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The effect of replicated selection for body weight in mice on vertebral shape

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Summary

The shapes of T1 and T2 vertebrae from unselected Q strain mice and from strains selected for large and small body size were studied by Fourier analysis in order to ascertain whether shape change was produced by size selection. The vertebrae of large, small and control strains were easily distinguishable, but between replicate groups shape differences were less marked. The main component of shape change was size related, but mice unselected for size also showed a non-size-related shape change.

1. Introduction

Those biological variables which are under genetic control are amenable to selection. It is possible to take a population, say of dogs, and to modify their shape and size by breeding from those which most closely approach an externally applied set of desiderata so as to produce a mastiff, a bulldog or a chihuahua.

Classical theory suggests that when we choose size as a criterion for selection we also modify shape. This size-related shape change is usually thought of as the result of mechanical influences: engineering mathematics dictates that because of surface area to volume relations a large cylinder must be proportionately thicker than a small one of the same strength. Galileo noted that the cylindrical long bones of mammals obeyed this rule. The problem was lucidly discussed by D'Arcy Thompson (1961, ch. 2). Although this approach is classically confined to comparison between species we see no reason why it should not also apply within a species: shape should not vary isometrically between small and large mice – a larger, heavier mouse is subjected to different forces to which the bone responds by assuming a different shape.

If this is so one might expect that selection for size might constrain shape. Replicated selection would therefore result in shapes more similar than in unselected controls. On the other hand different shapes might fulfil the necessary mechanical constraints: in this case repeated selection might lead to shapes less similar than in unselected controls.

Falconer (1973) took an outbred strain of mice, divided it into six groups and selected for large and small six week body weight in each line. Truslove (1976), who looked at skeletal preparations of Falconer's mice, commented that 'the skeletons of Large and Small mice have characteristic bones (i.e. size, shape, density, etc.) which presumably are the direct or indirect result of selection. The study of these differences... may well turn out to be of considerable interest'.

The availability of this material, in the form of skeletal preparations of the six Large and Small lines after some thirteen or fourteen generations of selection, together with unselected Controls, led us to ask the following questions. First, did the increase or decrease in size produced by selection lead to a measurable size-related shape change in the skeleton? Secondly, was any induced shape change similar amongst replicates of the experiment?

In this paper we have used the powerful technique of Fourier analysis to derive a representation of shape for each of Falconer's selected groups and examined the way in which selection for size has affected shape.

2. Materials and methods

We looked at the first and second thoracic vertebrae (T1 and T2) of fifteen males and fifteen females taken at random from the 13th or 14th generation of the selection experiment (Large QLA–QLF, Small QSA–QSF, Controls QCA–QCF). These preparations form part of the Grüneberg collection, and were loaned by the British Museum, Natural History. The

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original derivation of the Q strain and details of experimental procedure are given by Falconer (1973).

Vertebrae were videodigitized according to the protocol of Johnson *et al.* (1985). This may be briefly summarized as follows. Images of the anteroposterior projections of T1 and T2 vertebrae were obtained by placing them on the stage of a dissecting microscope, which was illuminated from below. An image, captured by a standard black and white television camera was fed to a simple video camera interface and digitized by a BBC microcomputer. The image was subjected to a series of routines which convert it to a binary form (black and white only), enhance the edge of the image (a commonly used procedure) and locate the edge. A stream of co-ordinate pairs (0, 1; 1, 2; 2, 3 etc.) representing the edge was transferred to the mainframe computer for further analysis. At the magnification used this stream of co-ordinates contained around 300 pairs per vertebra (Fig. 1*a*, *b*).

The Cartesian co-ordinates were converted to 128 polar co-ordinates aligned on their centres of area, rotated to give the best least squares fit and normalized for area. The opened-out graph of polar co-ordinates can be considered as a wave form and the technique of Fourier analysis applied. Jean Baptiste Fourier (1768–1830) described a method which splits a complex waveform into a series of sine and cosine components of varying amplitude. Lestrel (1974, 1982) applied this series to biological shapes. The general Fourier series can be represented:

$$F(\theta) = a_0 + a_1 \cos\theta + b_1 \sin\theta + a_2 \cos 2\theta + b_2 \sin 2\theta \ldots a_n \cos n\theta + b_n \sin n\theta, \quad (1)$$

where a_0 is a constant, a_1–a_n are known as cosine components, b_1–b_n are known as sine components and

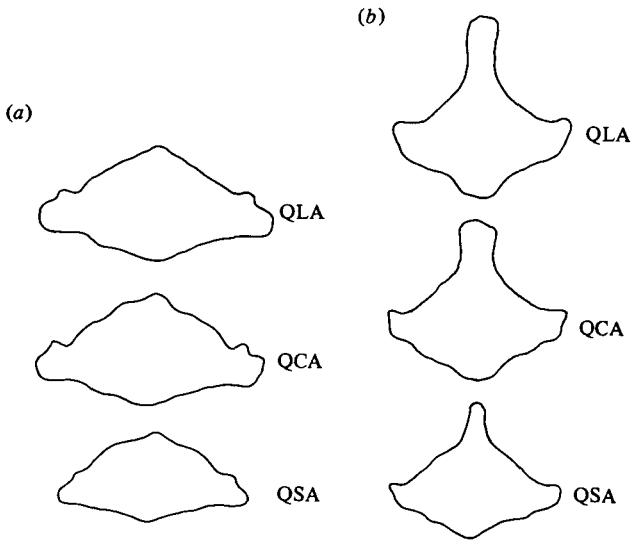

Fig. 1. Outlines of vertebral shapes reconstructed from polar co-ordinates. (*a*) First thoracic vertebrae (T1), (*b*) second thoracic vertebrae (T2). These outlines are the mean shapes for one replicate (A) of Falconer's experiment and have not been scaled to the same area. They thus differ from each other in both size and shape.

$F(\theta)$ is the magnitude of a polar radius r. Because the sine and cosine components are 90° out of phase the Fourier series can describe highly irregular waveforms by means of a series of numbers. The Fourier series is infinite: in practice a simple shape with low frequency outline undulations is adequately described by the early members of the series. More sophisticated shapes demand more components. The higher frequency components are influenced by errors in the measurement process. Since too few Fourier components produce an over-simplified version of the shape and too many introduce error in the form of random noise it is obviously necessary to find a compromise value for the number of Fourier components used. An objective test of the value of n is to perform a discriminant function analysis which compares all chosen variates simultaneously. In each of ten successive analyses random tenths were removed from our data sets. Each tenth was then compared with the remaining 90% by discriminant analysis using the first two Fourier components (SAS, 1982). The analyses were repeated using increasing numbers of Fourier components until the quality of discrimination began to decline (see O'Higgins & Williams, 1987). This number of components was then taken to best describe the observed shape differences, and used to calculate Mahalanobis' distances between group centroids. Canonical axes were calculated by the method of Gower (1966*a*, *b*) who extracted principle co-ordinates from the Mahalanobis' D matrix. Cluster analysis was performed using Ward's minimum variance method (Ward, 1963).

3. Results

The numerical value of the components of the Fourier series tends to zero while the accuracy with which they are computed is constant. Because of this later components are less reliably estimated than earlier ones (O'Higgins & Williams, 1987, p. 417). If too few components are used to describe a shape we oversimplify it: if too many are used we introduce error. In order to achieve a compromise we must find the number of Fourier components best suited to the data set. This is done in practice by repeated discriminant analyses using different numbers of components until discrimination is maximized.

When we removed successive random tenths of the data and looked for misclassification we found the lowest error with 30 Fourier components for both T1 and T2. Smaller or larger numbers gave worse classification.

The numbers of T1 and T2 vertebrae respectively classifying correctly and misclassifying with 30 components are given in Tables 1 and 2. Data from both sexes are pooled. For T1 (Table 1) a mean of 45·0% of Control vertebrae, 62·8% of Large and 56·6% of Small were classified correctly by replicate and 86–90% classified correctly by group. These

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Table 1. *Percentages of correct classification achieved for T1 vertebrae using Fourier components 2–31. n = number of mice per sample*

	Control						
	QCA	QCB	QCC	QCD	QCE	QCF	
n ...	29	29	29	29	30	28	Mean
Classifying correctly (%)	33·3	70·3	37·0	55·5	35·7	30·7	45·0
Classifying within Control (%)	96·3	88·9	92·5	92·5	96·4	61·5	88·0
Classifying as Large/Small (%)	3·7	11·1	7·5	7·5	3·6	38·5	12·0
	Large						
	QLA	QLB	QLC	QLD	QLE	QLF	
n ...	30	28	28	30	26	28	Mean
Classifying correctly (%)	57·1	61·5	57·6	57·1	62·5	80·8	68·8
Classifying within Large (%)	89·2	96·2	80·8	78·6	79·2	92·3	86·1
Classifying as Control/Small (%)	10·8	3·8	19·2	21·4	20·8	7·7	13·9
	Small						
	QSA	QSB	QSC	QSD	QSE	QSF	
n ...	28	28	28	29	29	23	Mean
Classifying correctly (%)	53·8	69·2	34·6	55·5	55·5	61·9	56·6
Classifying within Small (%)	92·3	100	73·1	88·9	88·9	100	90·5
Classifying as Control/Large (%)	7·7	0	26·9	11·1	11·1	0	9·5

Table 2. *Percentages of correct classification achieved for T2 vertebrae using Fourier components 2–31. n = number of mice per sample*

	Control						
	QCA	QCB	QCC	QCD	QCE	QCF	
n ...	29	30	30	25	30	26	Mean
Classifying correctly (%)	22·2	53·6	32·1	65·2	21·4	29·2	37·3
Classifying within Control (%)	74·0	64·3	82·1	86·9	71·4	50·0	71·5
Classifying as Large/Small (%)	26·0	35·7	17·9	13·1	28·6	50·0	28·5
	Large						
	QLA	QLB	QLC	QLD	QLE	QLF	
n ...	24	27	26	27	29	28	Mean
Classifying correctly (%)	36·4	52·0	50·0	60·0	19·0	42·3	43·2
Classifying within Large (%)	86·4	76·0	79·2	76·0	76·2	73·1	77·8
Classifying as Control/Small (%)	13·6	24·0	20·8	24·0	23·8	26·9	22·2
	Small						
	QSA	QSB	QSC	QSD	QSE	QSF	
n ...	20	28	26	27	24	20	Mean
Classifying correctly (%)	42·1	61·5	54·1	24·0	46·6	57·8	47·7
Classifying within Small (%)	89·4	80·7	75·0	84·0	68·2	89·4	81·1
Classifying as Control/Large (%)	10·6	19·3	25·0	16·0	31·8	10·6	18·9

results were mainly internally consistent with the exception of QCF which had 9 members classifying erroneously, 3 as Large, 6 as Small. QSC mice were also poorly classified.

Once the number of Fourier components had been optimized we were able to perform Canonical analyses of the data. Plots of the first versus second Canonical axis (Fig. 2) using the first 30 components showed large, control and small group centroids well separated. Clustering, by Ward's method (Fig. 3), grouped all replicates correctly into Control, Large or Small.

For T2 (Table 2) the classification was less good with only 37·3% of Control vertebrae, 43·2% of Large and 47·7% of Small having classified correctly by replicate and 72–81% classifying correctly by group. On the plot of first versus second and first versus third Canonical axes (Figs. 4 & 5) the divisions

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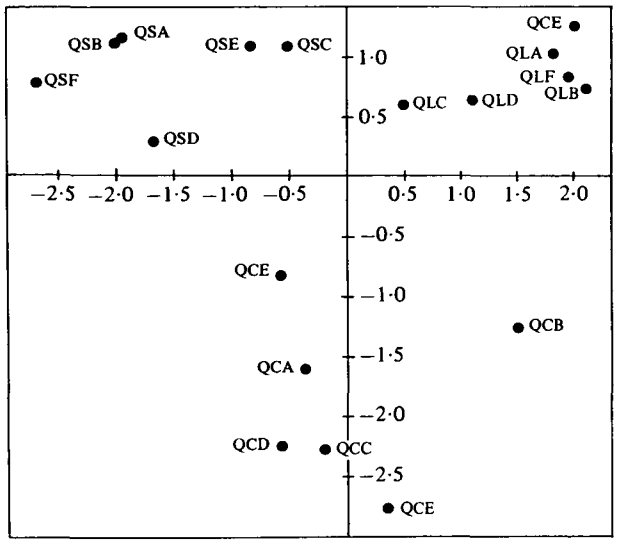

Fig. 2. Group centroids of Large (QLA–QLF), Control (QCA–QCF) and Small (QSA–QSF) T1 vertebrae. The *X* axis is Canonical axis 1, the *Y* axis Canonical axis 2. For T1 the proportions of the variance accounted for by the first three axes are as follows: 1, 26·4%; 2, 22·4%, 3, 12·6%.

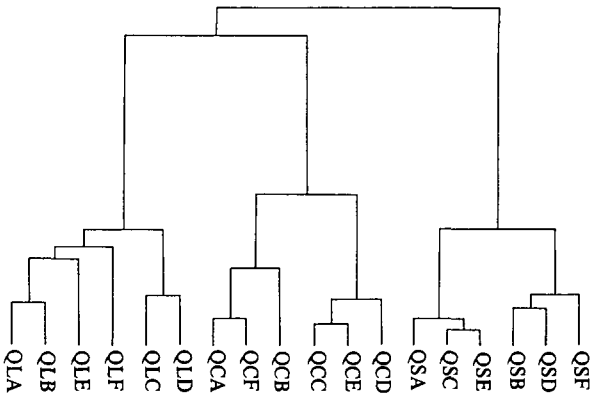

Fig. 3. Minimum spanning tree (Ward's method) for Large (QLA–QLF), Control (QCA–QCF) and Small (QSA–QSF) T1 vertebrae.

between Large, Small and Control are less marked than in T1. Ward's method (Fig. 6) shows two groups of Controls clustering outside their groups: QCB clusters early with Larges and QCF with the Smalls.

The mean generalized distance between replicates centres (Table 3) was similar within T1 and T2, but that the T2 figures were slightly smaller. The mean distance between group centres was also similar (Table 4).

4. Discussion

Fourier analysis expresses a shape (in this case the mean outline of a number of vertebrae) as a series of numbers. These numbers allow us to make a comparison between the mean shapes, and, if they differ, to give an estimate of the difference. The technique is very poor at identifying differences in

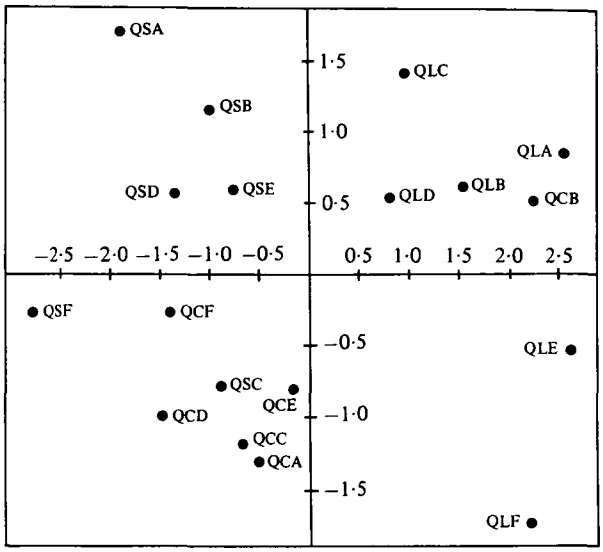

Fig. 4. Group centroids of Large (QLA–QLF), Control (QCA–QCF) and Small (QSA–QSF) T2 vertebrae. The *X* axis is Canonical axis 1, the Y axis Canonical axis 2. For T2 the proportions of the variance accounted for by the first three axes are as follows: 1, 37·1%; 2, 13·4%, 3, 11·3%.

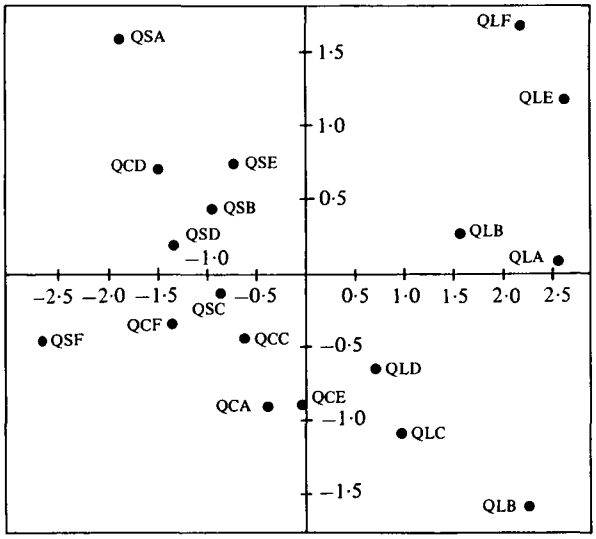

Fig. 5. Small (QSA–QSF) T2 vertebrae. The *X* axis is Canonical axis 1, the *Y* axis Canonical axis 3.

particular areas of a shape because such a difference will affect many Fourier components in a complex manner. Our results thus allow us to ask only one question: does selection for size affect shape? We are developing different analyses which will allow us to discuss regional differences in shape.

It is clear that in Falconer's experiment selection for size also changed shape. Even though we equalized the areas of the particular view of the vertebra with which we worked our discriminant analysis had no difficulty in classifying over 90% of T1s into Large, Small, or Control. The accuracy of classification between replicates was less and suggests that while replicates in each group differed from each other

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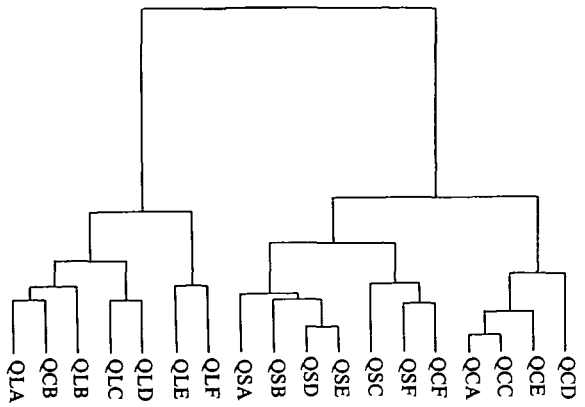

Fig. 6. Minimum spanning tree (Ward's method) for Large (QLA–QLF), Control (QCA–QCF) and Small (QSA–QSF) T2 vertebrae.

Table 3. *Mean generalized distance between replicate centres (SDU) for each group of T1 and T2*

	T1	T2
Control	3·30	3·31
Large	3·67	3·60
Small	2·92	3·03

Table 4. *Mean generalized distance between group centres (SDU) for T1 and T2*

	T1		T2	
	Large	Small	Large	Small
Large	—		—	
Small	2·96	—	2·78	—
Control	3·05	3·09	2·17	2·04

somewhat in shape, these differences were considerably less than differences between groups. The difference (% classifying correctly − % classifying within group) is a rough inverse measure of the variation within a group. This number was largest (and hence variation between replicates least) at 17·9% in Small mice, and less (11·6, 11·7%) in Controls and Large mice. This suggestion, that variance is less in the Small replicates, is borne out by the generalized distances between individual Control, Large and Small replicate group centroids (Table 3). Since the Large group were the most dispersed this might be taken to support weakly the argument that engineering constraints are most critical in the Small line where available material has to be placed to the best advantage. We might expect genetic drift, in the absence of selection in the Control group to emphasize the dispersal of the Control line.

In the T2s the situation was less clear cut. The accuracy of classification within groups and between replicates was a little less, but 82–88% still classified accurately as Large, Small or Control. A Canonical plot of group centroids (Fig. 4) shows overlapping of the groups and Cluster analysis showed that two groups of Control mice, QCB and QCF, joined at a low level with Large and Small respectively.

The poor classification of T2 clearly needs an explanation. This could be a real effect or an error due to some aspect of the shape of T2 vertebrae which does not respond well to our analysis. We discard the latter hypothesis. We have previously looked at T2 and other vertebrae from many strains of mice (Johnson *et al.* 1985; O'Higgins *et al.* 1986, 1987): the shapes of T2 vertebrae can usually be classified no better and no worse than those of other vertebrae. We believe that the misgrouping of QCB and QCF is a property of their shapes.

Truslove (1976) gives a table (her table 1) of the six week weights of the replicates at generation 13–14 when our skeletal preparations were obtained. QCB is the heaviest of the Control replicates with a mean weight of 25·5 g and QCF the smallest at 20·0 g. We think that this accounts for the misclassification seen in our data: the largest and smallest Controls classified with Large and Small respectively.

Canonical axes allow us to visualize high dimensional space as a series of projections into two dimensions. The first Canonical axis is calculated such that it takes up the largest between-group variance relative to within-group variance. The second and subsequent axes represent progressively smaller components of the variance. Canonical axes are orthogonal in Mahalanobis' D space. Studies in the primates (Ashton, 1981) have shown that in certain circumstances a biological meaning can be attributed to dispositions along these axes. Jolicoeur (1963) has suggested that the first principal component be used for (allometric) size correction (Canonical axes are equivalent to principal components). This seems to be a reasonable approach only when size accounts for the major part of the differences between groups.

In this study we have removed size as a variable by equalizing the areas of vertebral outlines. Any differences between vertebrae are therefore not due to size difference *per se*, but reflect the effect of size on shape. On Canonical axis 1 for T1 (Fig. 2) Large and Small vertebrae occupy opposite poles: the Controls are intermediate. On the second Canonical axis Small and Large occupy one pole and Control the other. This is also largely true for T2 (Fig. 4), if we neglect the groups which classify poorly. We suggest that selection for size produces vertebrae whose shape is size-related (along axis 1): controls, free from the constraint of selection vary along axis 2, i.e. in a non-size-related way, but are still arranged so that the largest (QCB) and smallest (QCF) tend towards the large and small poles of Canonical axis 1. Differences in size thus result in differences in shape irrespective of selection. These differences are almost universally of the same type in Controls as in selected vertebrae:

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there is only one size/shape trend. Any genetic differences in shape control in the selected lines are swamped by size differences, but can be seen in the Controls. If we plot the score on Canonical axis 1 (a measure of size-related shape change) against body weight (a measure of size) the relationship is linear (Fig. 7) for all groups. QCF, a poor classifier, lies close to the regression line and thus obeys the postulated size/shape relationship; QCB is an outlier of the Large group.

If the differences seen between T1 and T2 are real they must reflect pleiotropic effects of the selected genes which differ between these two vertebrae. We have shown elsewhere (O'Higgins *et al.* 1987) that vertebrae in the C1–T2 region of the mouse show this very local pleiotropism, with adjacent vertebrae in F_1s from inbred strains resembling different parents. Any future studies on pleiotropism must clearly bear this in mind: conclusions based on one group of measurements may not hold for measurements taken elsewhere.

We did not have a sample of the original founder populations: our Control group was 13–14 generations away from the founders, as were the Large and Small populations. The six week weights of these Control mice differed very little over 13 generations (Falconer, 1973); it seems reasonable to suggest that their mean vertebral shape was also fairly constant with time, although we must beware circularity of argument. If we equate founder population mean shape with 13th generation mean shape then it seems from the data in Table 4 that Smalls and Larges have diverged by similar amounts. Falconer found that six week weight was also increased or depressed by the same amount during selection.

Truslove (1976) noted that the frequency of minor skeletal variations within a population of mice was governed (amongst other things) by diet, and that about half of the diet-effected changes were correlated with increase or decrease in size. She attempted to investigate these variations in the Q strain to see if the effect of genetically induced size change paralleled that induced by dietary change. Unfortunately the incidence of suitable variants was much lower in Q than in the original work on C57BL, perhaps due to the outbreeding introduced prior to the experimental selection for size.

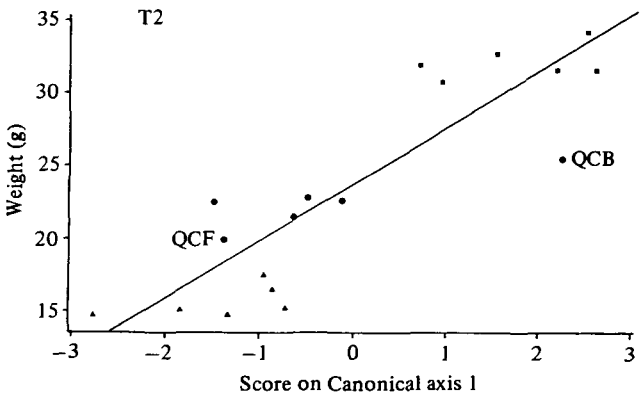

Fig. 7. Regression of a measure of shape (score on first Canonical axis) against body weight. ■, Large, ●, Control, ▲, Smalls. The two Control groups misclassifying are individually marked.

We must also ask if the changes due to size have anything to do with those due to increasing age. As the skeleton matures it changes its shape. Will a Small mouse vertebra resemble a Large one taken at a younger age? This result is predicted by a series of linked studies on the Q strain. Bryne, Hooper & McCarthy (1973) and Hooper & McCarthy (1976) worked on muscle, Clarke (1969) on fatness and Falconer, Gauld & Roberts (1978) on cell size and number in lung, liver, spleen and kidney. All these studies indicate that large size is produced by an accelerated passage through the growth process which affects both cell size and cell number. The effects of this process on the skeleton can only be ascertained by longitudinal study, or more realistically by killing of mice at a series of known ages.

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