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REVIEW

The genetic basis of emotional behaviour in mice

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The last decade has witnessed a steady expansion in the number of quantitative trait loci (QTL) mapped for complex phenotypes. However, despite this proliferation, the number of successfully cloned QTL has remained surprisingly low, and to a great extent limited to large effect loci. In this review, we follow the progress of one complex trait locus; a low magnitude moderator of murine emotionality identified some 10 years ago in a simple two-strain intercross, and successively resolved using a variety of crosses and fear-related phenotypes. These experiments have revealed a complex underlying genetic architecture, whereby genetic effects fractionate into several separable QTL with some evidence of phenotype specificity. Ultimately, we describe a method of assessing gene candidacy, and show that given sufficient access to genetic diversity and recombination, progression from QTL to gene can be achieved even for low magnitude genetic effects.
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Introduction

Emotionality is a psychological trait of complex aetiology, which moderates an organism's response to stress. Behavioural evidence of emotionality has been documented across a wide range of taxa from amphibians to rodents, and higher mammals; both in terms of a fear-like avoidance of perceived threats (eg predator–prey interactions and other environmental dangers),[1] and enduring personality-like variation in sensitivity to stress.[2] While evidence has only recently begun to emerge in favour of a capacity for non-human animals (including rodents) to experience[3] positive mood states, it is now accepted that a variety of species are likely possess an evolutionarily conserved capacity for fear and anxiety.

Emotionality, like other complex traits, is thought to result from the cumulative superimposition of low magnitude genetic effects and their interactions (both with other genetic loci (ie epistasis) and nongenetic (environmental) factors) ultimately producing a quasi-continuously distributed phenotype. As a combined consequence of the small individual genetic effects and their multifaceted patterns of moderation, genetic effects which contribute to variation in emotionality have, as in other complex traits proved remarkably difficult to identify.

The mouse exhibits a number of attributes, which may be useful to genetic research. These include a short gestation period, an early puberty, a short oestrus cycle and a tendency to produce large litters. The mouse genome is well characterised,[4] containing around 22 000 predicted genes, about 80% of which have a single identifiable human orthologue. These factors, along with a capacity for directed mating and rigid environmental control render the mouse an invaluable tool for complex trait dissection, with additional potential relevance towards human disease pathology. This relevance is illustrated through observations of mouse–human quantitative trait loci (QTL) concordance across a range of (primarily physiological) phenotypes (such as high-density lipoprotein (HDL)

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cholesterol concentrations[5]). While it is less likely that behavioural QTL will colocalise across species (not least due to difficulties in phenotype translation), it remains plausible that a small number of behavioural mechanisms, which carry a significant survival advantage may be conserved through evolution, and as such may depend, at least in part, on the same underlying genes.

Emotionality in mice

A range of behavioural phenotypes are currently utilised as informative measures of emotional reactivity in mice. These measures are founded on principals of avoidance, autonomic activation, and behavioural inhibition (the discontinuation of species-typical behaviours such as grooming, exploration and consumption in anxiogenic environments). These measures can be derived from a variety of paradigms, the most widely used of which is the open-field apparatus; a circular white, brightly lit and fully enclosed arena, within which behaviour can be remotely monitored.

Defecation and ambulation in the open-field exhibit a negative correlation.[6,7] Since these two measures are not thought to be under the control of the same peripheral nervous system, it follows that their consistent inverse relationship is likely to reflect the action of a central coordinating mechanism or psychological trait (such as anxiety).[8] Based on observations that intense fear can result in defecation, urination and immobility in humans, those animals found to demonstrate a combination of behavioural inactivity and heightened defecation have historically been considered to be more fearful or emotionally reactive. Consistent with this interpretation, repeated test exposures in the open-field result in a reduction in defection and decreased avoidance of the central (anxiogenic) areas[9] (ie a habituation).

The emotionality profile and phenotype heterogeneity

In addition to the open field, a number of other behavioural tests are available for the assessment and/or characterisation of an animal's emotionality profile. Those which we will pursue in this review include the elevated plus maze (EPM) and light–dark box (two environments consisting of contrasting anxiogenic (light and open) and safe (dark and enclosed) spaces); fear conditioning (evaluating an animal's behavioural response to cues or contexts previously associated with a negative experience (either in terms of avoidance or immobility)); the forced swim and tail suspension tests (measures of immobility in response to unavoidable aversive situations), and acoustic startle response. Although the measures derived from these tests are broadly consistent with one another, with for example behaviour in the EPM successfully predicting performance in other models of anxiety,[10] there is some evidence that behaviour across these tests can be divided into a small number of genetically separable dimensions.

A recent principal components analysis of over 100 behavioural phenotypes in nearly 1700 mice identified five genetically separable composite measures of anxiety. These include: (i) activity suppression in 'safe areas', (ii) avoidance of anxiogenic areas, (iii) suppression of rearing, (iv) latency to enter novel areas, and (v) autonomic activity in novel environments. Between 4 and 6 QTL were found to contribute towards variance on each measure, with only about 20% of loci contributing $>2\%$ of phenotypic variance to any given trait. These observations are consistent with previous multivariate mapping studies which have shown that a proportion of QTL in both mice[11] and rats[12] exhibit characteristic ethological profiles which transcend test type, and can be replicated across intercrosses.

Methods of mapping emotionality QTL in mice

Inbred mouse strains exhibit large differences in emotionality.[10,13,14] These differences can be exploited for the purposes of quantitative trait mapping through the construction of crosses between phenotypically divergent progenitor strains, and the subsequent use of directed breeding strategies with or without phenotype selection. As a general principal these approaches aim to maximise the level of genetic diversity at informative loci, and increase the number of recombination events intervening between genotyped markers and the QTL. Given an infinite marker density, crosses which contain higher levels of progenitor diversity, and which are separated from the parental strains by a larger number of meioses will provide the highest level of mapping resolution.

Several variations on this strategy exist, including the backcross and F_2 intercross, recombinant inbred lines (RILs), chromosome substitutions, recombinant congenics and heterogeneous stocks (HS). The backcross and F_2 intercross are the most widely used of these strategies. Two inbred strains (usually, but not necessarily with contrasting phenotypes) are crossed, the offspring genotyped and a test of the association between genotypic and phenotypic variation is performed. RILs involve a cross between two inbred strains, but in this case the F_2 generation are randomly intercrossed and inbred for around 20 generations yielding a diverse panel of inbred lines containing varying quantities of the two progenitor genomes. QTL can then be mapped by comparing the phenotypes of these lines, and establishing specifically which chromosomal regions they have in common.

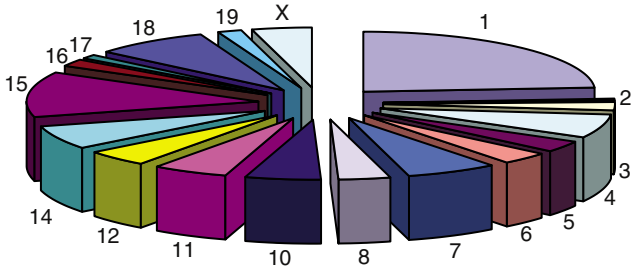
The chromosome substitution method of phenotype analysis represents an alternative first stage approach towards QTL detection. Single chromosomes from one inbred strain are introgressed into the background of

another by selective breeding, yielding a panel of inbred lines, which are genetically identical but for one chromosome. These lines can then be phenotyped for localisation of a QTL to the level of single chromosomes, and subsequently intercrossed to produce single chromosome F_2s with an enhanced mapping resolution and pure genetic background. Recombinant congenics are inbred lines, which contain a small proportion of one parental genome (the donor) relative to another (the recipient), typically produced by mating two inbred strains, backcrossing the descendents to one parental strain, and undertaking inbreeding without selection. Finally, HS are generated from a larger number of inbred strains (usually 8), which have been successively intercrossed for maximal diversity, and maintained over multiple generations through a program of pseudo-random mating. Of all the approaches discussed this method yields the greatest improvement in mapping resolution, but also necessitates an increase in maker density. Given current genotyping costs it may therefore prove more economical to utilise HS methodologies only once broad chromosomal regions have been identified, thereby limiting the number of markers required for QTL localisation (Figure 1).

Isolating broad regions of genetic involvement

The genetic mapping of rodent emotionality has historically focused predominantly on F_2 intercross strategies; derived either from phenotypically diverse inbred strains, or intercross progeny which have been subject to bidirectional selection over multiple generations and inbred to produce contrasting lines. To date there have been at least 13 attempts to map mouse emotionality using an F_2 intercross strategy,[8,15–26] relative to just one attempt using a backcross,[27] two using RILs,[28,29] one using chromosome substitution strains[30] and one using recombinant congenics.[28] Together these approaches have identified genome-wide significant linkage peaks (as determined either by permutation or application of the Lander and Kruglyak guidelines[31]) on 17 of the 19 mouse autosomes, and on one of the two sex chromosomes (chromosome X). Plot 1 (below) illustrates the distribution of genome-wide significant LOD (logarithm of the odds) scores (at $\alpha = 0.05$), which are available in the literature to date for those phenotypes described above. Regions which attained genome-wide significance only when mapped in parallel with other genetic cofactors (eg[28]) were excluded from this analysis, and likewise where data from a single cross and phenotype had been presented in more than one publication, only original findings were included.

This plot demonstrates a clustering of genome-wide significant LOD scores on chromosomes 1, 15 and 18, with the largest quantity of evidence (a total of 41 linkage peaks) pointing towards the presence of one or more QTL on chromosome 1. Simulations have shown that, depending on the size of the genotyped population, the extent of marker spacing and magnitude of QTL effect, the location of linkage peaks can fluctuate up to 20–30 centimorgan (cM) from the true locus,[32–34] and consistent with this observation over 80% of the genome-wide significant LOD



Plot 1 The distribution of significant anxiety-related LOD scores across the mouse genome by chromosome. This plot shows the number of genome-wide significant emotionality-related linkage peaks identified in the mouse to date, separated by chromosome.

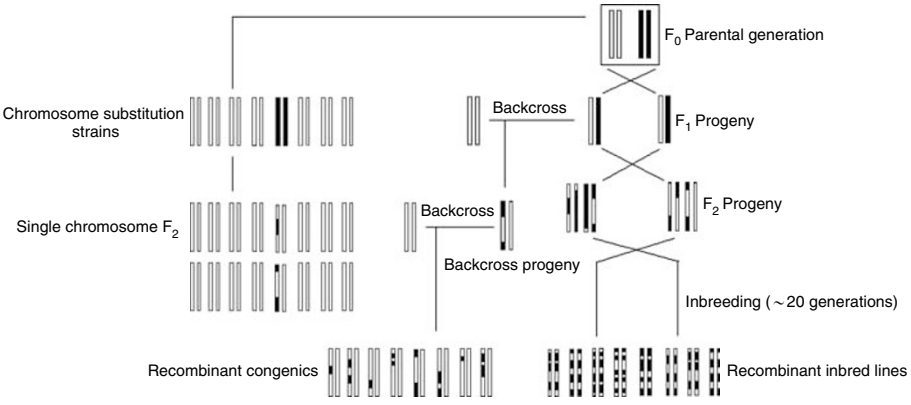


Figure 1 Approaches to mapping a quantitative trait. This figure illustrates the various crosses utilised in the genetic mapping of murine emotionality.

scores reported on chromosome 1 are located within a 30 cM interval (73–103 cM). However, from these data, which have been derived from multiple distinct strain contrasts and a range of different phenotypes, it is not possible to establish to what extent these peaks reflect common underlying QTL. Proximity alone is insufficient to enable a judgement of genetic equivalence, particularly in complex traits where single apparently large-effect QTL have often been found to fractionate into a multitude of smaller genetic effects.

Focusing on chromosome 1

The first evidence in favour of an emotionality locus on mouse chromosome 1 was derived from an F_2 intercross of the DeFries strains;[7] a set of phenotypically divergent inbred lines originally generated from a cross between C57BL/6J (a nonanxious strain) and BALB/cJ (an anxious strain), and then selected for trait extremity over multiple generations. Two of these lines (H1a and L1a) were then intercrossed, and the phenotypically most extreme progeny analysed across 84 microsatellite markers and four correlated measures of emotionality. In this way an emotionality QTL was identified on chromosome 1 located between markers D1MIT218 and D1MIT293 (spanning an interval of 65.6 Mb). The locus affected all four of the collected behavioural measures including both defecation and ambulation in the open-field, and entries into the open arms of the EPM.[8]

Similar analyses have since been carried out in a replicate population of the DeFries H1-L1 intercross progeny (H1b and L1b),[23] a second intercross between the remaining two DeFries strains (H2 and L2),[23] and a combined sample of two data sets (including over 1600 animals).[24] These analyses have consistently identified a linkage peak between 72 and 82 cM on mouse chromosome 1, using a range of primarily locomotor phenotypes including ambulation and defecation in the open-field, the number of open-arm entries in the EPM, the number of light–dark transitions made in the light–dark box, and the total amount of time spent in the light area. Consistent with these observations, a recent multivariate analysis of the complete DeFries data set showed that this chromosome 1 locus (located between 74 and 78 cM) operates in a phenotype specific manner, influencing a combination of safe-area ambulation and autonomic activity, and alone accounting for 4–10% of total activity variance within each apparatus.[35]

These data provide a relatively tight estimate of QTL location, and a homogenous description of QTL effect. However, all the evidence discussed thus far has been derived from a single inbred strain contrast between BALB/cJ and C57BL/6J. This limitation on the level of genetic diversity may mean that a proportion of QTL, that is, those which do not segregate between BALB/cJ and C57BL/6J, or which exert opposing effects on the phenotype despite association with the same allele, will not have been detected. Table 1 provides a list of those genome-wide significant linkage peaks, which have been detected on mouse chromosome 1 to date. These linkage peaks have an average effect size of 6.2% (with a range of 1.1–21%), and vary in location between 30 and 103 cM.

The use of additional genetic contrasts and phenotypes has led to a considerable expansion of the implicated regions, and a reduced homogeneity of effect. That is, QTLs on chromosome 1 have now also been shown to influence fear conditioning, activity in threatening areas and exploratory (rearing) behaviour, and furthermore, several potentially separable clusters of linkage peaks are now visible. Plots 2 and 3 illustrate the distribution of linkage peaks across chromosome 1 stratified both by phenotype and strain contrast. While there is no clear phenotype-dependent division between loci (short of a predominant emphasis on activity-based phenotypes), different strain contrasts seem to have isolated distinct linkage peaks. One cluster of peaks located between 72 and 82 cM on chromosome 1 appears to result from a contrast between C57BL/6J on the one hand, and A/J, C3H/HeJ and BALB/cJ on the other. In contrast, a second group of peaks located more distally between 93 and 103 cM appears to result from a contrast between only two strains (C57BL/6J and A/J), and a third cluster located more proximally (49–54 cM) reflects a contrast between C57BL/6J and both C3H/HeJ and 129SvEvTac. Furthermore, while the chromosome 1 locus represents the best replicated QTL for any mouse behaviour, a proportion of investigations have also failed to identify a QTL in the region (eg[36]).

Fine mapping of the chromosome 1 locus

While whole-genome mapping of two-strain intercrosses represents an effective strategy for the identification of QTL, accurate localisation (sufficient to enable molecular cloning) requires a higher level of resolution. Incorporating multiple inbred strains into a single heterogeneous stock, which has been maintained over multiple generations (accumulating recombinants) provides a both controlled and powerful method for the identification of QTL, which segregate in different ways between different strains. To date two HS have been utilised for the fine mapping of murine emotionality; the Boulder HS and the Northport HS. The older of these stocks (the Boulder HS) was generated from C57BL/6, BALB/c, RIII, AKR, DBA/2, Is, A/J and C3H, and had been bred for more than 58 generations at the time it was used for genetic mapping. Theoretically, the stock should offer a greater than 30-fold improvement in mapping resolution relative to F_2 intercross strategies.[37]

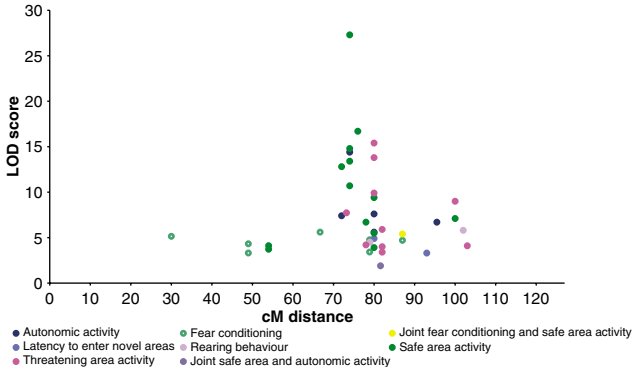
Table 1 Evidence for emotionality locus/loci on mouse chromosome 1

Phenotype	*Method*	*Strains*	*n*	*% Genotyped*	*Distance (cM)*	*Peak LOD score*	*% Variance*	*Reference*
FC: altered context	BC	C57BL/6J, C3H/HeJ	473	11	49	3.31	NR	Caldarone *et al*[27]
FC: altered context	BC	C57BL/6J, C3H/HeJ	473	11	78.9	3.42	NR	Caldarone *et al*[27]
FC: context	BC	C57BL/6J, C3H/HeJ	473	11	30	5.14	6.2	Caldarone *et al*[27]
FC: context	BC	C57BL/6J, C3H/HeJ	473	11	49	4.32	NR	Caldarone *et al*[27]
FC: context	BC	C57BL/6J, C3H/HeJ	473	11	78.9	4.76	NR	Caldarone *et al*[27]
EPM: OAE	F2	Defries (H1a, L1a)	879	22	100	9	21	Flint *et al*[8]
OFT: OFA	F2	Defries (H1a, L1a)	879	22	74	13.4	9.2	Flint *et al*[8]
OFT: OFD	F2	Defries (H1a, L1a)	879	22	80	5.6	5.3	Flint *et al*[8]
OFT: CT (5 min)	F2	A/J, C57BL/6J	518	12	73.2	7.73	6.8	Gershenfeld and Paul[18]
OFT: OFA (novel, 5 min)	F2	A/J, C57BL/6J	518	12	100	7.1	6.3	Gershenfeld *et al*[17]
OFT: R (novel, 5 min)	F2	A/J, C57BL/6J	518	12	79	4.5	5.9	Gershenfeld *et al*[17]
OFT: R (habituated, 5 min)	F2	A/J, C57BL/6J	518	12	102	5.8	5.4	Gershenfeld *et al*[17]
OFT: OFA (first 15 min)	F2	C57BL/6J, 129SvEvTac	289	100	54	3.72	6	Kelly *et al*[19]
OFT: OFA (5 min)	F2	C57BL/6J, 129SvEvTac	289	100	54	4.11	6	Kelly *et al*[19]
LD box: latency	F2	C57BL/6J, CSS-1	91	100	93	3.3	NR	Singer *et al*[21]
LD box: light box time	F2	C57BL/6J, CSS-1	91	100	103	4.1	NR	Singer *et al*[21]
OFT: EMO	F2	C57BL/6J, CSS-1	91	100	81.6	1.9	NR	Singer *et al*[21]
OFT: OFD	F2	C57BL/6J, CSS-1	91	100	95.5	6.7	NR	Singer *et al*[21]
Composite CA	F2	C57BL6/J, DBA2/J	396	99	87	4.7	NR	Steinberger *et al*[22]
Composite CA and OFT	F2	C57BL6/J, DBA2/J	396	99	87	5.4	NR	Steinberger *et al*[22]
EPM: CAE	F2	DeFries (H1,H2, L1, L2)	1636	100	80	9.4	3.1	Turri *et al*[24]
EPM: OAE	F2	DeFries (H1,H2, L1, L2)	1636	100	80	15.4	5.3	Turri *et al*[24]
EPM: OAT	F2	DeFries (H1,H2, L1, L2)	1636	100	82	3.4	1.1	Turri *et al*[24]
LD box: latency	F2	DeFries (H1,H2, L1, L2)	1636	100	80	4.9	1.7	Turri *et al*[24]
LD box: light box time	F2	DeFries (H1,H2, L1, L2)	1636	100	80	9.9	3.3	Turri *et al*[24]
LD box: transitions	F2	DeFries (H1,H2, L1, L2)	1636	100	76	16.7	6.2	Turri *et al*[24]
OFT: OFA	F2	DeFries (H1,H2, L1, L2)	1636	100	74	27.3	10	Turri *et al*[24]
OFT: OFD	F2	DeFries (H1,H2, L1, L2)	1636	100	74	14.4	5.5	Turri *et al*[24]
EPM: CAE	F2	Defries (H1b, L1b)	815	100	80	3.9	2.7	Turri *et al*[23]
EPM: CAE	F2	Defries (H2, L2)	821	100	80	5.5	3.7	Turri *et al*[23]
EPM: OAE	F2	Defries (H2, L2)	821	100	80	13.8	8.6	Turri *et al*[23]
EPM: OAE	F2	Defries (H1b, L1b)	815	100	82	4	3.3	Turri *et al*[23]
OFT: OFA	F2	Defries (H1b, L1b)	815	100	72	12.8	10.9	Turri *et al*[23]
OFT: OFA	F2	Defries (H2, L2)	821	100	74	14.8	10	Turri *et al*[23]
OFT: OFD	F2	Defries (H2, L2)	821	100	72	7.4	5.3	Turri *et al*[23]
OFT: OFD	F2	Defries (H1b, L1b)	815	100	80	7.6	5.8	Turri *et al*[23]
LD box: light time	F2	Defries (H2, L2)	821	100	82	5.9	3.9	Turri *et al*[23]
LD box: transitions	F2	Defries (H2, L2)	821	100	74	10.7	7.8	Turri *et al*[23]
LD box: light time	F2	Defries (H1b, L1b)	815	100	78	4.2	3.2	Turri *et al*[23]
LD box: transitions	F2	Defries (H1b, L1b)	815	100	78	6.7	5.1	Turri *et al*[23]
FC: cue	F2	C57BL/6J, DBA/2J	479	40	66.7	5.6	6.3	Wehner *et al*[25]

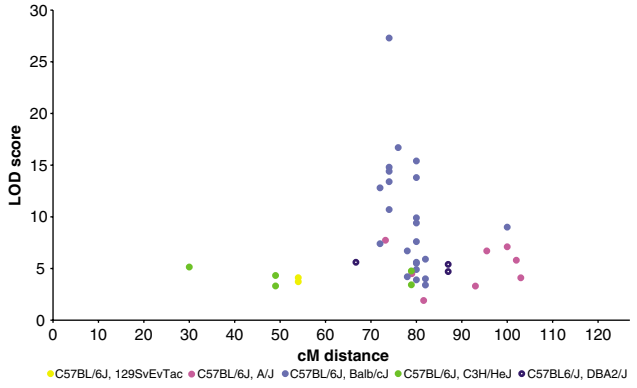
Presents the evidence in favour of an emotionality locus/loci on mouse chromosome 1.
BC = backcross; OFT = open-filed test; EPM = elevated plus maze; LD box = light dark box; FC = fear conditioning; OFA = open-field ambulation; OFD = open-field defecation; CT = centre time; R = rearings; OAT = open-arm time; CAT = closed-arm time; OAE = open-arm entries; CAE = closed-arm entries; CA = conditioned avoidance.

Consistent with this estimate, several fine-scale loci have now been mapped in this HS stock within the confines of what initially appeared to be a single locus. Talbot *et al*[37] mapped open-field activity and defection (along with a composite measure termed EMO) in 751 of these animals, revealing a conservatively estimated 2 Mb region surrounding the marker D1MIT264 (located at 71.5 cM on chromosome 1). The allele found to increase emotionality was shared by A/J and C3H, whereas AKR, RIII and DBA/2 all shared a decreasing allele. At this locus, however, the two strains utilised for the original F_2 mapping experiment could not be distinguished (ie QTL with opposite effects were associated with the same allele), indicating that this locus represents an independent effect.

As most marker loci have a small number of alleles, and the genomes of inbred mouse strains are very similar, it can be difficult to establish from which strain each allele originally descended. Consequently, a number of markers are likely to be uninformative, resulting in an inability to map opposing QTL, which associate with the same allele. Where full genealogical history and founder haplotypes are known, this problem can be circumvented by assigning each animal with a probability that a given marker is derived from a given founder strain, and testing the level of association between these probabilities and the phenotype of interest through analysis of variance. This approach was applied to the Boulder HS[38] and as expected revealed two QTL; one located between D1MIT103–D1MIT289



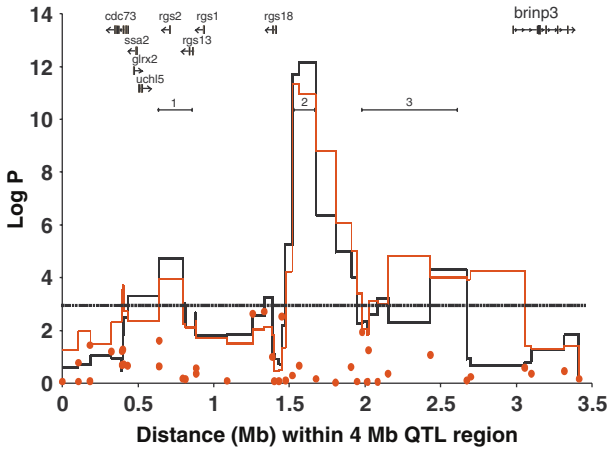
Plot 2 The distribution of significant anxiety-related LOD scores across mouse chromosome 1 by phenotype. This plot illustrates the distribution of genome-wide significant LOD scores (ie linkage peaks) across mouse chromosome 1, separated by phenotype (where possible using the classifications reported in[35]).



Plot 3 The distribution of significant anxiety-related LOD scores across mouse chromosome 1 by strain contrast. This plot illustrates the distribution of genome-wide significant LOD scores across mouse chromosome 1, separated by strain contrast (ie the types of strains used for mapping).

(73–74.3 cM); a locus which aligns with that previously described in this population,[37] and the other falling between D1MIT456-D1MIT115 (95.8–99.7 cM).

The second HS population (the Northport HS) was also derived from eight inbred strains (A/J, AKR/J, BALB/cJ, C3H/HeJ, C57BL/6J, CBA/J, DBA/2J and LP/J), with ~40 generations of breeding (yielding a slightly reduced mapping resolution relative to the Boulder HS).[39] Like the Boulder stock, a composite measure of open-field ambulation and defecation (EMO) was also mapped to two loci in this population; with one locus positioned at 82 cM (−10 Log $P = 5.4$), and the other at 72 cM ((−10 Log $P = 4.8$). While these localisations represent a significant advance in mapping resolution relative to two-strain intercrosses, because of the sheer number of recombination events required to identify single genes, in the majority of cases the QTL intervals remain too large to enable positional cloning.



Plot 4 Murine emotionality QTL on chromosome 1. Data derived from the MF1 heterogeneous stock. Single-point ANOVA results are shown as red circles. HAPPY multipoint analysis results are provided in two formats; based on four (black line) or eight progenitors (red line). The 95% confidence intervals of the three QTL identified are illustrated as horizontal lines numbered 1–3. The Bonferroni corrected 5% significance threshold is presented as a broken horizontal line.

The use of outbred stocks

True outbred stocks have one major advantage over HS populations; they have been randomly maintained over an extended number of generations accumulating an unprecedented level of recombination. One such outbred stock, the MF1, was created in the early 1970s by crossing two outbred lines (LACA and CF). Although the genealogy of the MF1 is unknown before this time-point, sequence comparisons have shown a high level of sequence similarity with other inbred strains, such that the MF1 genome can be treated as a mosaic of homology blocks utilising as few as four theoretical progenitors.[40] As a consequence of this relatively simple genetic architecture, ancestral descent mapping methods can be applied equivalent to those previously used in the HS. In this way emotionality has now been successfully mapped to three apparently independent QTL within the confines of the original HS chromosome 1 locus. The 95% confidence interval (CI) of peak 1 (Log $P = 5.0$) covered 0.38 Mb and just two genes; both regulators of G-protein signalling (RGS2 and RGS13). Peak 2 (Log $P = 10.9$, CI = 0.24) was juxtaposed to RGS18, and the CI of peak 3 (Log $P = 4.2$) covered 0.68 Mb of noncoding sequence (Plot 4).

Regulators of G-protein signalling modify the kinetics of G-protein-mediated signalling events, accelerating Gα-catalysed GTP hydrolytic turnover by up to 1000-fold,[41] without compromising the strength of steady-state signalling. Both RGS2, and to a lesser extent RGS13 are expressed in the brain,[42,43] and a null mutant is available for RGS2, which exhibits a greater preference for the dark in the light/dark test, and an increased response to the acoustic startle.[44] Consequently, RGS2 has been pursued as a candidate gene for the chromosome 1 emotionality locus.

Progressing from QTL to gene

Identifying the genes underlying QTL action is a major aim of modern genetics. Of the ~2000 rodent complex trait QTL identified to date, only around 1% have been successfully cloned, with the majority of these exhibiting uncharacteristically large effect sizes.[45] Compounding the difficulties associated with detecting small genetic effects, the causative variant may also lie some distance from its target gene, and may lack any association with gene expression (ie due to compensatory mechanisms/a direct effect on protein function).

Circumventing these difficulties, in order to test the candidacy of RGS2 to the chromosome 1 QTL, Yalcin *et al*[40] applied a novel method originally designed for use in *Drosophila melanogaster*;[46] quantitative complementation (also known as the knockout interaction test). This approach assesses the presence of a genetic interaction between a positional candidate gene and a homologous QTL using a candidate gene mutation (typically null or deficiency) and a simple analysis of variance. By using the RGS2 null mutant which was already available and two inbred strains between which the QTL was known to segregate, Yalcin *et al*[40] were able to show that RGS2 contributes towards emotionality in mice, interacting with the mapped QTL either through allelism or epistasis.

Implications and future directions

The data presented in this review demonstrate the potential for progression from broad QTL spanning many cM and hundreds of genes, to single genes of confirmed involvement. However, this confirmation comes with a number of caveats. First, given the methods described above it is impossible to distinguish between allelic and epistatic effects. In order to achieve any degree of confidence that a gene corresponds directly to a QTL further corroborating (preferably functional) evidence is required. Second, this gene represents the tip of a very large iceberg. Given that the QTL containing RGS2 was originally mapped some 9 years prior to the confirmation of RGS2's involvement, it is likely to be some time before the remaining QTL on chromosome 1 (let alone the whole genome) reach a similar level of dissection. However, with the advent of new and more powerful methodologies such as the collaborative cross[47] (a large reference panel of RILs derived from eight genetically diverse founder strains), whole-genome association and e-QTL (expression-QTL) analysis, accurate QTL localisations should become commonplace – enabling a rapid (and financially viable) shift from QTL mapping to assessments of gene candidacy. In addition, while only mice have been discussed in this review, investigations of QTL concordance across species are also likely to provide valuable insights into QTL localisation and function. The challenge for the future is then to develop economical methods of confirming QTL-gene equivalence, and assessing the relevance of these genes to human pathologies.

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