

X-linked and lineage-dependent inheritance of coping responses to stress

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Received: 1 May 2003 / Accepted: 8 July 2003

Abstract

Coping—or how one routinely deals with stress—is a complex behavioral trait with bearing on chronic disease and susceptibility to psychiatric disorders. This complexity is a result of not only underlying multigenic factors, but also important non-genetic ones. The defensive burying (DB) test, although originally developed as a test of anxiety, can accurately measure differences in coping strategies by assaying an animal's behavioral response to an immediate threat with ethological validity. Using offspring derived from reciprocal crosses of two inbred rat strains differing in DB behaviors, we provide convergent phenotypic and genotypic evidence that coping styles are inherited in an X-linked fashion. We find that first-generation (F₁) males, but not females, show maternally derived coping styles, and second-generation (F₂) females, but not males, show significant differences in coping styles when separated by grandmaternal lineage. By using a linear modeling approach to account for covariate effects (sex and lineage) in QTL analysis, we map three quantitative trait loci (QTL) on the X Chromosome (Chr) (*Coping-1*, *Approach-1*, and *Approach-2*) associated with coping behaviors in the DB paradigm. Distinct loci were associated with different aspects of coping, and their effects were modulated by both the sex and lineage of the animals, demonstrating the power of the general linear modeling approach and the important interplay of allelic and non-allelic factors in the inheritance of coping behaviors.

Introduction

Most people today acknowledge that behavior is the product of an interaction between genes and environment, yet few seem to tackle the complexity inherent in this assumption. Even on a purely genetic level, the study of complex traits is a relatively recent phenomenon, spurred on by the realization that many diseases, rather than being caused by a single highly penetrant gene, are likely caused by multiple genes of smaller effect. Quantitative trait loci (QTL) analysis has for the most part been used to dissect the multiple genetic underpinnings of complex traits, but analyses involving the modulating influence of non-genetic variables are necessary in order to capture more fully the complexities we seek to understand. In this study, we demonstrate X-linked inheritance of coping strategies in an animal model and show how the use of covariates in linear modeling greatly enhances the power of the QTL approach and our understanding of the complex behavioral trait of coping.

Coping strategies are those processes—cognitive, behavioral, or physiological—aimed at diminishing or terminating stress (Costa and McCrae 1989; Lazarus and Folkman 1984; Wechsler 1995). A coping style can also be defined as a coherent set of behavioral and physiological stress responses that is consistent over time, within a certain group; shaped by evolution, coping styles are general adaptive response patterns in response to challenges experienced in the natural habitat (Francis et al. 1999). More specific to our animal model of coping in the defensive burying (DB) test, burying behavior in the rodent is an adaptive coping response to threat or danger, representative of the endogenous, instinctual fear response necessary for its survival in the wild.

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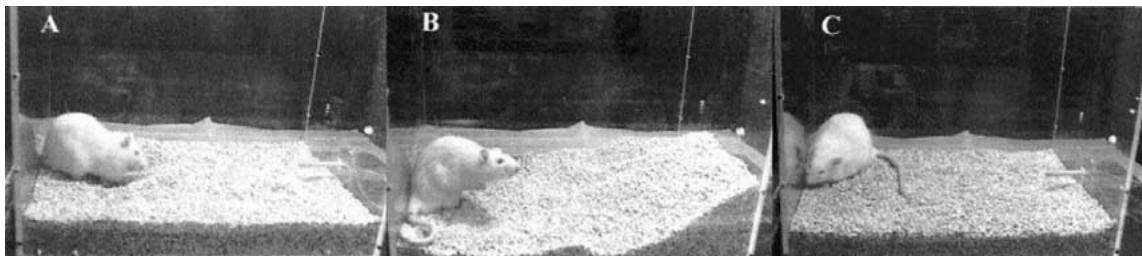


Fig. 1. Photographs depicting animals in the defensive burying test. (A) Some time after receiving a shock, animal begins spraying bedding toward the prod. (B) Typical of the F344, animal engages in extensive burying behavior such that bedding completely covers prod. (C) Unlike the F344, the WKY buries very little and after a series of prod approaches, remains immobile in a corner of the test chamber.

Different coping strategies elicit simultaneous changes in physiological variables such as autonomic nervous system tone, heart rate, and blood pressure, which can lead to an increased risk of chronic diseases such as hypertension, diabetes, and heart disease (Koolhaas et al. 1999; Busjahn et al. 1999; Rostrup et al. 1993). Furthermore, extremes in coping styles can influence susceptibility to psychiatric disorders, particularly depression and certain anxiety disorders (Huether 1996; Whatley et al. 1998). To better understand the relationship between stressors and their adverse physiologic consequences, the study of the mediating factor of coping is important (Vogele and Steptoe 1992).

The study of the successful use of coping strategies is also important since successful coping can be a protective factor in the face of stress. When exposed to stressful life events as children, a significant percentage emerge resilient, not only protected from the negative consequences of stress but also faring better in adulthood than their non-stressed counterparts (Werner 1989). These differences between resilient and non-resilient children, and between resilient and non-stressed children, seem to lie at least in part in the successful use of adaptive coping skills by resilient children (Rutter 1985). Thus, coping strategies are the link determining whether a stressor will have a positive or negative consequence, as well as being the mediator of those consequences.

It is generally believed that coping behaviors are made up of genetic, environmental, and learned components. Human studies determining whether the complex trait of coping is under genetic control have been reported (Mellins et al. 1996), but animal studies of the transmission of coping behaviors hold more molecular genetic potential and have not yet been attempted. The DB test, although originally developed as a test of anxiety (Treit et al. 1981), can accurately measure differences in coping strategies (Pare 1994; Korte et al. 1992; Sluyter et al. 1996; Treit et al. 1986) by assaying an animal's behavioral

response to an immediate threat with ethological validity (Treit 1991).

Materials and methods

Cross. Wistar Kyoto (WKY) and Fischer 344 (F344) rats served as parental inbred strains. Reciprocal parental crosses (F344 female \times WKY male, and WKY female \times F344 male) were used to generate 150 first-generation (F₁) offspring. These F₁ rats were intercrossed in brother-sister matings to produce a panel of 486 phenotypically and genotypically segregating second-generation (F₂) animals.

Defensive burying test. Rodents in the wild, when threatened by the attack of a predator, will spray sand toward the predator in an effort to ward it off (Treit 1991). The defensive burying (DB) test mimics this scenario in the laboratory. In this test, rats are habituated (four cagemates together) to a plexiglass chamber (40 cm \times 30 cm \times 40 cm) with bedding (wood shaving) for 15 min each day, for three consecutive days. On the fourth day, animals are singly and randomly introduced into the test chamber with a continuously electrified prod present. The prod delivers a shock (\sim 5 mA) when the rat touches it, thereby starting the 15-min test which is videotaped and subsequently scored by an observer blinded to the identity of the animals, particularly to their lineage of origin.

Once shocked, animals typically approach the prod a certain number of times [in a stretch-attend posture likely indicative of risk assessment (Rodgers 1997)], and at some point begin spraying bedding toward the prod (Fig. 1A), at times completely covering the prod (Fig. 1B). Behaviors measured include latency to begin burying, duration of burying, the number of times an animal approaches the prod, and the number of times a rat gets shocked. Animals engaged in active coping will have a shorter latency to bury, a longer duration of burying, and fewer prod approaches. Animals employing passive coping tend

to approach the prod more frequently, while having a longer latency to bury and a shorter duration of burying. After a period of prod approaches, these passive copers will typically remain immobile in a corner of the test chamber (Fig. 1C). For further details on the defensive burying test, see Neuroscience Protocols (Treit D 1994).

Primers. DXRat67, DXRat82, DXRat64, DXRat127, DXRat104 were determined to be polymorphic between F344 and WKY by using Rat Genome Database (URL: <http://rgd.mcw.edu/>) and were purchased from Research Genetics. The forward primer for DXRat104 was modified from its published sequence for better amplification in our cross. The modified primer (CCC TGC CAA ACA TAT CCA TG), along with its published reverse primer (TGC TCT CAG TGA TCC ATA GGC), was purchased through Integrated DNA Technologies (IDT; www.idtdna.com). DXWox30 and DXWox31 were among several markers found through a gene-based map of the X Chromosome (Chr) (Millwood et al. 1997) and were tested by us for polymorphism in our cross, then purchased through IDT.

Genotyping. The genotypes of the 486 F₂ animals were determined for the seven polymorphic markers spanning the X Chr. Animals were genotyped on two different occasions by different people, each time being scored by two independent observers. Tail samples were collected at weaning, and DNA was isolated by standard phenol-chloroform extraction; genotypes were resolved by autoradiography on polyacrylamide gels. For more details, see genotyping methods in Shimomura et al. (2001).

Quantitative genetic analysis. A female genetic map of the X Chr was constructed with R-QTL software (Broman et al., 2003) with allowance for 1% genotyping error rate. This map was used for interval mapping analysis. Single QTL and pairwise genome scans were carried out using pseudomarker software (Sen and Churchill 2001). The putative positions of either one or two QTLs were 'scanned' over the length of the chromosome in 2-cM increments, and a LOD score was computed. Single QTL scans generate a traditional LOD curve, whereas the pairwise scans generate a 2D surface of LOD scores. The maximum LOD score over all putative QTL locations is interpreted as evidence for the QTL location(s) and strength of effect.

The LOD scores obtained from a simple genome scan, as first described by Lander and Botstein (1989), compare the likelihood of a model with a single QTL at a given location to the likelihood of a model with

no QTL. The simple genome scan does not account for possible effects of covariates or other QTLs. The models being compared in a simple genome scan can be written as,

$$y = \beta_0 + \beta_1q + \epsilon$$

$$y = \beta_0 + \epsilon$$

where y is the phenotype, β_i are regression coefficients, q represents the QTL genotype, and ϵ represents normal error. The simple genome scan explicitly assumes that only one QTL is determining trait values. In reality, there may be multiple loci as well as other factors such as sex, treatment, lineage, or interactions between these variables that contribute to the phenotypic variation within a given population.

In this study, covariates, sex, and grandmaternal lineage were incorporated into genome scans with both additive and interactive effects of covariates being considered. We also considered a combined covariate with four discrete levels corresponding to the possible combinations of sex and lineage. The genome scans reported here are all based on this combined covariate.

LOD scores for a genome scan with covariates are based on the likelihood ratio of a model that includes the covariate plus the QTL, compared with one that includes only the covariate. The genome scan with additive covariates provides LOD scores contrasting the models,

$$y = \beta_0 + \beta_1x + \beta_2q + \epsilon$$

$$y = \beta_0 + \beta_1x + \epsilon$$

where x represents the covariate, and other variables are as above. These LOD scores are used to assess whether the QTL adds any explanation of the phenotypic variance beyond that explained by the covariate. The scan with additive covariates will detect QTL that affect all classes of covariates in a similar manner, such as a QTL that has the same effect in males and females while taking account of any overall difference in mean phenotype between the sexes.

Genome scans with interacting covariates allow for detection of QTL that have different or even opposite effects in classes of rats defined by the covariate. Analysis of an interacting covariate is carried out in two steps. First, we compare models with and without the joint effect of the QTL and the QTL by covariate interaction term

$$y = \beta_0 + \beta_1x + \beta_2q + \beta_3q^*x + \epsilon$$

$$y = \beta_0 + \beta_1x + \epsilon$$

to obtain the LOD scores for our genome scan. If a locus is found to be significant, we proceed to determine whether the QTL by covariate interaction contributes significantly by comparing models

$$y = \beta_0 + \beta_1x + \beta_2q + \beta_3q^*x + \epsilon$$

$$y = \beta_0 + \beta_1x + \beta_2q + \epsilon$$

This secondary test is carried out without the genome-wide correction for significance that has already been applied to the first test. If both these tests are significant, we can conclude that there is a significant interaction between our covariate and QTL. This sort of comparison allows us to detect a QTL that has distinct effects within different classes of a covariate, such as a QTL that affects only males but not females, or one that affects both males and females but in opposite directions. We note that because the degrees of freedom associated with the joint test (QTL and QTL by covariate interaction) are greater than those for the simple genome scan, the corresponding LOD thresholds will be higher. Additional complications arise in this study because the genome scans are restricted to the X Chr and the covariates are sex and lineage; thus, not all combinations of genotype and covariate can occur. This will alter the degrees of freedom associated with the LOD scores and requires that a permutation test be used to establish significance thresholds (see below).

Pairwise genome scans provide a means to detect and characterize the simultaneous effects of two QTLs (Sen and Churchill 2001). Pairwise genome scans with covariates follow a pattern similar to that described for the single QTL genome scans. With two covariates and two QTLs to consider, there are many possible models on which to base genome scans. The evidence provided by many of these scans is redundant, and we certainly cannot report the details of each one. The scans selected in the results section were deemed most appropriate for testing hypotheses needed to elucidate the genetic architecture of each individual trait.

Significance thresholds for the genome scans were computed by permutation analysis (Churchill and Doerge 1994) with modifications for the pairwise scans (Sen and Churchill 2001, Churchill and Doerge 1994). As we noted above, some combinations of sex, lineage, and X Chr genotypes cannot occur. In order to compute an appropriate threshold value, we must avoid impossible combinations such as heterozygous males in the permuted data. This is achieved by randomizing the genotype-phenotype relationships only within the subgroups defined by sex and lineage. The stratified and permuted data will retain the original covariate-phenotype

associations, but any genotype-phenotype associations will be destroyed.

Lastly, we applied multiple regression analysis to jointly assess the main effects and interactions of several QTLs and covariates in various combinations. Proportion of variance explained by a QTL is reported based on the adjusted (type III) sums of squares from these regression models. The contributions of covariates and QTLs are reported separately. In the case of covariate by QTL interactions, the variance explained by the interaction term is attributed to the QTL. Multiple regression analyses were carried out with pseudomarker software (Sen and Churchill 2001).

All traits were analyzed after taking logarithms [or $\log_e(x+1)$] to reduce skew in distributions. Alternative transformations, such as square root, were considered, but there was no substantial difference in the results obtained. A strong negative correlation between latency and duration ($r = -0.78$, on the logarithmic scale) is noted, hence these traits are discussed together. Complete data files and analysis scripts for the analyses carried out here are available at <http://www.jax.org/staff/churchill/labsite>.

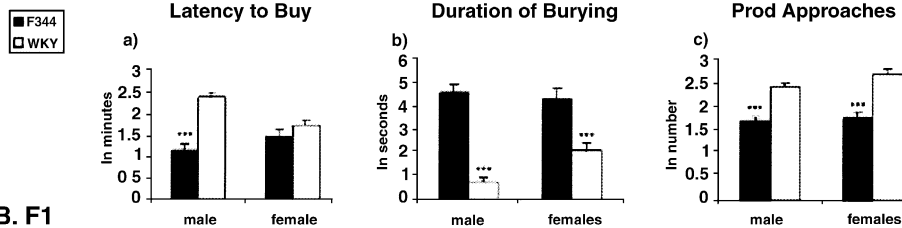
Results

Phenotypic evidence suggesting X-chromosomal pattern of transmission of coping behaviors. In our current study, Wistar Kyoto (WKY) and Fischer 344 (F344) rats served as parental inbred strains and showed significant phenotypic differences in the DB paradigm (Fig. 2A). In general, F344s engaged in a more active coping response to the electrified prod than WKYs, with a shorter latency to begin burying (2Aa), a longer duration of burying (2Ab), and less prod approaches (2Ac) than the WKYs. There were no differences in the number of shocks received between F344s and WKYs.

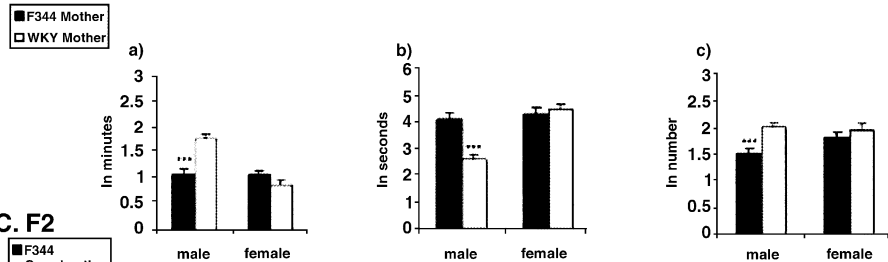
F₁ offspring were then obtained from reciprocal parental crosses (F344 female × WKY male, and WKY female × F344 male). Since inbred strains are assumed to be greater than 98% homozygous at all loci, reciprocal crosses between two inbred strains will produce F₁ offspring with the same complement of genes, heterozygous at all loci where the inbred strains differ (except for the sex chromosome in males, which will be hemizygous—only one copy of X, always from the mother).

When the DB behaviors of the F₁ offspring were assessed for parental influence (Fig. 2B), significant maternal inheritance only by the male F₁ offspring was found. Maternal strain had no impact on the behavior of F₁ females. F₁ males from an F344 mother had a shorter latency to bury (2Ba), longer

A. Parents



B. F1



C. F2

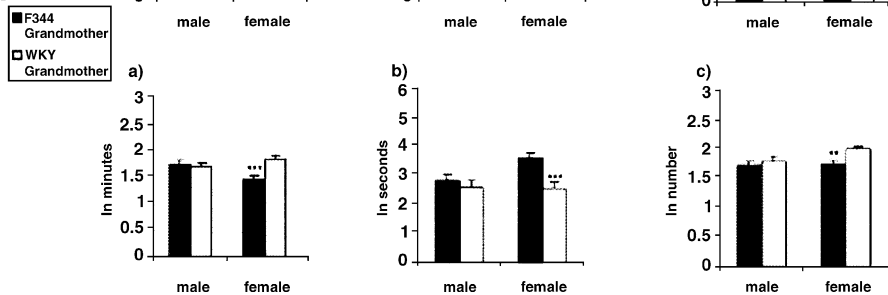


Fig. 2. Phenotypic differences in defensive burying parameters in parental, F₁, and F₂ generations. Bar graphs show means \pm SEM for ln transformed phenotypes of latency (minutes), duration (seconds), and prod approaches (number) in (A) Parental inbred strains, (B) F₁ progeny separated by maternal strain, and (C) F₂ progeny separated by grandmaternal strain. Note that strain differences observed in parental line emerges again in the F₁ male offspring (when separated by maternal strain), but not in F₁ female offspring. In the F₂ generation, there is no difference in any of the phenotypes between F₂ males of different grandmaternal lines, while significant differences exist when F₂ females are separated along grandmaternal lines. *** = $p < 0.001$; ** = $p < 0.01$.

duration of burying (2Bb), and less prod approaches (2Bc) than F₁ males from a WKY mother. The maternally derived differences seen in the F₁ male offspring were similar in nature and direction to the original strain differences observed between parental inbred strains. There was no difference in number of shocks received by both groups.

This maternal inheritance of coping strategies in the DB paradigm by F₁ males could be a result of either maternal environment (pre and postnatal) or genetic factors. If genetic, our findings most closely support an X-linked mode of inheritance, since only the F₁ males showed maternal inheritance of these behavioral traits. Imprinting and mitochondrial inheritance are less likely since, in both cases, male and female F₁ offspring should each be affected (Oakey and Beechey 2002; Alcolado et al. 2002).

To further determine whether this maternal inheritance of coping styles as measured by DB behaviors was X-linked, we analyzed the coping behaviors of the 486 phenotypically and genotypically segregating F₂ animals—representing equal numbers of males and females from each grandma-

ternal strain. If DB behaviors are passed on in an X-linked fashion, we expect our F₂ generation females, but not males, to retain differences in coping styles consistent with their grandmaternal strain. The phenotypes of the F₂ progeny are shown in Fig. 2C. Neither the latency to bury, duration of burying, nor the number of prod approaches differed between the F₂ males derived from F344 grandmothers and the F₂ males derived from WKY grandmothers. In contrast, F₂ females derived from F344 grandmothers had a much shorter latency to begin burying (2Ca) and buried for a far longer duration (2Cb) than F₂ females derived from WKY grandmothers. In addition, F₂ females from F344 grandmothers approached the prod less frequently than F₂ females from WKY grandmothers (2Cc). These phenotypic differences in F₂ females, along with a lack of phenotypic difference in F₂ males by grandmaternal strain, support an X-linked mode of inheritance.

We note that in the F₂ population (Fig. 3) male X Chrs can be recombinant and are maternally derived, with possible X-chromosomal genotypes of FO (F344 hemizygous) or WO (WKY hemizygous) at any locus.

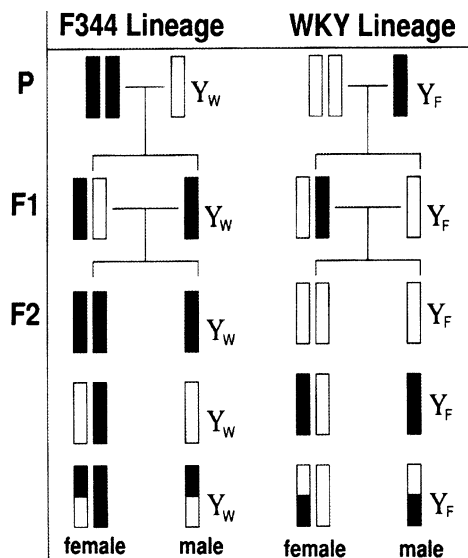


Fig. 3. Schematic showing the pattern of inheritance of the X Chr generated by the reciprocal intercross. Closed bars denote F344 X-chromosomal alleles, open bars denote WKY X-chromosomal alleles, mixed open and closed bars denote recombinant X chromosomes. Y_W and Y_F represent WKY and F344 Y Chrs respectively. 'F344 lineage' and 'WKY lineage' denote the strain of the grandmother in the original parental cross. P = parental generation; F_1 = first-generation offspring of the parental cross; F_2 = second-generation offspring generated by brother-sister mating of F_1 rats. The maternally derived chromosome is always drawn first.

F_2 females with F344 grandmothers can have genotypes FF or WF at any locus. Their paternal X Chrs are non-recombinant and from the F344 lineage, while their maternal X can be recombinant. Females with WKY grandmothers can be WW or FW, their paternal X being of WKY lineage and their maternal X being recombinant. If X-linked patterns of inheritance are associated with the non-recombinant paternal chromosome, this would not be possible to localize in our cross. However, genetic effects mediated by the recombinant maternal X Chr can be localized by genetic analysis. It is important to note that all rats with F344 grandmothers have F344 cytoplasm, and all rats from WKY grandmothers have WKY cytoplasm. Any traits acquired through maternal effect either in utero or postpartum, and stably transmitted intergenerationally, will follow this same cytoplasmic pattern of inheritance. Any traits transmitted through the Y chromosome would appear as differences in males by lineage, but males within the same lineage should not differ.

Quantitative genetic analysis confirms that coping behaviors map to loci on X-Chr. To test the hypothesis that genetic loci on the X Chr contribute significantly to the inheritance of coping strategies,

we conducted a QTL analysis on our F_2 population to determine whether the presence of specific X chromosomal marker alleles—polymorphic between the two inbred strains—co-segregated reliably with traits of latency, duration, and approach in the DB test. In order to study the role that the environment of the grandmother's lineage could have on our trait, we considered grandmaternal line-of-descent or 'lineage', in addition to sex, as covariates in our analysis.

'Latency' and 'duration' genome scans with and without an additive adjustment for sex and grandmother show a significant QTL in the region near *DXRat67* (Fig. 4A, 4B). (Latency: unadjusted LOD (no covariates considered) = 4.96, $p = 1.1 \times 10^{-5}$; adjusted LOD (covariates considered) = 3.10, $p = 7.9 \times 10^{-4}$. Duration: unadjusted LOD = 4.22, $p = 6.0 \times 10^{-5}$; adjusted LOD = 2.14, $p = 0.0072$.) QTLs for both latency and duration phenotypes overlap near *DXRat67*, indicating that at least some aspects of the underlying genetic architecture of these traits are shared. We will call this shared locus *Coping-1*. The shape of the LOD curves in the genome scans suggests the possibility of a second QTL near *DXRat64*, but the pairwise genome scan fails to provide support for a second QTL. There is no evidence for interaction between the QTL and sex or lineage.

A linear model including terms for sex, grandmother, and *DXRat67* explains 6.7% of the variance in log latency, of which 3.5% is attributable to the QTL. The same model explains 6.2% of the variance in log duration, of which 2.4% is attributable to the QTL. Figure 5 shows the allele-effect plots for the effects of *DXRat67* alleles on latency (with reciprocal effects on duration, not shown) at the *Coping-1* locus. Based on these plots, we conclude that there is a QTL near *DXRat67*, *Coping-1*, for which WKY alleles increase latency and decrease duration of burying, a profile similar to that seen in the passive coping response of the progenitor WKY animals in the DB test. Furthermore, the effect of *DXRat67* alleles at the *Coping-1* locus on latency and duration phenotypes appears to be independent of both sex and lineage.

For the prod approach phenotype, the unadjusted (simple) genome scan without consideration for covariate effects (blue line in Fig. 4C) shows a significant QTL effect in the region near *DXRat104* (LOD = 3.43, $p = 3.7 \times 10^{-4}$). However, the genome scan (green line) with sex and lineage as additive covariates shows a significant QTL peak shifted to the region near *DXRat127* (LOD = 3.40, $p = 4.0 \times 10^{-4}$). The genome scan with sex and lineage as interacting covariates has a significant peak near *DXRat104* (LOD = 4.61, $p = 0.0066$) with a second non-significant peak near *DXRat127*. These results suggest that

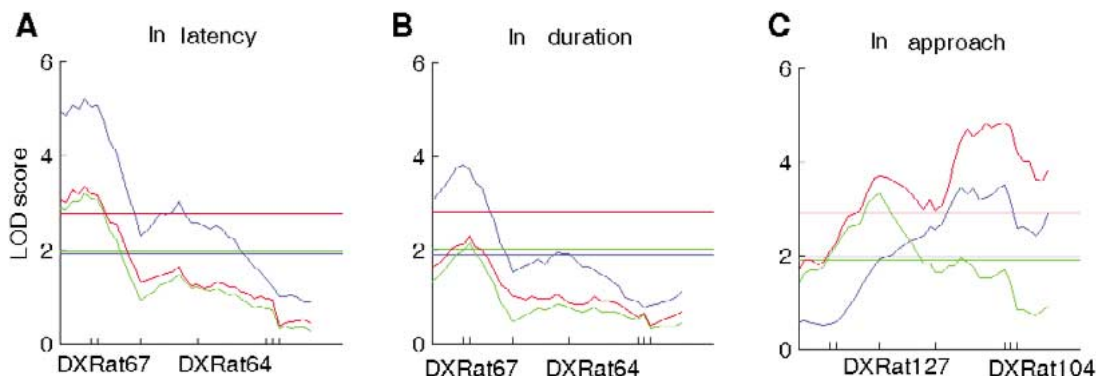


Fig. 4. Main effects genome scans for log transformed traits of A) Latency, B) Duration, and C) Approach. Blue line is standard (unadjusted) LOD score, representative of single QTL scan with no covariates. Green line is LOD score adjusted for additive effects of covariates, sex, and grandmaternal lineage. Red line is LOD score for model with covariates sex and grandmaternal lineage interacting with the QTL. Permutation-derived thresholds are shown as horizontal lines with same color-coding. Note that the interactive scan threshold is substantially higher owing to 4 degrees of freedom (df) in likelihood ratio versus 2 df for the other scans. Tick marks on the x-axis represent each of seven polymorphic markers typed on χ and are drawn to represent the relative map distances they occupy; from left to right (proximal to distal ends of χ) they are *DXRat67*, *DXRat82*, *DXRat64*, *DXRat127*, *DXRat104*, *DXWox30*, *DXWox31*.

there may be two linked QTLs for prod approaches and that their effects may vary according to sex and/or lineage. The pairwise genome scan allows us to determine whether two loci acting together either additively or interactively can explain the F_2 phenotypic variance in approach behavior better than either locus can alone. Figure 6A shows the results of the pairwise genome scan with grandmaternal lineage as an interacting covariate. This scan clearly indicates two QTL with peak locations near *DXRat127* (*Approach-1*) and *DXRat104* (*Approach-2*). The maximum LOD score ($LOD = 9.6$, $p = 1.4 \times 10^{-5}$) exceeds the permutation threshold of 8.0, and the localization of the two QTL is quite sharp. There is no evidence for interaction between the QTLs (indicated by blue color in upper half of Fig. 6A). A test statistic for the hypothesis of one QTL versus two can be computed by taking the difference in

peak LODs between the single- and two-QTL genome scans with grandmother as an interacting covariate. We find that this difference ($LOD_2 - LOD_1 = 3.62$, $p = 0.0022$) is significant, providing evidence in favor of the two-QTL model. From these results, we can conclude that *Approach-1*, near *DXRat127*, and *Approach-2*, near *DXRat104*, are two independent loci on the X Chr influencing approach behavior.

In order to refine the results from the genome scan, we used a regression analysis to fit a model with both QTL, the two covariates, and all pairwise interactions. We then carried out a backward elimination of non-significant terms and arrived at a model which suggests that there is a significant interaction with grandmaternal lineage that involves only the *DXRat104* locus. In order to further characterize the interactions, we stratified the F_2 population into four groups by sex and lineage and

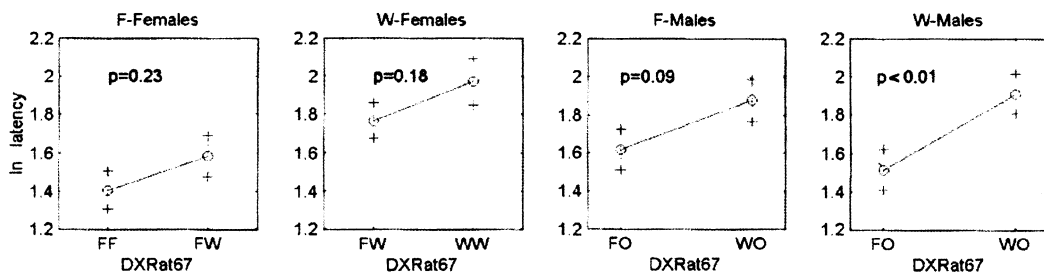


Fig. 5. Allele-effect plot showing the effect of alleles at *DXRat67* (*Coping-1* locus) on latency to bury, by sex and lineage. (The effect of alleles at *DXRat67* on duration of burying is similar but in the opposite direction to that of latency). Means \pm SEM of log latency phenotype in F_2 rats are shown. P -values obtained from two-sample t -test for difference in marker allele classes, with groups defined by sex and grandmaternal lineage. 'W-males' denotes F_2 males from WKY grandmaternal lineage; 'F-males' denotes F_2 males from F344 grandmaternal lineage. WO and FO denote the sex chromosomal genotypes of hemizygous males, respectively, WKY and F344 X-chromosomal alleles at the marker locus. The effect of the F344 allele at the *Coping-1* locus near *DXRat67* is to decrease latency (and increase duration of burying), while the effect of the WKY allele at the same locus is to increase latency (and decrease duration of burying); this effect is in the same direction as that seen for the WKY and F344 parental phenotypes and appears to be independent of sex and lineage.

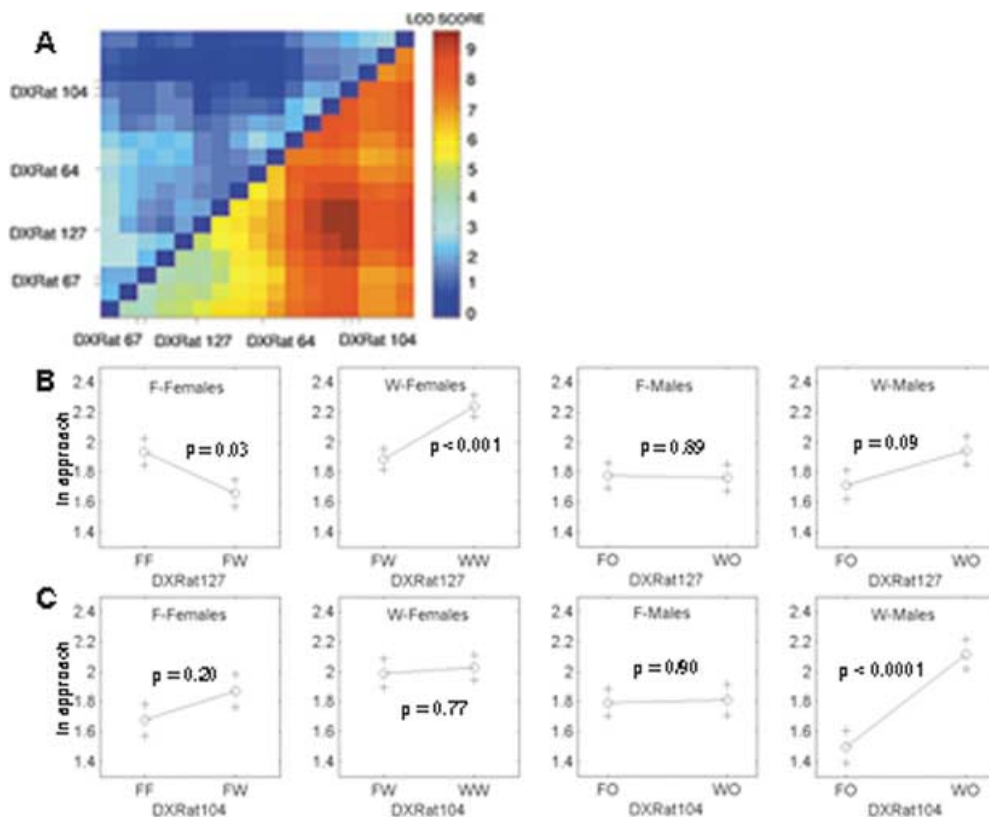


Fig. 6. (A) Pairwise genome scan for trait log approach and allele-effect plots showing effect of alleles at (B) *DXRat127* (*Approach-1*) and (C) *DXRat104* (*Approach-2*) loci on approach behavior by sex and grandmaternal lineage. (A) The pairwise scan detects whether two loci act either additively or interactively to explain the F_2 phenotypic variance in approach behavior, better than either locus can alone. The lower panel of the pairwise scan shows the LOD score for the two QTL model with grandmaternal lineage as an interacting covariate, significant in this scan, and confirming two independent loci contributing to the variance in approach behavior: *Approach-1*, near *DXRat127*, and *Approach-2*, near *DXRat104*. The upper panel shows the contribution to the LOD score owing to QTL \times QTL interaction, negligible in this scan. (B) Allele-effect plots of *Approach-1* at *DXRat127*, analyzed by sex and lineage, shows this locus to be female-specific, with heterozygotes displaying substantially fewer approaches than either homozygote, and the WW genotype predisposing to more approaches than the FF genotype. (C) Allele-effect plots for the *Approach-2* locus confirm the significant interaction of *DXRat104* alleles with grandmaternal lineage in determining approach behaviors. The plots further demonstrate that *Approach-2* is a sex-specific and lineage-specific locus, seen only in males from WKY lineage (W-males), far right.

computed t -tests for allelic effects of the marker loci within each group (p -values shown in panels 6B, 6C). From this analysis, it appears that *Approach-1*, near *DXRat127*, is a sex-specific QTL affecting only females, with heterozygosity being associated with fewer prod approaches than either homozygote, and the WW genotype predisposing to a greater number of approaches than the FF genotype (Fig. 6B). We also find that *Approach-2*, near *DXRat104*, is both sex and lineage specific, affecting only males from a WKY grandmaternal lineage (W-males) (Fig. 6C). We prefer the latter description of the QTL effects in this case. Although less formal than a linear regression model derived by backward elimination, it provides a more accurate picture of what is actually happening with respect to the QTL effects in each individual combination of sex and lineage.

Lineage significantly modulates allelic effects at Approach 2. An interesting pattern emerges when we look more closely at the nature of this interaction between grandmaternal lineage and the *Approach-2* locus near *DXRat104* in the F_2 males (Fig. 6C, right). Here we find that F_2 males with WKY alleles derived from a WKY grandmaternal lineage behave very much like parental WKY animals, with large numbers of prod approaches. Likewise, F_2 males with F344 alleles derived from a F344 grandmaternal lineage retain the behavioral pattern of F344 animals. We further find, however, that the F344 allele is a protective feature in a WKY lineage, with FO animals from WKY grandmaternal lineage retaining features of F344s. In addition, we find that the influence of the F344 lineage overrides that of the WKY allele, allowing WO animals from F344

grandmaternal lineage to be identical with FO animals from F344 grandmaternal lineage. Here, the interaction of allelic with non-allelic variables, passed on intergenerationally through lineage, more fully describes certain aspects of the trait of coping than analysis of allelic factors alone could provide.

Discussion

By studying inbred rat strains and their F₁ and F₂ progeny, we have shown convergent evidence, both genotypically and phenotypically, that differences in coping styles as measured by DB behaviors are inherited in an X-linked fashion. Genetic analysis of the segregating X Chr of the F₂ population reveals three quantitative trait loci (QTL) that are associated with coping styles: *Coping-1*, on the proximal end of the X Chr related to behaviors of latency and duration, and *Approach-1* and *Approach-2* on the distal end of the X Chr, related to prod approaches.

Furthermore, our study illustrates the critical role of lineage and sex in the genetic analysis of X-linked traits in particular, and of complex traits in general. In addition, our statistical analysis illustrates the power of a general linear modeling approach to QTL analysis. Standard interval mapping analysis, which generates unadjusted LOD scores that simply compare the hypothesis of QTL presence with that of no QTL presence, failed to account for the complexity of the genetic effects and their interplay with the factors sex and lineage. By incorporating covariates of sex and lineage into the models used for hypothesis testing and QTL detection, however, we were able to more fully explain the phenotypic variance or our F₂ population. In addition, pairwise genome scans (taking into account sex and lineage), multiple regression modeling, and the stratified analysis of allele-effects by sex and lineage, allowed us to gain a closer look at the underlying genetic architecture of different aspects of coping behaviors, demonstrating the range of simple (*Coping-1*, latency and duration) to more complex (*Approach-1*, *Approach-2*), sex-specific, and lineage-specific effects and inheritance patterns.

Since coping is a multigenic trait, we expect some autosomal loci are also involved in the manifestation of different coping styles, either independently or through interactions with the X-chromosomal loci. However, the fact that we were able to see evidence of X-linkage even on a phenotypic level, through differences in coping responses of F₁ and F₂ animals when separated by sex and lineage, illustrates the actual relevance of the X Chr to the inheritance of this trait.

In complex traits, genes alone do not always lead to the expression of phenotype. Environment can alter the manifestation of traits. This complexity was demonstrated in part by our *Approach-1* locus (near *DXRat127*), which was female specific, but probably best demonstrated by our *Approach-2* locus (near *DXRat104*), which was both male-specific and dependent on grandmaternal lineage. Indeed, for this aspect of coping, both the WKY allele and the WKY grandmaternal lineage are necessary for an F₂ male to manifest approach behaviors typical of its WKY progenitor. In contrast, either F344 allele or lineage is sufficient for heritability of F344-like behavior by an F₂ male. Our current study was not designed to decipher the exact mechanism by which "lineage" is influencing genotype in the manifestation of coping behaviors, but any cytoplasmic factors, prenatal or postnatal maternal environmental influences, or other epigenetic events that are stably transmitted across generations are candidates for such modulating effect.

It is not surprising that lineage is an important determinant of coping styles in our segregating population of rats. Since coping strategies are adaptive responses to threats to one's homeostasis, it makes evolutionary sense that the coping strategies necessary for an offspring's survival in the wild be modulated by allelic as well as non-allelic means, especially those linked to the more stable maternal influences. This gene-lineage interaction might ensure more rapid adaptation in the face of changes to the external environment than standard Darwinian evolutionary theory would suggest, and could conceivably be one way nature ensures that the processes of adaptation and flexibility are stably and appropriately inherited.

Acknowledgments

We thank Amber E. Baum for assistance with DNA isolation, and Hoda Mahmoudi for assistance with initial phenotype characterization and DNA isolation. Photographs used in Fig. 1 were kindly provided by William P. Pare. This study was supported by NIH grant MH60789. J.S. Takahashi is an Investigator in the Howard Hughes Medical Institute.

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