OPINION

Epistasis: too often neglected in complex trait studies?

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Interactions among loci or between genes and environmental factors make a substantial contribution to variation in complex traits such as disease susceptibility. Nonetheless, many studies that attempt to identify the genetic basis of complex traits ignore the possibility that loci interact. We argue that epistasis should be accounted for in complex trait studies; we critically assess current study designs for detecting epistasis and discuss how these might be adapted for use in additional populations, including humans.

In its broadest sense, epistasis implies that the effect of a particular genotype on the phenotype depends on the genetic background. In its simplest form, this refers to an interaction between a pair of loci, in which the phenotypic effect of one locus depends on the genotype at the second locus (BOX 1a). More generally, the effect of one locus might depend on the genotype at several or many loci. In the case of QUANTITATIVE TRAITS, epistasis describes the general situation in which the phenotype of a given genotype cannot be predicted by the sum of its component single-locus effects[1] (BOX 1b–e).

Extensive work on the control of qualitative genetic variation has highlighted the biological importance of epistasis at a 'locus-by-locus' level. On the basis of this work, several classic genotype–phenotype patterns that are caused by epistasis — such as comb type in chickens, coat colour in various animals, the BOMBAY PHENOTYPE in the ABO blood-group system in humans and kernel colour in wheat — have been characterized (see BOX 1a). Modifier genes in the human and mouse have provided further evidence of the importance of epistasis: the genetic background often influences the phenotypes of the susceptible genotype of MAJOR GENES, for example, by affecting the PENETRANCE of the gene[2]. Complex traits are also regulated by epistasis, as shown by the CANDIDATE GENE studies in which interactions between individual candidate genes are evaluated. One such example is the interaction between the *D*-allele of the angiotensin I converting enzyme (*ACE*) gene and the *C*-allele of the angiotensin II type 1 receptor (*AGTR1*) gene[3]. The risk of myocardial infarction is significantly increased by the *ACE D*-allele in patients who carry that particular *AGTR1* allele.

In the case of quantitative genetic variation, several or many genes of largely unknown function combine with environmental influences to control trait variation. This is the case for many complex traits that are of medical relevance in humans or of economic importance in plants and livestock. By combining quantitative genetic theory and molecular information on genetic marker maps, we can identify the individual genomic loci with the largest effects on quantitative traits (also known as quantitative trait loci or QTLs) and start to examine the genetic control of these traits. We therefore have the means to address the next important challenge in quantitative genetics — defining the interactions that occur among the genes that underlie these traits. The best source of information on the importance of epistasis in the regulation of complex traits comes from studies on model and other experimentally amenable organisms. Even so, most studies of model organisms have ignored epistasis; indeed, a recent review points out that epistasis is a hidden complexity in the regulation of complex traits that in general is not unravelled in QTL-mapping studies[4]. Similar opinions are expressed in other recent reviews[5–8], leading to the speculation that epistasis could be a factor that contributes to the failure to replicate the results of many human ASSOCIATION STUDIES[9], and could be one cause of QTL effects that diminish or disappear if they are isolated on fixed genetic backgrounds in experimental organisms. However, recent developments in QTL-mapping methodologies have allowed us to detect not only epistasis between QTLs with individual effects, but also novel epistatic QTLs that primarily mediate their effects on the traits through interactions with other genes (BOX 1).

The extent to which epistasis is involved in regulating complex traits is not known, and so we cannot assume that epistasis will be found for every trait in every population. However, we argue that epistasis has been overlooked for too long and that it now needs to be routinely explored in complex trait studies. Here, we use examples from the literature to show that much can be gained by considering epistasis in QTL-mapping studies. We explain how information about gene interactions will aid our understanding of complex traits, and provide an overview of the results obtained in several successful studies in model organisms. We discuss how the principles and challenges gleaned from these studies could be adopted for carrying out similar research in model species and in natural, including human, populations.

Overview of QTL mapping

Methods for detecting, or mapping, QTLs have been developed for a wide range of populations. This section, together with FIG. 1 and BOX 2, briefly addresses the principles of individual and epistatic QTL mapping as well as the challenges that they pose. For a more thorough review of QTL-mapping methodologies, we refer readers to REF. 6.

Individual QTLs. For illustration, we will consider a simple but widely used study design in which an F_2 population is derived from a cross between two different inbred lines (FIG. 1a). In their simplest form, QTL-mapping approaches work by contrasting the mean effects on the phenotype of alternative F_2 genotypes (for example, *QQ* versus *Qq* versus *qq*, where *Q* and *q* are alternative marker alleles derived from lines 1 and 2, respectively). However complicated the statistical analysis, most

experiments that aim to partition the genetic variation in a population have focused on detecting the genetic (that is, additive and dominance) effects of individual QTLs, irrespective of interactions[10,11] (FIG. 1b). This strategy has been successful for detecting QTLs with large effects on the quantitative traits[12,13], and, in several instances, causal mutations for QTLs have been identified in the coding[14] as well as the regulatory[15] regions of genes. These studies have therefore focused on the average genetic effect of the genotypes of a QTL, ignoring the possibility that these effects might be influenced by genetic background, either by other individual loci or by all other loci.

Epistatic QTLs. Epistatic QTL-mapping methods are more flexible than those for individual QTLs as they simultaneously consider the mean effects of multi-locus genotypes on the phenotype (FIG. 1). The use of the methodology poses more technical challenges and demands more from the data than individual QTL mapping (BOX 2). For these reasons, epistatic QTL mapping is not yet a standard tool in complex trait studies. Epistasis between pairs of QTLs in which both or one QTL have detectable individual effects has been reported[16–20], but the extent to which epistasis controls variation in quantitative traits has been poorly explored. There are several methods for mapping epistatic QTLs in human[21] and experimental populations[22–25]; some of the most recent methods are based on simultaneous scans and randomization tests that detect QTLs that do not have individual effects[23,24]. Such approaches have led to the identification of many, statistically reliable, novel epistatic QTLs.

Insights from model organisms

Epistatic QTL-mapping studies in model organisms have detected many new interactions and have therefore concluded that epistasis makes a large contribution to the genetic regulation of complex traits. Epistatic QTLs without individual effects have been found in various organisms, such as birds[26,27], mammals[28–32], *Drosophila melanogaster*[33] and plants[18,34]. However, other similar studies have reported only low levels of epistasis or no epistasis at all, despite being thorough and involving large sample sizes[35–37]. This clearly indicates the complexity with which multifactorial traits are regulated; no single mode of inheritance can be expected to be the rule in all populations and traits. Thorough genetic studies of complex traits therefore need to be flexible and need to accommodate various modes of inheritance, as we cannot currently define a specific mode of inheritance before

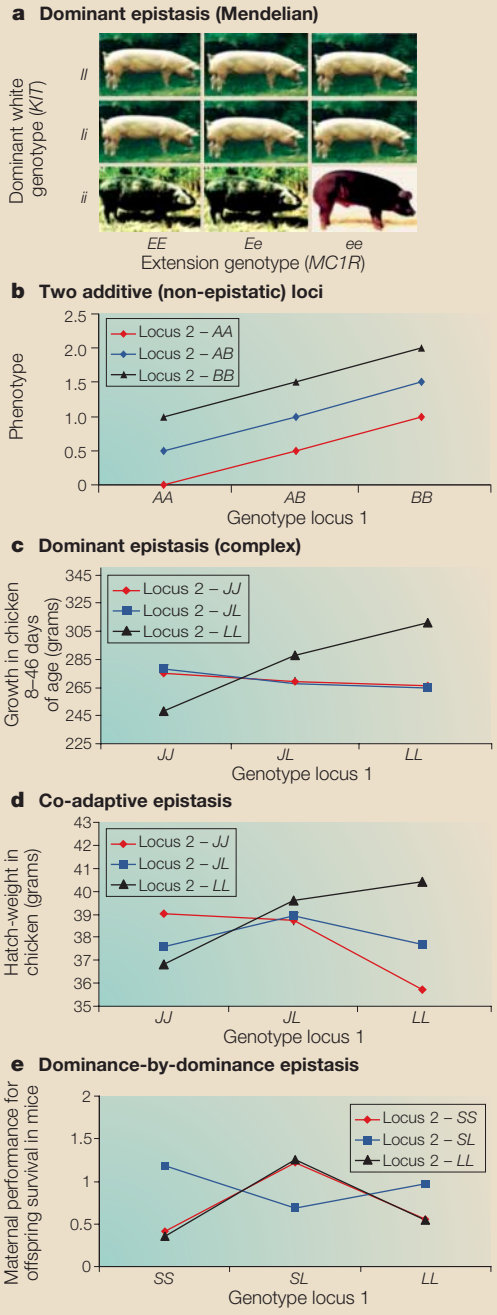
Box 1 | Defining epistasis

Mendelian traits

The term 'epistasis' was initially used in the context of Mendelian inheritance; environmental effects are relatively unimportant for Mendelian traits, so individuals can be clearly assigned to one of a limited number of classes according to their phenotype. Here, epistasis was used to describe the situation in which the actions of one locus mask the allelic effects of another locus, in the same way that completely dominant alleles mask the effects of the recessive allele at the same locus. A clear example of this can be seen in **a**, in which the dominant allele (*I*) at the *KIT* locus, which confers white-coat colour in the pig, is dominant over all alleles at the *MC1R* locus (*E*), which confer a darker coat colour. The effects of the various alleles at the *E* locus can only be determined in individuals with the recessive genotype *ii* at the *I* locus. This example was classically termed 'dominant epistasis', which gives a segregation ratio of 12:3:1 for white:black:brown, respectively.

Complex traits

For complex traits, epistasis describes any interaction between two or more loci, such that the phenotype of any genotype cannot be predicted simply by summing the effects of individual loci. A fictive example with two loci with no epistasis for a complex trait is shown in **b**. Here, the 3 lines for the effects of 3 genotypes at locus 1 run in parallel, indicating that the phenotypic effect is not influenced by the genotype at locus 2. Examples of epistasis for complex traits are shown in **c**–**e**. The first common pattern (**c**) is similar to Mendelian dominant epistasis shown in **a**, in which one locus in a dominant way suppresses the allelic effects of a second locus. In this example of growth in chickens, among-genotype variation for locus 2 is only expressed in the presence of the homozygous *LL* genotype at locus 1 (REF. 26). Such epistasis often leads to individual QUANTITATIVE TRAIT LOCI (QTLs) having small average differences among genotypes and therefore not being detected unless epistasis is incorporated into the analysis. The second epistatic pair (**d**) is an example of co-adaptive epistasis, in which genotypes that are homozygous for alleles of the two loci that originate from the same line (that is, *JJ* with *JJ*, or *LL* with *LL*) show enhanced performance. This type of gene interaction is particularly interesting as the loci have no significant individual effect (for example, the average effect of *JJ*, *JL* and *LL* do not differ) and it therefore cannot be detected without a SIMULTANEOUS SCAN for multiple QTLs[26]. The third epistatic example (**e**) shows dominance-by-dominance epistasis, in which the double heterozygote (*LS*, *LS*) deviates from the phenotype that is expected from the phenotypes of the other heterozygotes (—, *LS* or *SL*, —). The figure shows an example of a negative dominance-by-dominance interaction, which causes the double heterozygote to have a lower phenotype than expected[31]. Images in panel **a** are reproduced with permission from REF. 56 © (2001) Macmillan Magazines Ltd. and REF. 57 © (1998) Genetics Society of America.



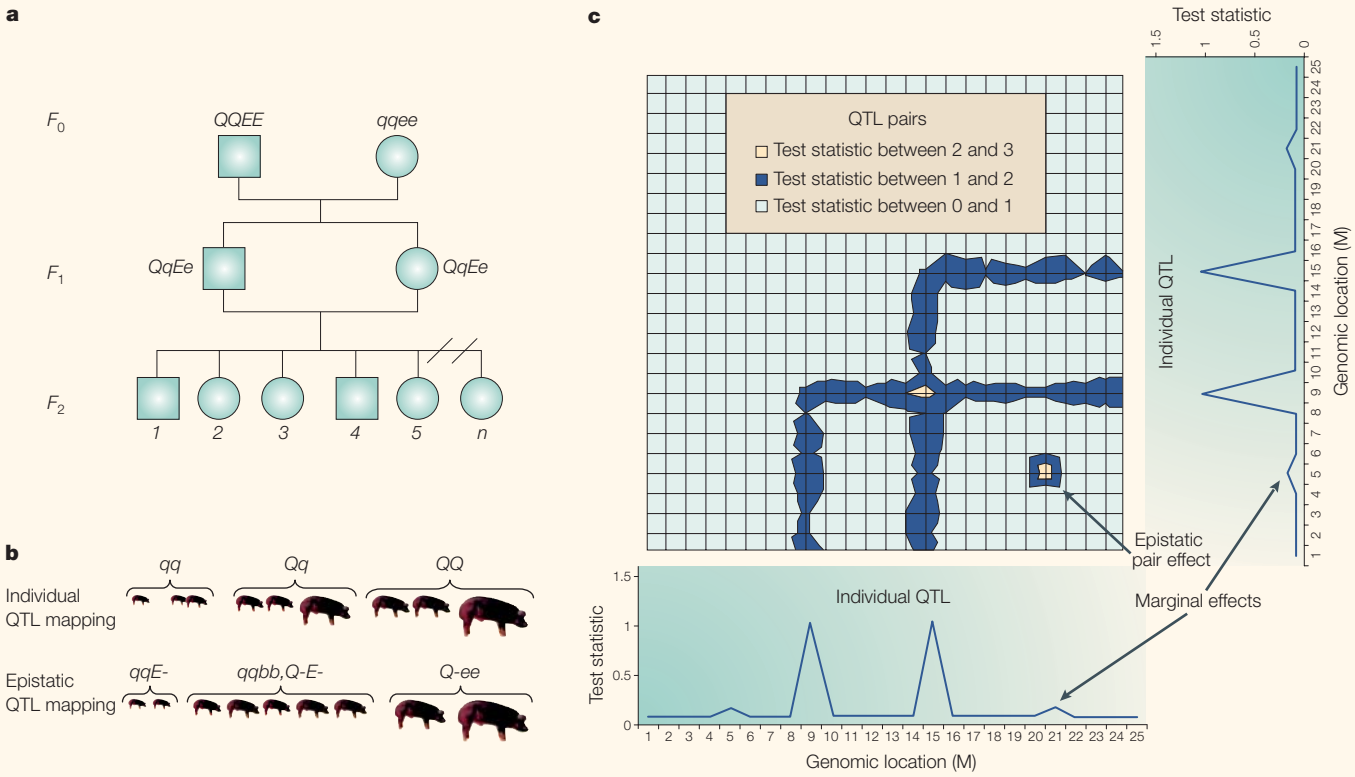


Figure 1 | **Principles of individual quantitative trait loci and epistatic quantitative trait locus mapping.** Quantitative trait locus (QTL) mapping aims to partition the total genetic variation for a trait into the effects of individual loci. **a** | The F_2 design for QTL mapping uses a three-generation pedigree, and requires two strains that differ in the trait of interest and a linkage map of polymorphic molecular markers. The hybrid F_1 generation derived from the parental lines is intercrossed to produce F_2 individuals; here, one full sibling family of *n* individuals that differ in the proportion of markers that they have inherited from the parental lines is shown. F_2 individuals are analysed statistically to determine whether there is a difference in phenotype between marker genotype classes. If there is, then the QTL alleles (here, *Q*,*q* and *E*,*e*) are linked to the marker. **b** | In principle, individual QTL mapping searches for the partitioning of the F_2 individuals according to the QTL genotype with the smallest phenotypic variation within the genotype classes and the largest differences among the genotype classes. Epistatic QTL mapping partitions the individuals according to the genotypes at multiple loci (for example, *Q* and *E*), which ensures a better fit if the phenotype depends on the genotypes of both loci. **c** | An individual QTL scan results in a statistical support curve for the individual effects of a QTL at each tested location in the genome (two green boxes). A simultaneous scan for QTL pairs results in a statistical support surface for all potential combinations of QTL locations in the genome (centre panel). QTLs with large individual effects will be seen as high peaks in the individual QTL support curve and as ridges in the QTL-pair support surface. A stepwise approach for detecting epistatic QTLs (for example, on the basis of FORWARD SELECTION) will only detect epistasis along these ridges. QTLs with mainly epistatic effects will usually appear only as small peaks in the individual QTL support curve, but as large peaks in the support surface for QTL pairs. Images of pigs in part **b** are reproduced with permission from REF. 57 © (1998) Genetics Society of America.

the analysis is done. The routine use of epistatic QTL-mapping methodologies will help to explore whether there are particular traits, population types or species for which epistatic regulation is more important than for others. Here, we focus on the results of the first genome-wide scans for interacting QTLs without individual effects[26–33]. These studies indicate that the more widespread use of QTL-mapping methods that are based on simultaneous scans for the joint effect of pairs of epistatic QTLs can give further insight into the genetics that underlie complex traits.

Population size and statistical power. As expected, the power to detect epistasis varies with the size of the population and the precision with which the analysed phenotypes are measured. So, results that are obtained from smaller populations in which single measurements are taken from each individual[29,30] detect less epistasis than studies of large populations[26] or populations for which several measures per genotype are considered[33]. In summary, although the results indicate that epistatic effects are often large enough to merit a full genome scan for epistasis regardless of the population size[26–30,33], the epistatic studies (or meta-analyses) of populations are at their most powerful if they use good quality data from 500 or more F_2 individuals.

Potential to detect novel epistatic QTLs. Simultaneous mapping of QTLs using an epistatic model can detect loci that mainly affect the quantitative trait through epistatic interactions with another locus, in addition to those QTLs that are detected through their average individual effects[26–30]. An example of this is shown in FIG. 2, which summarizes the results of mapping single QTLs and epistatic QTL pairs that affect growth differences between Junglefowl and White Leghorn chickens[26]. Four QTLs were detected by their individual effects, and four additional QTLs were found as part of an epistatic QTL pair. Two of the QTLs that were significant only as part of an epistatic QTL pair had near-significant individual effects, whereas the other two were novel epistatic loci that showed only minor individual effects. These last two loci could have been detected only through the simultaneous search approach. Similar results have been found in other studies: for example, in their analysis of functionally related physiological traits in 95 *D. melanogaster* RECOMBINANT INBRED LINES, Montooth *et al.*[33] identified both epistatic QTL pairs without individual effects and QTL pairs with near-significant individual effects.

Box 2 | The challenges of epistatic analyses

Methods for detecting epistasis can easily be derived from methods that have been developed for detecting individual QTL effects in experimental[58–60] and human populations[61–67]. The main obstacle to the more widespread use of these methods is therefore not the theoretical adaptation of QTL-mapping methods to accommodate epistasis, but, rather, the practical limitations of performing the analyses on experimental data. The principal limitations are outlined below.

The computational demand of thorough epistatic QTL analyses is generally high. The number of potential QTL combinations in a multiple-QTL model increases rapidly with the number of QTLs that are considered simultaneously. So, it quickly becomes computationally intractable to evaluate all of these potential combinations of QTLs. By using parallel computing and efficient algorithms for epistatic QTL mapping[68–70], the computational demands of the epistatic QTL analyses can be significantly reduced.

The standard procedure during significance testing in QTL mapping is to use stringent significance thresholds, which are derived, for example, through RANDOMIZATION TESTS, to correct for the multiple testing that is performed during genome scanning[23,24,71]. Owing to a markedly higher number of tests that are carried out in multi-dimensional scans for epistatic QTLs, the use of multiple-testing corrected thresholds will cause only large epistatic effects to be detectable. To detect more subtle epistasis, alternative testing approaches are needed.

Many data sets are not suitable for evaluations of epistasis. When searching for epistatic QTLs, the epistatic effects in the genetic models are derived from the genotype means of multi-locus genotypes instead of single-locus genotypes, as in individual QTL analyses. So, the power to detect QTLs depends on the number of individuals in the genotype classes on which the analysis is based. This means that considerably larger population sizes are needed to obtain the same power to detect an epistatic effect as an individual effect in the analyses.

Computationally improved QTL-mapping algorithms will become a powerful tool for detecting epistatic QTLs in experimental populations, which can be designed for epistatic QTL mapping. The obstacles to obtaining high power in epistatic QTL mapping are, however, high in human populations, and will hamper the use of traditional approaches to detect epistasis unless alternative routes to identifying and validating epistatic QTLs are found.

Potential to attribute a larger proportion of the genetic variance to QTLs. By estimating the consequences of both significant individual and interaction effects, it has been possible to better explain the total phenotypic variation in terms of individual loci and combinations of loci. The proportion of the total genetic variance in F_2 or recombinant backcross populations that results from epistasis was estimated in 5 available comparable studies: the variance ranged from 0 to 81% with a mean of 38% for the 18 traits studied. The results are summarized in TABLE 1 (REFS 26–30). Given that the average phenotypic variances associated with correctly identified individual QTLs can be overestimated if smaller numbers of progeny are analysed[38], there is a risk that the variance that results from epistasis for the smaller populations shown in TABLE 1 (REFS 28–30) has been overestimated; by contrast, the estimates from the larger populations are expected to be more reliable[26].

Epistatic versus biological interactions

The term epistasis has two related, but distinct, meanings in genetics[1]. It was originally coined to describe the action by which one Mendelian locus alters the allelic effects at another locus, similar to the way dominance alters the allelic effects within a locus. In quantitative genetics, epistasis relates to the improvement in predicting phenotypic variation from simultaneously considering multiple-locus genotypes, relative to predicting it from the sum of single-locus genotypes. Despite the success of some epistatic QTL studies, the estimates of epistasis do not, in general, have a direct relationship to biological mechanisms of gene interactions[1,5]. In this section, we illustrate how knowledge about statistical epistasis can be used to infer the biological mechanisms that underlie complex traits. We also highlight the problems in interpreting and applying QTL results that could arise by ignoring epistasis.

Most strategies for epistatic QTL mapping use quantitative genetic models, with the detected epistasis being an indication of a genotype–phenotype dependency between loci that cannot be explained by individual QTL effects. However, epistasis that is detected in this way is not always biologically relevant. For example, some types of statistical epistasis result from the scale that is used to model QTLs[39] rather than from biological gene interactions[40]. It is therefore necessary to evaluate all combinations of loci using other post-mapping analyses to further explore whether the results can be explained by biological gene interactions. One way to link statistical estimates of epistasis to their biological meaning is to plot two-locus genotype–phenotype patterns for epistatic pairs and to connect these to experimentally determined gene-interaction patterns[26,27,31,41]. Several frequently occurring epistatic patterns have been identified by this approach (BOX 1), and, in some cases, these resemble Mendelian epistatic relationships ('dominant epistasis' in BOX 1a,c); such patterns can then be used to identify the underlying molecular mechanisms. Another means of exploring the functional relationships that underlie epistasis is to use gene regulatory networks[42] to infer the genetic regulatory structures, such as positive- or negative-feedback loops, that can generate the detected epistatic patterns.

Functional relationships among loci have also been used to reconstruct the genetic pathways that are involved in regulating the complex trait. By creating networks in which QTLs are nodes and connections are epistatic

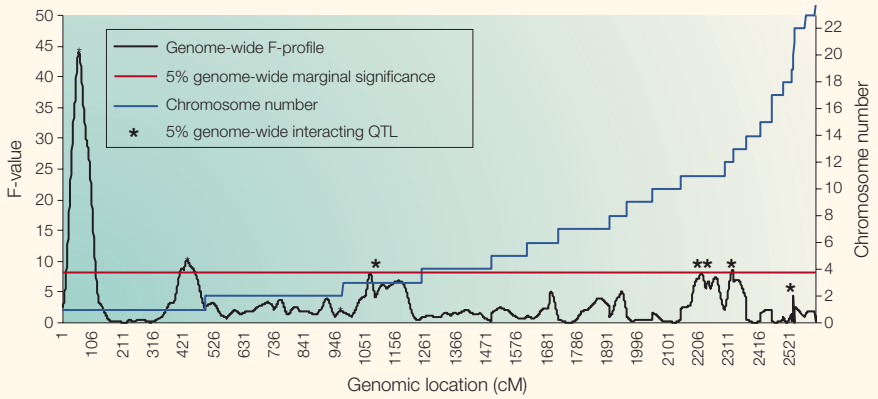


Figure 2 | **Comparing the results of searches for epistatic and individual quantitative trait loci.** Genome-wide F-PROFILE support and 5% genome-wide significance level from the mapping of quantitative trait loci (QTLs) by their individual (additive and dominance) effects on growth from 8 to 46 days of age in an intercross between Junglefowl and White leghorn F_2 chickens[26]. The best estimated locations for the epistatic QTLs that were significant at a 5% genome-wide level in a scan for interacting QTL pairs are indicated by asterisks. cM, centiMorgan.

Table 1 | **Epistatic genetic variation explained by quantitative trait loci***

Species	Genotyped population	Number of traits	Proportion of variance that results from epistasis			Reference
			Mean	Maximum	Minimum	
Chicken	F_2	5	46%	79%	0%	26
Chicken	F_2	3	26%	31%	19%	27
Mouse	BC1	4	27%	33%	19%	28
Mouse	F_2	5	49%	81%	16%	29
Mouse	RBC	1	16%	16%	16%	30

*Estimates are based on the 5 available comparable studies, which cover 18 traits[26–30]. BC1, first-generation backcross; RBC, reciprocal backcross.

interactions, large networks that contain many QTLs have been created for several traits, including adiposity (build up of fat) and tail length in mice[41] and growth in chickens[27], whereas several smaller networks have been reported for maternal performance for offspring survival[31].

Interactions among loci result in the genetic effects of alleles at one locus differing in magnitude or direction depending on the genotype at another locus. There is a risk that individual loci will remain undetected in cases in which epistasis is ignored, and that the estimated effects of detected QTLs could be severely biased. Overestimation of individual QTL effects leads to erroneous interpretations of the relative importance of detected QTLs, but also to problems with confirming QTL effects in further crosses and to lower economic gain if attempts are made to use the QTLs (for example, in MARKER-ASSISTED SELECTION; MAS). Epistasis therefore needs to be considered if choosing the validation strategy for detected QTLs, as the nature of the interaction can guide the researcher to the appropriate genetic background to obtain maximal power for replication. For example, improving the resolution of mapping a QTL through recombinant-progeny testing is only possible if the chosen parents have a genetic background that allows expression of the phenotype in the progeny. The same applies to MAS, in which the economic gain of introducing QTL alleles to new lines can be improved by using knowledge about important interactive effects that are mediated by specific alleles at other genomic loci.

Guidelines for effective analysis

As discussed in the previous section, large and powerful studies are needed to thoroughly examine the genetic basis of complex traits, as smaller studies only allow detection of larger epistatic QTLs. How can this knowledge be applied to improve understanding of epistasis in model organisms and how should epistasis be approached in inherently less powerful designs such as in human or other natural populations? In theory, the analytical principles described above should apply equally well to all populations; however, in practical terms, its application is hindered by technical limitations and the nature of the experimental data (see BOX 2). New technologies, such as high-throughput, high-density genotyping, more affordable expression analysis and improved bioinformatic tools, will help to address some of these technical problems. In this section, we consider some options for incorporating these new technologies in a joint framework for quantitative genetic analysis.

Model organisms. Epistatic QTLs with large enough effects can be detected even in small experiments[29,30], so more knowledge about epistasis could be obtained from many previously collected experimental data sets by re-analysing them with an epistatic QTL-mapping method. An even more rewarding strategy — one that has been used successfully for individual QTL effects — would be the joint analysis of data from similar or identical crosses carried out in different laboratories or at different times[43]. New analyses of already-collected data would be a cost-efficient way to obtain more experimental evidence for the

Glossary

ASSOCIATION STUDIES
A set of methods that is used to identify correlations between genetic polymorphisms and expression of phenotypes, such as diseases, in populations.

BOMBAY PHENOTYPE
A rare ABO blood group (O_h) in which the genotype at a locus other than the ABO gene locus makes the individuals seem to have blood type 'O' even if the 'A' or 'B' enzymes are present.

CANDIDATE GENES
Genes in which functional variation is thought to affect the trait under consideration, often on the basis of their physiological role or their effects in other species.

F-PROFILE
A plot of the statistical support (measured by an F-test) for quantitative trait loci at regular intervals throughout the genome.

FALSE DISCOVERY RATE
(FDR). The proportion of false-positive test results out of all positive (significant) tests (note that the FDR is conceptually different to the significance level).

FIRST-ORDER GENETIC INTERACTIONS
Interactions between pairs of genes or quantitative trait loci.

FORWARD SELECTION
A statistical procedure in which a multi-dimensional genome scan is reduced to a series of sequential one-dimensional genome scans.

HAPLOTYPE
The allelic configuration of multiple genetic markers that are present on a single chromosome of a given individual.

MAJOR GENE
A gene that is part of a polygenic or oligogenic system but for which alternative alleles have a large influence on the phenotype.

MARKER-ASSISTED SELECTION
(MAS). Genetic markers are used to indirectly select for specific alleles at closely linked trait loci by directly selecting for the marker.

PENETRANCE
The proportion of individuals with a specific genotype who manifest the genotype at the phenotypic level. For example, if all individuals with a specific disease genotype show the disease phenotype, then the disease is said to be 'completely penetrant'.

QUANTITATIVE TRAIT
A continuously distributed measurable trait for which variation depends on a single gene or on the cumulative action of many genes and the environment. Common examples include height, weight and blood pressure.

QUANTITATIVE TRAIT LOCUS
(QTL). Genetic loci or chromosomal regions that contribute to variability in complex quantitative traits, as identified by statistical analysis. Quantitative traits are typically affected by several genes and by the environment.

RANDOMIZATION TEST
A statistical test in which statistical significance is judged by comparison to a distribution that is generated by repeated random permutations of the actual data.

RECOMBINANT INBRED LINE
A population of fully homozygous individuals that is obtained by repeated selfing from an F_1 hybrid, and that comprises 50% of each parental genome in different combinations.

SIMULTANEOUS SCAN
A multi-dimensional genome scan in which several gene locations are selected simultaneously.

VARIANCE-COMPONENT APPROACH
Quantitative trait locus (QTL) analysis method, suited to complex family structures, in which variance that is attributable to a QTL is estimated rather than the mean effects of alternative genotypes.

role of epistasis in the regulation of complex traits.

Future study designs should, however, consider exploring epistasis more thoroughly. Collecting information on epistatic patterns and networks from many organisms and on a wide range of traits could be a valuable approach to understand more about what to look for in natural populations and potentially also to find new ways to model gene interactions. A thorough exploration of FIRST-ORDER GENETIC INTERACTIONS needs to be based on reasonably large populations, and new studies should aim for at least replicating the population sizes of the largest successful studies (of 850 F_2 individuals[26] or 100 unique genotypes with multiple phenotypic measurements[33]). It will also be necessary to further explore the contribution of higher-order epistasis, for which even larger populations are needed. For example, 4,000 F_2 individuals are needed to study three-way interactions in an F_2 population, based on having the same number of individuals in all three-locus genotype classes as in a study of two-way interactions using 1,000 F_2 individuals. However, before initiating such studies, it would be useful to explore large individual studies that have already been carried out or meta- or joint analyses of compatible data sets.

Natural populations. There is reason to assume that the importance and abundance of gene interactions in natural populations are of the same magnitude as those found in model organisms. Several studies of epistasis among major genes or candidate genes have found epistasis in the expression of complex traits of medical importance in humans, including type I diabetes[44], type II diabetes[45] and inflammatory bowel disease[46]. However, owing to lack of power, evaluations of epistasis are not included as a standard procedure in the genetic analysis of complex traits in natural populations. Could the algorithms that have successfully been used in experimental populations be adopted for analyses of more complex populations as well? In theory, the answer is yes, but in practice, it is difficult to collect data sets of sufficient size to obtain the full benefits of this methodology. However, current efforts in humans and other species to generate dense genetic maps using many polymorphic markers, such as SNPs, is encouraging; the aim of such projects is to use the maps to reconstruct common genome-wide HAPLOTYPES. In future, it should then, at least theoretically, be possible to obtain sufficient population sizes by sampling individuals from the general population and using high-density SNP maps to reconstruct haplotypic relationships[47]. The derivation of haplotypes across the genome opens new opportunities for mapping genes that underlie complex traits in natural populations[48]. It should be possible to perform genome scans for direct associations between haplotypes at one location or combinations of haplotypes at two locations with trait variation. However, testing for interactions between multiple haplotypes in two locations is probably intractable. As an alternative, the haplotypes could be used to reconstruct the genetic relationship between individuals, both at individual loci and for combinations of loci. QTL effects can then be predicted by using the VARIANCE-COMPONENT APPROACH to estimate the proportion of the genetic variance that results from the effects of individual loci and from the interactions between them[49]. The sample sizes of these populations will probably need to be considerably larger than those of the experimental populations to achieve similar power, owing to a lower signal-to-noise ratio in the phenotypic measurements[50,51]. The designs might therefore not be cost-effective for detecting novel epistatic patterns until the large-scale collection of haplotype data becomes feasible.

Integrated framework for detecting epistatic QTLs. Most QTL-mapping studies are based on stringent significance thresholds that are derived to control the rate of false positives in the study. It has been argued[52] that we ought to focus on optimizing our procedures for eliminating and controlling false positives instead of imposing these stringent criteria. A stepwise approach in which a FALSE DISCOVERY RATE (FDR)[53] calculation is used to control the rate of false positives in each step is efficient at removing false positives[52]. Furthermore, the combination of information from many data sources has improved the range and quality of conclusions that can be drawn, for example, in studies that are based on gene-expression analysis[54]. Here, we propose a stepwise approach to build confidence in epistatic QTLs using many independent data sources. A multi-dimensional genome scan is used to identify a set of potentially interacting QTLs, and, in subsequent steps, external information is used to increase or decrease confidence in each of the QTLs in the initial set (FIG. 3). This strategy will allow the detection of interacting QTLs of smaller effects and strong external support as well as novel QTLs with larger effects but less external support. What we present here is the outline of a strategy that, if fully implemented, exploits the ability of a multi-dimensional scan to detect novel epistatic QTLs without demanding large populations or effects. It is not a ready-made solution, which means that the full implementation of the strategy requires further research — for example, into how to use information from the different sources to avoid biasing the results towards previously well-studied systems and away from potential new findings.

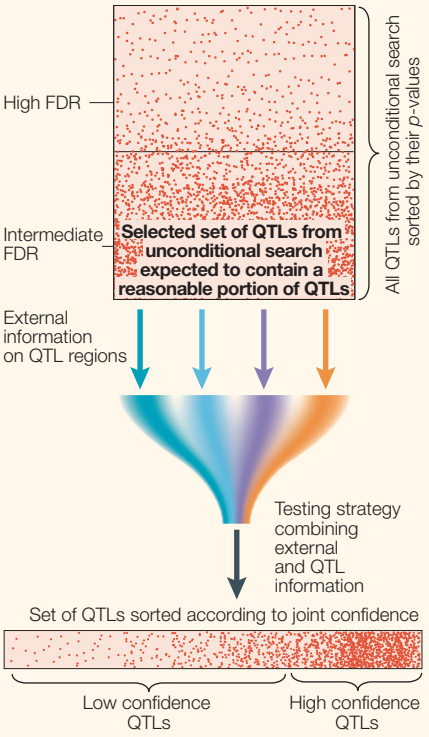


Figure 3 | **Proposed framework for acquiring confidence in quantitative trait loci.** The strategy involves integrating quantitative trait locus (QTL) mapping and external information. First, a (multi-dimensional) genome scan is performed to estimate the contribution of all possible combinations of genomic regions to the expression of the studied trait(s). From the complete results, a set of potentially biologically interesting QTLs is selected on the basis of a false discovery rate calculation (FDR). The FDR that is used does not need to be set at a particularly high level to identify potentially interesting regions[52]. The second step of the procedure aims to separate the set of potential QTLs into high- and low-confidence QTLs using external information. The classification of a QTL as a high-confidence QTL could be on the basis of very high significance in the QTL-mapping experiment, strong external evidence for an interaction or a combination of the two.

The rapid development of high-throughput techniques provides many potential sources of external information for the testing procedure. Genome-sequencing projects provide information on most genes that are located in the regions of QTLs. As few regions in the genome are expected to lack potential candidate genes, functional information about dependencies between the

genes needs to be added before the information can be used. Experimental evidence on gene dependencies can be extracted from the literature or databases on reported epistasis between QTLs or candidate genes, from knowledge of biochemical pathways and from studies on protein–protein interactions. Further experimental information can be obtained by studying gene expression, which provides evidence of regulatory relationships between groups of genes in the specific data set. Other bioinformatic evidence could come from, for example, mining literature databases for co-occurrences of gene names in published articles[55]. To further develop and evaluate this approach, we need to implement it for model organisms in which we can carry out large-scale studies and identify convincing evidence of epistasis with limited external evidence. These will then provide a means of learning what information is most valuable and how it can be applied most effectively in studies of natural populations such as those of our own species. Ironically, the extra restrictions that this procedure imposes on the type of loci involved could, in some circumstances, make it easier to identify candidate loci that underlie pairs of interacting QTLs than it is to identify a candidate gene that underlies a QTL with no interactions.

Future prospects

New technologies are continually evolving to give us more information about isolated components of biological systems. One of the most challenging tasks will be to integrate the information in a biological model that can predict the function of the entire biological system. Genetics is central in biological modelling, as it provides the framework in which all other components act. It is possible to identify the influence of individual genetic components on variation in a system, and we are now starting to supplement that knowledge with information about the interplay between genes and how they jointly affect the system. The identification of epistatic interactions between genes and/or QTLs is a valuable starting point for a more thorough understanding of these genetic networks. Our aim is to develop analytical frameworks that integrate information from many sources. It is with this in mind that we have proposed a general strategy for the detection of (epistatic) QTLs, in which information from many disciplines is integrated in one framework. It is to be hoped that this will provide more information on the nature of gene interactions, because unless we consider epistasis, we will not be able to fully understand the control of complex traits.

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Competing interests statement

The authors declare that they have no competing financial interests.

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