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Effects of alleles at the agouti locus on minor skeletal variants in C57BL/6 house mice

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Summary

In this study, five separate alleles at the agouti locus in house mice were tested for potential effects on a battery of 13 minor skeletal variants. Six genotypes (*aa*, a^ta, a^ta^t, *Aa*, $A^{vy}a$, and A^ya) were compared on a standard congenic background (C57BL/6). In log-linear analyses, three of the 13 characters showed significant genotype differences (another three were close to significance), and genotypes also exhibited overall significance in a multivariate randomization test. Both multidimensional scaling and clustering showed an association of *aa* with a^ta, *Aa* with $A^{vy}a$ and A^ya, and a general separation of a^ta^t from the other genotypes. Genotype differences averaged 0·63 in probit standard deviations, 0·09 when assessed by the mean measure of divergence. Since the general magnitude of effects of these major genes was quite similar to those previously estimated for presumptive polygenes from subline divergence studies, it was concluded that major genes may often act as polygenes and make important contributions to the variation in minor skeletal variants.

1. Introduction

Minor skeletal variants, typical examples of which are the presence or absence of various foramina and the fusion or nonfusion of various bones, are ubiquitous in skeletons of mammals. Although phenotypically discrete, these sorts of traits have an underlying continuous distribution which, according to recent heritability studies (Self & Leamy, 1977; Cheverud & Buikstra, 1981), has a significant genetic basis. It also is known that major Mendelian genes often exert significant pleiotropic effects on these traits, the magnitude of which may be considerable (Grüneberg, 1963). Thus in a classic study with the C57BL inbred mouse strain, for example, Grüneberg (1955) found that the mean effect of seven major mutant genes (versus wild type alleles) on a battery of minor skeletal characters was 1·16 standard deviations. Further, this estimate also was considerably higher than the mean of about 0·6 standard deviations consistently obtained, again using minor skeletal variants, from presumptive single 'minor' gene differences among sublines of the C57BL strain (Grüneberg, 1955; Deol *et al.* 1957; Carpenter, Grüneberg & Russell, 1957; Grewal, 1962; Hoi-Sen, 1972).

If the discrepancy between the two sets of estimates is real, it would imply that effects of major genes on these kinds of quantitative characters are fundamentally greater than those of minor genes responsible for differences among sublines (and by inference, inbred strains). It is true that the subline divergence studies used generally larger sample sizes which may have permitted the detection of smaller differences, but this would only account for part of the discrepancy in these estimates (Grüneberg, 1955). A more likely possibility is that the mutants chosen by Grüneberg (1955) were not really representative of most 'major' genes in that they had large, almost pathological, effects on the skeleton. Non-skeletal genes such as those at coat colour loci are known to exhibit scattered pleiotropic effects on various characters in mice (Searle, 1968; Silvers, 1979), but their effects on minor skeletal variants have not yet been systematically assessed.

It would therefore seem worthwhile to test whether such coat colour genes do in fact significantly affect minor skeletal variants, and if so, to what extent. Basically this paper describes a study in which this was done with alleles at the agouti locus in C57BL/6 house mice, these mice previously having been used in a study with continuous skeletal traits (Schwam, 1974; Leamy & Sustarsic, 1978). The results depict the comparative effects of six agouti genotypes on 13 minor skeletal variants and so are interesting in their own right, but also are useful in furthering our understanding of the relationship between major and minor genes.

2. Materials and Methods

(i) *The Population and Variables*

The C57BL/6 strain of house mice was used in this study, the original inbreds being obtained from the Jackson Laboratory. Besides the standard genotype at the agouti locus, *aa*, other mice received were black-and-tan ($a^t a$), viable yellow ($A^{vy} a$) and yellow ($A^y a$) heterozygotes. The agouti gene (A) also was put on this same background (forming the heterozygote, Aa) via the backcross system (Green, 1966), and all four heterozygotes were compared to the *aa* genotype. In addition, the black-and-tan homozygote ($a^t a^t$) was constructed from heterozygote crosses ($a^t a \times a^t a$) in order to assess the effect of two doses of the a^t allele. Six classes of agouti genotypes were thus created, all sharing the same congenic background.

Table 1 lists these six genotypes with their associated phenotypes, origins, the number of generations involved both at origin and at the time of utilization, and the number of mice used in each sex and genotype. The genotypes are listed in rank order of increasing dominance from the recessive (*aa*) to the top dominant ($A^y a$) although the black-and-tan homozygote ($a^t a^t$) may not strictly be ranked. Further details regarding these agouti alleles and their specific coat colour effects may be found in Silvers (1979). It should be noted in Table 1 that the number of generations of backcrossing varies from 18 to 40, and about 8 generations is sufficient to ensure that nearly all alleles not closely linked with the agouti locus will have been replaced with genes from the inbred strain (Green, 1966). It is of course still possible that some non-C57BL/6 genes remain in these congenic strains, but even if so, they would be few in number and are assumed to be of little importance here.

The mice were reared in standard fashion in an animal room at room temperature, water and food (Purina Laboratory Chow) being provided *ad libitum* (Schwam, 1974). For use in this study, both homozygous genotypes (*aa* and $a^t a^t$) were generated by brother-sister matings, and all four heterozygotes by crosses of these heterozygotes with the *aa* genotype. All mice were sacrificed at 90 days of age, at which time body weights were taken. Skeletonization was accomplished by a modification of the papain digestion technique (Luther, 1949). Seven osteometric characters were measured on each skeleton in the original univariate analysis of the effects of the agouti alleles (Schwam, 1974), these plus body weight also corresponding to the B set of traits used in a subsequent multivariate analysis (Leamy & Sustarsic, 1978).

A total of 19 quasi-continuous skeletal variants (all on the skull or mandible) was chosen at random from lists given in previous studies, and every skeleton was scored for each of these. Of the 19, six (foramen sphenoidale medium, foramen ovale double, foramen ovale open posteriorly, foramen pterygoideus, frontal foramen, and metoptic sutures) turned out to be monomorphic and were therefore discarded. The remaining 13 characters chosen for use are listed in Table 2 along with alphabetic abbreviations and with references in which their complete descriptions are given. Four of these variants (3,4,5,6) are unpaired, and the remaining paired ones were scored on both left and right sides. Mice were included in the analysis only if each had all 13 traits scorable, the resultant total number available being 242.

(ii) *Statistical analysis*

All 13 skeletal variants were first coded dichotomously with one representing presence (on either side for the paired characters) and zero corresponding to absence of the character. Although such coding is somewhat arbitrary, this particular method has been conventionally used in most past studies with these variants (Grüneberg, 1955; Grewal, 1962) and was therefore also followed here. The means for each variant were then calculated for each genotype (and expressed as percentages) for a preliminary examination of potential patterns of effects of the agouti alleles on the variants.

Next, in order to test whether genotypes (as well as sexes) significantly affect the incidences of the skeletal variants, it was decided to use log-linear models (Sokal & Rohlf, 1981). These are basically the counterpart for frequency or attribute data to linear models used in the analysis of variance of continuous variables. Unlike the analysis of variance, however, the significance of a given factor in the log-linear model is tested for

Table 1. *Genotype, phenotype, origin, number of inbred (F) or backcross (N) generations at origin and time of utilization, and sample size for each of the six classes of agouti mice used*

Genotype	Phenotype	Origin	Number of generations at:		Sample size	
			Origin	Utilization	Male	Female
aa	Black	Jackson Lab	F62	F85	20	7
$a^t a$	Black-and-tan	Jackson Lab	N23	N40	18	17
$a^t a^t$	Black-and-tan	C57BL/6-$a^t a$ × C57BL/6-$a^t a$	—	N30-F_{12}	20	21
Aa	Agouti	C3HeB/Fe onto C57BL/6	—	N18	13	12
$A^{vy} a$	Viable yellow	Jackson Lab	N10	N25	26	33
$A^y a$	Yellow	Jackson Lab	N22	N37	26	29

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Table 2. *The names of the 13 skeletal variants used with their abbreviations and references for descriptions*

Skeletal variant	Abbreviation	Reference
1. Accessory mental foramen	ACCMF	(Deol, 1955)
2. Mandibular foramen	MANDF	(Berry, 1963)
3. Interfrontal	INTER	(Truslove, 1952)
4. Fused frontal	FUSEDF	(Deol & Truslove, 1957)
5. Parted frontal	PARTF	(Truslove, 1952)
6. Frontal fontanelle	FFONT	(Deol & Truslove, 1957)
7. Maxillary foramen I	MAXF1	(Berry, 1963)
8. Maxillary foramen II	MAXF2	(Berry, 1963)
9. Foramen palatinus minor anterior	FPALMA	(Berry, 1963)
10. Foramen palatinus minor posterior	FPALMP	(Berry, 1963)
11. Preorbital foramen	PREFOR	(Berry & Searle, 1963)
12. Preoptic sutures	PRESUT	(Truslove, 1954)
13. Foramen hypoglossi	FHYPO	(Deol, 1955)

through its interaction with the response factor by fitting two models, one with and one without the corresponding interaction term. The significance of the factor is thus tested through it partial association with the response as indicated by the difference in goodness of fit between the two models. Goodness of fit is measured with the likelihood ratio χ^2 statistic, and the partial association tested by the χ^2 difference (Sokal & Rohlf, 1981). Three-way frequency tables with sex, genotype, and 'response' (e.g. 0 or 1) as the three factors were therefore constructed for each character, and these were analysed with log-linear models via BMDP4F (Dixon, 1983). χ^2 difference values were used to test for the significance of partial associations of response with sex (1 D.F.), genotype (5 D.F.), and the sex by genotype interaction (5 D.F.).

Beyond univariate tests of the significance of genotypes and sex on the incidences of the skeletal variants, it is useful to conduct multivariate tests of these factors as well to see whether their effects are significant over all variants. Unfortunately, there were too many variants to attempt this with the log-linear model, and because dichotomous data cannot closely approximate a multivariate normal distribution, a conventional multivariate analysis of variance also could not be used. Multivariate tests of significance for the two factors and their interaction were therefore accomplished with randomization procedures (Edgington, 1980). Basically, the randomization test is a non-parametric technique which generates a probability for a given sample based on all possible outcomes obtained by randomly rearranging variates or frequencies, and is becoming increasingly used in biological applications (Sokal & Rohlf, 1981).

The randomization test was carried out with an unbalanced two-way layout (sex by genotype) using equal cell weights. Ordinary *F*-values served as test statistics, with the multivariate test employing Rao's (1971) monotonic transform of Wilk's lambda. The test statistics for the trials were computed with the program BMDP4V (Dixon, 1983). Randomization was done separately for sex and genotype with an APL program designed specially for this purpose. The null distributions were based upon 1000 such randomizations of the data. The number of these 1000 *F*-values equal to or exceeding the value of the test statistic (*F*) obtained with actual data for each of the three categories (sex, genotype and sex by genotype interaction) was used to assign an appropriate probability to that *F*.

One of the major thrusts of this study was to measure the magnitude of effect of the agouti alleles on the skeletal variants, especially so they could be compared to past studies assessing major genes effects and subline divergence. For this purpose, it was necessary to use two measures to quantify differences in the incidence of the skeletal variants among the six genotypes. In conformity with many of the early investigations (for example, Grüneberg, 1955), differences between each pair of genotypes for each variant were first computed in standard deviations from probit transformations.

In line with more recent investigations (for example, Hoi-Sen, 1972), mean measures of divergence (MMD) also were calculated for each pair of genotypes. The MMD was devised by C. A. B. Smith, and more recently refined by Sjovold (1973). It is calculated as:

$$\text{MMD} = \Sigma(\theta_1 - \theta_2)^2/13 - (1/n_1 + 1/n_2).$$

Here θ is the angular value for each trait in populations 1 and 2, and is calculated as $\sin^{-1}(1-2p)$ in radians, where p is the frequency with which a given trait is found. The sum of the squared differences between the angular values above is divided by the number of traits, in this case 13. The expression $1/n_1 + 1/n_2$ (where n is the sample size in each of the groups 1 and 2), is subtracted from this quantity because it represents the amount of variance expected from random sampling fluctuations (Sjovold, 1973). Once calculated, all MMD's were tested for significance (from 0) with the use of the χ^2 statistic (Sjovold, 1973).

In order to provide a clear picture of the relationships among the six genotypes, the MMD values were subjected to both ordination and clustering. The

ordination method chosen was multidimensional scaling (MDS), a 'nonmetric' technique which requires no more than ordinal relations in the original correlation or distance matrix, but which provides quantitative, metric results (Shephard, 1962*a*, *b*). Basically, MDS produces a multi-dimensional picture of groups (the six genotypes), and as in principal components analysis, the distances between the groups are taken to represent the strengths of their associations. Usually several dimensions are required for a satisfactory solution (one with low 'stress' – see Kruskal, 1964), although only two-dimensional results typically are depicted. Clustering of the MMD values also was done by the group average (UPGMA) method (Sneath & Sokal, 1973).

3. Results

Before the actual tests of significance of genotypes and sexes on the skeletal variants are presented, it is instructive to examine their percentage incidences for each of the six genotypes (Table 3). The sex by genotype interactions overall were not significant (see Table 4 below), so the two sexes are combined for ease in interpretation. Marginal means are provided in Table 3 for males and females, however, and give some idea of differences between the two sexes. For the pooled genotypes and sexes, the overall level of incidences varies considerably among traits, with the lowest (FUSEDF) averaging only about 4%, and the highest (PARTF) about 87%.

The incidences of the skeletal traits given in Table 3 are most useful in discovering any trends of effects of the agouti genotypes. Comparing incidences among the genotypes, it is apparent (Table 3) that *aa* and $a^t a$ are quite similar overall and generally distinct from the other four genotypes. Another apparent feature of these data is that the percentage incidence in the $a^t a^t$ genotype often is quite different from that in the other genotypes, being either the highest or lowest for 10 of the 13 traits. In any event, the incidences of the 13 skeletal traits do appear to vary among the genotypes.

The results of the univariate (log-linear) and multivariate (randomization) tests of significance for the 13 skeletal traits are given in Table 4. The multivariate results are given as F values from the original multivariate analysis of variance, their associated probabilities generated from the randomization procedure being 0·001 for sex, 0·007 for genotypes, and 0·189 for the sex by genotype interaction. From the log-linear analyses it may be seen that 5 of the 13 characters show significant sex differences, and as might be expected, the multivariate test also confirmed the overall significance of sex. Only one character (INTER) shows a significant sex by genotype interaction, however, and this essentially may be ignored since the multivariate test did not show overall significance for this category. Three of the 13 characters (INTER, FFONT, MAXF2) show significant genotype differences, and three others (MAXF1, FPALMA, and FHYPO) also nearly reached significance ($P < 0{\cdot}08$). Since genotypes also reached significance in the multivariate randomization test, it may be concluded that the agouti genotypes are definitely exerting an overall influence on the skeletal variants.

It is worth noting that the three probabilities for sex, genotype, and the sex by genotype interaction obtained from these randomization tests were nearly identical to those produced by the ordinary multivariate analysis of variance (0·000, 0·007, and 0·192). Actually, univariate tests also were done via randomization, and this correspondence held throughout these tests as well. In fact, the two sets of probabilities rarely diverged by more than 0·02, and never differed by more than 0·01 in the critical tail with values below 0·10.

Although the agouti genotypes significantly affect specific skeletal variants as shown in Table 4, it also is of interest to discover the relative effects of the genotypes on the variants. This can be obtained by quantifying differences among the six genotypes, as previously explained. Table 5 gives mean differences

Table 3. *Percentage incidence of the 13 skeletal variants among the six agouti genotypes (pooled sexes)*

	aa	$a^t a$	$a^t a^t$	*Aa*	$A^{vy}a$	$A^y a$	M–F
1. ACCMF	18·5	25·7	14·6	16·0	17·0	20·0	24·4–12·6
2. MANDF	74·1	68·6	58·5	64·0	72·9	67·3	77·2–58·0
3. INTER	63·0	80·0	100·0	76·0	81·4	69·1	79·7–78·2
4. FUSEDF	3·7	5·7	9·8	4·0	0·0	1·8	4·1–3·4
5. PARTF	100·0	88·6	80·5	84·0	83·1	89·1	87·0–86·6
6. FFONT	37·0	37·1	17·1	12·0	40·7	20·0	34·2–21·9
7. MAXF1	59·3	62·9	31·7	44·0	44·1	36·4	52·0–37·0
8. MAXF2	89·0	82·9	51·2	68·0	71·2	67·3	67·5–73·1
9. FPALMA	63·0	65·7	34·2	56·0	61·0	58·2	61·0–51·3
10. FPALMP	22·2	11·4	22·0	20·0	18·7	18·2	16·3–21·0
11. PREFOR	33·3	51·4	31·7	44·0	54·2	43·6	37·4–51·3
12. PRESUT	25·9	25·7	29·3	12·0	17·0	20·0	21·1–21·9
13. FHYPO	89·0	71·4	70·7	60·0	84·8	72·7	76·4–74·8

Marginal mean incidences for the separate sexes (Male = M, Female = F) also are given.

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Table 4. *χ^2 values for sex, genotype, and the sex by genotype interaction (SXG) from the log-linear analyses for each of the 13 skeletal variants*

	Sex	Genotype	SXG
1. ACCMF	5·73*	1·91	9·19
2. MANDF	10·30**	2·89	3·90
3. INTER	0·46	26·65**	11·22*
4. FUSEDF	0·04	8·37	3·61
5. PARTF	0·08	10·18	4·17
6. FFONT	4·66*	15·17**	3·48
7. MAXF1	4·48*	10·33	8·49
8. MAXF2	1·96	16·21**	7·69
9. FPALMA	2·17	10·40	2·43
10. FPALMP	1·07	2·06	4·60
11. PREFOR	3·93*	6·34	5·88
12. PRESUT	0·07	4·43	6·72
13. FHYPO	0·02	9·97	4·42
F value	3·15**	1·50**	1·16

F values from the multivariate tests of significance for each of these factors also are given. *$P < 0·05$; **$P < 0·01$.

among the genotypes calculated as probit standard deviations (below diagonal), and as MMDs (above diagonal). The values in the Table were obtained by pooling over the sexes, this being permissible because of the absence of significant sexual dimorphism in these measures. The probit differences average 0·63 overall, ranging from 0·34 for the difference between the two yellow genotypes to over 1 for the *aa*-a^ta difference. Clearly, therefore, the black-and-tan heterozygote appears to be exerting the greatest overall effect on the skeletal variants.

The 15 MMD's range from 0·013 to 0·291, their overall average being 0·093. The highest five measures, all of which are significant at the 1% level, are associated with the a^ta^t genotype. If these five are omitted, the mean of the remaining 10 MMD's drops to 0·039, The *aa* genotype also shows significant differences (but at $P < 0·05$) from each of the three genotypes containing the dominant agouti (*A*) gene, and also the black-and-tan heterozygotes are significantly different from the viable yellow genotype. In general, there is an association of *aa* with a^ta, and another of the agouti, viable yellow, and yellow genotypes, whereas the black-and-tan homozygote is distinct from all others. Probit differences show basically this same pattern, and in fact the product-moment correlation between the two measures of differences is quite high (+0·88).

To depict the strength and patterns of effects of the six agouti genotypes on the skeletal variants, the MMD values from Table 5 were subjected to both ordination (multidimensional scaling) and clustering. Results from multidimensional scaling (A) and arithmetic average clustering (B) of the MMD values (Table 5) are shown in Fig. 1. Basically the same patterns already evident from the overall incidence and especially from the significance of the MMD's themselves are obvious in the Figure. That is, the *aa* and a^ta genotypes associate together, as do the *Aa*, $A^{vy}a$, and A^ya genotypes, but the black-and-tan homozygote is set apart from all others. This pattern is apparent both in the distance between the genotypes in the multidimensional scaling results and in the clusters formed in the arithmetic average clustering. Incidentally, other clustering strategies tried (complete linkage, single linkage) also gave comparable results.

4. Discussion

(i) *Agouti allele effects*

The results of this study indicate that alleles at the agouti locus significantly affect minor skeletal variants, at least in the C57BL/6 house mice used here. Only three of the 13 characters reached individual significance in the log-linear analyses (although another three were close), but the multivariate test clearly indicated overall differences in the incidences of these variants among the six genotypes. Although it is possible that this effect (or part of it) is really due to some other polygenes closely linked to the agouti locus, the pattern of the effect (see below) argues against this. Further, in two previous studies, the specific agouti genotypes used here were found to significantly affect both molar widths (Leamy & Hrubant, 1971) as well as three skull dimensions and body weight (Schwam, 1974). There also is a mounting literature which suggests that various coat colour genes exert pleiotropic

Table 5. *Mean measures of divergence (above diagonal) and probit standard deviation differences (below diagonal) among the six genotypes (sexes pooled)*

	aa	a^t	a^ta^t	*Aa*	$A^{vy}a$	A^ya
aa	—	0·015	0·291**	0·080*	0·056*	0·048*
a^ta	0·607	—	0·216**	0·038	0·038*	0·044
a^ta^t	1·112	0·763	—	0·137**	0·230**	0·136**
Aa	0·757	0·508	0·786	—	0·030	0·023
$A^{vy}a$	0·645	0·470	0·843	0·483	—	0·013
A^ya	0·644	0·395	0·681	0·458	0·344	—

*$P < 0·05$; **$P < 0·01$.

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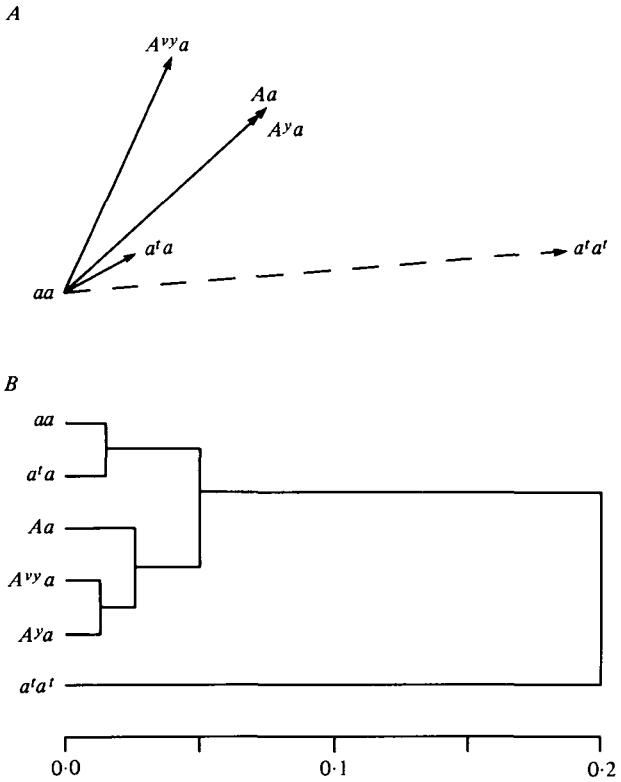

Fig. 1. Results from multidimensional scaling (*A*) and arithmetic average clustering (*B*) of the MMD values between the agouti genotypes. In *A*, solid lines connect the *aa* genotypes with all heterozygous genotypes, and a dashed line connects the *aa* genotype with the homozygous $a^{t}a^{t}$ genotype.

effects on a number of different kinds of characters (Searle, 1968; Silvers, 1979).

Whatever the pathway involved in the effect of the agouti alleles on the skeletal variants, it does not appear to be via overall body size. Thus a preliminary analysis of covariance conducted with body weight as a covariate yielded basically the same results (individual significance of genotypes for INTER, FFONT, and MAXF2; multivariate significance for genotypes) as already described. This same conclusion also was reached in both previous studies with the agouti alleles (Leamy & Hrubant, 1971; Schwam, 1974), for in spite of the fact that the viable yellow and yellow genotypes showed the highest overall body weight, both tooth and bone dimensions generally exhibited an orderly decrease in size from the recessive (*aa*) to the top dominant ($A^{y}a$) genotype.

The agouti genotypes themselves exhibited a clear pattern of effect on the minor skeletal variants. One major feature of this pattern was the distinctiveness of the $a^{t}a^{t}$ genotype compared to all others, and in fact, a preliminary multivariate analysis of variance run without this genotype failed to generate overall significance for the genotypes. It is true, of course, that of the five genotypes used to compare to the standard *aa* type, only $a^{t}a^{t}$ is homozygous. But it is difficult to say whether the other alleles, if made homozygous, would have had a similar effect on the skeletal variants. Of the four heterozygous genotypes, the general separation of $a^{t}a$ from the others is particularly interesting, for it suggests that a real pleiotropic distinction exists in the action of the 'recessive' *a* versus the 'dominant' *A* gene. This genotypic clustering pattern, incidentally, is roughly similar to that previously found for various metric characters (see Leamy & Sustarsic, 1979). The precise correspondence is a weak one, however, for product-moment correlations of the MMD values from this study with distances (Mahalanobis D values) among genotypes generated from the tooth (+0·09) and bone (+0·37) dimensions (Leamy & Sustarsic, 1979) both are non-significant.

(ii) *Magnitude of agouti allele effects*

Given that the agouti alleles do significantly affect the minor skeletal variants, comparison of the magnitude of their effects to those of previous studies is of special interest. The overall average effect of 0·63 (probit standard deviations) certainly is lower than the previously-cited estimate of 1·18 made by Grüneberg (1955) for the effects of 7 major genes. If only those eight agouti genotype differences reaching significance (Table 5) are used, the average increases to 0·74, but this is still less than 1·18. Grüneberg's (1955) estimate was undoubtedly biased to some extent, for the samples he used were probably too small to generate significance for differences less than about 0·5 S.D. But it also is possible that the mutants he chose may not have been entirely representative of the majority of major mutants in regard to their effects on minor skeletal variants. Only when we have more estimates of effects from a greater variety of mutants than just the limited sample used here, however, will we more fully be able to assess this.

What is particularly interesting about the overall mean magnitude of agouti allele effects is that it is precisely in the range of the estimates previously found in subline divergence studies (Grüneberg, 1955; Deol *et al.* 1957; Hoi-Sen, 1972). Nearly all of these studies have yielded estimates of around 0·6 standard deviations for presumed single 'mutations' in the C57BL inbred mouse strain. Grewal (1962) has plotted subline divergence measured in MMD's with the number of generations of separation, the slope of this plot suggesting an average divergence per generation per character of about 0·003. Thus the average divergence shown by the agouti genotypes, 0·09, might be expected from mutations occurring during roughly 30 generations (0·09/0·003) of subline separation. This would be enough time for significant subline divergence to have occurred, and in fact, the MMD average of about 0·09 is close to that of 0·11 generated between the 6p and 6JAX sublines of the C57BL strain (Grewal, 1962). The MMD value between the *aa* and $a^{t}a^{t}$ genotypes, 0·29, is comparable to the differences between British and American sublines of the C57BL strain ac-

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cumulated over some 90 generations of separation (Grewal, 1962).

(iii) *Major versus minor genes*

From the evidence at hand, it is apparent that the agouti alleles, which are 'major' genes, can and do contribute to the genetic variance in quantitative characters such as the skeletal variants used here. Further, the magnitude of their effect is so similar to that previously estimated for 'minor' genes, that distinguishing between the two kinds of genes in this particular case would be very nearly impossible. This suggests that some of the 'minor' genes involved in producing polygenic variation in these sorts of characters may in fact be at 'major' loci, or in other words, that in some cases polygenes and major genes are identical. This conclusion was reached many years ago by Grüneberg (1955) and also more recently by Leamy (1981).

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