Genet. Res., Camb. (2005), **86**, *pp.* 89–95. © 2005 Cambridge University Press
doi:10.1017/S0016672305007780 Printed in the United Kingdom

Minireview

QTL-based evidence for the role of epistasis in evolution

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Summary

The extent to which epistasis contributes to adaptation and speciation has been a controversial topic in evolutionary genetics. One experimental approach to study epistasis is based on quantitative trait locus (QTL) mapping using molecular markers. Comparisons can be made among all possible pair-wise combinations of the markers, irrespective of whether an additive QTL is associated with a marker; several software packages have been developed that facilitate this. We review several examples of using this approach to identify epistatic QTLs for traits of evolutionary or ecological interest. While there is variability in the results, the number of epistatic QTL interactions is often greater than or equal to the number of additive QTLs. The magnitude of epistatic effects can be larger than the additive effects. Thus, epistatic interactions seem to be an important part of natural genetic variation. Future studies of epistatic QTLs could lead to descriptions of the genetic networks underlying variation for fitness-related traits.

1. Introduction

The term epistasis has been used in several different, yet related, fashions in the various subdisciplines of genetics (Avery & Wasserman, 1992; Phillips, 1998). Each use of epistasis has the sense of a phenotype dependent upon interactions between alleles at different loci. In population, evolutionary or quantitative genetics, epistasis is broadly defined as non-additive interactions between alleles at different genes. The question of how much epistasis contributes to local adaptation, population differentiation and speciation dates back over 75 years to the differing views of Fisher (1958) and Wright (1984) on the genetic basis of evolutionary change. Wright viewed epistatic interactions as an essential component of moving from one adaptive peak to another. In contrast, Fisher emphasized the additive effects of genes, summarized in his Fundamental Theorem of Natural Selection as a population's response to natural selection being proportional to the additive genetic variance of fitness in the population (Fisher, 1958). Recently, there has been a resurgence of interest in the question of the role of epistasis in evolutionary biology as new theoretical and experimental approaches have been developed (Coyne *et al.*, 2000; Goodnight & Wade, 2000; Whitlock & Phillips, 2000; Wolf *et al.*, 2000).

One experimental approach to the study of epistasis is to use specific mutations as a starting point, then measure fitness effects with combinations of other mutations or genetic backgrounds; some examples include Elena & Lenski (1997*b*) with *E. coli*, deVisser *et al.* (1997) with *Aspergillus niger*, and the results from several groups working with viruses (Bonhoeffer *et al.*, 2004; Froissart *et al.*, 2004; Michalakis & Roze, 2004; Sanjuán *et al.*, 2004).

A second approach is based upon modified QTL mapping: one can identify two-way epistatic interactions by performing a complete pair-wise analysis of all the molecular markers. Shook & Johnson (1999) and Cheverud and colleagues (Cheverud, 2000; Peripato *et al.*, 2004; Routman & Cheverud, 1997) have been pioneers of this QTL approach. Carlborg & Haley (2004) provide an overview and assessment of the methods for epistatic QTL mapping. They reviewed several examples where the proportion of variance that results from epistasis was large (16–79%), and argued that more such studies are

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Table 1. *Five freely available programs that will search for epistatic QTLs*

Program	System	Web site	Reference
Epistat	MS Windows executable	http://64.226.94.9/epistat.htm	Chase *et al.* (1997)
Epistacy	Uses SAS	http://www4.ncsu.edu/~jholland/Epistacy/epistacy.htm	Holland (1998)
Pseudomarker	Uses MATLAB	http://www.jax.org/staff/churchill/labsite/software/pseudomarker/index.html	Sen & Churchill (2001)
MapManager QTX	MS Windows and MacOS executables	http://mapmanager.org/mmQTX.html	Manly *et al.* (2001)
BQTL	Uses R	http://hacuna.ucsd.edu/bqtl/	Borevitz *et al.* (2002)

needed to understand the genetic basis of complex traits. Zeng *et al.* (2005) have recently reviewed the quantitative models that are used for estimating quantitative traits with additive, dominance and epistatic variances and effects, pointing out statistical problems with some of the models used.

In this minireview we discuss examples of using the QTL approach to identify epistatic interactions of evolutionary interest. Our focus is on recent results with plants, but some comparisons with other systems are made. In particular, *Arabidopsis thaliana* has become one of the favoured model organisms for evolutionary functional genomic studies, including QTL analysis. These studies have uncovered epistatic QTLs for natural variation for a number of traits of evolutionary or ecological significance.

2. Software and analysis

Standard QTL analysis programs, such as QTL Cartographer (Basten *et al.*, 2004), estimate epistatic interactions among already identified additive QTLs as part of multiple interval mapping, but will not currently perform a complete pair-wise analysis of map segments without regard to already identified additive loci. At least five computer programs have been developed that will facilitate all pair-wise map segment scanning. These are briefly summarized in Table 1.

The statistical significance of the detected epistatic QTL interactions needs some consideration, since one is performing multiple tests. A simple Bonferroni correction, dividing significance level by the number of marker × marker tests, is not correct as the markers are linked, and hence not independent. Chase *et al.* (1997) incorporate Monte Carlo permutations into their Epistat programs to address this issue. Holland (1998) suggests using the number of linkage group comparisons, $G(G-1)/2$, as a 'liberal, but reasonable' correction factor. Cheverud (2000) calculated an effective marker number from the inter-marker correlation matrix; this is more stringent than using the number of linkage groups. Borevitz *et al.* (2002) use a Bayesian approach to develop a genome-wide significance level, instead of a Bonferroni-style correction.

Bonferroni corrections are statistically conservative; the actual probability of obtaining a result is less than the target 0·05 or 0·01, usually by an unknown amount. Put another way, Bonferroni corrections increase the rate of type II errors, false negatives (discussed in the SAS manual; SAS Institute, 2001). This problem has spurred interest in false discovery rate statistics (Benjamini & Hochberg, 1995). Here, this means that the power to detect epistatic interactions by this approach is much less than is desirable, certainly less than the power to detect the main effects of additive QTLs.

The epistatic mapping software will frequently handle data from either recombinant inbred lines or F2 populations. Recombinant inbred lines have been used in many studies, although they may underestimate the total amount of epistasis if additive × dominant or dominant × dominant interactions exist (Kearsey *et al.*, 2003). Generally, tests for higher-order epistatic interactions (above two-way) have not been performed, although examples of three-way epistasis are certainly known to exist (Templeton, 2000).

Malmberg *et al.* (2005) plotted their epistatic QTL results in a two-dimensional analogue of the familiar LOD plot along the genetic map that accompanies standard additive QTL maps. The genetic map was linearized forming both the *x*- and *y*-axes, and the probability of a detected epistatic interaction was indicated by points of varying intensities plotted at the intersection of the genetic map positions of the two markers involved. This plot helped resolve the output of the analysis programs into groups of neighbouring points that are likely to indicate a single underlying epistatic interaction. They also graphed the additive QTLs and epistatic QTLs detected on an effectively circular genetic map with lines connecting the epistatically interacting QTLs. This helped identify loci that were participating in both additive and epistatic effects, or that were participating in more than one epistatic interaction, representing the

genetic architecture underlying the quantitative traits as a network.

3. Epistatic QTLs for fitness components

Several studies have directly measured epistatic QTLs for fitness or fitness components in field-grown or greenhouse- or growth-chamber-grown plants.

Weinig *et al*. (2003) studied the inheritance of fitness components in the same set of Landsberg × Columbia recombinant inbred lines, grown in fields in North Carolina and Rhode Island. Fruit number was measured in three conditions: a spring cohort in North Carolina, and both a spring and autumn cohort in Rhode Island. They found two additive QTLs for fall fruit number in Rhode Island, six additive QTLs for spring fruit number in Rhode Island, and one additive QTL for spring fruit number in North Carolina. After identifying additive QTLs, they performed a search for epistatic interactions among combinations of markers linked to the additive QTLs as well as markers linked to a candidate gene of interest, *TFL1*. They found four significant epistatic interactions involving a total of five markers for spring fruit number in the lines grown in Rhode Island, but not in the other growth cohorts. Three of the markers and one of the interactions mapped to the upper arm of chromosome 5, in a region spanning about 40 cM which contained three additive QTLs.

Malmberg *et al*. (2005) studied fruit number, germination and seed size in field-grown *A. thaliana*, using the standard Landsberg × Columbia recombinant inbred set. The number of mapped additive QTLs varied from two to four for these traits; in each case the number of two-locus epistatic interactions mapped was approximately double, varying from five to eight. There were both positive and negative epistatic interactions. For fruit number the effect of the epistasis on the genotypes was large, ranging from −41% to +29%; for the other traits the effects were smaller, from −5% to +4%. These epistatic effects are roughly double in magnitude the effects of the additive QTLs for the same traits. These results indicated that epistasis played a large role in the variation for fitness differences between these two accessions in field-grown plants, both in numbers of interactions and in genotypic effects. The map locations of the additive and epistatic QTLs suggested that some of the loci identified are participating in more than one fashion. In some cases, the locations of the additive QTLs closely corresponded to the location of one partner in an epistatic interaction. In other cases, one genetic map location appeared to participate in more than one epistatic interaction, within the limits of the mapping resolution. The genetic architecture underlying fitness was represented as a network of epistatic and additive effects.

Similar studies have been performed in animals. Shook & Johnson (1999) examined life history traits in recombinant inbred lines (81 lines from an F6 of Bristol-N2 by Bergerac-BO, 40 molecular markers) of *Caenorhabditis elegans* as part of their investigation of genes affecting ageing. They used a two-factor ANOVA, all pair-wise marker comparison, to uncover epistatic interactions. There was one significant and one suggestive epistatic interaction for bagging (death by internal hatching of progeny), two significant interactions for fertility, three significant and one suggestive interaction for age of first reproduction, and one significant and one suggestive interaction for population growth. There was one suggestive QTL for survival, none for bagging, two significant QTLs for self-fertility, two significant and one suggestive QTLs for age of first reproduction, and one significant and one suggestive QTL for population growth. The number of significant epistatic interactions is similar to the number of additive QTLs. Peripato *et al*. (2004) studied the genetic architecture of mouse litter size in 166 females from an F2 intercross of the SM/J and LG/J inbred strains. They found two additive QTLs from interval mapping and eight epistatically interacting QTLs from a two-way comparison of all chromosome map segments. The additive QTLs and epistatic QTLs mapped to different chromosomes. Since they were examining an F2 population, they were able to determine that all forms of epistasis occurred: additive × additive, additive × dominance and dominance × dominance.

Although the total number of studies is limited, the genetic architecture underlying fitness shows a similar pattern in these studies. The number of additive QTL loci identified is usually small (1–6); the number of epistatic QTLs is usually slightly larger (5–10). The number of loci detected doubtless is also a function of the size of experiments that are currently practical.

4. Other epistatic QTLs for traits of evolutionary or ecological interest

Flowering time is an adaptive trait that is known to have wide natural variation; not surprisingly, there have been a number of studies of QTLs for this variation, dating back at least to Kowalski *et al*. (1994). Several of the more recent examples specifically test for the presence of epistatic interactions, either by screening for interactions among identified additive QTLs or by the complete pair-wise comparison approach. For example, Kuittinen *et al*. (1997) have examined the genetic basis of adaptation in *Arabidopsis thaliana* with respect to flowering time in growth-chamber-grown plants, identifying one major and six minor QTLs, as well as epistasis between one pair of the additive QTLs.

Juenger *et al*. (2000) used the Landsberg × Columbia recombinant inbred lines, grown in the greenhouse, to map eight quantitative floral characters, as well as comparing these traits across a worldwide sample of 15 *A. thaliana* accessions. They found 18 QTLs scattered across all five chromosomes. They also looked for epistasis among QTLs, finding two significant interactions. In both cases the loci were on separate chromosomes. The additive effects of the QTLs involved were substantially larger than the epistatic interactions. They noted, however, 'It is likely that our analyses have seriously underestimated the possibility of epistatic effects because we limited our screen to pair-wise combinations of QTL with previously identified additive effects on at least one of the floral traits.' Juenger *et al*. (2005) report a complete pair-wise marker by marker search for epistatic QTLs using the Pseudomarker program (Sen & Churchill, 2001) as part of their study of flowering time quantitative loci in the Landsberg × Columbia and Landsberg × Cape Verde recombinant inbred lines. They found two significant epistatic interactions in the Landsberg × Cape Verde lines, and none in the Landsberg × Columbia lines.

Borevitz *et al*. (2002) mapped QTLs responsible for natural variation in light and hormone response between the Cape Verde Islands (Cvi) and Landsberg erecta (Ler) accessions of *Arabidopsis thaliana* using recombinant inbred lines. Twelve QTLs were identified that mapped to loci, some of which had not been identified as candidate genes. Some of the QTLs acted in all environments but others showed genotype-by-environment interaction. In this case, there were fewer epistatic interactions detected than additive QTLs.

Kearsey *et al*. (2003) compared biometrical quantitative approaches with analyses of recombinant inbreds of *A. thaliana*. They examined 22 quantitative traits relating to leaf and flower bud size and developmental time, in plants grown in an unheated polytunnel. They found evidence for additive and/or dominance effects for 19 of the traits, and evidence for epistasis in 15 of the traits. In a further analysis of backcross generations, they determined that nearly all the epistasis present was additive × dominant, rather than additive × additive. The direction of the additive × dominant interaction on the phenotype was always negative. They mapped one to five QTLs for most of the traits using the recombinant inbred lines.

Ungerer *et al*. (2003) examined the effect of genetic background on selection response on advanced generations from a cross of the *A. thaliana* accessions Landsberg and Niederzenz. Their results indicated that genetic background did not have a strong consistent effect on the adaptive evolution they studied; allelic fitnesses were not strongly dependent upon genetic background. Ungerer & Rieseberg (2003) used the program Epistacy to examine the same set of Landsberg and Niederzenz lines for epistatic interactions. Under a very conservative $P = 0{\cdot}00017$ threshold they failed to detect any pair-wise marker interactions; however, at a liberal $P = 0{\cdot}005$ threshold they found multiple interactions for five of the six measured traits. They could explain the selection responses they observed by the additive QTLs and hypothesized that the epistatic interactions may also have been involved.

Kroymann & Mitchell-Olds (2005) have recently studied a QTL for plant mass (growth rate) in *A. thaliana* derived from the Landsberg × Columbia recombinant inbred lines in controlled growth chamber studies. They performed a genetic dissection of a 1 cM/210 kb interval on chromosome 5 to create near-isogenic lines. They identified two QTLs within this region, and also demonstrated a significant epistatic effect of 34% on the total biomass depending upon the parental background used for the same segment. The magnitude of this effect is similar to the magnitudes we noted for fruit number. Kroymann & Mitchell-Olds also detected high levels of nucleotide polymorphism in this region indicative of balancing selection. The authors predict that complex traits in *A. thaliana* will have a highly polygenic and epistatic architecture.

Although these studies differ from each other in a number of ways, each found some evidence for epistatic QTLs. In some cases the number of epistatic interactions found was greater than the number of additive QTLs, in other cases it was less.

5. Examples of epistasis associated with reproductive isolation or speciation

Epistatic interactions associated with reproductive isolation barriers were found in rice by Li *et al*. (1997*a*, *b*). They examined hybrid breakdown in the cross between rice *Oryza sativa japonica* cultivar Lemont and the *indica* cultivar Teqing to examine a possible case of incipient speciation. They examined a variety of quantitative traits related to sterility as well as plant height. For spikelet sterility, they found four QTLs and 21 epistatic interactions with varying degrees of significance. A notable result was that they could group many of the loci participating in additive and epistatic QTLs into supergenes, co-adapted gene complexes, based upon their map position. There were four such supergenes for spikelet sterility and two for plant height. 'Hybrid breakdown appeared to be largely due to incompatibilities between *indica* and *japonica* alleles at many unlinked epistatic loci in the genome' (Li *et al*., 1997*b*).

For comparison, Orr & Irving (2001) examined the genetic basis of hybrid sterility in crosses of populations of *Drosophila pseudoobscura* collected from Bogotá, Colombia and the United States. They

identified the underlying genetic architecture using a crossing scheme that involved a minimum of three separate backcrosses to identify chromosomal regions involved in the hybrid sterility, and to refine the map regions and replicate the results. They estimated that 15 genes were involved in the male sterility that separated the two populations. Strikingly, hybrid sterility required having a particular allele at four separate loci – a four-locus epistatic interaction!

Lukens & Doebley (1999) studied epistatic interactions between two genes involved in the differences between cultivated maize and teosinte, its wild ancestor. Two QTLs on separate chromosomes from teosinte had been identified that had large effects on plant and inflorescence architecture. These were introgressed into the standard maize inbred genetic background W22 by six generations of backcrossing. For two of the three traits measured, tillering and internode length, there were no significant interactions; the two chromosome segments contributed additively. For the third trait, staminate inflorescences, there was a high degree of synergistic interaction that was visually obvious. One segment had 21% staminate flowers in isolation; the second had 0·5% staminate flowers, while the combination had 90% staminate flowers. The two segments together 'produce the nearly complete conversion of the ear on the tip of the branch into a tassel' (Lukens & Doebley, 1999). They also observed this interaction at the level of changes in mRNA abundance by an examination of *tb1*, teosinte-branched, mRNA; *tb1* is considered to be one of the two QTLs involved in this trait. Lukens & Doebley speculated that once the locus of larger additive effect was identified during maize domestication, the effects of the second locus would then have been obvious and come under human selection as well.

6. What have we learned and which way do we go from here?

Not enough data yet exists to resolve the questions of how much epistasis contributes to natural variation for fitness between populations, and how much epistasis contributes to the process of speciation. From the studies completed to date, there is clearly variability in the amount of epistatic interactions detected, but the number of epistatic interactions also seems to be similar to, and frequently is larger than, the number of additive QTLs. The approach of performing all pair-wise comparisons, without restriction to just the known additive QTLs, has been performed in only a very small number of studies of the genetic basis of fitness. Hence, it is difficult to make generalizations about the relative importance of epistasis versus additivity.

Does the mating system of the organism affect the number of epistatic interactions? Shook & Johnson (1999) noted that the self-fertilizing *Caenorhabditis elegans* might be expected to have a build-up of co-adapted gene complexes and epistatic interactions; the same is true for the largely self-fertilizing *Arabidopsis thaliana*. The results of Paterson and colleagues (Li *et al*., 1997*a*, *b*, 2001; Luo *et al*., 2001; Mei *et al*., 2003) indicated substantial epistatic interactions occur in rice, which is also a largely selfing species. Matioli & Templeton (1999) found co-adapted gene complexes from a parthenogenetically reproducing strain of *Drosophila mercatorum*. It seems reasonable that a selfing or clonal species might have more epistatic interactions than an outcrossing species. A similar point was made by Malmberg (1977) with respect to epistasis as a function of genetic recombination. However, Routman & Cheverud (1997) and Cheverud (2000) used an all pair-wise comparison approach to identify more than 100 candidates for epistatic interactions in mouse body weight, and Peripato *et al*. (2004) found eight epistatic QTLs in comparison to two additive QTLs for mouse litter size. The recent studies of epistasis in RNA viruses found evidence for epistasis either in a virus that does not recombine (Sanjuán *et al*., 2004) or in one that does (Bonhoeffer *et al*., 2004). Thus, it is currently unclear whether mating system or extent of recombination is a determinant in whether epistasis is an important component of fitness.

Epistatic QTL analysis has some parallels with microarray data analysis: both can be methods for investigating genetic networking, and both involve a large number of data comparisons. In microarray analysis it is common to verify the expression patterns observed for selected sequences using PCR or other methods. A similar approach could be used with epistatic QTLs, viewing the interactions identified in the initial analyses as hypotheses, then verifying them by additional genetic tests. This amounts to applying false discovery rate statistics (Benjamini & Hochberg, 1995) to the problem of epistatic QTLs, and then using external information as a further test as recommended by Carlborg & Haley (2004). The method used by Lukens & Doebley (1999), introgression, could be a suitable follow-up to initial detection of epistatic interactions. Since the epistatic QTLs will normally have been identified by molecular markers, it should be possible to test the interaction after separately introgressing the segments that have been indicated to interact.

In the last few years, the QTL paradigm has been applied to microarray-measured transcript levels (for example, Brem *et al*., 2002), so that the quantitative trait is defined to be the level of transcript, and one can search for genes that control that level, termed eQTLs. Storey *et al*. (2005) have extended this

approach to look for epistasis as two genes controlling the level of a transcript. They found evidence for two-gene control in 37% of the gene expression traits studied. They developed a sequential test method to search for epistatic interactions that is different from the standard all-two-locus models usually used. They first searched for the single locus with the largest LOD score, then searched among all the other loci for a secondary locus that gave the largest LOD score conditioned on the selection of the first locus. Part of the argument in favour of this approach is that the lack of statistical power of the all pair-wise scans makes them less likely than the sequential approach to find epistatic interactions. Logically there must be epistatic interactions for pairs of loci that both lack significant main effects, but Storey *et al*.'s (2005) results suggest it is difficult to find them, at least for the eQTL traits they studied.

For model systems, the measurement of epistatic QTLs may eventually be superseded by the kind of genetic network analysis currently possible in yeast and bacteria (for example, Ihmels *et al*., 2005; Laub *et al*., 2000; Storey *et al*., 2005; Tong *et al*., 2004). While we need additional surveys of the amount of epistatic QTLs underlying fitness in various organisms, we should not stop with simply surveying the landscape. Addressing the role of epistasis in evolution will require understanding the mechanisms that underpin the genetic architectures discovered.

We thank Dr Greg Gibson and Professor W. G. Hill for useful suggestions to strengthen the manuscript. Research in the authors' laboratories is funded by DOE Grant DEFG02-97ER20286 to R.L.M. and NSF Grant DEB-0129191 to R.M.

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