locus are in opposite directions in the different

genetic backgrounds (FIG. 2d). Additive genetic variance therefore accounts, in theory, for most of the total genetic variance for a wide range of allele frequencies in the presence of epistasis[10,11,14] (FIG. 2e).

Most observed genetic variance for quantitative traits is additive. Such genetic variance could be either 'real', if most loci that affect the trait have additive gene action, or 'apparent' from non-zero main effects that arise from epistatic gene action at many loci. This distinction is not important if the goal is to estimate heritability, to predict phenotype from genetic relationships among individuals[16,17] or to predict short-term response to artificial and natural selection because all of these depend on additive variance that is specific to the population of interest[10,11]. However, knowing whether additive variance is an emergent property of underlying epistasis becomes crucial if the goals are to functionally dissect the genotype–phenotype map, to determine genetic interaction networks, to understand the effects of mutational perturbations on standing variation, to predict long-term responses to artificial and natural selection, and to understand the consequences of genetic drift and inbreeding on quantitative traits.

To distinguish between real and apparent additive genetic variance, we need to obtain evidence for the existence of epistasis, as well as to estimate genotypic values at causal, potentially epistatic, pairs of loci (or indeed at loci that are involved in higher order interactions). Genetically tractable model organisms allow analyses of epistatic interactions using: mutations that are generated in a common homozygous genetic background; quantitative genetic analyses of both inbred lines and outbred populations; chromosome substitution, introgression and near-isogenic lines; and induced mutations as foci for exploring such interactions with segregating variants. The ability to construct mapping populations from crosses of inbred lines in which all allele frequencies are 0.5 is particularly powerful, as this maximizes both epistatic variance and frequency of the rarer two-locus genotypes.

Epistasis between mutations

Mutations that have been induced in the same homozygous genetic background are excellent resources for estimating the magnitude and the nature of digenic epistatic interactions. Epistasis occurs if the difference in phenotype of the double mutant cannot be predicted from the combined effects of the single mutants. The double-mutant phenotype can be either more mutant than expected (which is known as synergistic, enhancing, aggravating or negative epistasis) or less mutant than expected (which is known as antagonistic, suppressing, alleviating or positive epistasis). The advantage of this method is that the interacting partners are known, which facilitates the construction of genetic interaction networks. A disadvantage is that it does not easily scale beyond pairwise interactions and to large numbers of mutations, as a comprehensive evaluation of n pairwise interactions requires the generation of $\sim n^2$ genotypes which, in practice, prevents exploration of the entire interaction space.

Figure 2 | **Quantitative genetics of additive-by-additive interactions.** The four double homozygote genotypes at two hypothetical bi-allelic loci (X and Y) are depicted. Model 1 is an epistatic model in which the effect of locus X is greater in the Y_1Y_1 genetic background than that in the Y_2Y_2 genetic background; Model 2 is an epistatic model in which the effect of locus X is of similar magnitude but in the opposite direction in the Y_1Y_1 genetic background, compared with that in the Y_2Y_2 genetic background (part **a**). The additive effect of locus X depends on the frequency at locus Y (part **b**). Additive genetic variance (V_A; part **c**), additive-by-additive genetic variance (V_{AA}; part **d**) and the ratio of additive genetic variance to the total genetic variance ($V_A/(V_A + V_{AA})$; part **e**) for Models 1 and 2 are shown.

Epistasis between small numbers of mutations. Studies using limited numbers of random mutations, or mutations that affect the same trait, show that epistasis is common. In *Escherichia coli*, 14 of 27 (52%) pairs of random mutations that were tested showed epistasis for fitness[18]. In *D. melanogaster*, 35 of 128 (27%) tests for epistasis among pairs of random mutations had significant effects on quantitative traits that are involved in intermediary metabolism. These epistatic effects were large and occurred between mutations without significant main effects[19]. Diallel cross designs among small numbers of *P*-element mutations that affected olfactory, locomotor, aggressive behaviour and lifespan in *D. melanogaster* revealed extensive epistasis and defined new genetic interaction networks[20–23]. These interaction networks were influenced by environmental conditions, sex and the presence or absence of an additional interacting mutation[21,22].

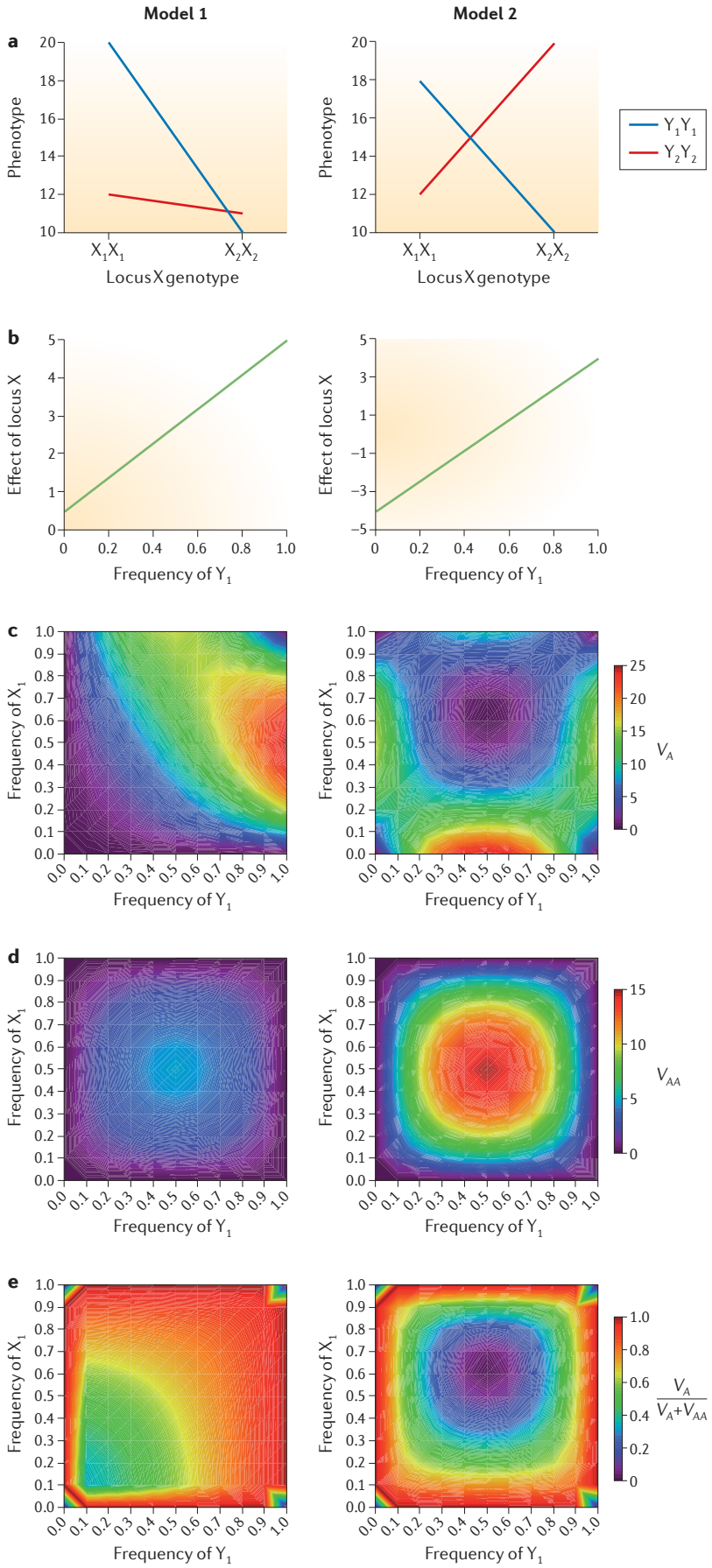
Genome-wide interaction screens. A few model systems are amenable to experimental analyses of genome-wide genetic interaction networks. An analysis of deletions for all 6,000 genes in *Saccharomyces cerevisiae* revealed that only 20% of the genome is essential for survival, at least under optimal growth conditions[24]. This observation attests to the robustness of biological networks to mutational perturbation and sets the stage for synthetic enhancement genetics in this species[25]. The collection of deletion mutants, together with high-throughput methods for generating and selecting double mutants, measuring growth rate and quantifying fitness[25], has facilitated large-scale genetic interaction screens in yeast[26–30]. Carrying out tests for all ~18 million possible pairwise interactions remains a practical impossibility even in this genetically tractable model system. Therefore, the yeast global genetic network architecture was investigated using a set of query mutations that were chosen to represent biological pathways of interest. Interactions were examined either between each of the query mutations and a larger number of target mutations[26,29,30] or for all possible pairwise combinations of the query mutations[27,28]. Similar strategies have been adopted for systematic mapping of genetic interactions in *Caenorhabditis elegans*[31,32] and in *D. melanogaster* cell lines[33] using RNA interference (RNAi).

Standing variation
Allelic variation that is currently segregating within a population, as opposed to alleles that appear as the result of new mutation events.

Introgression
The substitution of a genomic region from one strain with that of another, typically by repeated backcrosses.

Diallel cross
A class of experimental designs that are used to estimate both additive and non-additive variance components for a quantitative trait from all possible crosses among a population of inbred lines. Full diallel designs include reciprocal crosses, whereas half-diallel designs do not; parental lines can be included or excluded in either case.

Synthetic enhancement
A type of epistatic interaction whereby the phenotype of a double mutant is more severe than that predicted from the additive effects of the single mutants.



Larger-scale studies[26,27,29–31,33] that use qualitative assays typically find that ~1–3% of interactions have significant effects, whereas smaller-scale studies[28,32] that use quantitative assays identify a larger number of interactions (~13–35%). These studies have been instrumental in determining the general properties of genetic interaction networks (BOX 1). The scale-free and small-world properties of these networks imply that the major features of network topology can be inferred by focusing on major hub genes and on interactions among the genes with which they interact.

Gene expression-based screens. Mutations typically have pleiotropic effects on many phenotypes; therefore, focusing on only one phenotype will not uncover the full spectrum of possible interactions. Genome-wide analyses of differences in gene expression in the presence of single and double mutations relative to the control can be used to place genes in an interaction network in the absence of organismal-level phenotypes[23,34–36]. This approach is particularly powerful for higher eukaryotes that have long generation intervals and that lack high-throughput methods for generating double mutants and for accurately measuring complex organismal quantitative traits, but for which large collections of mutations are available[37–41]. In *D. melanogaster*, single mutations have pleiotropic effects on hundreds of gene expression traits[22,23,42]. The genes for which expression is altered in the mutant genetic background are thus candidate genes for inclusion in a genetic interaction network that affects the organismal phenotype associated with the focal mutation. A large proportion of such candidate genes indeed show epistasis with the focal mutation[42]. Thus, combining mutational perturbations with gene expression is a powerful approach to iteratively reverse-engineer networks. The large numbers of candidate genes that are implicated by gene expression profiling indicates that the interaction space is large.

Epistasis between QTLs

To what extent does the extensive epistasis that is implicated by analyses of induced mutations translate to epistatic interactions in natural populations? The ability to construct inbred lines, artificial selection lines and chromosome substitution lines, as well as to map quantitative trait loci (QTLs) that affect complex traits by linkage and association, facilitates analyses of epistasis between naturally occurring variants in model organisms.

Comparing broad- and narrow-sense heritability. Narrow-sense heritability (h^2) refers to the fraction of the phenotypic variance of a quantitative trait that is due to additive genetic variance, whereas broad-sense heritability (H^2) refers to the fraction of the phenotypic variance that is due to all components of genetic variance. In model organisms that can be crossed and inbred, one can obtain unbiased estimates of h^2 either from half-sib family designs or from the response to directional artificial selection. The genetic component of H^2 that

Box 1 | **Properties of genetic interaction networks**

Genetic interaction networks in *Saccharomyces cerevisiae*[25–30], *Caenorhabditis elegans*[31,32] and *Drosophila melanogaster*[33] share common properties that are likely to be generalizable to genetic interaction networks in other species.

- The fitness of the double mutant tends to be lower than expected if the genes act in separate but compensatory pathways, and higher than expected if the genes act in the same pathway.
- The distribution of the number of interactions per gene (that is, connectivity) follows a power law distribution, such that many genes have no or few interactions, and a few genes have many interactions. Genes that have many interacting partners are hubs in the interaction network.
- Genetic interaction networks are small-world networks, such that the shortest path between a pair of genes is small, which results in dense local neighbourhoods of genes that interact with each other.
- Genetic interactions occur among functionally related genes that belong to the same pathway or biological process. The 'guilt-by-association' principle can thus be used to infer the function of a computationally predicted gene from the function of the genes with which it genetically interacts.
- Network hub genes have the following characteristics compared with genes that have fewer interactions: they are more important for fitness; they are more pleiotropic; their mRNAs are expressed at higher levels; they are more sensitive to environmental perturbations; and they are more evolutionarily conserved.
- Genetic interaction networks are mostly decoupled from protein–protein interaction networks.
- Although properties of genetic network architecture are conserved across species, the network connectivities are not conserved.

is estimated from fully inbred lines is due to additive variance and additive-by-additive epistatic variance[10] (BOX 2). Thus, epistatic variance can be inferred to contribute to the genetic architecture of traits for which H^2 is much greater than is expected from strictly additive variance (BOX 2). However, further gene mapping studies are necessary to identify the individual loci that affect the traits.

QTL–QTL interactions. QTLs are mapped either by linkage to, or by association with, molecular markers. In model organisms, linkage mapping is typically carried out using line cross analyses. Linkage-mapping populations are established by crossing two lines that differ genetically for the trait of interest and by generating backcrosses, F_2 or advanced intercross individuals, or recombinant inbred lines (RILs)[10,11] (FIG. 3a). Association mapping uses samples of individuals or inbred lines from a natural population. In both cases molecular marker genotypes and quantitative trait phenotypes are obtained for members of the mapping population. Standard statistical methods are used to determine whether there is a significant difference in phenotype between marker genotypes, in which case the QTL that affects the trait is either linked to, or in linkage disequilibrium (LD) with, the marker locus[21]. These tests are carried out for each marker in turn, and genomic regions for which the *p*-value of the test passes an appropriate threshold that accounts for multiple tests correspond to the position of the QTL. Association mapping can be carried out either for candidate genes or genome wide. Compared with linkage mapping that uses a population of the same size, association mapping captures more genetic diversity and has increased mapping precision; however, it is prone to artefactual LD that is induced by population structure and has reduced power to detect QTLs that have minor allele frequencies <0.5 (REF. 21).

Epistasis between QTLs is estimated by fitting a statistical model that includes both the main effects of each QTL and the effects of the QTL–QTL interaction term (FIG. 4). The use of multifactorial perturbations in epistasis screens has the advantage that many interactions can be tested using genotypes and phenotypes that are determined for a reasonably small number of individuals. As a result, it is more efficient for exploring interaction space than laboriously constructing all possible pairwise combinations of mutant alleles. The power to detect epistasis between QTLs in mapping populations that are derived from inbred lines is maximal because all polymorphic alleles have frequencies of 0.5. However, in small mapping populations the number of lines that carry the rarer double-homozygous genotype classes is small, which increases the variance in the mean value of the trait within each class. In addition, other segregating QTLs can confound the estimate of epistasis for the tested pair of loci. These factors, together with the severe multiple testing penalty for pairwise epistasis screens, make it difficult to detect all but extremely strong interactions, particularly in association mapping populations in which allele frequencies are not balanced. Given these inherent biases against detecting epistasis, most studies only evaluate additive QTL effects. However, epistasis is often found when it is evaluated in linkage-mapping populations. Epistatic effects can be as large as main effects and can occur between QTLs that are not individually significant.

Traits for which epistatic interactions have been detected in QTL-mapping experiments include sporulation efficiency[43,44] and gene expression traits[45] in yeast; thermal preference in *C. elegans*[46]; bristle number, wing shape, longevity, enzyme activity, metabolic rate and flight velocity in *D. melanogaster*[20,21]; body weight and adiposity traits[47–50], litter size[51] and serum insulin-like growth factor 1 (REF. 52) in mice; growth rate[53,54] in chickens; growth rate[55] and metabolites[56,57] in *A. thaliana*; and differences in whole-plant and inflorescence architecture between maize and teosinte[58]. Although these studies show that epistasis cannot be ignored when describing the genetic architecture of complex traits, QTL mapping alone does not identify the causal interacting genes because the QTL intervals contain many loci.

Model organisms allow further dissection of QTLs. First, one can construct near-isogenic lines, in which a region that contains the QTL is introgressed into the isogenic background of one of the parental lines, and successive generations of recombination are used to narrow the QTL down to a small genomic interval (FIG. 3d). This approach was used to confirm the epistatic effects of two QTLs that do not have individual main effects for *C. elegans* thermal preference, but for which the interaction accounted for 50% of the total variance in this behaviour[46]. Similarly, genetic dissection of *A. thaliana* near-isogenic lines for a region that has no overall effect

Multiple testing penalty
The downward adjustment of the significance threshold for individual statistical tests that is required when multiple hypothesis tests are carried out on a single data set; for *n* independent tests, the Bonferroni-adjusted 5% significance threshold is 0.05/*n*.

on growth rate revealed two epistatically interacting QTLs that affect growth rate, for one of which the effect on growth rate was in opposite directions in the different genetic backgrounds[55]. Second, one can carry out transformation and allelic replacement to prove that variants are causal, as well as to engineer all possible combinations of causal variants to investigate epistasis at nucleotide resolution. These approaches were used in *D. melanogaster* to show that each of three domains in the *Alcohol dehydrogenase* (*Adh*) gene, as well as an intragenic epistatic interaction, contributed to the difference in Adh protein levels between the Fast and Slow electrophoretic alleles[59]. Similarly, in *S. cerevisiae*, strong epistasis for causal variants that affect sporulation efficiency[43,44] was revealed.

A powerful QTL mapping design is to introgress genomic regions from one strain into the genetic background of another. This can be done either at the level of entire chromosomes to create a panel of chromosome substitution strains[60] (FIG. 3b) or for introgressions that tile across the genome of the donor line (FIG. 3c), as for genome-tagged mice[61]. A fairly small number of introgression lines can be used to map QTLs with high precision. Epistasis occurs if the sum of the effects of the introgressed fragments is significantly greater than, or significantly less than, the mean difference in phenotype between the two parental strains. In rodents, introgression designs detect more QTLs, as well as QTLs that have larger effects than classical mapping populations for a wide variety of blood chemistry, bone and behavioural traits. In addition, the sum of the effects of individual QTLs is several orders of magnitude greater than the difference in phenotype between the parental strains[60–62]. Similar results are found for aggressive behaviour in *D. melanogaster*[63]. These results indicate that the combined effects of individual introgressed regions in the genome of the donor line are less than additive. Less-than-additive effects of introgressed QTLs have also been demonstrated for several fruit quality traits in tomato[64,65].

For different allele frequencies of interacting loci, epistatic interactions lead to different main effects of each of the interacting loci (FIG. 2b). Thus, they also lead to a lack of replication of estimated QTL effects in populations in which allele frequencies of causal interacting loci differ[66]. In model organisms one can construct mapping populations that have different QTL allele frequencies to determine how often allelic effects vary; in this case, the lack of replication of QTL effects can identify potentially interacting loci. The *D. melanogaster* Genetic Reference Panel (DGRP) is a collection of ~200 sequenced inbred lines that are derived from a single population, which allows genome-wide association mapping for quantitative traits using all polymorphic molecular variants[67]. Flyland is a large outbred advanced intercross population that is derived from 40 DGRP lines[68]. In this population, QTLs can be rapidly mapped by phenotyping large numbers of individual flies and by sequencing pools of individual flies from the phenotypic extremes of the distribution; QTLs have significant differences in allele frequencies between the two pools of sequenced flies[68,69]. None of the QTLs that were detected in the DGRP for each of three quantitative traits were replicated in extreme QTL mapping in the Flyland population. However, 50–60% of the QTLs that were detected for the three traits in either population were involved in at least one epistatic interaction, and these interactions perturbed common, biologically plausible and highly connected genetic networks[68]. Although these analyses indicate pervasive epistasis, the challenge remains to determine which of the statistically predicted interactions are biologically important.

Box 2 | Evidence for epistasis from narrow- and broad-sense heritability

The response to a single generation of artificial selection for a quantitative trait is given by the breeder's equation: $R = h^2S$. R is the difference between the mean of the parental generation and the mean of the offspring generation. h^2 is the narrow-sense heritability: $h^2 = (V_A + ½V_{AA})/V_P$, where V_A is the additive genetic variance, and V_{AA} is the additive-by-additive genetic variance, ignoring higher order epistatic interactions for simplicity. V_P is the phenotypic variance: $V_P = V_A + V_{AA} + V_E$, where V_E is the environmental variance. The selection differential (S) is the difference between the mean of the parental population and the mean of the selected group[10].

The narrow-sense heritability is thus $h^2 = R/S$. If selection is carried out over several generations, the narrow-sense heritability can be estimated from the regression of the cumulated response (ΣR) on the cumulated selection differential (ΣS); that is, $h^2 = \Sigma R/\Sigma S$. By contrast, broad-sense heritabilities that are determined from variation among completely homozygous inbred lines, ignoring higher order additive-by-additive epistatic interactions, are $H^2 = (2V_A + 4V_{AA})/V_P$, where $V_P = 2V_A + 4V_{AA} + V_E$ (REF. 100). Note that in this scenario there is no dominance variance and no epistatic interaction variance terms that involve dominance, as there are no heterozygotes. If all variation is additive (that is, $V_{AA} = 0$), then H^2 among inbred lines is related to h^2 from artificial selection from the outbred populations from which the inbred lines were derived: $H^2 = 2h^2/(1 + h^2)$[101]. h^2 and H^2 values for *Drosophila melanogaster* behavioural traits are given in the table[102–106]. In all cases H^2 values are greater than those expected from strictly additive variance, which implies that epistatic variance contributes to the genetic architecture of these traits.

Trait	Observed		Expected
	h^2	H^2	$H^2 = 2h^2/(1+h^2)$
Copulation latency	0.07	0.25	0.13
Startle response	0.16	0.58	0.28
Aggressive behaviour	0.09	0.78	0.17
Ethanol knock-down time	0.08	0.24	0.15

Epistasis between mutations and QTLs

Analyses of epistasis between induced mutations do not scale well to large numbers of mutations but have the advantage that the interacting partners are specified. Analyses of epistasis between QTLs have the advantage that interactions among large numbers of polymorphisms and genes can be evaluated; however, owing to the severe multiple testing penalty, there will be large numbers of false-positive associations among the top interactions for which there is the highest level of statistical support. An alternative strategy is to carry out one-dimensional screens that evaluate the phenotypic effects of a known mutation in different genetic backgrounds. Although these designs have not yet been implemented on a large scale, many studies indicate that this will be a powerful approach.

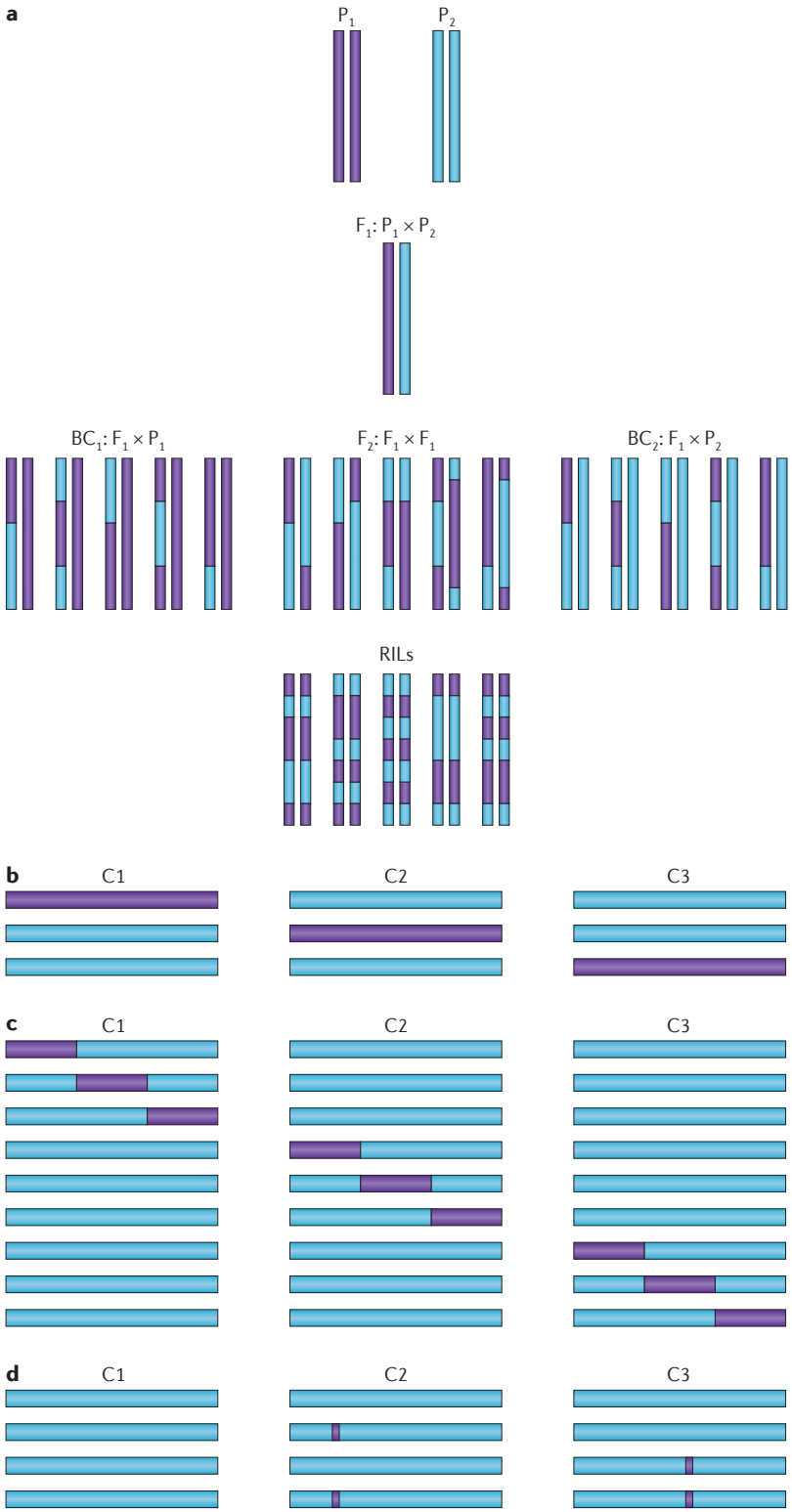


Figure 3 | **Genotypes for mapping QTLs between two genetically divergent lines.** Parental lines (P_1 and P_2) are crossed to produce an F_1 generation (part **a**). Common segregating generations that are used for quantitative trait locus (QTL) mapping are backcrosses of the F_1 to either parental line (BC_1 and BC_2); the F_2 generation, which is derived from mating F_1 individuals; and recombinant inbred lines (RILs), which are derived by inbreeding F_2 families. Experimental designs that are based on introgression use chromosome substitution lines (part **b**), introgression lines (part **c**) or near-isogenic lines (part **d**); three chromosomes (C1–C3) are depicted for each type of lines.

Waddington[3] noted the contrast between the large effects of mutations and their phenotypic variability, as well as the apparent stability of wild-type strains, despite exposure to naturally occurring genetic and environmental perturbations. He coined the term 'canalization' to refer to the buffering of natural variation against such perturbations. In modern parlance, genetic canalization refers to suppressing (that is, less-than-additive) epistatic interactions between naturally segregating variants. To the extent that these interactions occur between different genetic loci, one can probe both the nature and the magnitude of the naturally occurring epistatic modifier loci by asking to what extent they modify the effects of a mutant allele.

One of the first experiments to show the occurrence of naturally segregating epistatic modifiers of a mutation was Rendel's introgression of a *scute* (*sc*) mutation into a wild-derived background[70]. *D. melanogaster* has four large scutellar bristles on the dorsal thorax, and this number is invariant in nature. Mutations at *sc* reduce this number to an average of one or less. In a wild-type genetic background that is segregating for *sc* and sc^+ alleles, the number of scutellar bristles changed to ~3 in *sc* mutants and to 5–6 in sc^+ individuals following artificial selection for increased bristle number. These results are consistent with the selection of epistatic modifiers of *sc* that were segregating in the initial population and that suppressed the mutant *sc* phenotype. However, the genetic backgrounds for this experiment were not well defined. More recently, introgressions of mutant *Ultrabithorax*, *Antennapedia*, *sevenless* and *scalloped* alleles into different wild-derived *D. melanogaster* backgrounds have demonstrated variation outside the invariant wild-type phenotype for, respectively, haltere size, shape and bristle number[71,72]; the antenna-to-leg transformation homeotic phenotype[72]; eye roughness and size[73]; and wing morphology[74]. The epistatic effects ranged from complete suppression to enhancement of the mutant phenotype.

A variant of the mutant introgression design is to cross the mutant allele to a sample of wild-derived lines and to evaluate phenotypes of F_1 genotypes. The advantage of this method is that it is easier to implement than constructing introgression lines; the disadvantage is that any phenotypic variation cannot be attributed to allelic complementation (that is, dominance effects) or to non-allelic complementation (that is, epistasis) unless the experiment is carried out in a QTL-mapping population. In *D. melanogaster*, crosses of a dominant *Epidermal growth factor receptor* mutation to wild-derived lines give a range of eye roughness phenotypes[73,75]. Approximately 1–2% of F_1 progeny from crosses of *D. melanogaster* strains that carry mutant alleles of the heat shock protein gene *Hsp90* (also known as *Hsp83*) to outbred strains had a wide variety of morphological abnormalities, which suggests that *Hsp90* normally suppresses alleles that affect multiple phenotypes[76]. These results indicate that populations harbour a hidden reservoir of genetic variation for invariant traits that is only revealed in the 'decanalizing' background of the mutation. Such variation has been called potential variance or cryptic genetic variation[77].

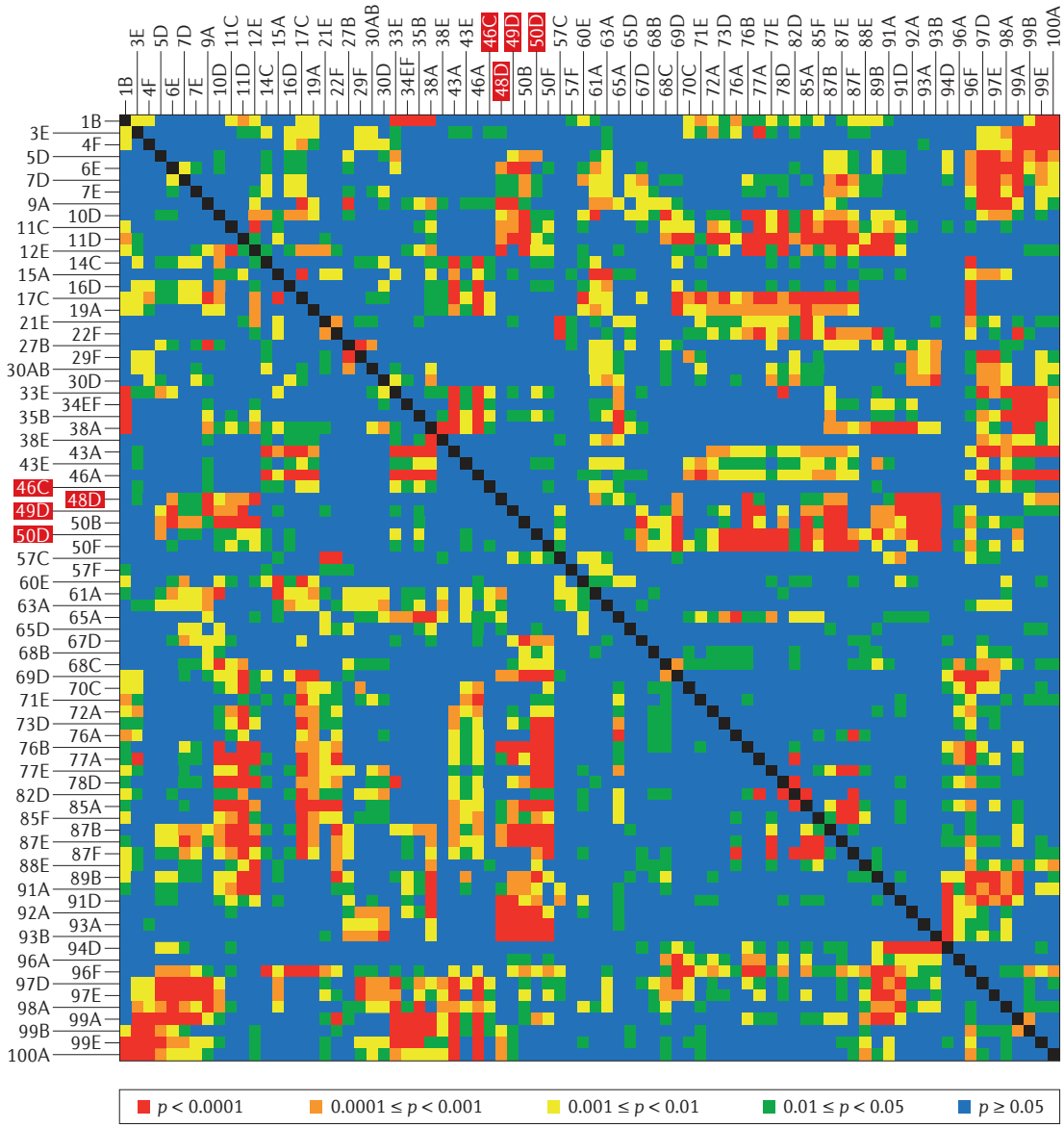


Figure 4 | **Two-dimensional search for epistatic interactions.** Data from an experiment mapping QTLs that affect *Drosophila melanogaster* lifespan in a recombinant inbred line population are depicted[107]. The *x* and *y* axes show the marker loci. Two main-effect QTLs are indicated at cytological positions 46C–49D and at 50D (shown in red boxes). The body of the graph depicts the *p*-values of the QTL–QTL interaction terms. Main-effect QTLs do not interact with each other, but they do interact with QTLs that do not have significant main effects. QTLs without significant main effects show significant interaction effects.

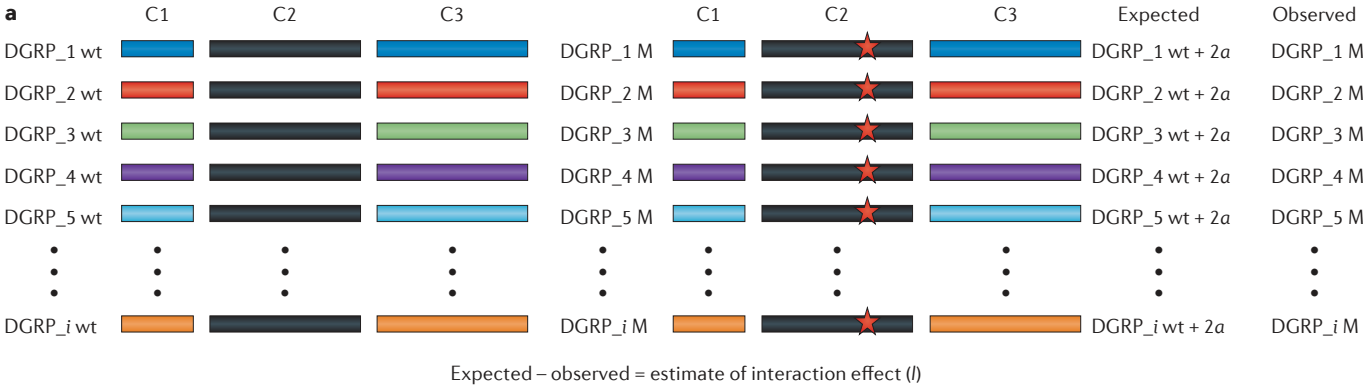
Experimental designs for assessing epistasis between mutations and QTLs can be adapted to determine the effects of naturally segregating epistatic modifiers of mutations that affect traits which show quantitative phenotypic variation in natural populations. In this case, the effects of both the mutant and the wild-type alleles of the locus in question need to be assessed for the quantitative trait phenotype in different genetic backgrounds in either an introgression or an F_1 design. Epistasis occurs if the additive effect of the mutation varies with genetic background, which is detected as a significant interaction between the mutant and background genotypes. These designs have been used in *D. melanogaster* to demonstrate epistasis for the extended-lifespan phenotype that is caused by the overexpression of a human superoxide dismutase transgene in motorneurons[78]. Similarly, epistasis was found between several mutations that affect startle response[79] (FIG. 5), olfactory behaviour and sleep traits[80] in different DGRP line backgrounds. Moreover, there is epistasis between a null myostatin allele and genetic background for growth traits in mice[81]; between the disease resistance mutation *Rp1-D21* and genetic background for the hypersensitive response in maize[82]; and for an RNAi knockdown *HSP90* allele and genetic background for both morphological and life history traits in *A. thaliana*[83].

Only a few studies so far have analysed QTL-mapping populations to map, either by linkage or by association, loci that interact with focal mutations[72,74,75,81–83]. Some studies have used candidate gene association analyses to test whether the mutant allele interacts with naturally occurring alleles at the mutant locus[75] or with naturally occurring variants at a known interacting locus[74]. Others carried out unbiased genome scans in a QTL-mapping population[72,81–83], which typically uncovered unlinked interacting loci that do not have significant main effects.

Implications of pervasive epistasis

The studies reviewed here indicate that epistasis is a common feature of the genetic architecture of quantitative traits in model organisms. By extension, the same is likely to be true for quantitative traits in other organisms in which gene–gene interactions are more difficult to detect, including humans. The epistatic interactions that have been detected define previously uncharacterized, highly interconnected genetic networks that are enriched for biologically plausible gene ontology categories, and metabolic and cellular pathways. Analyses of epistasis reveal that much quantitative genetic variation is hidden and is not apparent from analyses of main effects of causal variants, and that additivity is an emergent property of underlying epistatic networks. Furthermore, several types of observations suggest that natural populations have evolved suppressing epistatic interactions as homeostatic (that is, canalizing) mechanisms. These observations include less-than-additive interactions between QTLs; cryptic genetic variation for invariant phenotypes in natural populations that can only be observed in the presence of a decanalizing mutation; and naturally segregating variation that generally suppresses the effects of induced mutations for quantitative traits.

This realization is paradigm shifting. Rather than perceiving phenotypic variation for quantitative traits in natural populations as highly variable, it may be more



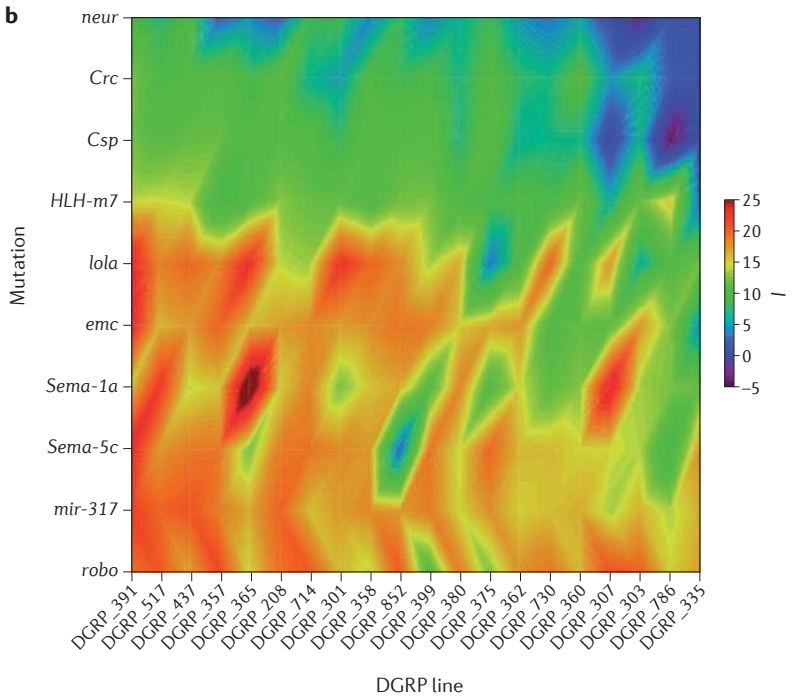


Figure 5 | **Epistasis between naturally occurring variation and mutations in *D. melanogaster*.** A graphical representation shows genotypes of *i* homozygous *Drosophila melanogaster* Genetic Reference Panel (DGRP) lines (DGRP_*i*), in which C1, C2 and C3 represent the three major chromosomes (part **a**). Co-isogenic C2 chromosomes that contain either a wild-type allele (wt) or a mutant allele (M; red star) of a focal gene that affects a quantitative trait have been introgressed into each DGRP line. The quantitative trait is measured for all pairs of wild-type and mutant DGRP introgression lines. The difference in phenotype between the wild-type and mutant alleles in the background on which the mutant was generated is 2*a*. If there are only additive effects on the phenotype, then the expectation is that the effect of the mutation will be the same on each DGRP line background, and the expected phenotype of the ith DGRP line with the mutant C2 allele is DGRP_*i* wt + 2*a*. If this is not the case, then the difference between the expected and the observed phenotypes is due to epistasis. Estimates of epistatic interactions (*I*) for ten mutations that affect startle response in 20 DGRP backgrounds[79] are shown (part **b**). The interaction effects vary among mutations and DGRP lines. These effects are large and predominantly positive; that is, naturally occurring variation suppresses the effects of the mutations.

accurate to wonder why there is not more variation in organismal phenotypes, given the large amount of segregating molecular genetic polymorphism. Genome-wide association studies in both model organisms[67] and humans[84] typically find an inverse relationship between minor allele frequency and additive effect, such that the rarer alleles are associated with larger effects than the more common alleles. Statistically, rare alleles must have larger effects than common alleles to be detected in a mapping population of the same size; the puzzle is why so few common alleles of large effect are found to segregate within natural populations. One possible explanation is that rare alleles have large effects because they are relatively new mutations compared with common alleles, and epistatic modifiers that ameliorate their effects have not yet occurred in the population. Common alleles are presumably older and could achieve an intermediate frequency owing to a modifier mutation at another locus that suppresses the effect of the polymorphism.

However, QTLs that are detected by linkage mapping in populations derived from crosses of inbred lines typically have moderately large effects[20,21]. Possibly, these loci were not common in the populations from which the parental inbred lines were derived. Alternatively, the lines that survived inbreeding could be enriched for compatible epistatic interactions that were decanalized by crossing to a different genetic background. In this case, one would predict that adding further parental lines to linkage-mapping populations might incorporate additional canalizing alleles, such that more QTLs with smaller effects will be found in these populations than in populations that are derived from crosses of two inbred lines. This prediction seems to be borne out in an outbred advanced intercross population that is derived from eight inbred mouse strains[85], as well as in the maize nested association-mapping population that consists of 200 RILs from each of 25 crosses between diverse inbred lines and a single common parental line[86].

Pervasive epistasis has consequences for plant and animal breeding, evolutionary biology and human genetics. Applied breeding programmes rely on artificial selection within populations, as well as on transfer of exotic genetic material to elite lines, to improve quantitative traits of agronomic importance. In the presence of epistasis, the genetic architecture of response to artificial selection from the same base population could differ among replicate lines, as well as within the same line over time, owing to allele frequency drift and to changes in frequency of causal alleles as a result of selection. Loci that have beneficial effects in one genetic background will not have the same effects when they are introgressed into another background, unless interacting loci are identified and co-introgressed. Many modern breeding programmes use additive models that are based on both dense molecular markers and estimates of trait phenotypes from a reference population to predict breeding values of selection candidates on the basis of only genotypic information[16,17]; in the presence of epistasis, genomic prediction may be poor if the frequency of causal alleles varies between the reference and the test populations.

Two major unresolved questions in evolutionary biology concern the mechanisms that maintain quantitative variation in natural populations, and the causes of adaptation and speciation. The puzzle of maintaining quantitative variation[10,87,88] arises because heritabilities of quantitative traits are appreciable in natural populations such that the magnitudes of genetic and environmental variation are approximately equal. However, most quantitative traits seem to be under strong stabilizing selection[89], which reduces genetic variation. Direct estimates of mutational variance for quantitative traits in many model organisms are ~0.001 of the environmental variance[90]. Most theoretical models that assess the possibility that quantitative genetic variation is maintained by a balance between elimination of variation by stabilizing selection and re-introduction by mutation cannot simultaneously account for the empirical estimates[89,91]. Estimates of mutational variance are too low to generate the observed levels of genetic variance under strong selection. Suppressing epistasis between QTLs could cause overestimates of the strength of stabilizing selection, and suppressing epistasis between mutations could lead to underestimates of the magnitude of mutational variation. This necessitates a revision of the inference that mutation–selection balance does not account for much segregating variation for traits under stabilizing selection[79]. Furthermore, both inbreeding and genetic drift cause variation in allele frequencies from the parental population. With epistasis, this can result in the 'conversion' of epistatic variance to additive variance, which potentially enables rapid adaptation to new environments[92–94]. Epistasis is central to Wright's[13] models of the genetic basis of evolution and to founder-effect speciation models[92]. With epistasis, the genetic architecture of response to natural selection will be different in different populations and will potentially increase the likelihood of the evolution of Dobzhansky–Muller incompatibilities[5,6] and consequent speciation events.

Epistasis is one of several non-mutually exclusive explanations for the small effects, missing heritability and the lack of replication of top trait-associated variants in different populations in human genome-wide association studies[84]. First, with suppressing epistasis, additive effects of common interacting loci will be small. Second, estimates of h^2 in humans are obtained from twice the difference in the correlation of monozygotic and dizygotic twins[10], and these estimates are biased upward in the presence of dominance and epistasis. Thus, suppressing epistasis could potentially account for the high levels of heritability and the small amounts of additive genetic variation that have been estimated from mapped loci in human populations. Third, estimates of additive effects of causal alleles will differ between populations that have different allele frequencies but the same underlying epistatic genetic architecture[66,68]. Additive genomic prediction methods that use all variants explain a much higher proportion of phenotypic variance in human genome-wide association studies than that obtained by summing the variance explained from individual markers that exceed the genome-wide significance threshold[95], but the prediction accuracy of these methods is low in independent

Minor allele frequency
The frequency of the less common allele at a bi-allelic locus.

Founder-effect speciation models
A class of models for the evolution of reproductive isolation that is based on changes in selection pressures and on allele frequencies of epistatically interacting loci, which result from the establishment of a new population in a new environment from a small number of individuals.

Dobzhansky–Muller incompatibilities
Substitutions that occur during divergence of two lineages; these substitutions are neutral in the respective genetic backgrounds of the two lineages but cause a reduction in fertility and/or viability in hybrids between the two lineages.

Genomic prediction methods
Models that are derived from a discovery sample which consists of individuals with measured phenotypes and genome-wide marker data; these models are used to predict individual phenotypes in an independent sample from the same population using only genome-wide marker data.

populations[96,97]. Genomic prediction methods that allow non-additive effects[98,99] are likely to increase the accuracy of individual risk prediction, but understanding the biology of human quantitative traits and complex diseases will require knowledge of the underlying loci.

Conclusions and future prospects

Mapping epistatic interactions is statistically and experimentally challenging. Much progress in understanding and predicting genetic interaction networks that affect quantitative traits has been made by taking advantage of the unique resources and experimental designs that are available for model organisms. Epistasis is common and can cause cryptic genetic variation for quantitative traits in natural populations; however, the mapping of causal interacting variants is in its infancy. Future advances will be made by using these experimental designs on a much larger scale and by taking advantage of decreasing costs of sequencing individual genomes, as well as prospects for high-throughput and accurate measurements of quantitative trait phenotypes[21]. Molecular variants, both singly and in combination, perturb transcriptional, metabolic and protein–protein interaction networks which, in turn, causally affect organismal phenotypes[21]. However, systems genetic models so far only consider additive effects of variants on transcripts and traits[21]. In the future, we must assess the effects of pairwise and higher order epistatic interactions between polymorphic DNA variants on molecular interaction networks and, in turn, evaluate their effects on organismal phenotypes to understand the mechanistic basis of epistasis. Only then will we be able to go beyond describing the phenomenon of epistasis to predicting and testing its consequences for genetic systems.

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Competing interests statement
The author declares no competing interests.