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Quantitative trait loci for directional but not fluctuating asymmetry of mandible characters in mice

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Summary

Non-directional variation in right minus left differences in bilateral characters, referred to as fluctuating asymmetry (FA), often has been assumed to be largely or entirely environmental in origin. FA increasingly has been used as a measure of developmental stability, and its presumed environmental origin has facilitated the comparisons of populations believed to differ in their levels of stability. Directional asymmetry (DA), in which one side is consistently larger than the other, has been assumed to be at least partially heritable. Both these assumptions were tested with interval mapping techniques designed to detect any quantitative trait loci (QTLs) affecting FA or DA in 15 bilateral mandible characters in house mice resulting from a cross of the F1 between CAST/Ei (wild strain) and M16i (selected for rapid growth rate) back to M16i. For purposes of the analysis, all mandibles were triply measured and 92 microsatellite markers were scored in a total of 350 mice. No significant QTLs were found for FA, but three QTLs significantly affected DA in several characters, confirming both assumptions. The QTLs for DA were similar in location to those affecting the size of several of the mandible characters, although they accounted for an average of only 1% of the total phenotypic variation in DA.

1. Introduction

The genetic basis of variation in the subtle differences typically found between right and left sides of bilateral characters continues to be a matter of considerable interest. This is particularly so for fluctuating asymmetry (FA), a ubiquitous type of non-directional asymmetry that sometimes has been considered to be one of those rare characters that truly may not be heritable (Palmer *et al*., 1994). Theoretically at least, this could be possible if the same gene or genes uniformly control development of both sides of a given bilateral character and no other genes act on the separate sides. Certainly the presumed environmental basis for FA has helped fuel its recent rise as the preferred measure of developmental stability in populations (Parsons, 1990; Graham, 1992; Zakharov, 1994). Thus FA is expected to increase in populations subjected to any of a variety of stressors, and the comparison of FA in these versus other populations less stressed is facilitated if it can be assumed that direct genetic effects on FA itself are not responsible for any observed differences (Palmer *et al*., 1994).

In contrast to FA, a genetic basis has long been assumed for directional asymmetry (DA), another commonly found type of asymmetry in which one side is consistently larger than the other (Van Valen, 1962). For characters such as the mammalian heart that exhibits DA, this assumption seems reasonable because of the obvious functional advantage for this kind of asymmetry. Further, a heritable basis would have to be present for DA if, as hypothesized, it has given rise to macroscopic asymmetries such as seen in claw size in various crabs (Palmer *et al*., 1994). In any event, the presumed heritable basis for DA has contributed to the general view that it is not useful as an indicator of developmental stability (Palmer, 1994),

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although some investigators (Graham *et al*., 1993) have argued otherwise.

There have been a number of studies that have measured the heritability of FA in various characters, although many have been flawed for one or more reasons (see reviews in Leamy, 1997; Palmer & Strobeck, 1997; Markow & Clarke, 1997). However, a reasonable synopsis of several of the more carefully conducted studies suggests a very low (typically less than 0·1) heritability of FA in various characters that is significantly detectable only with considerable statistical power (Leamy, 1997; Whitlock & Fowler, 1997). Heritability estimates for DA are fewer in number, although those that have been conducted often also show a low, but sometimes significant level (for example, Cheverud *et al*., 1990; Leamy, 1999). So in some cases we might expect the segregation of at least a few genes that affect DA or FA in an appropriate bilateral character.

Two attempts to search directly for such genes (quantitative trait loci, or QTLs) affecting DA or FA have recently been conducted, and have shown somewhat mixed results. Leamy *et al*. (1997) found a significant number of QTLs for DA, but not FA, in mandible dimensions in F2 mice produced from an original intercross of the Large and Small inbred strains, although the opposite result was obtained for discrete skeletal characters in these mice (Leamy *et al*., 1998). The purpose of the study described in this paper was to search once again for QTLs significantly affecting DA or FA in mandible dimensions in mice, this time using a backcross population originating from different strains. Given the results of these previous QTL studies, the working hypothesis was that a significant number of QTLs might be found for DA, but not FA, in this particular population of mice.

2. Materials and methods

(i) *The population and characters*

The mice used in this study had their origins in two genetically diverse inbred strains, M16i and CAST/Ei (CAST). The M16i strain was produced from long-term selection for rapid postweaning (3 to 6 week) weight gain in ICR mice (Hanrahan *et al*., 1973; Eisen, 1975), followed by relaxed selection and, most recently, by 15 generations of full-sib mating. The minimum inbreeding coefficient for the M16i strain was 0·95, and probably was close to 1·0 given the effective inbreeding that took place during selection. Several quantitative genetics studies have previously been conducted using M16i mice prior to their full-sib mating period (Timon & Eisen, 1970; Eisen & Leatherwood, 1978*a, b;* Robeson *et al*., 1981; Eisen, 1986, 1987). CAST was originally derived from a wild population of the subspecies *Mus musculus castaneus*, and had undergone at least 35 generations of inbreeding via full-sib mating prior to use in this study.

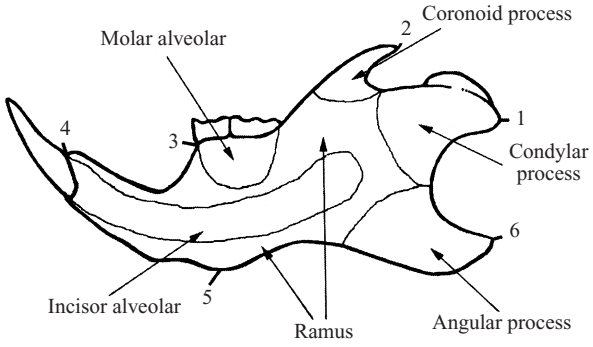

Fig. 1. Outline of a mouse mandible showing the six landmark points that were digitized. M1, 1 to 2; M2, 1 to 3; M3, 1 to 4; M4, 1 to 5; M5, 1 to 6; M6, 2 to 3; M7, 2 to 4; M8, 2 to 5; M9, 2 to 6; M10, 3 to 4; M11, 3 to 5; M12, 3 to 6; M13, 4 to 5; M14, 4 to 6; M15, 5 to 6.

To produce the mice used here, CAST males were mated to M16i females, and nine F1 males were then backcrossed to M16i females. This backcross eventually yielded 54 litters (3–7 litters per sire) and a total of over 400 mice that reached 12 weeks of age.

All mice were reared in an environment of 21 °C, 55% relative humidity, and a light:dark cycle of 12h:12 h, all according to NIH guidelines for animal care in the Mouse Genetics Laboratory at North Carolina State University. Water and food (Purina Mouse Chow 5015 from mating until weaning, and Purina Laboratory Chow 5001 from weaning until sacrifice) were provided *ad libitum*. All litters were standardized to 10 individuals on the day (0) of their birth. Pups were individually identified by toe-notching at day 12, weaned at 21 days of age and housed separately by sex. For each mouse, body weights were recorded at 12 days and again at 3, 6, 9 and 12 weeks of age, and at 6 weeks a tail clip ($\sim$ 1 cm) was collected and frozen at -80 °C for later DNA extraction.A total of 92 microsatellite markers located on 19 autosomes (see Appendix) were scored in each mouse by standard methods using the polymerase chain reaction (PCR) and gel electrophoresis. Genotypes were independently scored by two individuals prior to being entered in a computer database (Excel). Any discrepancies, ambiguities or failed amplifications were rectified by repeating genotyping of the samples.

All mice were killed at 12 weeks of age and their skeletons prepared by exposure to dermestid beetles. After skeletonization was completed, left and right sides of the mandible in each of the backcross mice were separated at the mandibular symphysis, placed under a microscope, and scanned into a computer with the use of Adobe Photoshop. Six points around the periphery of each mandible (Fig. 1) were recorded

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in millimetres in x, y space with the NIH program IMAGE. These six points were chosen because of their proven repeatability (see below). Fifteen inter-landmark distances, referred to as mandible characters M1–M15 (see Fig. 1), were then calculated between each pair of points. Each mandible was measured three times from the same scan so that three separate estimates of the 15 distances were available for both left and right mandibles in each mouse.

(ii) *Asymmetry analysis*

Before the actual calculation of both DA and FA (see below), their significance for each of the 15 mandible characters was obtained from a mixed model, two-way analysis of variance. In this model, individuals is a random factor that assesses variation in size or shape among individual mice, sides is a fixed factor that assesses DA, the individuals × sides interaction assesses FA, and the error assesses variation in replicate measurements, or measurement error (Leamy, 1984; Palmer, 1994). In all these analyses, sex differences were taken out with the use of sex as a classification variable. Sex × sides interactions were not statistically significant, and were not included in the model. As appropriate to this mixed model (Sokal & Rohlf, 1995), mean squares for sides were tested over the individuals × sides interaction whereas mean squares for the interaction were tested over the error mean squares. In determining significance, all probabilities generated from F-tests in these analyses were assessed via the sequential Bonferroni procedure (Rice, 1989). A significant interaction effect was taken to mean that significant FA beyond measurement error was present and thus that the analysis of FA could proceed (Palmer, 1994). Measurement error was also assessed by the ratio of the error variance relative to the interaction (FA) variance for each character (see Palmer, 1994).

After this preliminary assessment of the magnitude of DA, FA and measurement error, right minus left differences for each of the characters were examined in greater detail. Among all replicate measurements for each of the 15 characters, a total of 11 of these differences qualified as outliers (Sokal & Rohlf, 1995). Where outliers were present, all data for that mouse were deleted since the QTL mapping technique (see below) made use of the multivariate technique of canonical correlation that required a complete data set. Combined with a number of the mandibles that were chipped or broken during the skeletonization or measurement process, this resulted in a final sample size of 350 mice (182 males and 168 females).

All subsequent analyses made use of the mean of the three repeat measurements for each mandible character. The mean of the two sides, or size, for each character was calculated first so that QTL results for these characters could be compared with those for their asymmetries. All such means were averaged over all repeats, and then tested (using the sequential Bonferroni procedure; Rice, 1989) for sex, sires, litters within sires, and litter size effects in an analysis of covariance with sex as one factor, litter size as a covariate, and with litters nested within sires. (Sex × sire and sex × litter interactions were not included in the model since they were not significant). Sire and litter size effects were not statistically significant, but sex and litter effects were significant for most of the size characters, and so were adjusted for by obtaining residuals from the analysis of covariance. Basic statistics, including correlations, were calculated for the adjusted values of these characters to provide some description of their variation and covariation.

Right minus left side differences were calculated for each of the 15 characters, and then averaged over repeat measurements. These differences were tested for the effects of sex, sires, litters and litter size, and only sex was significant for some characters and therefore adjusted as before. If the mean of these *signed* differences significantly differed from zero, this indicated the presence of DA (Van Valen, 1962; Leamy, 1984, 1997). Even if a given character did not show significant DA, however, signed differences for that character were used to assess DA in the QTL analysis since different alleles of a QTL could act to produce DA in opposite directions (right side larger than left side and vice versa) that could cancel each other and produce a mean difference of zero (see Leamy *et al*., 1997). Skewness and kurtosis statistics for the signed differences also were calculated to discover whether these differences were normally distributed as would be expected if this variation represents classical FA (Van Valen, 1962; Palmer, 1994). Kurtosis statistics were helpful in testing for antisymmetry, another type of bilateral asymmetry suggested by a significant platykurtotic distribution of signed differences between sides (Palmer & Strobeck, 1992). Signed differences were also tested to see whether they significantly scaled with size (Palmer, 1994) by evaluating the significance (Rice, 1989) of regressions of each character with its size.

To assess fluctuating asymmetry, most investigators use the unsigned right minus left differences of sides when it is found that the mean of the sides is zero (Palmer, 1994). Significant DA was present in the mandible characters (see below), however, so FA was calculated via a method recently proposed by Graham *et al*. (1998) that corrects for DA. Specifically, measures of FA for each character in each mouse were calculated as the absolute (unsigned) scores of the second component generated in principal component analyses of covariance matrices of the values for left and right sides. In these component analyses, both

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sides for each character were first logarithmically transformed to promote homoscedasticity of errors (Graham *et al*., 1998), although the results (see below) were the same without this transformation. Distributions of FA in these characters, however, were half-normal (Palmer, 1994) and were subjected to Box-Cox transformations (Swaddle *et al*., 1994) of the form $(FA + 0{\cdot}0005)^{0{\cdot}33}$ that were successful in achieving normality. These FA characters showed no significant sex, sire, litters or litter size effects, and therefore were not adjusted for any of these factors.

(iii) *QTL analyses*

QTL analyses were carried out for the mandibular size, DA and FA characters using the interval mapping method described by Haley & Knott (1992). To implement this method, genetic distances between the molecular markers on each chromosome first were determined using the Mapmaker 3.0 program (Lincoln *et al*., 1992). This generated distances that are expressed as Haldane cM units (see Appendix). Then, at a given marker, genotypic deviations were set at 1 for the M16i homozygote and 0 for the CAST/M16i heterozygote. Genotypic deviations for all locations 2 cM apart between flanking microsatellite markers on each chromosome were imputed using the recombination percentages derived from the Mapmaker program and the equations for backcross populations in Haley & Knott (1992). In this backcross generation, genotypic deviations at each location are estimates of the probability of a M16i/M16i homozygote.

QTL runs were done using multivariate canonical correlation via the CANCORR procedure in SAS (SAS Institute, 1989). Separate analyses were run for the 15 mandible size characters. the 15 mandible DA characters and the 15 mandible FA characters. 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. Conditioning markers located on chromosomes other than the one being analysed were also used as partialling variables in each analysis to account for the effect of background genes and other QTLs (Jansen, 1993; Zeng, 1994). The markers chosen for conditioning were those reaching significance in preliminary multiple regression analyses (using all 15 mandible characters with each of the 92 markers). Where several markers on one chromosome reached statistical significance, the one with the highest squared multiple correlation value was chosen for use (Lynch & Walsh, 1997). Thus a maximum of one marker per chromosome was used in conditioning so that the power of the analysis would not be significantly reduced (Zeng, 1994; Jansen & Stam, 1994).

Linkage odds (LOD) scores were calculated from the probabilities associated with the F approximations to Rao's statistic generated for each 2 cM interval in the canonical correlation runs. LOD scores are ratios of the $\log_{10}$ likelihood that a QTL exists to the $\log_{10}$ likelihood that it does not exist, and were therefore used to test the null hypothesis that no QTL was present. If the highest LOD score calculated for a given chromosome exceeded the appropriate critical value, a QTL was considered to be present at the position of that LOD score. The critical values used for determining the presence of QTLs were determined by 1000 permutation (Churchill & Doerge, 1994) runs done for each chromosome in each of the three groups of characters. In this process, the mandible character 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. For each chromosome, the fiftieth and tenth highest LOD scores generated from these 1000 runs provided the 5% and 1% suggestive linkage threshold values. Experimentwise threshold values also were calculated as the fiftieth and tenth highest LOD scores among the highest LOD scores for all 19 chromosomes generated in each of the 1000 permutation runs. Confidence intervals were defined as the distances in centimorgans on either side of the QTL locations where there was a drop in the LOD score of 1·0. Thus the limits for these intervals describe positions 10 times less likely than the peak position to represent a QTL.

If a single QTL was found on a given chromosome, it was possible to test for the presence of two QTLs on that chromosome. This was done once again by canonical correlation runs using all mandible variables, genotypic deviations and appropriate conditioning markers, but this time for all possible pairs of locations. Bartlett's V statistic, distributed as χ^2 with 30 d.f., was computed from the canonical correlation output (Green, 1978) for each run, and the highest such value generated was compared with its counterpart from the one-QTL run (distributed as χ^2 with 15 d.f.). If the difference between these χ^2 values exceeded the critical χ^2 value for 15 d.f. (24·996), the improvement in fit was considered significant and it was concluded that two QTLs were present on that chromosome at the locations indicated by the highest χ^2 value. Confidence intervals around both QTLs were determined as already described, but using LOD scores generated from new canonical correlation runs that partialled out the effect of one QTL and fitted a one-QTL model for the other QTL.

Once QTL positions were determined for each chromosome, multiple regressions of each character on the genotypic deviations for the QTL(s) at that point on each chromosome were run, again including

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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 difference between the homozygote and heterozygote. These differences are denoted as *a* throughout Sections 3 and 4, although it should be noted that they are not the same as the additive genotypic value typically defined as one-half the difference between the homozygotes (Falconer & MacKay, 1996). For this backcross, these values are estimates of the effect of replacing one CAST allele (in the heterozygote) with an M16i allele (in the homozygote). The significance of these *a* values was tested by *t*-tests of the regression coefficients themselves, with conventional (rather than adjusted) 5% and 1% critical values because multivariate significance had already been established for the QTLs at each location. Standard errors for the *a* values were provided by the standard errors of the regression coefficients. Finally, the percentage of the total variation explained (after removal of covariate effects) by each QTL was given by 100 times the squared partial multiple correlation value associated with each multiple regression.

3. Results

(i) *Basic statistics*

The results of the two-way analysis of variance of the repeated mandible measurements for each of the 15 characters are given in Table 1. The percentage contributions to the total variance for both the individuals × sides interaction and the error variance components are also given so that precision of measurement can be assessed. Differences among individual mice are highly significant for all mandible characters, and differences of sides are significant for 14 of the 15 characters. Thus significant DA is detectable in all characters except M4. The individuals × sides interactions are significant for all characters, suggesting that FA is present and detectable in all cases. The percentage contribution of FA to the total variation certainly is not trivial, ranging from 8% (M1) to nearly 27% (M13), and averaging 16·9%. Measurement error, assessed by the percentage contribution of the error variance, averages only 2·2% of the total variation or 2·2/16·9 = 13% of the within-individual, non-directional (FA) variation across all 15 characters.

Table 2 gives means and standard deviations for the mean of the sides (size) and for DA and FA in each of the 15 mandible characters. The average size of these characters ranges from just over 3 mm (M11) to over 12 mm (M3 and M14), with standard deviations over all 350 mice averaging about 0·24. Coefficients of variation for the size of the mandible characters average 2·89, suggesting that these characters exhibit a fairly low level of overall variation. Correlations of the size of the characters range from −0·27 (M1 with M6) to +0·94 (M12 with M14), averaging +0·45. All but four of these 102 total correlations are significantly different from zero in sequential Bonferroni tests.

Signed differences between sides (Table 2) are significant for 14 of the 15 characters, suggesting that, as was seen earlier in the analysis of variance results (Table 2), all characters except M4 exhibit significant DA. Six of the mandible characters (M1, M2, M3,

Table 1. *The analysis of variance* (*mean squares and components of variance* × 10^4) *of the 15 mandible characters. Percentage contributions for the interaction* (*non-directional asymmetry*) *and error* (*replicate measurement variation*) *sources of variation for all mandible characters are also given*

		I × S (d.f. = 349)			Error (d.f. = 2240)		
	Individuals (I)	Sides (S)	MS	$\sigma^2_{I\times S}$	%	σ^2_E	%
M1	6924·8**	159 394·2**	320·5**	104·1	8·1	8·6	0·7
M2	3101·9**	45 827·7**	308·1**	99·2	16·3	10·8	1·8
M3	5892·1**	10 130·9**	456·1**	147·7	13·1	13·3	1·2
M4	3787·4**	29·9	625·9**	200·8	25·5	24·2	3·1
M5	2798·1**	122 458·2**	356·1**	115·2	20·5	10·8	1·9
M6	3380·7**	8 563·2**	349·7**	112·9	17·0	11·3	1·7
M7	6348·7**	97 052·8**	492·7**	161·2	13·3	9·4	0·8
M8	4046·1**	84 944·5**	499·1**	161·5	20·0	14·9	1·8
M9	6950·4**	1 539·7**	356·1**	115·5	8·9	9·9	0·8
M10	1118·1**	6 026·5**	190·0**	60·0	25·5	10·3	4·4
M11	944·3**	66 154·1**	123·9**	37·5	19·2	11·4	5·9
M12	4880·5**	6 942·4**	291·9**	95·0	10·3	7·2	0·8
M13	1982·8**	3 660·7**	358·9**	112·9	26·7	20·6	4·9
M14	8178·6**	3 084·7**	411·1**	132·8	8·7	13·0	0·8
M15	4339·7**	15 725·9**	572·2**	183·3	20·7	22·9	2·6

** $P < 0{\cdot}01$.

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Table 2. *Means and standard deviations (SD) for the mean of the sides (in mm) and for directional (DA) and fluctuating asymmetry (FA) between sides for each of the 15 mandible characters*

	Mean of sides		DA		FA	
	Mean	SD	Mean	SD	Mean	SD
M1	3·97	0·168	0·154**	0·146	0·197**	0·055
M2	9·15	0·174	0·092**	0·143	0·153**	0·038
M3	12·52	0·228	0·041**	0·175	0·149**	0·035
M4	8·92	0·202	0·001	0·204	0·173**	0·044
M5	3·34	0·176	−0·144**	0·154	0·212**	0·059
M6	6·84	0·204	−0·039**	0·153	0·168**	0·045
M7	9·84	0·267	−0·129**	0·181	0·160**	0·041
M8	8·03	0·219	−0·123**	0·182	0·172**	0·044
M9	6·44	0·199	−0·022**	0·154	0·176**	0·045
M10	3·42	0·106	−0·035**	0·113	0·189**	0·053
M11	3·24	0·109	−0·105**	0·091	0·180**	0·051
M12	9·18	0·217	0·044**	0·140	0·153**	0·037
M13	5·63	0·140	−0·022**	0·155	0·181**	0·047
M14	12·56	0·278	0·030**	0·166	0·147**	0·034
M15	7·78	0·224	0·057**	0·195	0·176**	0·047

** $P < 0{\cdot}01$.

Table 3. *Locations and confidence intervals (CI) of significant QTLs for the size (mean of the two sides) of the mandible characters, given as map distances from the nearest proximal marker and from the centromere. LOD scores from the tests of significance are also given*

QTL	LOD	Proximal marker	Marker distance	Centromere distance	Marker CI	Centromere CI
QTL-M1.1	5·39**	*D1Mit4*	14	26	*D1Mit4+6–D1Mit4+26*	12–38
QTL-M1.2	—	*D1Mit9*	6	51	*D1Mit4+32–D1Mit140+10*	49–65
QTL-M2.1	14·51**	*D2Mit61*	2	36	*D2Mit120+6–D2Mit61+6*	21–40
QTL-M2.2	—	*D2Mit224*	2	67	*D2Mit133+2–D2Nds1+0*	62–70
QTL-M3.1	10·30**	*D3Mit46*	4	17	*D3Mit46+0–D3Mit46+10*	14–24
QTL-M3.2	—	*D3Mit31*	0	75	*D3Mit10+24–D3Mit31+0*	59–75
QTL-M4.1	11·74**	*D4Mit27*	14	50	*D4Mit27+4–D4Mit27+24*	40–60
QTL-M5.1	2·91**	*D5Mit48*	54	55	*D5Mit48+8–D5Mit24+10*	29–70
QTL-M6.1	7·86**	*D6Mit50*	6	9	*D6Mit50+0–D6Mit50+16*	3–19
QTL-M7.1	8·47**	*D7Mit55*	34	49	*D7Mit55+22–D7Mit37+18*	37–75
QTL-M8.1	7·42**	*D8Mit25*	2	23	*D8Mit4+2–D8Mit75+10*	16–36
QTL-M9.1	5·49**	*D9Mit10*	12	55	*D9Mit2+24–D9Mit10+22*	41–65
QTL-M10.1	7·70**	*D10Mit16*	4	20	*D10Mit16+0–D10Mit16+12*	16–28
QTL-M10.2	—	*D10Mit31*	8	37	*D10Mit31+2–D10Mit13+0*	31–57
QTL-M11.1	4·30**	*D11Mit63*	26	28	*D11Mit63+2–D11Mit5+4*	4–47
QTL-M12.1	10·06**	*D12Mit5*	0	41	*D12Nds11+26–D12Mit5+8*	32–49
QTL-M13.1	5·37**	*D13Mit51*	6	47	*D13Mit36+0–D13Mit51+8*	37–59
QTL-M14.1	9·69**	*D14Mit10*	16	19	*D14Mit10+2–D14Mit10+24*	5–27
QTL-M14.2	—	*D14Mit42*	0	48	*D14Mit32+8–D14Mit42+0*	38–48
QTL-M15.1	4·06**	*D15Mit3*	0	30	*D15Mit121+2–D15Mit29+4*	27–43
QTL-M16.1	5·49**	*D16Mit29*	10	23	*D16Mit29+2–D16Mit14+0*	15–33
QTL-M17.1	6·96**	*D17Mit7*	6	39	*D17Mit22+10–D17Mit39+0*	29–45
QTL-M18.1	2·08*	*D18Mit51*	24	51	*D18Mit10+8–D18Nds1+0*	25–73

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

M12, M14, M15), all of which involve points 1 and 6 and are generally length dimensions, have larger right sides than left sides. The opposite is true for the other eight characters, all of which primarily involve points 3, 4 and 5 and are often height dimensions. The relative amount of DA expressed as a percentage of the size of the character averages 1·3%, with the greatest DA being apparent for M1, M5, and M11.

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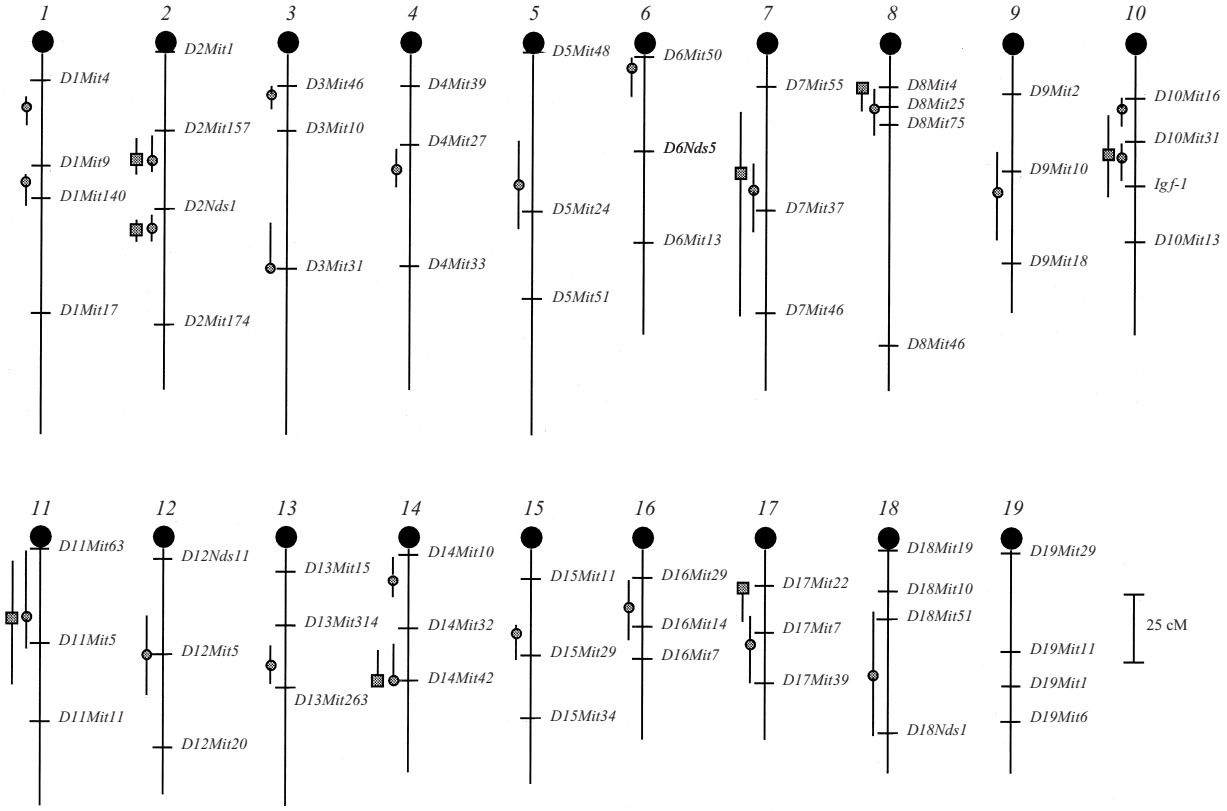

Fig. 2. Relative positions and confidence intervals of QTLs significant for the size (round symbols) of the mandible characters as well as DA (square symbols) in the mandible characters. The microsatellite molecular markers on each chromosome (four representative ones only on chromosomes 2, 13 and 15) are also shown.

None of the skewness ($-0{\cdot}18$ to $+0{\cdot}28$) and kurtosis statistics ($-0{\cdot}26$ to $+0{\cdot}67$) for the signed differences between sides reached significance, suggesting that these differences are normally distributed for all 15 characters. Further, regressions of these differences on the mean of the sides also showed no significance differences, so no scaling corrections were necessary. Correlations of these signed differences ranged from $-0{\cdot}56$ to $+0{\cdot}77$, averaging $+0{\cdot}18$. As evaluated by the sequential Bonferroni procedure, 69 of the 105 pair-wise correlations between these DA values are significantly different from zero.

FA values for the 15 mandible characters (Table 2) all are positive in sign since they were calculated from the absolute values of the scores of the second component from principal components analysis, as previously described. They are all also significantly different from zero, as expected. Correlations of these FA values ranged from $-0{\cdot}14$ to $+0{\cdot}50$, averaging $+0{\cdot}11$. Of the 102 possible correlations, 30 are significantly different from zero.

(ii) *Interval mapping*

Table 3 lists all QTLs found to affect the size of the mandible characters significantly. Each QTL in the table is designated as QTL-M 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. Preliminary multiple regression analyses indicated that at least one marker on each of the 19 autosomes was significant, so the canonical correlation analyses that generated these results for each chromosome made use of 18 conditioning markers in all cases. LOD scores exceeded the suggestive critical values at the 1% level determined from permutation runs (range 2·41 to 3·26, mean = 2·68) in all autosomes except numbers 18 (which exceeded the 5% level) and 19. Further, all LOD scores except that for the QTLs on chromosomes 5 and 18 exceeded the 5% experimentwise threshold value of 3·252. Two QTLs were also detected on chromosomes 1, 2, 3, 10 and 14, making a total of 23 QTLs significantly affecting the size of one or more mandible characters. Locations of these QTLs are given by the distance in centimorgans from both the proximal marker and the centromere, and are also depicted in Fig. 2. Confidence intervals for all QTLs are also given in terms of the proximal marker and the centromere distances. The length of these confidence intervals varies considerably among the QTLs, averaging 23 cM.

Table 4 lists the individual mandible characters

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Table 4. *Means for the percentage of variation accounted for, and absolute, standardized a values for all QTLs significantly affecting the size of the 15 mandible dimensions. Characters with positive or negative a values also are listed*

QTL	Mean %	Mean absolute *a*	Characters with $+a$ value	Characters with $-a$
QTL-M1.1	2·60	0·536	10, 13	—
QTL-M1.2	2·30	0·430	—	3, 9, 10, 12, 13, 14, 15
QTL-M2.1	4·06	0·394	4, 6, 7, 8, 10,12, 14, 15	1
QTL-M2.2	3·76	0·386	5, 9, 11, 13	6
QTL-M3.1	2·23	0·280	5, 9, 10, 13	—
QTL-M3.2	1·98	0·253	4, 5, 15	7
QTL-M4.1	5·18	0·487	3, 6, 7, 8, 10, 12, 14, 15	—
QTL-M5.1	1·85	0·264	6, 7, 8, 10	—
QTL-M6.1	3·38	0·352	5	2, 3, 6, 7, 8, 11, 13
QTL-M7.1	5·62	0·466	1	3, 4, 6, 7, 8, 10, 12, 14, 15
QTL-M8.1	2·60	0·402	1, 2, 3, 9, 12, 13, 14	6
QTL-M9.1	2·31	0·313	1, 4, 5, 9, 15	11
QTL-M10.1	1·65	0·379	13	—
QTL-M10.2	1·69	0·369	11	4, 8, 10, 15
QTL-M11.1	3·07	0·347	2, 3, 6, 7, 8, 12, 13, 14	—
QTL-M12.1	7·28	0·487	—	3, 6, 7, 8, 9, 10, 12, 14, 15
QTL-M13.1	2·39	0·277	10, 11, 13	5, 12, 15
QTL-M14.1	2·23	0·363	—	3, 4, 5, 8, 9, 12, 14, 15
QTL-M14.2	1·50	0·255	—	7
QTL-M15.1	3·46	0·330	1, 2, 3, 4, 5, 8, 9, 15	—
QTL-M16.1	3·59	0·366	1, 2, 3, 4, 12, 14, 15	—
QTL-M17.1	2·55	0·284	6, 8, 11, 12, 15	1
QTL-M18.1	2·11	0·337	—	2, 4, 11, 12, 15

Table 5. *Locations and confidence intervals (CI) of significant QTLs for directional asymmetry in the mandible characters, given as map distances from the nearest proximal marker and from the centromere. LOD scores from the tests of significance are also given*

QTL	LOD	Proximal Marker	Marker distance	Centromere distance	Marker CI	Centromere CI
QTL-D2.1	5·62**	*D2Mit61*	2	36	*D2Mit57 + 10–D2Mit37 + 0*	25–43
QTL-D2.2	—	*D2Mit224*	4	69	*D1Mit164 + 2–D2Mit166 + 2*	65–72
QTL-D7.1	2·38*	*D7Mit55*	26	41	*D7Mit55 + 6–D7Mit46 + 0*	21–91
QTL-D8.1	3·67**	*D8Mit4*	0	14	*D8Mit4 + 0–D8Mit25 + 2*	14–23
QTL-D10.1	3·18**	*D10Mit31*	4	33	*D10Mit16 + 6–Igf-1 + 8*	22–49
QTL-D11.1	2·17*	*D11Mit63*	28	30	*D11Mit63 + 8–D11Mit5 + 16*	10–53
QTL-D14.1	2·19**	*D14Mit42*	0	48	*D14Mit32 + 10–D14Mit42 + 0*	40–48
QTL-D17.1	2·17*	*D17Mit22*	0	19	*D17Mit22 + 0–D17Mit22 + 12*	19–31

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

whose size is significantly affected by each of the 23 QTLs. It also provides estimates of the percentage of variation accounted for and the difference between the homozygote and heterozygote (*a*) for each QTL, standardized by division by the standard deviation of the character (Table 2) in each case. For summary purposes, these effects are averaged over all affected characters rather than displayed individually. There is considerable diversity in the number of mandible characters significantly affected by these QTLs, two QTLs (QTL-M10.1 and QTL-M14.2) affecting only one character and others (QTL-M7.1) affecting as many as 10 of the 15 mandible characters. The distribution of affected characters is quite uniform, however, all mandible characters being significantly affected by seven to 12 different QTLs. The mean percentage of variation accounted for by each of the 23 QTLs varies from 1·2% to 14·5% (mean = 3·4%) in the individual mandible characters, although this range is more restricted (about 1·5–7·3%) when averaged over all characters affected by each QTL. The standardized homozygote/heterozygote differ-

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Table 6. *Correlations of each of the DA characters with their canonical vectors generated for five QTLs that showed multivariate significance but for which no individual character reached significance* (*see text*)

	QTL-D2.1	QTL-D2.2	QTL-D7.1	QTL-D10.1	QTL-D14.1
DA1	−0·066	−0·201	−0·042	0·038	−0·160
DA2	0·015	0·256	−0·071	0·049	−0·153
DA3	−0·039	0·222	0·101	0·082	−0·067
DA4	0·164	0·235	0·048	0·102	0·128
DA5	0·102	0·368	−0·335	0·148	0·235
DA6	−0·200	0·192	0·028	−0·007	−0·189
DA7	−0·213	0·177	0·130	0·093	−0·065
DA8	−0·080	0·089	0·051	0·040	0·046
DA9	−0·115	−0·220	−0·228	0·217	0·199
DA10	−0·060	0·039	0·266	0·026	0·090
DA11	−0·057	−0·138	−0·234	−0·133	0·047
DA12	−0·035	−0·383	0·053	0·196	0·210
DA13	−0·124	0·026	0·036	−0·272	−0·131
DA14	−0·049	−0·275	0·267	0·141	0·200
DA15	0·144	−0·278	0·276	0·218	0·298

Table 7. *The percentage of variation accounted for, and a values and their standard errors for all QTLs significantly affecting DA in the mandible characters*

QTL	Trait	%	*a*	Standard error
QTL-D8.1	DA11	3·36	0·033**	0·0096
QTL-D11.1	DA5	1·85	−0·048*	0·0189
	DA11	2·52	0·041**	0·0137
QTL-17.1	DA1	2·98	−0·051**	0·0157
	DA9	3·09	−0·054**	0·0165

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

ences (*a*) produced by these QTLs range from −0·775 to +0·769, the mean of their absolute values being 0·375 (Table 4). The positive signs for 80 of the 139 total *a* values suggests that the effect of the M16i allele is to increase the size of many of the mandible characters, although this allele also acts to decrease mandible characters in the other 59 cases. Finally, it should be noted that alleles at 13 of the 23 QTLs consistently increase (or decrease) the size of all mandible characters that they significantly affect. Alleles at the other 10 QTLs increase the size of some mandible characters while decreasing the size of others, although only one of these (at QTL-M13.1) affects more than one character in each direction.

Table 5 lists all putative QTLs found to affect DA in the mandible characters significantly. Preliminary multiple regression analyses indicated that at least one marker on each of 11 chromosomes (numbers 2, 7, 8, 9, 10, 11, 13, 14, 15, 17 and 18) was significant and so these markers were used for conditioning in the canonical correlation runs. These runs identified eight QTLs (designated QTL-Ds) whose LOD scores exceeded the suggestive threshold values at the 5% (range 1·64 to 2·30, mean = 1·89) or 1% level (range 2·51 to 3·08, mean = 2·69), although only three of these (QTL-D2.1, QTL-D2.2 and QTL-D8.1) exceed the 5% experimentwise threshold value of 3·281. Thus there appear to be eight QTLs significantly affecting DA: two on chromosome 2 and one each on chromosomes 7, 8, 10, 11, 14 and 17. Locations of these QTLs are given as before (Table 3), and are also illustrated in Fig. 2. Except for QTL-D17.1, locations of these QTLs appear to be about the same as those already found for the size of the characters themselves (Fig. 2).

Five of these QTLs (QTL-D2.1, QTL-D2.2, QTL-D7.1, QTL-D10.1 and QTL-D14.1), however, did not generate significant *a* values for DA in any of the individual mandible characters. To explore the possible reasons for this, correlations of the 15 DA characters with the canonical vector generated in the canonical correlation analyses were inspected (Table 6). As may be seen, these correlations tend to be quite mixed in sign in all five vectors, suggesting that each of the vectors produced is a complex contrast of the DA characters. In addition, none is particularly high

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in magnitude, the (absolute) value of the highest correlation being about 0·38.

The effects of the remaining three (QTL-D8.1, QTL-D11.1, QTL-17.1) of these eight putative QTLs that did generate significant *a* values for DA in the individual mandible characters are shown in Table 7. These three QTLs significantly affect DA in four characters: M1, M5, M9 and M11. On average, they explain 2·76% of the variation in DA, and they also produce an average absolute *a* effect of 0·045 mm.

Canonical correlation runs were also done for FA in the 15 characters, this time conditioning with only one marker (on chromosome 1) that reached significance. None of the highest LOD scores produced for any of the chromosomes (range 0·07 for chromosome 17 to 1·72 for chromosome 4) exceeded the suggestive linkage scores at the 5% level (range 1·41 to 1·93, mean = 1·58) derived from permutation runs as previously described. It was therefore concluded that no QTLs could be detected for FA in the mandible characters in this population of mice.

4. Discussion

The aim of this study was to discover any QTLs affecting either DA or FA in the 15 mandibular characters measured in the backcross mice. It should therefore be borne in mind that the QTLs found reflect only those loci whose alleles differ between the M16i and CAST inbred strains. Mice from these strains were originally chosen because they differed considerably in mean body weight and body weight gain and thus the use of their backcross progeny should have optimized the search for QTLs affecting these sorts of characters (Pomp *et al*., in preparation). Mandibular asymmetry levels in these inbreds were not measured, however, so the extent of the differences in asymmetry between these two strains is unknown. However, the inbreeding process ought to have randomly fixed alleles that differ between the two strains, including alleles at loci other than those affecting body size. Thus the prospects for detection of QTLs affecting asymmetry in the mandible characters would seem as reasonable in this population as they were in the F2 progeny from a cross of the Large and Small strains previously used by Leamy *et al*. (1997).

(i) *QTLs for fluctuating asymmetry*

Whatever the true prospects for QTLs affecting asymmetry in the backcross mouse population, none were found for FA in the mandibular characters. This result was originally hypothesized because it is basically the same as that found by Leamy *et al*. (1997). In that study, 11 QTLs significantly affected FA in individual mandibular characters, but this was close to the number (9·5) expected by chance alone (Leamy *et al*. 1997). With the multivariate approach used here, roughly one QTL (19 chromosomes $\times$ 0·05 = 0·95) would be expected to reach significance at the 5% level by chance alone, so several QTLs significantly affecting FA would have to be discovered before they could be accepted as genuine. However, it might be argued that this multivariate approach was not optimal for identifying QTLs for these FA characters since it is more effective for characters that are moderately to highly correlated (Leamy *et al*., 1999). It is true that levels of FA in different characters often are relatively independent (see Palmer, 1994), but correlations between the FA values in the closely related mandible characters sometimes were significant and thus these values exhibited a reasonable degree of integration (Leamy, 1994). In addition, original QTL runs done for FA in each of the 15 characters using the MAPMAKER/QTL program (Lincoln *et al*., 1992) resulted in only seven QTLs significantly affecting FA, well below the number (0·05 $\times$ 15 characters $\times$ 19 chromosomes = 14·3) expected by chance alone.

We are therefore left with the conclusion that, at least for the mandible characters in this population of mice, there is no detectable genetical basis for FA. This is not particularly surprising since it is consistent not only with the QTL study by Leamy *et al*. (1997) already discussed, but with a recent study that assessed the heritability of FA in mouse mandibles (Leamy, 1999). In that study (Leamy, 1999), midparent heritability estimates for FA in 10 mandible characters averaged only 0·03 and none were statistically significant. A single QTL could account for this low proportion (3%) of the total variation in a given character, so perhaps at most we should have expected only one or two QTLs affecting FA in the mandibles used here. If so, this suggests that credible detection of genetic variation for a character such as FA would be difficult even with the rather sensitive QTL mapping approach.

The role of dominance may be relevant in understanding the failure to detect QTLs for FA. Dominance has often been implicated as a causative factor of FA (Livshits & Smouse, 1994), in part because FA levels in various characters (including mandible dimensions) are often greater in inbred compared with hybrid individuals (Leamy, 1984, 1992). Dominance was certainly important in the study by Leamy *et al*. (1997), where putative QTLs for FA generally exhibited statistically significant levels of dominance that were greater in magnitude than those seen for DA in the mandible characters used. Further, Klingenberg & Nijhout (1999) recently used a developmental model to simulate character variation and devel-

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opmental noise, and discovered that dominance for FA was an important consequence of the model. If dominance was important for FA in the mandible characters used in this study as well, it is possible that some QTLs for FA may have gone undetected if they exhibited a magnitude and direction of dominance that resulted in little difference between the homozygous (M16i/M16i) and heterozygous (M16i/CAST) genotypes.

It also is possible that epistatic interactions among some QTLs may have affected FA in the mandible characters. Epistasis previously has been surmised to play a role in generating FA (McKenzie & Clarke, 1988), and along with dominance as already mentioned, it showed up as an important source of genetic variation of FA generated in the developmental model used by Klingenberg & Nijhout (1999). Epistatic effects of QTLs for FA could not be tested since no such QTLs were found, but it was possible to test for potential epistasic effects on FA generated by the 23 QTLs that affected the size of the mandible characters. To do this, the multivariate significance of interactions of the regressions of the 15 FA values on the imputed genotypic values for the 253 pairwise combinations of (imputed genotypic values for) the 23 QTLs was calculated. Twelve of these 253 tests reached multivariate significance, about the same as the number (12·7) expected by chance alone, suggesting that epistasis at least of these QTLs is not significantly affecting FA in the mandible characters.

The concept that FA is largely or entirely environmentally determined is often taken as a working hypothesis by investigators who use FA, especially in comparisons of stressed and unstressed populations (see Palmer *et al*., 1994). Although this assumption is clearly supported by our results, it should be emphasized that heritable genetic variation for FA has been found for some characters in various organisms (Leamy, 1997; Whitlock & Fowler, 1997). No doubt the choice of characters is critical, and mandible dimensions may not be optimal for detecting genetical variability in FA, especially if it is expected to be at such a low level. A few QTLs for FA were detected, for example, for discrete skeletal characters in mice (Leamy *et al*., 1998). Another possibility is that the expression of any genes for FA is best seen only in a stressful environment. If so, approaches such as this one may have little real chance to detect QTLs for FA unless an appropriate stressor is applied during development of the characters chosen for use. On the other hand, Klingenberg & Nijhout (1999) argue that there simply may be few, if any, genes for FA that are distinct from those that govern the size (mean of the two sides) of a given bilateral character, because genetic variation in FA may be a natural consequence of genetic variation in the development of the character.

(ii) *QTLs for directional asymmetry*

In contrast to the situation for FA, the canonical correlation results suggested that eight QTLs on seven different chromosomes (including two on chromosome 2) significantly affected DA. But it will be recalled that the regression analyses showed that five of these QTLs did not significantly affect DA in the individual mandibular characters. This apparently happened because, as judged by the correlations of the DA values with the canonical vectors generated in the canonical correlation analyses (Table 6), the associations among the DA values were complex. In contrast, the 15 correlations of the mandible size characters with their canonical vectors (not shown previously) typically were mostly of the same sign and one or more were moderate to high (0·5+) in magnitude. Incidentally, canonical correlations of the first three components generated from principal components analysis of the DA characters with the imputed genotypic values calculated at the sites of these putative QTLs on each of the four chromosomes, failed to show statistical significance. Thus it is apparent that these are not sites of QTLs affecting either individual DA characters or independent characters generated from these DA values.

In any event, the result was that QTLs were found on three chromosomes only (8, 11 and 17) that significantly affected DA in four different characters: M1, M5, M9 and M11. It is not particularly surprising that significant QTLs were found for DA in M1, M5 and M11, for these were the three characters that showed the highest overall levels of DA (see Table 2). The overall level of DA in M9 was considerably lower, although still statistically significant. Leamy *et al*. (1997) discovered QTLs significantly affecting signed differences in two mandible characters which did not even exhibit significant DA. This is possible if different alleles at these QTLs act in opposite directions to generate a mean right minus left difference close to zero (Leamy *et al*., 1997).

Although significant QTLs for DA in the mandible characters were hypothesized primarily because they were discovered in the QTL study by Leamy *et al*. (1997), their numbers and effects were quite small. The average percentage contribution of these few QTLs ranged from 1·9 % to 3·4 % (Table 6), averaging 2·8 %. And if we assume no contribution for DA in the 11 characters not significantly affected, this average drops even further to 0·9 %. Thus the average genetic variance of DA accounted for in these characters is about 1 %, even less than the quite low average of 3–6 % for comparable estimates previously made for DA in mandible characters (Leamy, 1984, 1999; Leamy *et al*., 1997).

What might be the nature of these three QTLs affecting DA in the mandible characters? First, it

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seems apparent that they do not correspond to those previously found by Leamy *et al*. (1997) in the F2 intercross of the Small and Large strains. In that study, 16 QTLs on seven different chromosomes significantly affected DA, although use of the multivariate canonical approach (without conditioning) for those same data produced QTLs for DA that were restricted to four chromosomes (3, 7, 10 and 15) entirely different from the three (8, 11 and 17) identified in this study. Inspection of the locations of the QTLs on chromosomes 8 and 11 affecting DA (Fig. 2), suggests that they are fairly close to those (QTL-M8.1 and QTL-M11.1) affecting the size of several of the mandible characters. Thus it is possible that at least two of these three QTLs for DA are the same as those affecting size of the mandibles. Leamy *et al*. (1997) found this same trend as well, although discovered that the QTLs for DA in a given character generally were in locations close to QTLs affecting the size of other mandible characters. Perhaps QTLs for DA in a given character may often turn out to be those that control the overall size in that character or another closely related character.

(iii) *QTLs for size*

Beyond these three QTLs for DA, many other QTLs were discovered which affected the size of one or more of the mandible characters in the backcross mice. QTLs on chromosomes 1, 2, 4, 6, 12, 13, 15 and 18 correspond roughly in location with those already found to affect weight and weight gain in these mice, and thus it is possible that some may be genes influencing growth generally. The remaining QTLs (on chromosomes 3, 5, 7, 9, 10, 14 and 16) do not match up with those already found for overall growth, however, and thus may be other general growth genes or genes that are more specific in their effect on the mandible characters. Some of the QTLs for mandible size may also be the same as those discovered (on chromosomes 1, 2, 5, 7, 10, 12, 17 and 18) that affect the lengths of the limb bones in these mice (Pomp *et al*., in preparation). Additional analyses that involve fine-scale mapping, however, will be necessary to resolve the precise nature of these QTLs.

(iv) *Conclusion*

In conclusion, QTLs were found for DA, but not FA, in the mandible characters and thus both original hypotheses were supported. For bilateral characters such as these mandible dimensions, therefore, FA could safely be used as a measure of developmental stability. Genetic variation was present for DA, but only in some of the mandible characters, and its level was so low as to make it virtually negligible. Although DA is not generally considered useful as a measure of developmental stability (Palmer *et al*., 1994), it does

Appendix.

Microsatellite markers genotyped and their chromosomal location in Haldane units (cM). The location of the first marker on each chromosome was taken from the Mouse Genome Database. Markers for *Agouti*, *Ghrh*, and *Ppara* were PCR-RFLP.

Marker	cM	Marker	cM	Marker	cM	Marker	cM
D1Mit	12	*D2Mit200*	105	*D10Mit16*	16	*D15Mit131*	12
D1Mit9	45	*D3Mit46*	14	*D10Mit31*	29	*D15Mit86*	19
D1Mit140	55	*D3Mit10*	35	*Igf-1*	41	*D15Mit121*	23
D1Mit17	97	*D3Mit31*	75	*D10Mit13*	57	*D15Mit3*	30
D2Mit1	1	*D4Mit39*	11	*D11Mit63*	2	*D15Mit64*	35
D2Mit79	13	*D4Mit27*	36	*D11Mit5*	37	*D15Mit29*	39
D2Mit120	15	*D4Mit33*	78	*D11Mit11*	67	*D15Mit107*	44
D2Mit157	30	*D5Mit48*	1	*D12Nds11*	6	*Ppara*	48
D2Mit61	34	*D5Mit24*	60	*D12Mit5*	41	*D15Mit34*	62
D2Mit37	43	*D5Mit51*	92	*D12Mit20*	75	*D16Mit29*	13
D2Nds1	53	*D6Mit50*	3	*D13Mit15*	10	*D16Mit14*	33
D2Mit103	58	*D6Nds5*	36	*D13Mit181*	16	*D16Mit7*	45
D2Mit133	60	*D6Mit14*	70	*D13Mit311*	20	*D17Mit22*	19
D2Mit164	63	*D7Mit55*	15	*D13Mit314*	29	*D17Mit7*	33
D2Mit224	65	*D7Mit37*	57	*D13Mit160*	31	*D17Mit39*	45
D2Mit166	70	*D7Mit46*	97	*D13Mit36*	37	*D18Mit19*	2
D2Mit22	73	*D8Mit4*	14	*D13Mit51*	41	*D18Mit10*	17
Agouti	75	*D8Mit25*	21	*D13Mit53*	50	*D18Mit51*	27
Ghrh	76	*D8Mit75*	26	*D13Mit263*	52	*D18Nds1*	73
D2Mit49	80	*D8Mit42*	110	*D14Mit10*	3	*D19Mit29*	4
D2Mit25	90	*D9Mit2*	17	*D14Mit32*	30	*D19Mit11*	38
D2Mit147	93	*D9Mit10*	43	*D14Mit42*	48	*D19Mit1*	52
D2Mit174	101	*D9Mit18*	75	*D15Mit11*	10	*D19Mit6*	64

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also seem to be overwhelmingly environmental in its origin, at least in these characters in this particular population of mice.

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