Analysis of Quantitative Trait Locus Effects on the Size and Shape of Mandibular Molars in Mice

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ABSTRACT

While >50 genes have been found to influence the development of teeth in mice, we still know very little about the genetic basis for the adaptive characteristics of teeth, such as size and shape. We applied interval mapping procedures to Procrustes size and shape data obtained from 10 morphological landmarks on the mandibular molar row of the F_2 progeny from a cross between the LG/J and SM/J strains of mice. This revealed many more QTL for molar shape (18) than for molar centroid size (3), although levels of dominance effects were comparable among QTL for size and shape. Comparisons of patterns of Procrustes additive and dominance shape effects and ordination of QTL effects by principal components analysis suggested that the effects of the shape QTL were dispersed among the three molars and thus that none of these molars represents a genetically distinct developmental structure. The results of an analysis of co-occurrence of QTL for molar shape, mandible shape, and cranial dimensions in these mice suggested that many of the QTL for molar shape may be the same as those affecting these other sets of characters, although in some cases this could be due to effects of closely linked genes.

MAMMALIAN teeth represent structures of considerable taxonomic, anthropological, and evolutionary significance (Vernon 1995; Suwa *et al.* 1996; Carrasco 2000; Schwartz 2000; Stafford and Szalay 2000), and therefore it is not surprising that they have been the focus of a number of genetic studies (Bleicher *et al.* 1999). In recent years especially, developmental geneticists have discovered a number of genes that regulate specific processes leading to the formation of teeth (Cho and Garant 1996; Aberg *et al.* 1997; Thesleff and Jernvall 1997; Bei and Maas 1998; D'Souza *et al.* 1999; Yamazaki *et al.* 1999). Mutations at these loci can cause rather drastic effects, such as loss of certain teeth (Johnson *et al.* 1992; Thomas *et al.* 1997) or gross misalignment of the teeth and deformation of the jaw (Fantl *et al.* 1995).

Although such studies have been useful in adding to our understanding of tooth development, they tell us little about the genetics of specific measures on teeth (such as their size and shape) that tend to be of greater interest especially to evolutionary biologists. Some early quantitative genetical studies did make use of various dimensions in mouse teeth such as mandibular molar widths, and these studies showed that the heritability for these characters, as well as the genetic correlations among them, are moderate to high in magnitude, although more so for the first two molars than the third molar (Bader 1965a,b; Bader and Lehmann 1965; Leamy and Bader 1968; Leamy and Touchberry 1974). This suggests that there may be abundant genetic variability for various tooth dimensions, that genes producing this variability may often have pleiotropic effects among these dimensions, and that the third molar may be at least partially genetically independent from the other two molars, but such studies cannot take us any further than these generalizations.

Fortunately, interval mapping techniques (Thoday 1961; Lander and Botstein 1989) now are available that enable us to locate and assess specific quantitative trait loci (QTL) affecting characters of interest. QTL studies have been successfully applied to various dimensions in mouse mandibles (Cheverud *et al.* 1997; Leamy *et al.* 1997) and skulls (Leamy *et al.* 1999). Recently, Klingenberg *et al.* (2001) used QTL mapping to analyze the entire geometric configuration of a set of landmark points. Using a Procrustes geometric approach with five landmark points in mandibles of mice, they were able to identify a number of QTL for overall (centroid) size and even more QTL for shape in these mandibles, with dominance effects being relatively more important for the QTL influencing shape. Further, they showed that the variation of shape effects among these QTL was continuous, with no evidence for distinct groups of QTL that had similar effects on mandible shape (Klingenberg *et al.* 2001). This suggested that the effects of these genes were not restricted to the developmentally distinct ascending ramus and alveolar

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regions of the mandible, as had been found previously by Cheverud *et al.* (1997) in their analysis of interlandmark distances.

In the study reported in this article, we searched for QTL affecting size and shape of the mandibular molar row of the mice used by Klingenberg *et al.* (2001). We wanted to know if, as was found by Klingenberg *et al.* (2001) for the mandible, we would find more QTL for tooth shape than for size and whether the QTL for tooth shape also would exhibit more dominance. Our primary interest, however, was in discovering whether the effects of the QTL for tooth row shape would be localized in one or two of the three molars that comprise the tooth row. According to the concept of morphological integration (Olson and Miller 1958), such clustering of QTL effects might be expected if any of these molars represents a developmentally distinct unit. If the effects of QTL influencing tooth row shape were not restricted to one or two molars, however, we also were interested to know whether these QTL influenced characters other than the tooth row. We therefore made use of available mandible and skull QTL data in these mice to test whether any of the QTL found for tooth shape might co-occur with those influencing these other sets of characters.

MATERIALS AND METHODS

The population and variables: The study made use of the F_2 progeny from a cross between the Large (LG/J) and Small (SM/J) inbred strains that originally had been selected for large and small body size and subsequently inbred upon receipt at the Jackson Laboratory. Previous investigations have shown that the mean 60-day body weights are 37.4 g (LG/J) and 13.6 g (SM/J) for these strains of mice (Goodale 1941; MacArthur 1944; Chai 1956a,b). Single-pair matings of Large females by Small males produced 41 F_1 hybrids that were single-pair mated and eventually produced a total of 535 F_2 mice. After 21 days of age, all F_2 litters were weaned and sexes were caged separately. All F_2 mice were sacrificed at 70 days of age, their spleens were removed, and their skeletons were prepared by exposure to dermestid beetles.

DNA was extracted from the spleens of mice in the F_2 generation, and a total of 76 polymorphic microsatellite loci were scored in all 535 F_2 mice following a protocol that has been previously described (Routman and Cheverud 1995). Although these 76 loci adequately covered all 19 autosomes (see Figure 1), the X chromosome was not included because of its low incidence of polymorphic microsatellite loci (Routman and Cheverud 1995). In addition, some loci could not be well resolved on the gels, so the loci varied in their total sample sizes (Cheverud *et al.* 1996). The positions of the 76 microsatellite loci based on recombination percentages derived from the MAPMAKER 3.0b program (Lander *et al.* 1987; Lincoln *et al.* 1992) have previously been given (Cheverud *et al.* 1996; Leamy *et al.* 1997). These 76 loci defined a total of 1500 cM of map distance and included 55 intervals between loci with an average interval length of 27.5 cM.

Both left and right sides of the mandible in each mouse were separated at the mandibular symphysis and coordinates of 10 landmarks on each mandibular molar row (see Figure 2) were measured. These points were chosen to ensure some representation for the first (M_1), second (M_2), and third molar (M_3), which comprise the molar row, and because they appeared to be the most repeatable in early measurement trials (see below for an assessment of measurement error). This procedure was repeated twice for the teeth on each side of the mandible, creating a set of four replicate measures for each of the F_2 progeny. Altogether, 502 mice (254 males, 242 females) measured in this manner were available for the analysis.

Morphometric analyses: Individual variation in tooth shape was analyzed using an adaptation of the Procrustes superimposition technique that has been previously described by Klingenberg and McIntyre (1998). This procedure starts with a set of *x*, *y* coordinates; eliminates the effects of size, location, orientation, and reflection; and produces a new set of coordinates that retains all remaining aspects of the original geometric configuration. This was accomplished by the following: (a) changing the sign of the *x* coordinates for both replicates of the left molar row for each mouse (creating its mirror image); (b) scaling all four replicates for each mouse to the mean of their respective centroid sizes (this is the standard measure of size for geometric morphometrics and is defined by the square root of the sum of the squared distances between each landmark and the mean *x* and *y* values for the entire configuration); (c) subtracting the mean *x* and *y* value for each replicate configuration from each of the landmark points within that configuration (this superimposes the four replicates); and (d) rotating each of the four replicate configurations about its own centroid to minimize the sum of the squared distances between corresponding landmarks.

The Procrustes procedure applied to the tooth row data produced values for the tooth row centroid size and 20 new shape variables for each of the four replicate measures for each mouse. In all analyses described below, centroid size was used as an overall measure of tooth row size and was treated separately from shape as measured by the 20 shape variables. Although the original morphospace has two dimensions (*x* and *y*) for each landmark, the shape variables have only $2(10) - 4 = 16$ dimensions because the Procrustes procedure eliminates 4 d.f. when size, location, orientation, and rotation are eliminated from the original geometric configurations. It should be noted that the tooth size and shape measures were produced geometrically by superimposition, and this is not equivalent to standard statistical procedures (such as principal components analysis, PCA), which might render these variables independent. In fact, there can be a correlation between the Procrustes size and shape tooth variables; and if this exists, it would indicate allometry.

We first adjusted tooth row centroid size and the 20 shape variables for potential effects of sex, dam, block, and litter size (see Cheverud *et al.* 1996) by obtaining the residuals from multiple regression and then adding these values to the overall mean for the individual *x* and *y* values for each landmark. To assess measurement error, these adjusted values for centroid size and the shape variables were subjected to mixed-model, two-way ANOVAs where the main factors were individuals and sides (Leamy 1984; Palmer 1994). Centroid size was analyzed using a conventional two-way ANOVA while the new Procrustes coordinates were analyzed using a two-way Procrustes ANOVA, which has been adapted for shape data (Klingenberg and McIntyre 1998). Since Procrustes shape data have more degrees of freedom than conventional morphometric data, *F*-tests for the Procrustes ANOVA were evaluated using $n(2k - 4)$ d.f. (where n is the degrees of freedom from an ordinary ANOVA and k is the number of landmarks; see Klingenberg and McIntyre 1998).

In these analyses, measurement error was assessed by variation in the replicate measurements for each side (Leamy 1984;

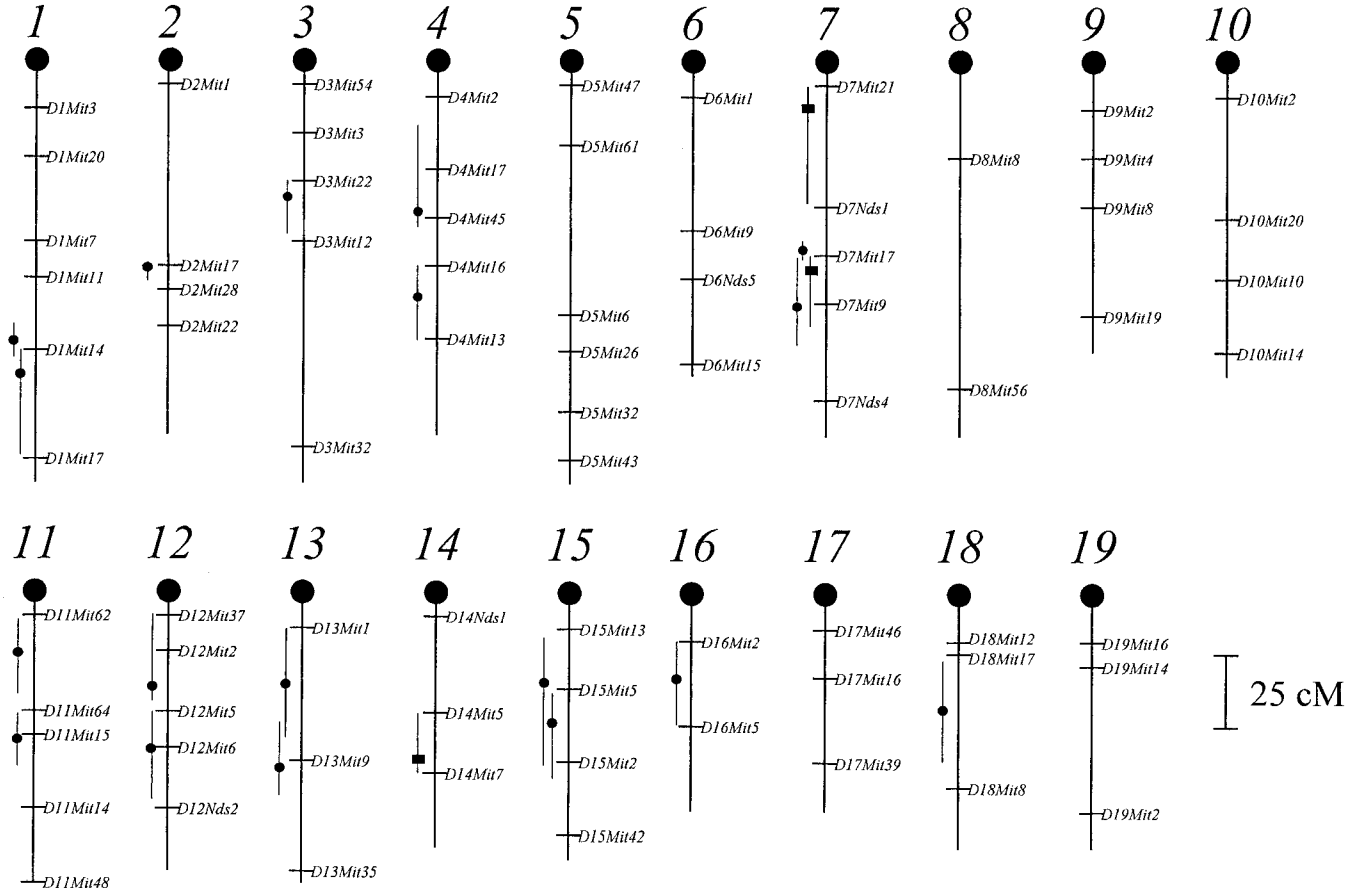

FIGURE 1.—Locations of marker loci and QTL for molar size and shape. All 76 marker loci are shown along with the locations and confidence intervals for QTL that influence molar row shape (circles) and molar centroid size (squares).

PALMER 1994) and accounted for 8.9% of the total variation for centroid size, but nearly 22% of the total variation in shape. Differences among individuals in tooth size and shape assessed here were significantly greater than those due to the size by individual interaction, and this interaction was significantly greater than the measurement error; therefore, this source of error does not appear to represent a problem for this study. Once this preliminary assessment of measurement error was completed, we used the mean of the four values (both replicates for left and right sides) for both centroid size and for each of the 20 shape variables in all subsequent analyses. This resulted in effective repeatabilities of 98% for tooth row centroid size and 93% for tooth row shape (FALCONER and MACKAY 1996).

Interval mapping procedure: Interval mapping was applied to both the centroid size and to the 20 shape variables using an approach described by HALEY and KNOTT (1992). Additive {+1, 0, −1} and dominance genotypic deviations {0, 1, 0} were assigned for the LG/LG, LG/SM, and SM/SM genotypes at each marker location. We then calculated the imputed genotypic deviations for each 2-cM interval between flanking markers on each chromosome by using the recombination frequencies between these markers and the formulas in HALEY and KNOTT (1992). Canonical correlation analyses were used to estimate the degree of association between the morphometric variables and the genotypic deviations at each 2-cM interval (LEAMY *et al.* 1999; KLINGENBERG *et al.* 2001). For each position 2 cM apart on a given chromosome, these analyses generated linear combinations of the genotypic deviations and mandible character values that resulted in pairs of canonical variables whose correlations were maximal. We conducted separate canonical correlation analyses for the size and shape data and, for shape, used only 16 of the 20 coordinates to obtain the appropriate dimensionality (KLINGENBERG *et al.* 2001).

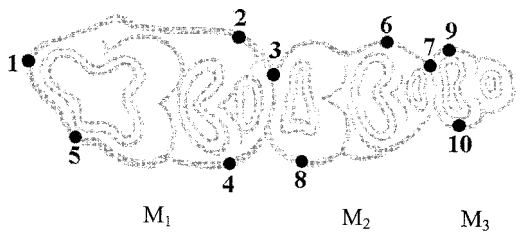

FIGURE 2.—Locations for the 10 molar row landmarks.

Microsatellite markers located on chromosomes other than the one being analyzed also were used as conditioning variables in each analysis to account for the effect of background QTL (JANSEN 1993; ZENG 1994). This did reduce the effective sample size for each chromosomal run, however, since the number of available markers varied from 458 to 495 (with the exception of *D5Mit47* for which only 196 individuals were available). The markers chosen for conditioning for the analysis of tooth centroid size were those reaching significance in preliminary stepwise multiple regression analyses. For tooth shape, we used canonical correlation to identify significant markers (although with *D5Mit47* omitted to maximize the

sample size), and where several markers on one chromosome reached statistical significance, the one with the highest squared multiple correlation value was chosen for use (Lynch and Walsh 1998). We did not use conditioning markers on the chromosome being analyzed, however, because the average number of markers on each chromosome was too low for this to be practical. Instead, we tested for the presence of two QTL on each chromosome (see below).

For each 2-cM interval, the canonical correlation analyses provided F approximations to Rao's statistic with their associated probabilities that were converted to linkage odds (LOD) scores. LOD scores represent ratios of the $\log_{10}$ likelihood that a QTL exists to the $\log_{10}$ likelihood that it does not exist in that interval and were therefore used to test the null hypothesis that no QTL was present at a given position. Significance for each of the putative QTL on each chromosome was tested by comparing the LOD scores to an empirically determined threshold value. Threshold values were obtained from permutation tests that were conducted for each variable (tooth size and shape) and for each individual chromosome (Churchill and Doerge 1994). Each permutation test consisted of 1000 iterations where the tooth size/shape values for each individual mouse were randomly permuted, merged with the imputed genotypic deviations and appropriate conditioning markers, and then run through the canonical correlation analysis. In these canonical correlation runs, the highest LOD score was recorded for each chromosome, and the 5% and 1% chromosomewise threshold values were obtained from the 50th and the 10th highest LOD scores among each of these 1000 LOD scores for each chromosome. Experimentwise threshold values were obtained from the 50th and 10th highest LOD scores that were observed on any chromosome during each of 1000 iterations.

Once a single QTL had been found, we applied a two-QTL model to determine if a second QTL was also present on that chromosome. Canonical correlation runs were computed for the size and shape variables with the genotypic deviations (and appropriate conditioning markers) from all possible pairs of locations on each chromosome. We subtracted Bartlett's V statistic (distributed as χ^2) that was obtained from the one-QTL model from Bartlett's V obtained from the two-QTL model. If this value exceeded the critical χ^2 value for $2n = 2$ d.f. for centroid size or $2(2n - 4) = 32$ d.f. for shape, we concluded that two QTL were present at the pair of locations that produced the maximal LOD score for that chromosome (Leamy *et al.* 1999).

Confidence intervals for each QTL were constructed using the one-LOD rule (Lynch and Walsh 1998). Using this rule, 95% confidence limits were determined by the interval on either side of the putative QTL location, where there was a 1.0-unit drop in the LOD score. For chromosomes that contained a second QTL, we ran one-QTL models that partialed out the effects of one of the QTL and then applied the one-LOD rule to these LOD scores to establish the 95% confidence interval for the remaining QTL (Leamy *et al.* 1999). All QTL locations and confidence intervals were expressed by the distance from the nearest proximal marker and by the distance from the centromere. The distance from the centromere to the most proximal marker was obtained from the Mouse Genome Database (2000).

Estimation and depiction of QTL effects: Once QTL positions were determined for each chromosome, multiple regressions of each character on the genotypic deviations for the QTL at that point on each chromosome were run, again including the same appropriate conditioning markers as were used in the canonical correlation analyses. The individual partial regression coefficients of each character on the imputed genotypic deviations provided an estimate of the additive (a) and dominance (d) genotypic values for each of the QTL. The additive genotypic value is one-half of the difference between the average phenotypic values of the two homozygotes and the dominance genotypic value is the difference between the average phenotypic value of the heterozygotes and the midpoint between the two homozygote genotypic values (Falconer and Mackay 1996). This procedure yielded single a and d values (and their standard errors) for the QTL for centroid size, but $\boldsymbol{a}$ and $\boldsymbol{d}$ vectors for shape that have both a magnitude and direction. The multiple regression analysis also yielded squared partial multiple correlation values that were multiplied by 100 to estimate the percentage of the total variation explained by each QTL.

Since the shape data are inherently multidimensional, the total magnitude of the $\boldsymbol{a}$ and $\boldsymbol{d}$ vectors for each shape QTL was quantified by calculating its length in units of Procrustes distance (Klingenberg *et al.* 2001). These additive ($\|\boldsymbol{a}\|$) and dominance ($\|\boldsymbol{d}\|$) shape effects were calculated as follows: $\|\boldsymbol{a}\| = (\boldsymbol{a}'\,\boldsymbol{a})^{0.5}$ and $\|\boldsymbol{d}\| = (\boldsymbol{d}'\,\boldsymbol{d})^{0.5}$ (Klingenberg *et al.* 2001). The overall significance of the Procrustes additive and dominance shape effects was tested for each QTL via a multivariate regression of the additive and dominance genotypic deviations at the site of the QTL on 16 of the 20 shape variables.

We also constructed diagrams using the entries for the $\boldsymbol{a}$ and $\boldsymbol{d}$ vectors for each QTL to depict the magnitude and direction of changes in shape at each landmark. Thus at each landmark, a line was drawn from the mean of the shape coordinates to a point equal to the mean plus 75 times the appropriate entry from the $\boldsymbol{a}$ (or $\boldsymbol{d}$) vector. In this way, the total shape effect of each QTL could be viewed in relation to the anatomical context of the entire molar row. Since all of the QTL effects were rather subtle, multiplication of the additive and dominance entries in each vector by the arbitrary factor of 75 was done simply to make these effects more visible. Thin-plate splines as used by Klingenberg *et al.* (2001) to depict landmark shifts in the mandible were not used here because they represent deformations that are only approximate between points, and the irregularity of the mandibular molar row outline would have made these between-point estimations even more subject to error.

Patterns of QTL effects: Once tooth shape QTL had been identified, we tested whether the effects of these QTL were primarily restricted to individual molars (morphological integration) or were dispersed fairly equally among all three of the molars. To accomplish this, for all QTL we first calculated Procrustes $\|\boldsymbol{a}\|$ and $\|\boldsymbol{d}\|$ values for each of the three molars. This was done for each molar by using only the landmark points on that molar (although point 3 was used for both M_1 and M_2, and point 7 for both M_2 and M_3; see Figure 2). Then we calculated Pearsonian correlations of these $\|\boldsymbol{a}\|$ (and $\|\boldsymbol{d}\|$) values for each pair of molars (M_1-M_2, M_1-M_3, M_2-M_3) and evaluated their significance using the sequential Bonferroni procedure (Rice 1989). A significant correlation was interpreted to mean that the magnitude of the $\|\boldsymbol{a}\|$ (or $\|\boldsymbol{d}\|$) effects of the QTL was similar across the two molars and thus that they were not genetically independent, whereas a nonsignificant correlation suggested genetic independence of the two molars (Leamy *et al.* 1999).

We also ran a PCA on the entries of the $\boldsymbol{a}$ and $\boldsymbol{d}$ vectors for each of the shape QTL (Jolliffe 1986; Klingenberg *et al.* 2001) to determine whether the QTL effects on tooth shape were clustered into distinct groups. If found, this would suggest that there are recurrent patterns that compress most of the variation among the QTL effects into a very small number of dimensions (Klingenberg *et al.* 2001). Separate PCAs were run on the covariance, rather than correlation, matrices of the $\boldsymbol{a}$ and $\boldsymbol{d}$ vectors from the individual QTL because these matrices preserve the Procrustes metric and thus do not elimi-

nate this common scale for shape variation (Dryden and Mardia 1998; Klingenberg and McIntyre 1998). Component scores for the first principal component (PC) were plotted against those of the second PC to facilitate inspection of the patterns of these effects.

QTL co-occurrence tests: QTL for mandible shape (Klingenberg *et al.* 2001) and skull dimensions (Leamy *et al.* 1999) previously have been discovered in our mice, and it seemed natural to ask whether some of these QTL were the same as those we identified as affecting tooth shape. To accomplish this, we first searched for all QTL affecting tooth shape that mapped within the confidence intervals of the QTL for the other characters. Then for each appropriate pair of QTL, we made use of an approach recently developed by Cheverud (2000) that tests whether QTL for two sets of characters map to the same position on a chromosome or to different positions.

This approach commenced by first determining the most likely chromosomal positions for each character set (tooth shape, mandible shape, and skull characters) as well as that for each combination of two-character sets (tooth shape with mandible shape, for example), using the canonical correlation procedure with conditioning markers as already described. For all chromosomes exhibiting two QTL, conditioning also was done for the genotypic deviations at the position of the QTL not being analyzed. A chi-square value for the model fitted to one character set was obtained at its most likely position, and a second chi-square value was obtained at the most likely combined trait position, both by controlling for variation in the second set of characters. This process was repeated for the second set of characters while controlling for variation in the first set, and again two chi-square values were identified. The differences between the pairs of chi-square values so generated were added to yield the final chi-square test statistic that was considered to have 1 d.f. (Cheverud 2000). A significant chi-square value indicated it was likely that there were two separate QTL involved, whereas a nonsignificant chi-square value suggested that a single QTL may be affecting both groups of characters (Cheverud 2000). In applying this test, the sequential Bonferroni procedure (Rice 1989) was used to ensure an experimentwise error rate of no greater than 5% among the comparisons.

It should be emphasized that the test described above is designed to detect common effects of a gene in a specific interval on a chromosome, which is the conventional interpretation of pleiotropy in QTL studies (Knott and Haley 2000). However, the test cannot distinguish pleiotropy in the strict sense (that due to common effects of a QTL at the nucleotide level) from effects potentially due to closely linked QTL in that specific region. This is especially true for F_2 populations derived from original intercrosses of inbred lines that exhibit linkage disequilibrium upon which QTL studies depend. Because significant associations of character sets found in these tests could in some cases be due to closely linked genes rather than to pleiotropy in the strict sense, we refer to these associations as evidence of "co-occurrence of QTL" rather than of pleiotropy.

RESULTS

QTL for centroid size: The locations and confidence intervals for all QTL significantly affecting tooth centroid size are summarized in Table 1 (see also Figure 1). Each QTL in Table 1 is designated as QTL-CS followed by its chromosome number and an extension of 1 or 2 to indicate whether it was the first or second QTL on that chromosome. Results of the interval mapping

TABLE 1

QTL for molar centroid size

QTL	LOD	Proximal marker	Marker distance	Centromere distance	Marker CI	Centromere CI	%	*a*	SE*a*	*d*	SE*d*
QTL-CS7.1	5.41**	*D7Mit21*	10	11	*D7Mit21+0–D7Nds1+0*	1-53	2.14	1.088*	0.448	1.352	0.750
QTL-CS7.2		*D7Mit17*	8	75	*D7Mit7+0–D7Mit9+10*	67-91	6.57	−2.110**	0.392	0.489	0.591
QTL-CS14.1	5.00**	*D14Mit5*	14	58	*D14Mit5+6–D14Mit7+0*	50-64	5.46	1.783**	0.393	1.173	0.632

Each QTL is designated as QTL-CS followed by its chromosome number and an extension of 1 or 2 to indicate whether it was the first or second QTL on that chromosome. Locations and confidence intervals (CI) are given in terms of the distance from the nearest proximal marker and from the centromere. LOD scores from the significance tests and the percentage of effect for each QTL are provided. Additive (*a*) and dominance (*d*) genotypic values and their standard errors (SE; all $\times 10^2$) obtained from multiple regressions also are given. $*P < 0.05$; $**P < 0.01$.

analyses revealed three QTL for centroid size, two on chromosome 7 and one on chromosome 14, whose LOD scores exceeded the 1% experimentwise value of 4.004. (Six other QTL reached chromosomewide significance levels, including five of them at the 1% level, but we report here only QTL reaching the experimentwise level of significance). Confidence intervals for these three QTL range between 14 and 53 cM with an average value of 30 cM, although this average is a slight underestimate because the confidence interval for the QTL on chromosome 14 includes an extreme marker.

These three QTL account for 2.1–6.6% of the total variation in centroid size or, on average, 4.7% (Table 1). The additive genotypic values for two of the three QTL are positive (and statistically significant), indicating that the alleles from the Large strain increase the centroid size of the mandibular molars for these QTL whereas the reverse is true for the other QTL. Absolute a values range between 0.011 and 0.211 mm and average 0.016 mm, greater than the average of 0.010 mm for the absolute dominance genotypic values. The ratio of the mean (absolute) dominance and additive (d/a) genotypic values is 0.60, which suggests that the larger-effect alleles of the QTL for centroid size are, on average, partially dominant to the smaller-effect alleles. However, none of the three d values are statistically significant, so we must conclude that there is no evidence for dominance for these QTL for tooth centroid size.

QTL for shape: Tooth shape is influenced by 18 QTL that reached the 5% (3.476) or 1% (4.185) experimentwide significance levels (Table 2 and Figure 1). These QTL are located on 11 of the 19 chromosomes, 7 of which carry 2 significant QTL. The confidence intervals for these 24 QTL average 28 cM and range between 10 and 56 cM. Again, this average is an underestimate because several of the QTL have confidence intervals that include one extreme marker.

The Procrustes $\|\boldsymbol{a}\|$ values ($\times 100$ in Table 2) for all 18 QTL are significant, ranging from 0.00309 to 0.1287 and averaging 0.00686. The Procrustes $\|\boldsymbol{d}\|$ values average 0.00687, but only one value is statistically significant, suggesting that there is little detectable dominance in the tooth shape QTL. The mean $\|\boldsymbol{d}\|/\|\boldsymbol{a}\|$ ratio for these shape QTL is 1.00, which is not significantly greater than the d/a ratio for centroid size of 0.60 ($P = 0.11$; one-tailed Kruskal-Wallis test). Dominance values are larger than additive values for only 1 of the 3 QTL for centroid size and for 9 of the 18 QTL for shape, although again this difference is not significant ($P = 0.41$). Thus there is no evidence that dominance is more important in the QTL for shape than in those for size.

Diagrams that depict the landmark shifts quantified by the $\boldsymbol{a}$ and $\boldsymbol{d}$ vectors for each of the 18 shape QTL are shown in Figure 3. As may be seen, there is great variability in the shape changes caused by the additive and dominance effects for these QTL. However, no QTL appears to have additive effects on only one molar such as the M_3, even though some QTL, such as QTL-TSH1.1, QTL-TSH1.2, and QTL-TSH18.1, for example, have obviously large effects on the M_3. Dominance effects for these QTL also show great variability, although the overall magnitude of these effects is quite prominent for some QTL such as QTL-TSH1.1, QTL-TSH1.2, QTL-TSH12.1, and QTL-TSH13.1. Again, however, these effects do not appear localized in any one molar.

There are some discernible trends among these shape changes, however, one being a combination of an anterior-posterior decrease in the M_1 with an increase in the M_2. This trend is present among the $\boldsymbol{a}$ vectors of three QTL (QTL-TSH1.2, QTL-TSH2.1, and QTL-TSH12.2), although the opposite effect (anterior-posterior increase in M_1 and decrease in M_2) is seen for QTL-TSH1.1, QTL-TSH3.1, and QTL-TSH7.1. The dominance effects appear relatively less coordinated than the additive effects for most QTL, even for those exhibiting large dominance effects. Dominance effects for one QTL (QTL-SH11.1) do show anterior-posterior expansion of the M_1 with contraction of the M_2, but, in general, patterns among these dominance effects are more difficult to discern.

Analysis of shape QTL patterns: Table 3 gives the Procrustes additive and dominance values generated by each of the shape QTL for each of the three molars. The $\|\boldsymbol{a}\|$ values vary from 0.0011 to 0.0105 (values in Table 3 are $\times 100$), although the means for each tooth are not significantly different ($P > 0.05$). Correlations of these $\|\boldsymbol{a}\|$ values for the M_1-M_2, M_1-M_3, and M_2-M_3 combinations are +0.63, +0.62, and +0.55, all of which are significant ($P < 0.05$) after sequential Bonferroni adjustment. The $\|\boldsymbol{d}\|$ values for the 25 QTL also vary considerably (from 0.0009 to 0.0135), but again their means do not differ among the three molars ($P > 0.05$). Their pairwise correlations among the three molars, +0.71, +0.59, +0.71, are somewhat higher than those for the $\|\boldsymbol{a}\|$ values, and, again, all three are significant ($P < 0.05$). These results suggest that both the additive and dominance effects of the shape QTL are similar among the three molars and thus that these molars are not genetically independent structures.

The first two principal components generated from a principal components analysis of the additive and dominance shape vectors account for 68.2% of the variation among the $\boldsymbol{a}$ vectors and 69.8% of the variation among the $\boldsymbol{d}$ vectors. This suggests that most of the variation among the shape effects is concentrated in 2 of the 16 available dimensions (recall that 4 dimensions were lost as a result of Procrustes superimposition). The phenotypic effects of the first two PCs from the separate analyses of additive and dominance effects are depicted in Figure 4. The first PC from the analysis of additive effects reflects expansion of the M_1 primarily in an anterior-posterior direction along with an anterior-posterior contraction of the M_2 and a counterclockwise shear of

TABLE 2

QTL for molar shape

QTL	LOD	Proximal marker	Marker distance	Centromere distance	Marker CI	Centromere CI	$\|\|a\|\|$	$\|\|d\|\|$
QTL-TSH1.1	7.40**	*D1Mit11*	20	88	*D1Mit11+10–D1Mit14+4*	78–98	1.137**	1.109
QTL-TSH1.2		*D1Mit14*	14	108	*D1Mit14+4–D1Mit14+36*	98–130	1.287**	1.584
QTL-TSH2.1	9.02**	*D2Mit17*	2		*D2Mit17+0–D2Mit17+4*		0.612**	0.311
QTL-TSH3.1	4.89**	*D3Mit22*	10	53	*D3Mit22+2–D3Mit22+16*	45–59	0.614**	0.463
QTL-TSH4.1	7.80**	*D4Mit17*	8	46	*D4Mit2+14–D4Mit45+4*	22–54	0.485**	0.263
QTL-TSH4.2		*D4Mit16*	14	84	*D4Mit16+0–D4Mit13+0*	70–92	0.395**	0.591
QTL-TSH7.1	6.17**	*D7Nds1*	12	65	*D7Nds1+8–D7Mit17+4*	61–71	0.603**	0.329
QTL-TSH7.2		*D7Mit19*	2	83	*D7Mit17+2–D7Mit19+18*	69–99	0.481**	0.395
QTL-TSH11.1	8.33**	*D11Mit62*	20	21	*D11Mit62+4–D11Mit62+30*	5–31	0.741**	0.762
QTL-TSH11.2		*D11Mit15*	4	61	*D11Mit64+4–D11Mit15+14*	51–71	0.506**	0.508
QTL-TSH12.1	6.25**	*D12Mit2*	12	31	*D12Mit37+0–D12Mit2+16*	1–35	0.645**	0.673*
QTL-TSH12.2		*D12Mit6*	0	51	*D12Mit5+0–D12Mit6+16*	41–67	0.309*	0.560
QTL-TSH13.1	8.01**	*D13Mit1*	30	37	*D13Mit1+0–D13Mit1+46*	8–53	0.819*	1.363
QTL-TSH13.2		*D13Mit9*	6	69	*D13Mit1+34–D13Mit9+14*	41–77	0.776**	0.823
QTL-TSH15.1	4.96**	*D15Mit13*	18	23	*D15Mit13+0–D15Mit2+4*	5–61	0.688*	0.794
QTL-TSH15.2		*D15Mit5*	12	41	*D15Mit5+4–D15Mit2+8*	33–65	0.823**	0.791
QTL-TSH16.1	3.71*	*D16Mit2*	14	30	*D16Mit2+0–D16Mit5+0*	14–44	0.587**	0.480
QTL-TSH18.1	5.23**	*D18Mit17*	20	85	*D18Mit17+4–D18Mit17+32*	69–97	0.833**	0.564

QTL for shape (QTL-TSH) are listed with the notation for locations, confidence intervals, and percentages of effect being the same as in Table 1. Procrustes additive ($\|\|a\|\|$) and dominance ($\|\|d\|\|$) shape effects, both $\times 100$, for each QTL also are given. $^*P < 0.05$; $^{**}P < 0.01$.

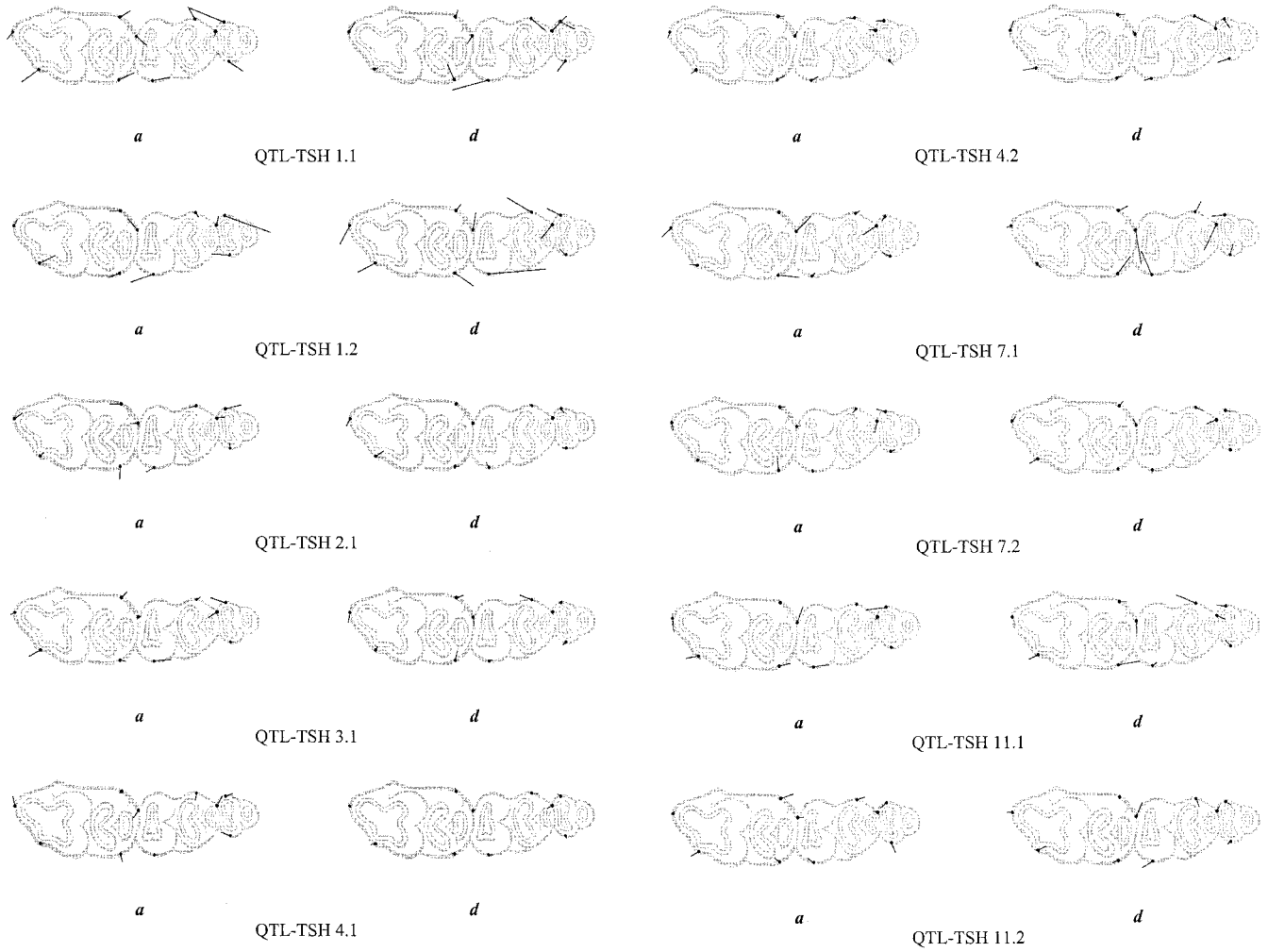

FIGURE 3.—Additive and dominance effects for all QTL that produce a significant effect on molar shape. Additive effects are shown in the left column while the corresponding dominance effects are shown in the column to the right. All effects are scaled ×75.

the M_3. The second PC from this analysis reflects expansion of the anterior portion of the M_1, posterior expansion of the posterior portion of the M_2, lateral shifts in the junctions between M_1-M_2 and between M_2-M_3, and a medial shift in the location of the M_3. The first PC from the analysis of dominance effects reflects a lateral shift in the M_1, an anterior-posterior expansion in the M_2, and a clockwise shear of the M_3. The second PC reflects a clockwise shear of the M_1 and a counterclockwise shear of the M_2 and M_3. Scatter plots of the first two PCs for the ***a*** and ***d*** vectors (Figure 5) do not show any clustering, which suggests continuous variation among the individual QTL effects.

Co-occurrence of QTL: Of the 18 tooth shape QTL, 12 had confidence intervals overlapping those of QTL for mandible shape (KLINGENBERG *et al.* 2001), and tests of co-occurrence of QTL showed that all of these could be genes commonly affecting both sets of characters (Table 4). Of 12 tests, 10 resulted in nonsignificant ($P > 0.05$) chi-square values even at the conventional significance level, whereas the remaining 2 tests were not significant when interpreted with the sequential Bonferroni procedure. Of the 18 shape QTL, 14 had confidence intervals overlapping those of QTL for cranial measures (LEAMY *et al.* 1999), and 12 of these showed nonsignificant results in the QTL co-occurrence tests, although 4 of these 12 reached significance at the conventional level. Thus many of the tooth shape QTL (or other closely linked genes) may be the same genes that affect mandible shape and cranial dimensions, and 8 of these apparently affect all three sets of characters. Only 2 of the 18 tooth shape QTL (QTL-SH1.1 and QTL-SH15.1) do not appear to affect either mandible shape or the cranial bones (Table 4).

DISCUSSION

The basic purpose of this study was to discover any QTL affecting tooth size and especially tooth shape in the F_2 mice in order to examine their patterns of effects. We found a total of 21 such QTL, which is perhaps an unexpectedly high number given that these QTL reflect only those loci whose alleles differ between the Large and Small inbred strains. Mice from these strains differ

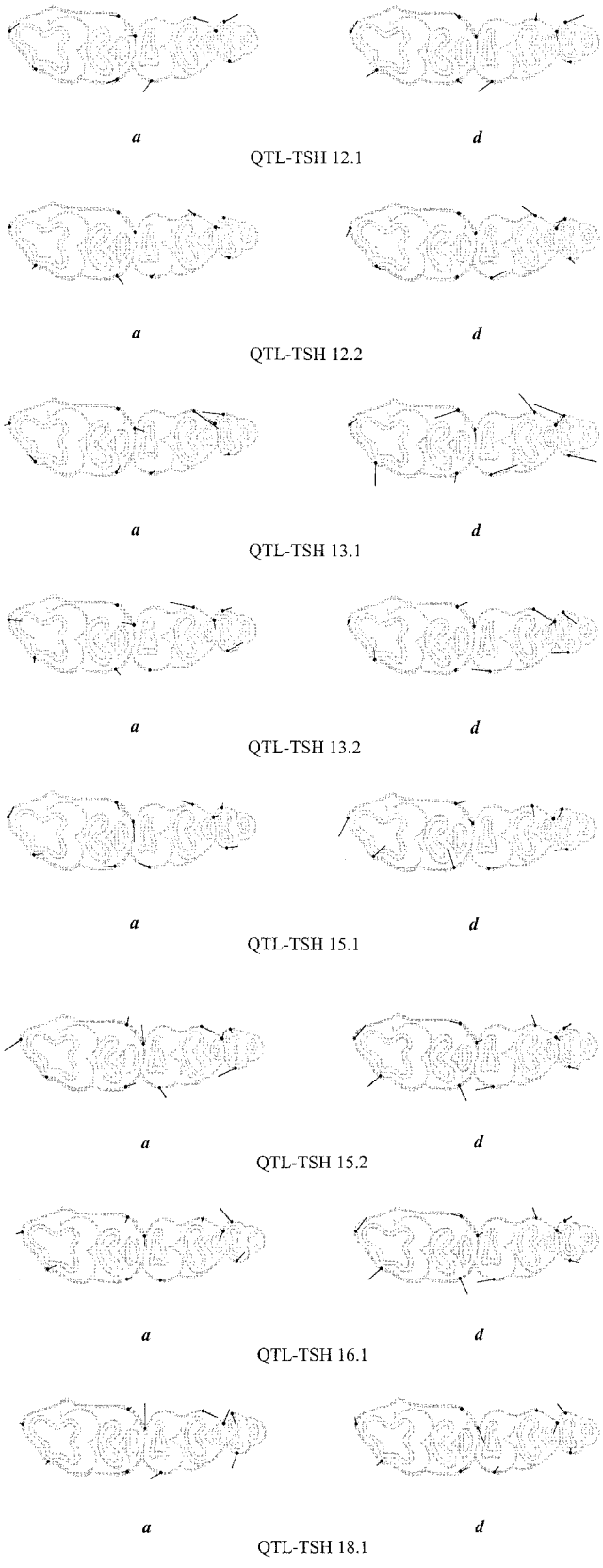

FIGURE 3.—*Continued.*

considerably in body weight, as already explained, and obviously were not chosen to optimize the search for QTL affecting tooth characters. The mandible tooth row of mice in the parental strains was not digitized (because of the labor involved), so the extent of the differences in tooth size and/or shape between these two strains is unknown. But it clearly must have been sufficient for us to detect so many QTL affecting these kinds of characters. On the other hand, KLINGENBERG *et al.* (2001) found a total of 37 QTL for size and shape of the mandibles in these mice, so perhaps we should have expected a large number of QTL for the teeth that, after all, form a part of the mandibles.

QTL for tooth size *vs.* shape: The results of this study showed that there were many more QTL for molar shape (18) than for molar centroid size (3). A similar result was found by KLINGENBERG *et al.* (2001) who identified 12 QTL for centroid size and 25 QTL for shape of the mandibles in these mice. And, using more landmark points on the mandible, CHEVERUD *et al.* (1997) discovered 26 QTL that affected various distances between these landmarks, only 6 of which were distances across the entire mandible (size measures). These results all suggest that the genetic basis for size, at least in teeth and mandibles, is simpler than that for shape. Although many explanations are possible, perhaps this has come about because the development of overall size is largely controlled by the endocrine system (SHEA 1992). Tooth development itself is regulated by a complex interaction between epithelial cells of the gubernaculum dentis and cells from the cranial ectomesenchyme (MARKS and SCHROEDER 1996), so it is easy to imagine that the final shape of the mandibular molar row requires the contribution of many genes.

In Drosophila, LAURIE *et al.* (1997) found several QTL that appeared to influence both size and shape differences in the posterior lobe (a male-specific genitalic structure) in several species, although their measure of shape may have been mechanistically connected with size in this structure (LIU *et al.* 1996; LAURIE *et al.* 1997). More recently, ZIMMERMAN *et al.* (2000) used both recombinant inbred (RI) lines and a backcross population of Drosophila to search for QTL affecting wing size and shape. They discovered 37 QTL in the RI lines and 13 QTL in the backcross population that affected shape, but only 8 QTL that affected overall wing size. They concluded that different genes controlled different aspects of shape in each region of the wing and that overall wing shape probably is determined by the length and positioning of wing veins that, in turn, are regulated by various growth factors (ZIMMERMAN *et al.* 2000).

Beyond the differences in the number of QTL exerting effects on tooth size and shape, it should be recalled that we compared their dominance effects as well and found that those for tooth shape QTL (mean $\|\boldsymbol{d}\|/\|\boldsymbol{a}\| = 1.00$) were not significantly greater than those for size QTL (mean $d/a = 0.60$). It is possible that dominance effects are more important in the shape

TABLE 3

Procrustes additive and dominance shape effects for each of the three molars

	$\|\|a\|\|$			$\|\|d\|\|$		
	M_1	M_2	M_3	M_1	M_2	M_3
QTL-TSH1.1	0.676**	0.513	0.809**	0.473	0.936	0.587
QTL-TSH1.2	0.565	0.592*	1.049**	0.828	1.353	0.472
QTL-TSH2.1	0.431**	0.315**	0.372**	0.260	0.125	0.119
QTL-TSH3.1	0.336**	0.411**	0.373**	0.369	0.319	0.930
QTL-TSH4.1	0.323**	0.326**	0.323**	0.146	0.203	0.151
QTL-TSH4.2	0.280	0.234**	0.228	0.370	0.347	0.345
QTL-TSH7.1	0.399**	0.400**	0.411**	0.123	0.263	0.219
QTL-TSH7.2	0.351**	0.266	0.309**	0.273	0.288	0.222
QTL-TSH11.1	0.539**	0.456*	0.398**	0.553	0.530	0.293
QTL-TSH11.2	0.360**	0.266*	0.276**	0.374	0.425*	0.188
QTL-TSH12.1	0.408**	0.426**	0.298**	0.383	0.396	0.425*
QTL-TSH12.2	0.236**	0.190	0.106	0.225	0.454*	0.267
QTL-TSH13.1	0.326**	0.648**	0.450	0.735*	0.821	0.881
QTL-TSH13.2	0.397**	0.595**	0.425**	0.378	0.585	0.481*
QTL-TSH15.1	0.555**	0.515	0.256	0.674*	0.313	0.296
QTL-TSH15.2	0.614**	0.543	0.414	0.625	0.434	0.263
QTL-TSH16.1	0.334**	0.279*	0.475**	0.221	0.331	0.295
QTL-TSH18.1	0.538	0.715**	0.493**	0.394	0.427	0.379
Means	0.405	0.397	0.369	0.406	0.487	0.329

Procrustes additive ($\|\|a\|\|$) and dominance ($\|\|d\|\|$) effects for each QTL are $\times 100$. $^*P < 0.05$; $^{**}P < 0.01$.

(compared to the size) QTL for the teeth, as was found by Klingenberg *et al.* (2001) for the mandible, but this hypothesis is not statistically supported by our data. Only 1 of the 18 $\|d\|$ values reached statistical significance, perhaps because of the limited statistical power for detecting dominance in this kind of QTL study (Klingenberg *et al.* 2001). Interestingly, the QTL for Drosophila wing size discovered by Zimmerman *et al.* (2000) were largely dominant in their effects, whereas those affecting wing shape exhibited mostly additive effects. Thus the relative importance of dominance in the QTL influencing size and shape in morphological structures clearly remains an open question and may vary among different structures.

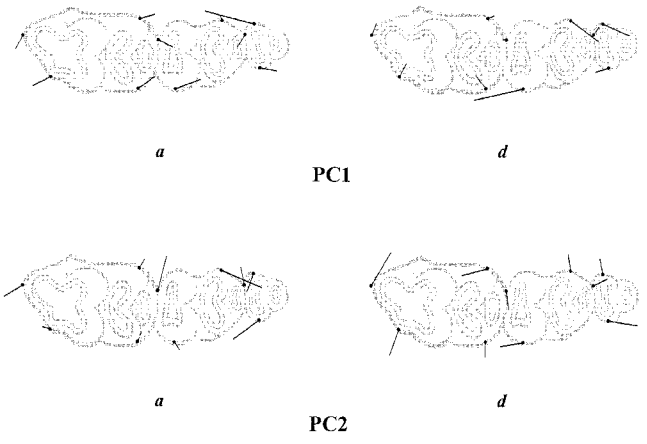

Figure 4.—First and second PCs calculated from the additive and dominance effects of the 25 QTL for molar shape.

Spatial patterns of shape effects: A major thrust of this study was to determine if the three molars represent genetically independent structures. We thought that the M_3 especially might show some independence in these tests since in house mice it lags behind the other two molars in its development (Cohn 1957) and is sufficiently small that it often is regarded as semivestigial (Bader 1965b). Further, phenotypic and/or genetic correlations between M_1 and M_2 tend to be higher than those between M_1 and M_3 or M_2 and M_3, and the width of the M_3 generally has a smaller heritability than widths of the M_1 or M_2 (Bader 1965a,b; Bader and Lehmann 1965; Leamy and Touchberry 1974). For example, Bader (1965a) found that the genetic correlation between the widths of the M_1 and M_2 (0.81) in house mice was greater than that between the M_1 and M_3 (0.50) or the M_2 and M_3 (0.57). Finally, some genes already known in the mouse (*Cd* and *Arg 31*, a missense mutation in the homeodomain of *Msx-1*) affect the development of the M_3 differently than the M_1 and M_2 (Grewal 1962; Gruneberg 1965; Vastardis *et al.* 1996).

But there is no evidence that the M_3 or any of the molars in our population of mice is genetically independent from the others, at least as judged by the significantly high correlations of $\|a\|$ and $\|d\|$ values between each pair of molars. These correlations were slightly lower in magnitude for the M_1-M_3 and M_2-M_3, compared with the M_1-M_2 combination, but the fact that all were

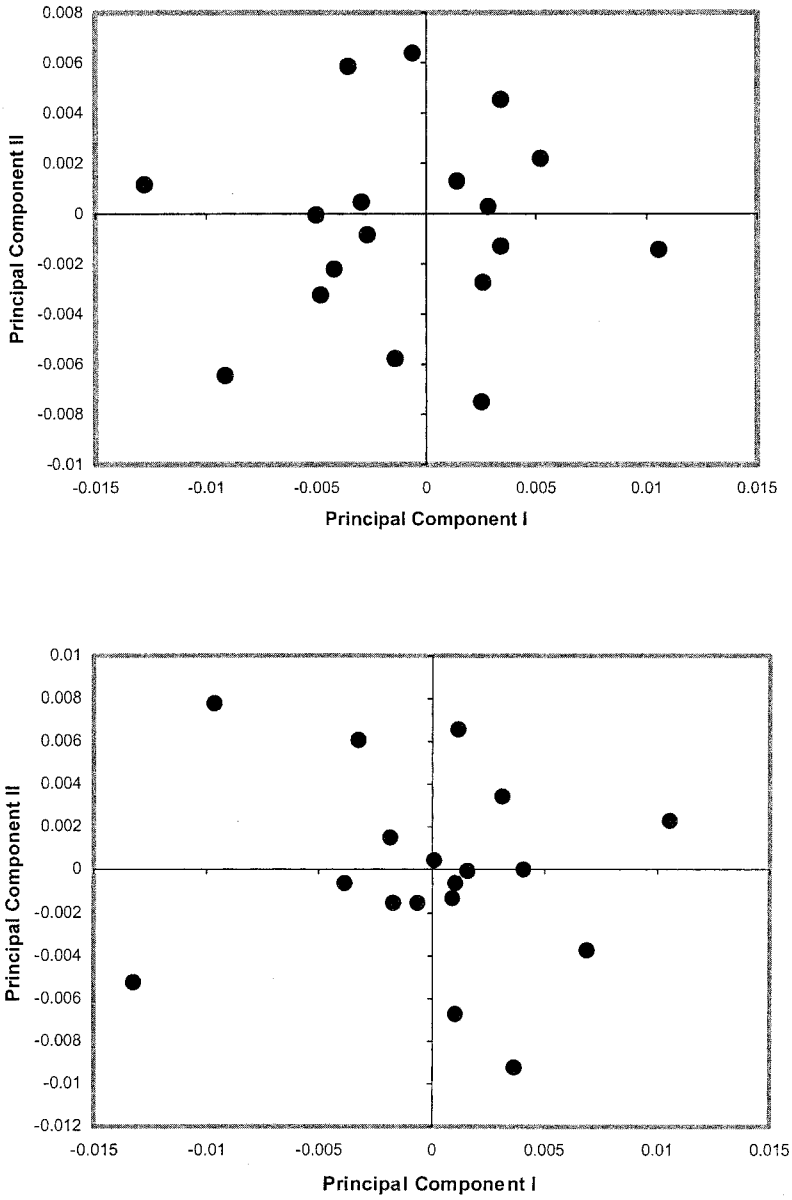

FIGURE 5.—Scatterplot of first and second principal component scores for the additive and dominance values for molar shape. (Top) Results from the additive values; (bottom) results from the dominance values.

significant suggests that both the additive and dominance effects of most of the shape QTL were common to all three molars. This result seems somewhat surprising in view of the developmental and size differences between the M_1 or M_2 *vs.* the M_3. But it is the differences in the magnitude of genetic correlations among these pairs of molars that are more relevant to our expectation that some QTL might affect primarily only one (or two) molars, and these genetic differences (BADER 1965a) are perhaps not that great when we take into consideration the well-known difficulties associated with their precise estimation (FALCONER and MACKAY 1996). It is also possible that some QTL that affect the M_3 more so than the M_1 or M_2 simply were not segregating in our F_2 mice (or had effects too small to be statistically detectable) and might be found in a QTL study making use of an intercross population derived from some other pair of inbred strains.

Ordination of QTL effects via principal components analysis also did not show any separate clustering of effects on the M_1 and M_2 *vs.* those on the M_3. Such clustering might have been expected if these two (or other) groups of characters represent morphologically integrated, developmentally distinct units (OLSON and MILLER 1958). Thus if the concept of morphological integration holds, pleiotropic effects of genes should produce phenotypic effects that form clusters according to the developmental or functional relationships among the characters that are influenced by these genes. Since we found no clustering among the shape effects of the 18 QTL, these ordination results are consistent with the conclusion above that the M_1 and M_2 do not represent

TABLE 4

Results of QTL co-occurrence tests

Tooth shape QTL	Mandible shape	Cranial bones	Tooth shape QTL	Mandible shape	Cranial bones
QTL-SH1.1			QTL-SH11.2	X	X[a]
QTL-SH1.2	X	X	QTL-SH12.1	X	X
QTL-SH2.1	X	O	QTL-SH12.2	X	X
QTL-SH3.1	X[a]	X[a]	QTL-SH13.1		X
QTL-SH4.1	X[a]	X	QTL-SH13.2		X
QTL-SH4.2		X	QTL-SH15.1		
QTL-SH7.1	X		QTL-SH15.2	X	X
QTL-SH7.2		X[a]	QTL-SH16.1	X	X[a]
QTL-SH11.1	X	O	QTL-SH18.1	X	

X indicates that co-occurrence was detected between the shape QTL listed at the left and a QTL affecting mandible shape or the cranial bones. "O" indicates a pair of QTL with overlapping confidence intervals that do not show co-occurrence.

[a] Chi-square values reaching conventional, but not sequential, Bonferroni significance.

a genetic or developmental unit that is distinct from the M_3. Instead, it seems clear that the QTL effects on molar shape are continuously distributed along two primary patterns in shape variation (denoted by the first two principal component axes). These findings are similar to those of the previous study on mandible shape in mice (Klingenberg *et al.* 2001).

Comparisons with known genes: Developmental biologists have identified >50 genes that are known to influence the development of teeth (Mouse Genome Database 2000). Although many of these genes facilitate events that are basic to the development of all teeth, several genes may influence dental adaptations. For example, *Activin beta-A* and the distal-less genes *Dlx-1* and *Dlx-2* have all been found to influence the maxillary molars differently than the mandibular molars (Thomas *et al.* 1997; Ferguson *et al.* 1998). This type of gene effect is important because it may facilitate functional integration between the occlusal surfaces of mandibular molars and their maxillary counterparts. Other important examples include the crooked (*cd*) gene and transforming growth factor (*TGF beta-2*), both of which influence molar size (Grewal 1962; Gruneberg 1965; Sofaer 1977; Chai *et al.* 1994), and the effects of bone morphogenic proteins (*BMP-2*, *-4*, and *-7*), fibroblast growth factors (*FGF-4*, *-8*, and *-9*), and epidermal growth factor (*EGF*), all of which are active in the enamel knot, which is thought to regulate shape and cusp patterns among developing teeth (Vaahtokari *et al.* 1996; Aberg *et al.* 1997; Thesleff and Jernvall 1997; Jernvall *et al.* 1998; Kettunen and Thesleff 1998).

In spite of the rather large number of genes that influence tooth development, there appear to be relatively few that map fairly closely to the QTL that we have found for molar size and shape. One such candidate is *Ccnd1*, which has been shown to influence tooth alignment and deformations of the jaw (Fantl *et al.* 1995) and maps in the region of both QTL-CS7.2 and QTL-TSH7.1. Another example of a potential candidate gene is *Col1a1*, which maps reasonably close to QTL-TSH11.2. This gene codes for the procollagen precursor molecule of the α1 chain of type I collagen, which is of particular importance in the extracellular matrix of dentine (Li *et al.* 1995). These and other genes may be possible candidates for the QTL that we have found affecting tooth row size and/or shape, but much more mapping in an advanced intercross or other such population subjected to greater amounts of recombination will be necessary before we can be more certain of the locations of these QTL.

In addition to these potential candidate genes, our tests for QTL co-occurrence suggested that a number of QTL for tooth row shape may have effects on overall mandible shape (Table 4) as defined by the 5 landmark points used by Klingenberg *et al.* (2001). In addition, Cheverud (2000) used 21 landmark points in the mandibles of these same mice and discovered a total of 17 QTL that had general alveolar or specific molar alveolar effects (Cheverud 2000). Many of these 17 QTL correspond in location to QTL found here for molar shape (or centroid size), providing further evidence for the existence of genes affecting both teeth and mandibles. This general result is not particularly surprising, because most of the genes that influence tooth development do so by regulating physiological interactions between the mesenchyme of the developing alveolar bone and the epithelial tissue of the enamel organ.

It was interesting that we found a potential commonality of genes affecting tooth shape and the cranial dimensions previously measured in these mice by Leamy *et al.* (1999). This may reflect the developmental origin of the teeth and skull from the cranial ectomesenchyme (Marks and Schroeder 1996), but whatever the case, this suggests that these QTL could have effects well beyond the individual teeth. Perhaps this is why most of these QTL did not map to the locations of the major

genes (described above) known to affect teeth, although some major genes that affect teeth, such as *cd*, are known to have pleiotropic effects on various skeletal dimensions (Gruneberg 1965).

Conclusions: The results of this study parallel those previously found for mandibles in these mice (Klingenberg *et al.* 2001). Thus we found more QTL for shape than for size, although we discovered that the magnitude of dominance effects was comparable between the size and shape QTL. The effects of the shape QTL also were dispersive and did not appear to be localized for any given molar. The tooth shape QTL appeared to be distinct from most of the genes presently known to affect development of mouse molars, but many may well be the same as those affecting the shape of the mandible and various cranial dimensions.

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