

A Common Polygenic Basis for Quinine and PROP Avoidance in Mice

David B. Harder and Glayde Whitney

Department of Psychology, Florida State University, Tallahassee, FL 32306-1270, USA

Correspondence to be sent to: David B. Harder, Department of Psychology, Florida State University, Tallahassee, FL 32306-1270, USA

Abstract

Inbred strains of mice (*Mus musculus*) differ greatly in ability to taste various bitter compounds. For some compounds, the differences result from allelic variation at a single locus. However, segregation patterns incompatible with monogenic inheritance have been found for quinine avoidance. The *Soa* bitter sensitivity locus exerts some influence on this phenotype, but an unknown number of other loci also contribute. Relative avoidance patterns for quinine sulfate in panels of naive inbred strains resembled avoidance patterns for 6-*n*-propyl-2-thiouracil (PROP), suggesting a common genetic basis. In particular, C57BL/6J mice strongly avoided both 0.1 mM quinine sulfate and 1 mM PROP in two-bottle preference tests, whereas C3H/HeJ mice were indifferent to both. Therefore, 12 BXH/Ty recombinant inbred strains, derived from these strains, were tested with both solutions to begin identification of the unknown bitter loci. Naive mice were tested for four consecutive days with each compound (order counterbalanced). Some BXH/Ty strain means resembled those of the parent strains, but others were intermediate. This indicated recombination among loci affecting avoidance, and therefore polygenic inheritance. The strain means were highly correlated across compounds ($r = 0.98$), suggesting that the same polygenes controlled both phenotypes. The BXH/Ty means for both compounds were then compared with the strain genotypes at 212 chromosome position markers distributed throughout the genome. Eight markers on five chromosomes (3, 6, 7, 8 and 9) yielded significant correlations. Six of the markers were correlated with both phenotypes, again suggesting common polygenic inheritance. The marker with the highest correlation was *Prp*, tightly linked to *Soa* on chromosome 6. The correlated marker regions likely contain quantitative trait loci affecting bitter avoidance. The phenotypic similarity of PROP to quinine, rather than to phenylthiourea, apparently stemming from a common polygenic basis, indicates a difference between mice and humans in gustatory organization related to bitters.

Introduction

Among inbred strains of mice (*Mus musculus*), avoidance of quinine sulfate in two-bottle tests correlates with avoidance of 6-*n*-propyl-2-thiouracil (PROP), though neither phenotype correlates with avoidance of phenylthiourea (PTC) (Harder *et al.*, 1996). In humans, PROP and PTC bitter sensitivities are highly correlated, and PROP is often used as a substitute for PTC in taste tests (e.g. Lawless, 1980). Human sensitivity differences for both compounds are usually attributed to pleiotropic effects of monogenic variation. Quinine sensitivity in humans does not display the monogenic segregation patterns and bimodal distributions characteristic of PTC/PROP sensitivity (Fischer and Griffin, 1964). Monogenic control of PTC avoidance in mice has been reported (Klein and DeFries, 1970), though not confirmed (Lush, 1986b). The *Soa* bitter sensitivity locus has been shown to affect PROP and quinine avoidance, but does not affect PTC avoidance (Boughter *et al.*, 1992; Whitney and Harder, 1994; Boughter and Whitney, 1997). Altogether, the intercorrelation patterns and genetic data suggested a species difference between mice and humans in gustatory organization related to bitterness.

Both monogenic and polygenic control of quinine

avoidance/consumption in mice have been suggested. A major single-gene influence has been evident in most studies, and some inbred strains may differ at only one locus affecting quinine sensitivity (Lush, 1984). However, patterns incompatible with monogenic control of quinine sensitivity have been found in several segregating generations (Lush, 1984; Boughter *et al.*, 1992; Bachmanov *et al.*, 1996), among congenic strains (Boughter and Whitney, 1997) and, despite the monogenic interpretation offered, among recombinant inbred (RI) strains (Lush, 1986a). Only one of the genes involved in quinine avoidance has been localized. Called both *Soa* and *Qui*, this gene is on distal chromosome 6, very near *Prp* (proline-rich protein locus) at 63.0 cM (Azen *et al.*, 1986, 1989; Capeless *et al.*, 1992; Lush *et al.*, 1995; Blizard *et al.*, 1996). The number and identities of the other genes are undetermined. [Quantitative trait loci (QTL) affecting quinine consumption difference among BXD/Ty RI strains have been reported (Gora-Maslak *et al.*, 1991). However, the solution used was actually a quinine-saccharin mixture, so the relevance of these QTL to bitter sensitivity is unclear (see also Results below).]

Little is known about the genetic basis for PROP

sensitivity differences in mice. Avoidance differences among congenic strains were affected by the locus on distal chromosome 6 (*Soa*) and by an unknown number of background loci (Boughter and Whitney, 1997). No PROP segregation studies in mice have been reported.

In the present study, the BXH/Ty set of RI strains was tested with both quinine sulfate and PROP to begin identification of other loci affecting bitter sensitivity, and to assess the degree of commonality between the genetic bases for avoidance of the two compounds. The BXH/Ty set was derived from the C57BL/6J (B6) and C3H/HeJ (He) inbred strains. These strains displayed contrasting avoidance phenotypes to both compounds in pilot tests (strong avoidance and relative indifference respectively). They were also known to carry the *Soa^b* (nontaster) and *Soa^c* (demitaster) alleles respectively (Harder *et al.*, 1992). Polygenic control of quinine avoidance had been found in serial backcross generations derived from B6 and C3HeB/FeJ inbred mice (Boughter *et al.*, 1992). The He strain is closely related to the latter strain, so multiple genetic differences affecting quinine avoidance appeared likely in the BXH/Ty set.

RI strains embody homozygous reshufflings of the genomes of their two progenitor inbred strains. The phenotypic pattern produced by an RI set can be compared with its genotypic pattern for chromosome position markers (both named loci and anonymous DNA sequences) to locate genes (QTL) contributing to the phenotypic variation (Gora-Maslak *et al.*, 1991; Plomin *et al.*, 1991; Belknap *et al.*, 1996). An initial genome-wide search can be done with phenotypic testing alone. RI strains have fixed genotypes, so genotypic patterns for the set can be accumulated over time and across laboratories. These patterns are available via the Internet. QTL indications in the genome-wide search can then be confirmed (or disconfirmed) in a targeted search in a segregating generation (each individual of which must be genotyped at every marker used). The quinine and PROP means for the BXH/Ty strains were, therefore, compared with their genotypes at markers distributed throughout the genome to locate bitter avoidance QTL.

Materials and methods

The mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and had been kept in our laboratory for at least 2 weeks before testing began. They were 37–239 days old at the start of testing, with considerable age variation within some strains. Naive mice from 12 BXH/Ty strains (five males and five females per strain, except four males and four females in strain #2) plus the B6 and He progenitor inbred strains (five males and five females per strain per run) were given two consecutive 48 h, two-bottle preference tests with 0.1 mM quinine sulfate and two similar tests with 1 mM PROP. The mice were tested in three separate runs, each consisting of four RI strains plus B6 and He mice. In each run, half the mice of each strain were tested with

quinine first, the other half with PROP first. The mice had 72 h with water only between compounds. The quinine sulfate and PROP (Sigma Chemical Company, St Louis, MO) were dissolved in deionized water. All fluids were presented to the mice at room temperature (~22°C).

The mice were tested individually, in suspended stainless-steel cages with wire-mesh fronts and floors, in a temperature-controlled room on a 14 h light:10 h dark cycle. Purina Mouse Diet #5015 was available *ad libitum* from hoppers at the rear of the cages. Two inverted 25 ml graduated cylinders, with neoprene stoppers and curved stainless-steel spouts, were clipped to the front of each cage during the tests. The spouts protruded through the wire-mesh ~2 cm above the floor and 6 cm apart. One cylinder contained tastant solution, the other contained deionized water. The fluid level in each cylinder was recorded at the start of each test, and again after 24 h. The cylinder positions were then exchanged to control for any side biases, and the levels recorded once more. The final fluid levels were recorded 24 h later. Fresh fluids, cylinders and spouts were used for the second 48 h test with each tastant. A preference ratio (= solution consumption/total fluid consumption) was calculated for each mouse for each 24 h period. The preference ratio for a given 48 h test was the mean of its two 24 h ratios. The overall preference ratio for a tastant was the mean of the two 48 h test ratios.

Relevant QTLs were searched for by calculating point-biserial correlations (Pearson product-moment correlations with one variable coded as 0 and 1) between the avoidance means for the 12 RI strains and their genotypes at 212 chromosome position markers distributed across the genome (average marker spacing = 7 cM). The marker positions and strain genotypes were obtained from the Mouse Genome Database, via The Jackson Laboratory homepage (<http://www.jax.org>). The markers were chosen from >500 named loci and anonymous DNA sequences (SSLP's) genotyped in the BXH/Ty set. Named loci were given preference where possible and markers with incomplete data were excluded. Adjacent markers were spaced at least 2 cM apart. *P*-values < 0.01 were required for significant correlations. This α -level has been recommended for initial genome-wide RI searches when confirmatory targeted searches are anticipated (Lander and Kruglyak, 1995; Belknap *et al.*, 1996). It protects against false-negatives while still excluding most false-positives (any remaining are eliminated in the targeted search). Its use generally results in a subsequent targeted search of ~10% of the total genome. Significant correlations found in the genome-wide search should be considered as only suggestive evidence for QTL until confirmed.

Results

The parental strains displayed contrasting phenotypes as expected (see Figure 1). The B6 mice strongly avoided both

compounds while the He mice were relatively indifferent to both. The BXH/Ty means ranged from avoidance through indifference for both compounds. Preliminary STRAIN $\times$ TEST-ORDER $\times$ SEX analyses of variance of the BXH/Ty preference ratios showed no significant main or interaction effects involving test order or sex for either compound (all $P > 0.05$). Significant strain differences were found for each compound in single factor analyses of variance [for quinine, $F(13,163) = 20.65$; for PROP, $F(13,163) = 21.76$, both $P < 0.0001$]. The BXH/Ty means were not all closely grouped near the parental means (as would be expected of a

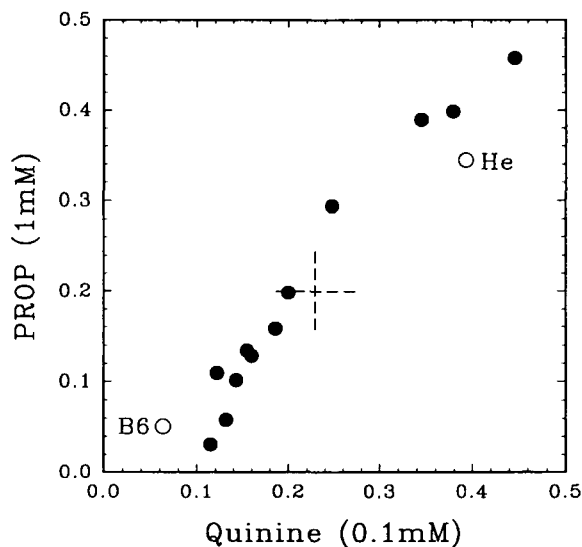


Figure 1 Mean preference ratios for quinine sulfate versus PROP in 12 BXH/Ty recombinant inbred strains and their B6 and He progenitor strains. The crossed dashed lines indicate midparent values.

monogenic phenotype) for either compound. Instead, some appeared to be intermediate, including some very near the midparent values. Newman–Keuls multiple comparisons confirmed the intermediate status of at least one RI strain for each compound (see Table 1). Two homogeneous subsets ($P \geq 0.05$), each containing one of the parental strains, could not encompass all of the RI strains. At least three subsets were required for each compound. Intermediate RI strains are inconsistent with monogenic inheritance, and thus indicate polygenic variation. Intermediate strains do not rule out major gene effects, however, and such an effect appeared to be reflected in the modest bimodality of the BXH/Ty mean distributions. The BXH/Ty means were highly correlated across compounds (Pearson $r = 0.98$, $P < 0.001$), suggesting a common polygenic basis for differences in both phenotypes.

The BXH/Ty quinine distribution strongly resembled the quinine distribution reported for 14 BXD/Ty RI strains (Lush, 1986a) (see Figure 2). The latter distribution, however, was interpreted (without analysis) as truly dichotomous, not just bimodal. Even strains with means near the midparent value were assigned parental phenotypes, and a monogenic strain distribution pattern (SDP) was reported. With seven more BXD/Ty strains added (means unpublished), the quinine SDP was compared with SDPs for other bitter compounds (e.g. RUA) and for *Prp* (Lush and Holland, 1988; Azen, 1991). One strain (#15) was stated to have a discordant quinine phenotype (mean unpublished). This discordancy was the entire basis for mapping a separate *Qui* locus 0.9 cM from *Prp*. The B6 and D2 inbred strains, like the B6 and He strains, have different *Soa* alleles (nontaster and demitaster). The BXD/Ty and BXH/Ty quinine distributions both appear to reflect the

Table 1 Newman–Keuls multiple comparisons of mean quinine sulfate and PROP preference ratios in 12 BXH/Ty recombinant inbred strains and their C57BL/6J and C3H/HeJ progenitor strains

Quinine				PROP			
Strain	Mean	SE	N–K ^a	Strain	Mean	SE	N–K ^a
BXH-11	0.446	0.025		BXH-11	0.458	0.032	
He	0.393	0.024		BXH-14	0.398	0.047	
BXH-14	0.379	0.035		BXH-6	0.389	0.052	
BXH-6	0.345	0.045		He	0.344	0.023	
BXH-8	0.248	0.040		BXH-8	0.294	0.055	
BXH-7	0.200	0.045		BXH-7	0.198	0.032	
BXH-12	0.186	0.033		BXH-12	0.158	0.040	
BXH-9	0.160	0.026		BXH-2	0.134	0.050	
BXH-2	0.155	0.030		BXH-9	0.128	0.030	
BXH-10	0.143	0.035		BXH-3	0.109	0.036	
BXH-4	0.132	0.035		BXH-10	0.101	0.042	
BXH-3	0.122	0.021		BXH-4	0.058	0.017	
BXH-19	0.115	0.044		B6	0.051	0.007	
B6	0.064	0.007		BXH-19	0.031	0.004	

^aHomogeneous subsets not different ($P \geq 0.05$) in Newman–Keuls multiple comparisons.

influence of the *Soa* locus, plus effects of other unlinked loci.

In the QTL search, a total of eight markers on five chromosomes generated significant correlations (see Table 2). Only two would be expected by chance from 212 markers at a 0.01 α -level. Quinine and PROP avoidance were both correlated with six of the markers. For the remaining two, *Tnfr* and *Fgf3*, one of the correlations did not quite reach significance. The *Prp* locus, at 63 cM on chromosome 6, yielded the highest correlation. That this marker, closely linked to a gene known to influence bitter avoidance (*Soa*), was detected tended to validate the QTL search procedure. None of the other marker loci had any obvious connection to bitter sensitivity. *Ea10* at 49.5 cM on chromosome 6 was correlated with avoidance of both bitters. It may indicate a

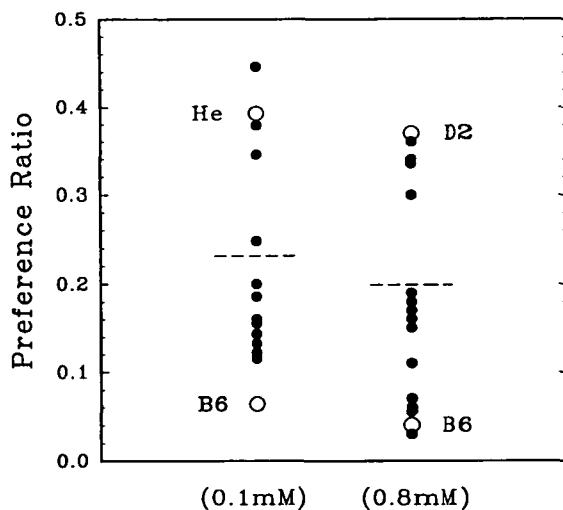


Figure 2 Mean preference ratios for quinine sulfate in 12 BXH/Ty recombinant inbred strains and their B6 and He progenitor strains, compared with means in 14 BXD/Ty recombinant inbred strains and their B6 and D2 progenitor strains (replotted from Lush, 1986a). The dashed lines indicate midparent values.

second, more proximal, QTL on this chromosome. Only one QTL each was indicated for chromosomes 3, 7 and 8. Two SSLP markers 6 cM apart on chromosome 9 were correlated with both phenotypes. An intermediate SSLP marker, *D9Nds1* at 46.0 cM, was not correlated with either phenotype ($P > 0.05$). Two linked QTL are therefore possible in this region.

The correlations for markers on chromosomes 3, 6 and 7 were positive, while those on chromosomes 8 and 9 were negative. For the latter markers, the B6 alleles (coded as 0) were associated with higher phenotypic means and the He alleles (coded as 1) with lower means. A mixture of sensitivity-increasing and sensitivity-decreasing alleles would be compatible with intermediate quinine sensitivity in the B6 strain (cf. Lush, 1984). The stronger avoidance of both bitters by B6 mice than by He mice suggested that the QTL on chromosomes 3, 6 and 7 accounted for most of the phenotypic variance.

The quinine-saccharin consumption QTL marker on proximal chromosome 6 (*Met*), reported in Gora-Maslak *et al.* (1991), was used in the present study, but was not correlated with avoidance of either quinine or PROP (both $P > 0.05$). The four quinine-saccharin QTL markers on chromosome 1 from the same report were not used, but nearby markers were not correlated with either phenotype (all $P > 0.05$). A 0.05 α -level was used in Gora-Maslak *et al.* (1991). The number of markers correlated with quinine-saccharin consumption at that level did not exceed the number expected by chance (from 163 total markers). Given the non-confirmation in the present study, these appear to have been false-positive QTL indications.

Discussion

The present report adds to the evidence for a quinine sensitivity gene very near *Prp*. Its effect on quinine avoidance/consumption has now been shown in three different types of populations; RI strains (Azen *et al.*, 1986; present study), segregating generations (Boughter *et al.*,

Table 2 Chromosome position markers showing significant ($P < 0.01$) pointbiserial correlations with mean quinine sulfate and PROP preference ratios in 12 BXH/Ty recombinant inbred strains

Marker			Quinine		PROP	
Chromosome	Position ^a	Symbol	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
3	72.0	<i>Mpmv9</i>	0.817	0.0012	0.724	0.0077
6	49.5	<i>Ea10</i>	0.715	0.0049	0.748	0.0051
6	57.0	<i>Tnfr</i>	(0.679)	(0.0151)	0.729	0.0072
6	63.0	<i>Prp</i>	0.799	0.0018	0.823	0.0009
7	72.4	<i>Fgf3</i>	0.719	0.0084	(0.676)	(0.0158)
8	32.0	<i>Cpe</i>	-0.748	0.0051	-0.796	0.0019
9	42.0	<i>D9Mit8</i>	-0.729	0.0072	-0.756	0.0045
9	48.0	<i>D9Mit9</i>	-0.751	0.0049	-0.748	0.0051

^aCentimorgan distance from centromere.

1992, Blizard *et al.*, 1996), and congenic strains (Boughter and Whitney, 1997). Its existence appears firmly established, although its exact identity is uncertain. It has been called both *Soa* and *Qui*, but as discussed above, *Qui*'s separate map position is questionable (and *Soa* has nominal priority). Of greater interest is whether the gene is identical to, or just very near, *Prp*. No recombination has been seen between *Prp* and *Soa* in any population. A definitive answer awaits further study, perhaps using *Prp* knockout or transgenic mice.

The present study also adds to the evidence for polygenic control of quinine avoidance in mice, and provides the first evidence for the number and locations of quinine avoidance genes not linked to *Prp*. Many genes near the QTL markers on chromosomes 3, 7, 8 and 9 have the potential to affect gustatory phenotypes (e.g. genes coding for ion channel proteins or neurotransmitter receptor proteins). However, confirmation of each QTL, and better position resolution for those confirmed, are needed before any of the linked genes are seriously considered as candidate genes. Nor are genes closest to the markers necessarily the most likely candidates. Point correlations do not distinguish between QTL with small effects near the marker and QTL with larger effects farther away. It is also possible that no known genes are involved. Polygenic control of PROP avoidance was demonstrated as well. PROP and quinine avoidance were found to be highly correlated in this population, with most of the QTL affecting both phenotypes. A species difference between mice and humans in the genetic basis for PROP sensitivity was thus indicated.

Avoidance in long-term behavioral tests is not, of course, a direct measure of gustatory sensitivity. There is evidence that the gene on distal chromosome 6 acts via peripheral gustatory mechanisms. However, the other quinine/PROP QTL may affect avoidance via other means. Olfactory, post-ingestive and motivational differences could all influence avoidance (though no such effects have been demonstrated for these compounds in mice). Quinine and PROP are virtually nontoxic at the concentrations used, and have little if any odor (to humans). Strain differences in avoidance of both compounds are stable across 10 days of two-bottle testing (Harder *et al.*, 1996). In contrast, PTC is considerably more toxic to mice, has a much stronger odor and, perhaps as a consequence, generates quite labile avoidance differences (Lush, 1986b; Harder *et al.*, 1996).

A cluster of bitter-aversion loci (*Qui*, *Cyx*, *Rua* and *Glb*) linked to *Prp* on chromosome 6 has been hypothesized based on similar, but not completely concordant, RI strain SDPs from two-bottle tests (Lush, 1989). The questionable nature of the dichotomous SDP reported for quinine avoidance in the BXD/Ty set has already been discussed. The *Qui* locus based on this SDP appears unjustified. The BXD/Ty SDP reported for cycloheximide avoidance is even more problematic. Identified strain means have not been published, but the strain distribution included decidedly

intermediate means (Lush and Holland, 1988). Furthermore, the parental strains did not differ, seemingly precluding division of the RI strain means into two parental phenotype classes. A dichotomous SDP was nevertheless reported. The *Cyx* locus is based solely on this arbitrary SDP.

The BXD/Ty SDP for raffinose undecaacetate (RUA) avoidance does appear to be dichotomous, but unlike the others is completely concordant with *Prp*. In other RI sets, SOA-avoidance SDPs are also completely concordant with *Prp* (Capeless *et al.*, 1992). *Rua* was proposed to account for avoidance of high RUA concentrations by a few strains considered to be SOA-nontasters (Lush, 1986a). The apparent discrepancy was resolved when these strains were later found to be SOA-demitasters (Harder *et al.*, 1992). A second acetylated sugar avoidance locus (*Rua*) at the position of *Soa* would be redundant.

The position of the final member of the cluster, *Glb* (copper glycinate aversion), rests on a slender foundation. The SDP for this compound was as dichotomous as that for RUA, and one discordant strain was noted (Lush and Holland, 1988; Lush *et al.*, 1995). One recombinant individual in a backcross generation was also found. Additional evidence of recombination between copper glycinate avoidance and RUA (or SOA) avoidance, especially in segregating generations, would be desirable. Also desirable would be more detailed characterization of the taste of this adventitious gustatory stimulus. In summary, despite inclusion in published linkage maps, there is no convincing evidence for at least three of the four bitter-avoidance loci in the postulated cluster on distal chromosome 6.

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