Behavioral genetics: **Anxiety under interrogation**

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Recent genetic mapping experiments in the mouse have made significant inroads into understanding the complex genetics of behavior and, in particular, anxiety.

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Current Biology 2001, 11:R473–R476

0960-9822/01/$ – see front matter

Our understanding of genes that influence behavior, including anxiety, is central to our quest to understand the genomic basis of variation in human behavioral pathologies. Such studies are essential for the development and refinement of therapeutic interventions, and the diagnosis and management of mental health. Although it has long been recognized that variation in human and mammalian behavioral patterns is in part determined by genes, the exact nature of the relevant genes and the relative contribution of each gene to behavior have been rather more difficult to decipher. There have been some successes [1] but these represent just the tip of an iceberg, and there remain a large raft of behavioral genes that are undiscovered. The relative lack of success in identifying behavioral genes is due to the complexity of the trait being studied. Recent developments in genome research — the use of animal models of human behavior, the availability of high-density genetic maps, the development of computational tools, and the use of technology to develop high throughput partially automated behavioral assays — have prompted a revisit to the subject by Turri *et al.* [2] and other workers [1,3–6].

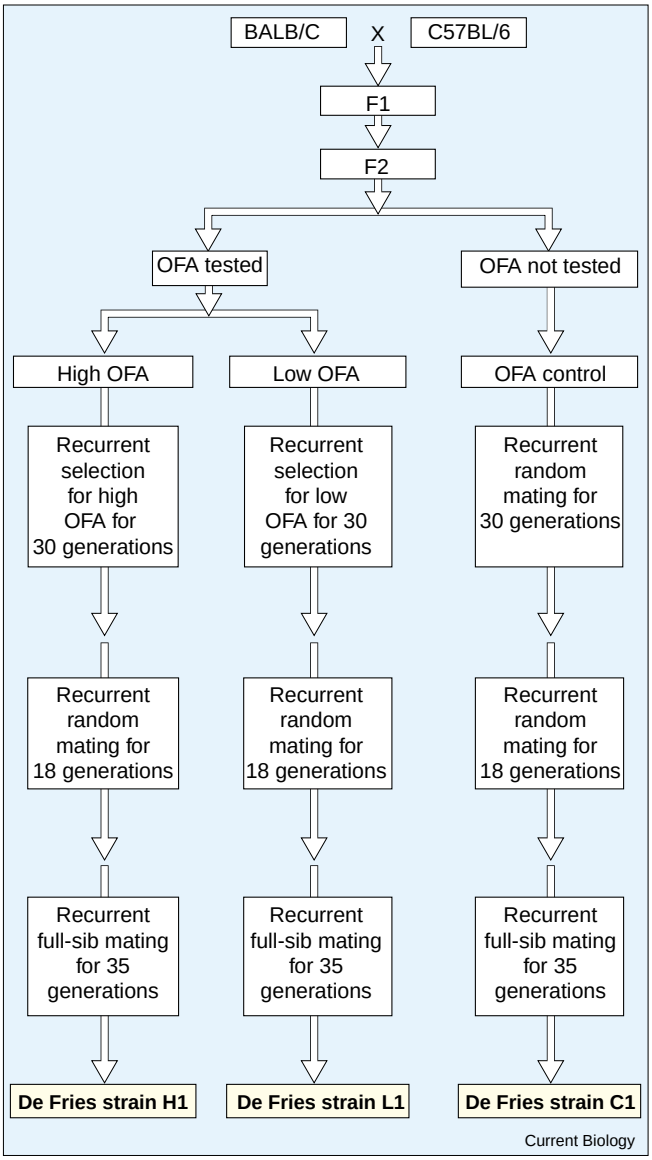
The definition of anxiety and its genetic determinants is at the core of the experiments reported recently by Turri *et al.* [2] in *Current Biology*. These authors asked whether anxiety is a unitary phenomenon, which and how many genes are involved, and whether the same genes influence behavior after habituation to a threatening environment. For the purpose of testing anxiety in animals, several tests have been developed. Such tests are usually intended to measure animal behavior that looks like human anxiety — that is, in an anthropomorphic context — and these tests have been validated by studies which show that tests on animals can be used to determine altered behavior following administration of anxiolytic drugs, as in humans. These and other bodies of evidence also indicate that the same or similar neural structures are involved in determining anxiety in humans and rodents [7,8].

There are, however, a number of obvious differences between the specific details of each test apparatus, and different studies that are aimed at deciphering gene function use some but not all the different available tests. This raises questions about the validity of conclusions that can be drawn from experiments where animals have been subjected to a subset of the available tests for anxiety. In this context, Turri *et al.* [2] asked whether or not anxiety is a unitary phenomenon determined by a common set of sub-phenotypes expressed in all anxiogenic compartments of five different tests, or alternatively, whether it is a pluralistic phenomenon determined by a set of sub-phenotypes whose expression varies in different anxiogenic environments. If the former is true, then any of the possible tests will suffice for classifying animals with respect to anxiety. If the latter is true, then the specific sub-components of anxiety determined by each test need to be identified in order to refine testing and interpretation of test results in future. Turri *et al.* [2] also asked whether habituation alters the expression of anxiety, and whether the same genetic determinants of anxiety in the first encounter of an anxiogeneic environment are implicated in behavior after habituation.

Turri *et al.* [2] chose a genetic mapping approach to tackle these questions. Their experiments used two lines of mice that exhibit very large differences in anxiety — a 30-fold difference on the open field activity (OFA) anxiety index (Figure 1) — named De Fries strains H (high) and L (low). These lines were crossed to produce offspring (F1) which were, in turn, intercrossed to produce the mice (F2) that were the subject of Turri *et al.*'s [2] experiments. Genetic markers and the genetic determinants of anxiety segregated in the cross that produced the F2 generation. Turri *et al.* [2] submitted each of over 1600 F2 mice to a battery of five ethological tests, comprising 21 subtests, of anxiety — the above-mentioned OFA index; the elevated plus maze (EPM); the elevated square maze (SQM); the light–dark box (LD); the mirror chamber (MC) — and three additional tests — home cage activity (HCA); tail suspension (TS); and defecation (OFD).

In order to identify the genetic regions responsible for the ethological test scores, Turri *et al.* [2] constructed a genetic map of each mouse. They then applied statistical approaches to determine the genetic map regions that were linked — co-segregated with and by implication determined — the ethological test scores. The statistical approach that they employed, though powerful, is not without problems; in particular, the false-positive rate increases as the number of tests rises. Hence, Turri *et al.* [2] empirically determined the appropriate test statistical

Figure 1



Origin of De Fries strains H1, L1 and C1 [6,9]. OFA refers to open field activity ethological test. Selection led to a 30-fold difference in OFA scores between strains H1 and L1 at the end of the experiment. The experiment was repeated to yield strains H2, L2 and C2.

threshold for declaring linkage in their experiments by applying a permutation test (see Box 1). By this approach, they identified 12 chromosomes that determined at least one of 21 different components of the five ethological tests studied (see Figure 2).

The fact that Turri *et al.* [2] were able to identify linked loci is not surprising, given the size of their experiment and the precedence set in their earlier work [3–6]. It is, however, noteworthy that they identified a different set of loci in

Box 1

Glossary of terms.

Interval mapping and composite interval mapping: likelihood-based analysis that fits the whole data set to a statistical model which describes the positions, effects and pairwise interactions between relevant loci [10].

Test statistics: The authors use a LOD score in the interval mapping and composite interval mapping. LOD is the total relative probability, expressed on a logarithmic scale, that a linkage relationship exists among selected loci.

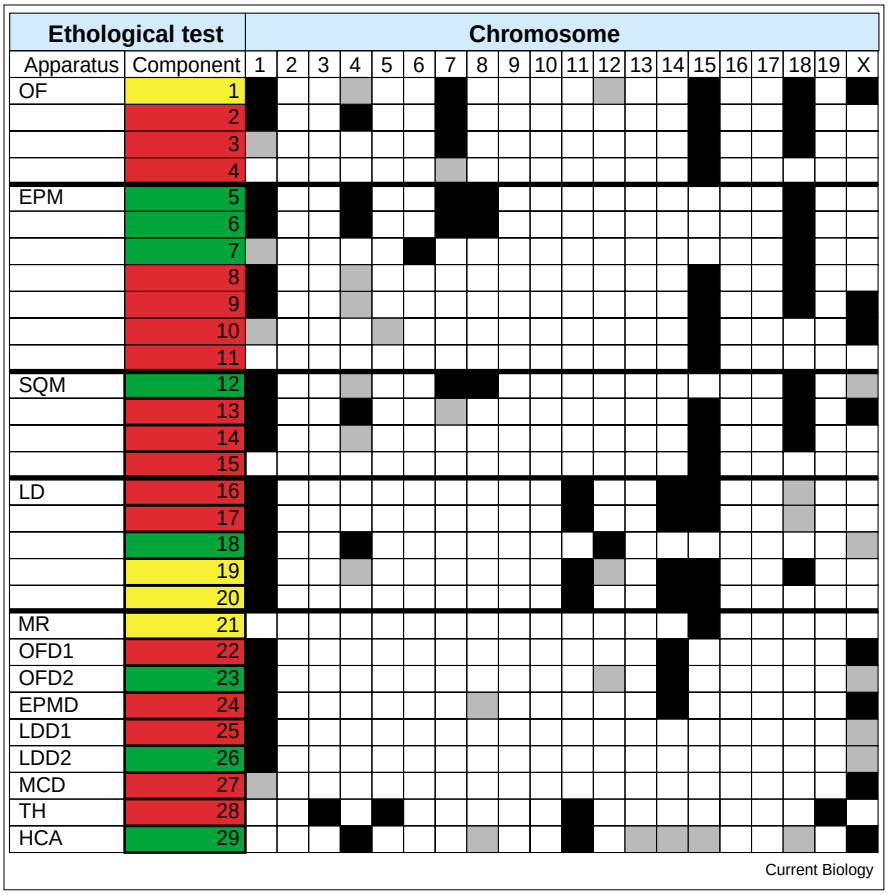
Permutation test: Establishes empirical significance thresholds in QTL mapping experiments. There are five steps: (1) Shuffle (permute) the trait values among the progeny. (2) Perform the same interval mapping analysis as on original data. (3) Record the highest test of significance score – this is the false-positive outcome of a random event. (4) Repeat the procedure (steps 1–3) hundreds or thousands of times. (5) Rank the recorded test scores. In 100 permutations, the 5th highest ranking test score is the empirically determined 0.05 alpha threshold (or the test score above which you are assured less than a 5% false-positive outcome).

Bootstrap analysis: Establishes properties of a distribution from actual data by use of a 'sampling with replacement' approach. Typically, assume your existing sample of mice (S_0) are representative of the population from which they were drawn. To empirically determine the 95% confidence interval of the map position of a phenotype, randomly sample mice from S_0 to obtain S_1. Record the exact phenotype and genotype of each mouse against its name. Repeat the procedure hundreds ($S_{10?}$) or thousands ($S_{100?}$) of times. Perform relevant analysis (interval mapping) on each of your sample populations (S_1 to …S_n) and record the map position predicted for the phenotype. Rank the recorded map positions. In 200 bootstraps, the 5th highest and lowest ranking map positions are the empirically determined 95% confidence limits (or the map limits within which 95% of attempts to map a phenotype will localize it).

different ethological tests on the same animals. At least one locus on chromosome 15 was identified in all ethological tests (see Figure 2) and suggests a common determinant of ethological test scores in all five tests; other loci, however, showed incomplete overlap between tests. These results suggest that different ethological tests measure different genetic determinants of behavioral outcome.

The results of three additional tests — OFD, TS and HCA — provided further support for their earlier conclusions on five ethological tests. In further analysis of their data, Turri *et al.* [2] took a hypothetico-deductive approach to ascribing function to the loci that they had identified. They identified generalised locomotor activity, anxiety-specific locomotor activity, emotional elimination and avoidance behavior as potentially dissectible components of the behaviors observed in their tests. By comparing the mapping data obtained in 21 different components of the five ethological tests and three additional tests, they were able to ascribe functions to some of the loci detected. For example, loci that are detected in anxiogenic and also anxiolytic components of the tests are likely to be

Figure 2



Grid-box abridged representation of the results of Turri *et al.* [2]. Green rectangles represent test component that should report lower states of anxiety than components in red rectangles. Yellow rectangles defy classification in the current scheme. Each shaded square reports the strength of the relationship between a test component and chromosome. Black squares represent relationships that are significant at the 5% statistical test threshold; grey squares are suggestive (lower than genome-wise but higher than point-wise significance) at this level; and white squares are clearly not significant. Test components are: 1, open field (OF) arena total activity; 2, OF centre activity; 3, OF centre time; 4, OF latency to approach centre; 5, elevated plus maze (EPM) closed arms entries; 6, EPM closed arms activity; 7, EPM closed arms time; 8, EPM open arms entries; 9, EPM open arms activity; 10, EPM open arms time; 11, EPM latency to enter open arms; 12, elevated square maze (SQM) closed arms activity; 13, SQM open arms activity; 14, SQM open arms entries; 15, SQM latency to enter open arms; 16, light dark box (LD) light box time; 17, LD light box activity; 18, LD dark box activity; 19, LD transitions; 20, LD latency to emerge; 21, mirror chamber (MC) latency to emerge; 22, OF defecation (OFD) on day 1; 23, OFD on day 2; 24, EPM defecation; 25, LD defecation (LDD) on day 1; 26, LDD on day 2; 27, MC defecation; 28, tail hang test score; 29, home cage activity score.

non-specific contributors to test results. In this manner, Turri *et al.* [2] deduced that the chromosome 4 locus is responsible for generalised locomotor activity independent of anxiogenic environment. Conversely, quantitative trait loci (QTLs) on chromosome 1 and 15 are firmly implicated in influencing anxiety (Figure 2). Further dissection of the test results suggested that the chromosome 15 locus acts to promote avoidance behavior, and that the QTL on chromosome 1 affects exploratory behavior. The role of other loci in anxiety is more difficult to interpret.

Taken together, these results suggest that the genetic determination of anxiety and other psychological traits is complex, more so than was hypothesized by the current workers in their earlier work [4]. This complexity is even more striking when one considers that the detectable genes in the current experiment are likely to represent only a proportion of the potential raft of genes involved in anxiety. This is because an integral property of the approach used by Turri *et al.* [2] is that only genes that were polymorphic in the founder strains of the H and L De Fries strains could have been found. There are potentially more, as yet unidentified genes that determine behavioral variation in anxiety in nature, but that lie undetected in this and other QTL mapping experiments because they lack polymorphism in the common inbred mouse strains. The use of wild mice, random mutagenesis of inbred strains and specific gene knock-in and knock-out experiments is likely to reveal a much larger repertoire of genes involved in behavioral pathologies, including anxiety, in future studies.

A question that arises out of the work of Turri *et al.* [2] is whether multiple components of ethological tests that map to the same chromosome are determined by the same gene, or by several closely linked genes. It is impossible to answer this question by directly comparing the exact map positions predicted for each test component in the interval mapping and composite interval mapping statistics (see Box 1). This is because the predicted positions are inexact, and characterized by a broad confidence interval (a genetic map interval in which the gene will localize in 95% of the attempts to do so). In other words, repeating the experiments several times might localize the gene slightly to the left or right of the currently predicted position. Turri *et al.*

[2] attempted to resolve this problem empirically by performing a 'bootstrap' analysis (see Box 1). They found that, in each case examined, they could not rule out the possibility that multiple test components were determined by a single gene.

In conclusion, Turri *et al.*'s [2] ground-breaking meta-analysis of the results of several ethological tests on the same set of mice reveals the complexity of the genetics that underpin anxious behavior, emphasises the need for clearer definition of the trait (in terms of measurement) and cautious interpretation of results, and in identifying some map locations and ascribing functions to certain genes provides encouragement that despite its complexity, anxiety can be dissected and functions ascribed to detected loci by use of current genetics tools. We are excited by the prospect promised by future research in this critical area of scientific endeavour.

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