Relative growth of the skull and postcranium in giant transgenic mice

BRIAN T. SHEA[1]†, ROBERT E. HAMMER[2]*, RALPH L. BRINSTER[2]
AND MATTHEW R. RAVOSA[1]

[1] *Departments of Cell, Molecular and Structural Biology and Anthropology, Northwestern University, 303 East Chicago Avenue, Chicago IL 60611 USA*
[2] *Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia PA 19104 USA*

Summary

Cross-sectional allometric growth patterns of the cranial and postcranial skeleton were compared between giant transgenic (MT-rGH) mice and their normal littermate controls. Body weights, external body dimensions, and a series of cranial and postcranial linear dimensions of the skeleton were determined for samples of known age. Comparative bivariate and multivariate allometric analyses were completed in order to determine whether (1) the larger transgenic mice differed significantly from the normal controls in aspects of body and skeletal proportions, and (2) any such proportion differences resulted from general allometric effects of overall weight or skeletal size increase. Results demonstrate that the transgenic mice do exhibit significantly different body and skeletal proportions than normal control adults. Allometric comparisons of the skeletal dimensions relative to body weight reveal similar coefficients of growth allometry but several differences in y-intercept values in the transgenic *vs.* control groups. The comparisons among the skeletal dimensions of the skull and postcranium generally reveal the sharing and differential extension of common growth allometries in the two groups. Thus, the elevated levels of growth hormone (GH) and insulin-like growth factor I (IGF-I) in the transgenic mice appear to result in increased overall growth for the various skeletal elements, but in the relative proportions determined by intrinsic growth controls within that system.

1. Introduction

The genetic and epigenetic bases of differences in growth rate, final size, and body proportions among animals are of interest to biologists in a number of areas. Investigators have utilized numerous models in addressing these questions, including mutant dwarf mice (van Buul-Offers, 1983; Bartke, 1979), size-selected strains of mice and rats (Eisen, 1976; Falconer, Gauld & Roberts, 1978; Pidduck & Falconer, 1978; Atchley, Rutledge & Cowley, 1982; Rutledge, Eisen & Legates, 1975), formation of mouse chimaeras (Falconer, Gauld & Roberts, 1978), and experimental manipulation of the endocrine system (Bartke, 1965; van Buul & Van den Brande, 1978; Simpson, Aisling & Evans, 1950; Nielsen, 1953).

The recent successful production of giant transgenic mice (Palmiter *et al.* 1982, 1983) provides an additional system which can be utilized to investigate the genetic and epigenetic control of body size and proportions (epigenetic is used here as in Hall, 1983, to refer to the mechanisms by which genes express their phenotypic effects.) These transgenic (MT-rGH) mice have been produced by the microinjection of a fusion gene (comprised of the metallothionein-I (MT) promoter/regulator region joined to the rat growth hormone (rGH) structural gene) into the pronuclei of the mouse. The MT-rGH mice harbouring these fusion genes are characterized by high levels of the fusion messenger RNA in several organs, elevated levels of foreign growth hormone and insulin-like growth factor I (somatomedin C) in serum, and markedly increased growth rates and final adult size (Palmiter *et al.* 1982, 1983; Hammer, Palmiter & Brinster, 1984; Hammer, Brinster & Palmiter, 1986). Pedigree analysis reveals that the MT-rGH fusion gene has been transmitted into subsequent generations with continued expression in all offspring carrying the gene (growth rates are

* Present address: Howard Hughes Medical Institute, Y5-322, Southwest Medical School, 5323 Harry Hines Blvd., Dallas, Texas 75235
† Corresponding author.

accelerated and average adult size is approximately twice normal size). The adult transgenic mice have been characterized as 'normally proportioned' by Palmiter *et al.* (1983), which presumably implies an isometric variant, although detailed allometric analyses have not previously been completed.

In this paper we report the results of investigation of skeletal growth and proportions in the transgenic mice compared to their littermate controls of normal size. We utilize cross-sectional analyses of patterns of ontogenetic allometry or relative growth (Huxley, 1932) to analyse changing body proportions, in conjunction with Wright's (1932) hierarchy of general, group and special factors influencing the size of structures. Our goal is to characterize the allometric growth patterns of the skeletal system in these two groups, and to determine if the larger adult transgenic mice indeed exhibit proportions similar to the adult controls.

2. Materials and Methods

(i) Samples

Details concerning the production of the MT-rGH mice have been given elsewhere (Palmiter *et al.* 1982, 1983) and will not be repeated here. Control and transgenic mice were shipped frozen to B. T. S., and skeletons were cleaned in a dermestid beetle colony and measured by M. M. R. Although the numbers may vary slightly depending on a particular bony element or comparison, the basic sample size is 41 for the transgenic mice and 36 for their littermate controls. Mice of different ages were chosen so that a reasonable cross-sectional representation of skeletal growth could be attained. The age distributions for both samples may be observed in Figure 1. Adults were defined as those individuals greater than 190 days old.

(ii) Measurements

A series of cranial and postcranial linear dimensions were taken to the nearest 0·1 mm using a sliding vernier calipers. All measurements were taken on the right half of the specimen when possible. The skeletal measurements taken are defined as follows, based on the studies of Atchley, Rutledge & Cowley (1981), Hughes & Tanner, (1970), and Green & Fekete (1933). Four linear measurements of external body dimensions described and analysed by Shea, Hammer & Brinster (1987) are included with the skeletal dimensions. These are nose–rump length, tail length, total length and hindfoot length.

(iii) Analyses

Ontogenetic and adult inter-group proportion differences are analysed allometrically using the log-transformed version of Huxley's (1932) bivariate formula, or
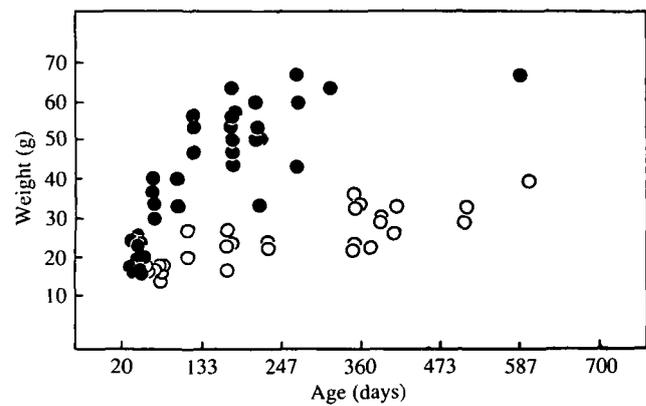
$$\log y = k \log x + \log b, \tag{1}$$

Fig. 1. A cross-sectional plot of growth in total body weight against time (in days) in the giant transgenic (filled circles) and normal control (open circles) mice.

where *y* equals the length or breadth of a skeletal element, *x* equals total body weight or the length of a second skeletal element, *k* is the slope of the bivariate regression of *y* on *x*, and *b* is the *y* intercept. An isometric relationship between *y* and *x* is indicated by a slope of 1·0 in a linear–linear comparison or 0·33 in a linear–weight comparison. A slope significantly greater than these values indicates positive allometry, and a progressively increasing ratio for *y* to *x*. Negative allometry is defined as slopes significantly less than the isometric values. In ontogenetic comparisons, the slope value (*k*) actually represents the ratio of specific growth rates; if the logarithmic bivariate plot is curvilinear, this reflects changing ratios of specific growth rates throughout ontogeny (Huxley, 1932; Laird, 1965).

Bivariate comparison of ontogenetic allometries in the transgenic and normal groups is based on analyses of regression and covariance, supplemented by careful visual examination of scatter plots. The analyses of covariance (ANOCOVA) permit tests for the homogeneity of slopes and differences in position or *y*-intercept values. Visual examination of scatter plots is a necessary supplement to these statistical outputs for two reasons. First, growth trajectories may be slightly curvilinear, in which case statistical results derived from linear regression analyses may indicate differences in slope and position even though the larger group follows a simple extrapolation of the curvilinear trajectory seen in the smaller. In addition, statistical testing for position differences at the *y* intercept often involves a distant and biologically suspect extrapolation from the observed range of the variables. For this reason, a careful visual examination of data scatters over the observed range of the *x* and *y* variables must be completed.

Bivariate allometric comparisons were supplemented by Jolicoeur's (1963) multivariate allometric generalization using principal components analysis. Principal components analysis is a multivariate technique where rigid, geometric rotation of the axes

Cranial

Face length (bspr)	basion to the anteriormost point of the inter-nasal suture
Biauricular width (ba)	smallest distance between the lateral/outer surface of the external auditory meati
Basicranial length (bsns)	basion to the intersection between the frontals and the inter-nasal suture
Bizygomatic width (bz)	greatest distance between zygomatic arches (usually near the temporal root)
nasal length (nl)	length along inter-nasal suture from the frontal to anteriormost point of nasal contact
nasal breadth (nb)	smallest transverse dimension of the snout between the infraorbital foramina
temporal fossa length (tpf)	greatest length (superiorly) from the temporal root to the maxillary root of the zygomatic arch
bitemporal width (bt)	smallest distance between the medial borders of the temporal fossae
palate length (pl)	staphylion to prosthion
anterior palate width (apw)	distance between the buccal surfaces of 1st maxillary molars
posterior palate width (ppw)	distance between the buccal surfaces of the 3rd maxillary molars
bicondylar width (bc)	maximum distance between the lateral surfaces of the mandibular condyles
mandibular height (mdh)	maximum perpendicular distance from a line formed by the superior tips of the coronoid process and the condyle to the gonial angle
mandibular length (mdl)	distance from posterior surface of mandibular condyle to anterior base of the lower central incisors

Post-cranial

scapula length (scl)	greatest distance from the glenoid cavity to the medial border (usually near inferior angle)
scapula breadth (scb)	greatest distance (perpendicular to spine) from the inferior angle to the superior border
ilium length (ill)	greatest length from the antero-medial notch of the acetabulum to the iliac crest
ilium width (ilw)	greatest dimension of the iliac crest in the coronal plane
ischium length (isl)	greatest length from the antero-medial notch of the acetabulum to the tuberosity marking the junction of ischio-pubic ramus
pubis length (pbl)	greatest length from the antero-medial notch of the acetabulum to the superior corner of the pubic crest
sacral width (sw)	maximum width of the 1st sacral vertebrate between the transverse processes
humerus length (hl)	greatest length from the head to the lateral condyle
humerus width (hw)	smallest midshaft diameter distal to the lateral flange
ulna length (ul)	greatest distance from the olecronon process to the radial head
femur length (fl)	greatest distance from the head to the lateral condyle
femur width (fw)	smallest midshaft diameter
tibia length (tl)	maximum perpendicular distance from the intercondylar processes to a line connecting the medial and lateral malleoli

describing the original variables results in a new set of axes such that the first axis or principal component is positioned to account for the maximum amount of variance, with subsequent components positioned to be orthogonal to one another (Albrecht, 1979). Jolicoeur (1963) noted that if principal components were extracted from a covariance matrix of logarithmic values, then the first component could be interpreted as a general vector of growth allometry, and the respective loadings of the variables on this component would accurately summarize isometric and allometric proportion changes. In a multivariate contrast of growth allometries in two closely related strains or taxa, the first principal component should summarize

the size *and* shape variance resulting from shared (but extrapolated) growth trajectories, while subsequent components summarize variance resulting from divergent or dissociated growth patterns (Shea, 1985). Statistical and biological issues associated with this approach have been discussed in greater detail elsewhere (e.g. Jolicoeur, 1963; Shea, 1985; Albrecht, 1979).

Regression analysis, analyses of covariance, and principal components analyses were all completed using the Systat Statistical Software package (Wilkinson, 1988 *a, b*).

Wright's (1932) hierarchical division of size influences into *general, group* and *special* factors provides a useful approach to comparative allometric analyses such as that between the transgenic and normal mice. However, since Wright's (1932) original tripartite division was developed for path analysis and not explicitly for allometric comparisons, some discussion of how it would translate and be tested in an allometric context is required. General, group and special factors may be defined on various levels depending on the investigation. In this study, differences in the skeleton due to *general* size factors would result from a simple extension or truncation of ontogenetic allometries (hereafter referred to as *ontogenetic scaling* – Gould, 1975) relative to overall size or body weight. In bivariate comparisons, ontogenetic scaling is tested on a case-by-case basis by checking for concordance of slopes and intercepts in the two groups. In multivariate comparisons, a rigid hypothesis of ontogenetic scaling predicts that almost all variance will be accounted for by the first principal component, and that subsequent components will affect no significant separation of the groups in question. *Group* size factors relate to a particular region or body system (in the present case, the skeletal system). In such cases, differences *within* the skeleton result from simple extension or truncation of ontogenetic allometries relative to other skeletal dimensions, even while the skeletal system as a whole may be dissociated from overall body weight. This can be tested in both bivariate and multivariate contexts by simply excluding weight (or other overall size variables) from the analyses and using the traditional tests for ontogenetic scaling on the remaining variables. *Special* size factors refer to particular changes in individual bony elements or local regions (e.g. the skull base) that do not result from general or group factors; predictions here would be for a clear departure from ontogenetic scaling for a particular feature within a region. In multivariate analyses, we expect a group separation on second or subsequent components that is 'driven' by those variables exhibiting the divergent growth trajectories.

3. Results

(i) Mean adult size and shape differences

Table 1 summarizes the means, standard deviations and results of significance tests for mean differences in weight and the skeletal dimensions of the adult transgenic and normal groups. On average the adult transgenic mice are almost twice as heavy as the adult controls. All the cranial and postcranial skeletal dimensions are also significantly larger in the transgenic mice. Table 1 reveals that the percentage increases in growth of the cranial *vs.* postcranial skeletons are quite different, however. Values for the cranial dimensions range from 1·05 to 1·14, with a mean of 1·10; values for the postcranium range from 1·14 to 1·28, with a mean of 1·20. Therefore, given the overall adult size increase in the transgenic mice, the cranium undergoes relatively less growth than the postcranium.

Table 2 presents means, standard deviations and results of significance tests for a selected series of ratios in adults of the two groups. The within-skull ratios exhibit significant differences at a 0·01 level for only one comparison (4 at a 0·05 level). For the selected series of postcranial skeletal ratios, 9 differ significantly at the 0·01 level and 11 at the 0·05 level.

Taken together, the data presented in Tables 1 and 2 demonstrate that the skeletons of the adult transgenic mice are both larger and differently shaped than the adult control normal mice. The degree of statistically significant size and shape differentiation is greater for the postcranium than it is for the cranium.

(ii) *Bivariate growth allometries vs. body weight*

Table 3 summarizes the results of the regression analyses relative to body weight for the individual groups, plus results of the analyses of covariance for the combined sample. These results also make clear the reduced growth of the cranial dimensions against body weight as compared to the postcranial dimensions. The average allometric coefficients for the 14 cranial dimensions are 0·178 for the transgenics and 0·179 for the normals, while the average for the 15 postcranial dimensions are 0·319 and 0·322, respectively. Thus, on average, the postcranial dimensions grow in nearly isometric fashion relative to weight, while the cranial dimensions exhibit considerable negative allometry.

Statistical results from the analyses of covariance indicate that the postcranial dimensions reveal 1 significant slope difference and 6 (of 14) intercept differences. For the cranial regressions, there are no slope differences and 4 (of 16) intercept differences between the transgenic and control groups.

Visual examination of the cranial and postcranial point scatters reveals that many exhibit varying degrees of curvilinearity, with a terminal phase during which the skeletal element has ceased (or dramatically

Table 1. *Sample sizes, means[1] and standard deviations for adult (sex-pooled) transgenic and normal control mice*

Measurement	Transgenic			Normal			Tx/Nx[2]
	n	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	
Body wt (bw) (g)	20	53·0	9·2	13	28·8	5·1	1·84
Postcranial (mm)							
scapula l. (scl)	19	12·3	0·5	13	10·8	0·2	1·14
scapula br. (scb)	19	9·7	0·5	13	8·2	0·3	1·18
ilium l. (ill)	19	13·8	1·0	13	11·3	0·6	1·22
ischium l. (isl)	19	9·0	0·6	13	7·3	0·4	1·23
pubis l. (pbl)	19	8·1	0·7	13	7·1	0·5	1·14
ilium w. (ilw)	19	3·1	0·2	13	2·7	0·2	1·15
sacral w. (sw)	19	6·8	0·7	13	5·3	0·3	1·28
humerus l. (hl)	19	14·6	0·8	13	11·9	0·3	1·23
ulna l. (ul)	19	15·9	0·7	13	14·0	0·4	1·14
femur l. (fl)	19	18·8	1·2	13	15·7	0·6	1·20
tibia l. (tl)	19	20·7	1·1	13	17·9	0·4	1·16
humerus w. (hw)	19	1·1	0·1	13	0·9	0·04	1·22
femur w. (fw)	19	1·5	0·2	13	1·2	0·07	1·25
Cranial (mm)							
face l. (bspr)	15	23·9	0·9	10	21·3	0·4	1·12
palate l. (pl)	19	13·2	0·6	13	12·0	0·5	1·10
face br. (bz)	19	13·5	0·7	13	12·5	0·3	1·08
bitemporal br. (bt)	14	4·3	0·1	10	4·1	0·2	1·05
ant. palate w. (apw)	19	5·2	0·2	13	4·9	0·1	1·06
post palate w. (ppw)	19	4·8	0·1	13	4·5	0·1	1·07
bicondylar br. (bc)	19	11·2	0·4	13	10·5	0·2	1·07
mandibular ht. (mdh)	19	6·3	0·2	13	5·6	0·1	1·12
nasal l. (nl)	18	9·1	0·6	13	8·3	0·4	1·10
temporal fossa l. (tpf)	19	8·3	0·4	13	7·3	0·2	1·14
nasal br. (nb)	19	3·9	0·2	13	3·5	0·2	1·11
mandibular l. (mdl)	19	14·1	0·5	13	12·8	0·2	1·10
biauricular br. (ba)	18	9·3	0·2	13	8·6	0·2	1·08
basicranial l. (bsns)	13	16·5	0·7	10	14·7	0·5	1·12

[1] All means are significantly different at $P < 0.001$, except bitemporal breadth ($P < 0.006$).

[2] Transgenic mean divided by normal (control) mean to show percentage increase.

slowed) its growth while increase in overall weight continues. Figure 2 illustrates both this curvilinearity and the general pattern characteristic of many of the skeletal dimensions when plotted against body weight. This general pattern is one of relatively minor but nonetheless clear differences in the point scatters relative to body weight, at least during the middle ranges of the allometric growth trajectories, and thus an apparent departure from the predictions of rigid ontogenetic scaling. We will consider in the Discussion below whether such patterns may in fact represent underlying extensions of similar patterns of curvilinearity. Statistical results of the analyses of covariance indicate a pervasive, if not total, fit with the predictions of ontogenetic scaling against body weight, however (see Table 3).

Figure 3 illustrates with two representative plots the general pattern observed in the cranial regressions relative to body weight. As with the postcranium, the bivariate plots evidence varying degrees of curvilinearity, and the control mice have higher y/x ratios throughout the middle portion of the relative growth regressions. Thus, the cranial comparisons against overall body weight coincide with the postcranial ones, both in the curvilinearity and partial divergence of the allometric trajectories, and the statistical results of analyses of covariance suggesting a pervasive fit with the predictions of ontogenetic scaling (Table 3).

(iii) *Within-skeleton bivariate growth allometries*

Table 4 summarizes results of the bivariate regressions and analyses of covariance *within* the postcranial and cranial skeleton (i.e. with a skeletal length rather than body weight as the x variable). The postcranial comparisons reveal 1 (of 11) differences in slope and 4 (of 11) differences in intercept between the two groups; there are no slope differences and 4 (of 19) intercept differences for the cranial regressions.

Figure 4 illustrates 4 of the postcranial plots. These tend to exhibit less curvilinearity than in the skeletal plots *vs.* weight, although this varies somewhat. These postcranial plots, as well as those not illustrated, fit

Table 2. *Sample sizes, means and standard deviations for ratio comparisons between adult transgenic and normal control mice. Significance levels from t tests are given. Values lower than 0·01 are underlined (see Table 1 for measurement abbreviations)*

Ratio	Transgenic			Normal			Signi-ficance level
	n	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	
Cranial							
bspr/bsns	13	1·44	0·04	10	1·44	0·03	0·894
pl/bsns	13	0·81	0·04	10	0·81	0·03	0·938
bz/bsns	13	0·83	0·06	10	0·85	0·03	0·326
bt/bsns	10	0·26	0·02	8	0·28	0·02	0·020
apw/bsns	13	0·31	0·01	10	0·33	0·01	0·000
ppw/bsns	13	0·29	0·01	10	0·30	0·01	0·013
bc/bsns	13	0·69	0·04	10	0·71	0·02	0·089
mdh/bsns	13	0·39	0·02	10	0·38	0·01	0·355
nl/bsns	13	0·56	0·03	10	0·56	0·04	0·830
tpf/bsns	13	0·51	0·02	10	0·49	0·02	0·030
nb/bsns	13	0·23	0·01	10	0·24	0·01	0·321
mdl/bsns	13	0·86	0·03	10	0·87	0·03	0·863
ba/bsns	13	0·56	0·03	10	0·58	0·02	0·075
Postcranial							
scl/hl	19	0·84	0·03	13	0·91	0·02	0·000
scl/ill	19	0·89	0·06	13	0·96	0·06	0·002
fl/ill	19	1·36	0·05	13	1·39	0·04	0·134
scl/fl	19	0·65	0·04	13	0·69	0·03	0·008
(hl+ul)/(fl+tl)	20	0·77	0·02	13	0·77	0·02	0·735
tal/nr	20	0·46	0·05	13	0·48	0·03	0·198
hf/nr	20	0·18	0·01	13	0·20	0·01	0·000
scl/nr	19	0·10	0·01	13	0·11	0·01	0·003
scl/scb	19	1·27	0·04	13	1·32	0·04	0·001
ill/ilw	19	4·51	0·40	13	4·18	0·42	0·030
isl/pbl	18	1·11	0·09	13	1·03	0·11	0·039
ilw/sw	19	0·45	0·05	13	0·51	0·04	0·002
hl/fl	19	0·78	0·03	13	0·76	0·02	0·095
ul/hl	19	1·09	0·04	13	1·18	0·03	0·000
tl/fl	19	1·10	0·03	13	1·14	0·03	0·004

the expectations of ontogenetic scaling in spite of the 4 significant position differences indicated in Table 4 (this presumably reflects the fact that even insignificant slope differences will often produce significant position differences when extrapolated to the distant y axis). The plot of humerus length *vs.* femur length (Fig. 4*a*) is a particularly interesting case. Although both slope and intercept differ according to Table 4, the curves of the two groups are quite similar in exhibiting a 'flattening' of the curve during late ontogeny. This appears to occur at about the same *time* in the two groups, though of course at markedly different *sizes*. This probably reflects the earlier closure of the humeral epiphyses than those of the femur. Here also there is underlying similarity in the curves in spite of the statistical results of simple line-fitting techniques. When the biological basis of the curve is understood, the plot of humerus *vs.* femur fits the predictions of ontogenetic scaling, or the sharing and extension of a common pattern of growth allometry.

Figure 5 illustrates four representative plots of the cranial dimensions. These (plus those not illustrated here) exhibit varying degrees of curvature, but in all cases the transgenic and normal mice appear to share a common growth trajectory, with the transgenics extending this into larger size ranges.

The regression data presented in Tables 3 and 4 demonstrate that for numerous comparisons the bivariate slopes depart from predicted values for isometry (0·33 in Table 3 and 1·00 in Table 4). This means that as the mice get larger, both within and between the two groups, they are simultaneously undergoing continuous and complex shape changes. It is of interest, however, that none of the slope values departs very markedly from isometric values.

(iv) *Cranial vs. postcranial bivariate comparisons*

Comparisons of allometric trajectories for postcranial *vs.* cranial linear dimensions were also completed. Statistical results from analyses of covariance indicate no dissociation of the cranial *vs.* postcranial patterns of allometric growth in the skeleton. The fact that in a contrast of the adults, postcranial dimensions increase on average at a greater percentage than do cranial dimensions, is not reflective of *special* factors

Table 3. *Results of logarithmic bivariate regression analyses for selected dimensions* (y) *against body weight* (x). *Sample sizes, slopes, standard errors of the slope and correlation coefficients are given. Results of ANOCOVA's comparing the two groups are given, indicating whether regression slopes and intercepts differ significantly. Values lower than 0·01 are underlined* (see Table 1 for measurement abbreviations)

Comparison	Transgenic				Normal				ANOCOVA	
	n	Slope	S.E.	*r*	*n*	Slope	S.E.	*r*	Slope	*y* int.
Postcranial										
nr*bw	41	0·33	0·01	0·97	33	0·35	0·02	0·93	0·495	0·176
tal*bw	41	0·19	0·05	0·56	35	0·13	0·06	0·38	0·506	0·671
tot*bw	41	0·28	0·02	0·93	33	0·26	0·03	0·88	0·481	0·252
hf*bw	41	0·06	0·01	0·66	35	0·05	0·02	0·39	0·867	0·003
scl*bw	40	0·37	0·02	0·97	34	0·41	0·03	0·93	0·211	0·000
scb*bw	40	0·45	0·02	0·96	34	0·54	0·04	0·91	0·053	0·000
ill*bw	40	0·50	0·03	0·94	35	0·49	0·05	0·85	0·933	0·001
isl*bw	39	0·41	0·02	0·95	35	0·38	0·03	0·90	0·458	0·005
pbl*bw	40	0·29	0·03	0·86	35	0·26	0·05	0·70	0·507	0·175
ilw*bw	40	0·27	0·03	0·87	35	0·43	0·05	0·84	0·003	0·889
sw*bw	40	0·35	0·03	0·88	35	0·33	0·04	0·81	0·716	0·374
hl*bw	40	0·37	0·01	0·97	34	0·31	0·02	0·92	0·065	0·035
ul*bw	40	0·22	0·01	0·97	34	0·24	0·02	0·89	0·351	0·601
tl*bw	40	0·39	0·02	0·95	35	0·38	0·03	0·90	0·736	0·003
fl*bw	40	0·30	0·02	0·96	35	0·27	0·02	0·89	0·331	0·014
Cranial										
bspr*bw	35	0·23	0·01	0·94	27	0·26	0·02	0·95	0·401	0·059
pl*bw	40	0·22	0·01	0·94	35	0·23	0·02	0·89	0·602	0·002
bz*bw	40	0·15	0·01	0·87	35	0·17	0·01	0·92	0·408	0·500
bt*bw	33	0·10	0·01	0·83	31	0·09	0·02	0·62	0·586	0·026
apw*bw	40	0·12	0·01	0·90	35	0·11	0·01	0·81	0·513	0·029
ppw*bw	40	0·12	0·01	0·88	35	0·13	0·02	0·76	0·691	0·236
bc*bw	39	0·08	0·01	0·78	35	0·07	0·01	0·68	0·423	0·022
mdh*bw	40	0·22	0·02	0·91	35	0·20	0·02	0·88	0·453	0·061
tpf*bw	40	0·24	0·02	0·93	34	0·22	0·02	0·92	0·532	0·293
nb*bw	40	0·17	0·01	0·91	35	0·16	0·03	0·70	0·632	0·427
ba*bw	39	0·13	0·01	0·92	34	0·14	0·01	0·88	0·547	0·200
nl*bw	36	0·29	0·02	0·92	35	0·33	0·03	0·88	0·217	0·000
bsns*bw	31	0·21	0·02	0·91	25	0·21	0·02	0·94	0·975	0·089
mdl*bw	40	0·21	0·01	0·94	35	0·19	0·02	0·90	0·478	0·006

affecting the skull and postcranium differentially, with major differences between the two regions. Rather, the percentage differences simply result from the higher coefficients of relative growth for the postcranial *vs.* cranial dimensions. The means of the coefficients of growth allometry relative to body weight given in Table 3 are 0·32 for postcranial dimensions and 0·18 for cranial dimensions (in neither case does the mean for transgenics differ from the controls).

(v) Multivariate analyses

Figure 6 presents a plot of scores for transgenic and normal mice on the first two principal components extracted from a covariance matrix of the postcranial linear dimensions, external body dimensions, and body weight. The first and second component account for 91·4 and 2·7 % of the total variance, respectively. All variables load positively on the first component, with non-normalized component loadings ranging from 0·07 (for tail length) to 0·48 (for body weight),

though most cluster between 0·15 and 0·20. These varying loadings reflect differences among the coefficients of relative growth, and they correspond closely to the bivariate coefficients in Tables 3 and 4 (once divided by that for body weight – see Jolicoeur, 1963, and Shea, 1985). The first principal component thus both distributes the specimens along an axis of multivariate 'size', but also accurately summarizes the shape variation which results from differential positioning along this multivariate growth allometric axis (see Shea, 1985, for additional details and discussion).

The second principal component accounts for a much smaller percentage of the variance, as expected, but it appears to produce no separation between the transgenic and control groups (Fig. 6). However, an analysis of covariance for between-group differences in regression of component II *vs.* component I scores yielded a significant ($P < 0.002$) position difference between the two groups. It should be stressed that in this case the test for position differences at the *y* axis (i.e. when $x = 0$) is much more meaningful
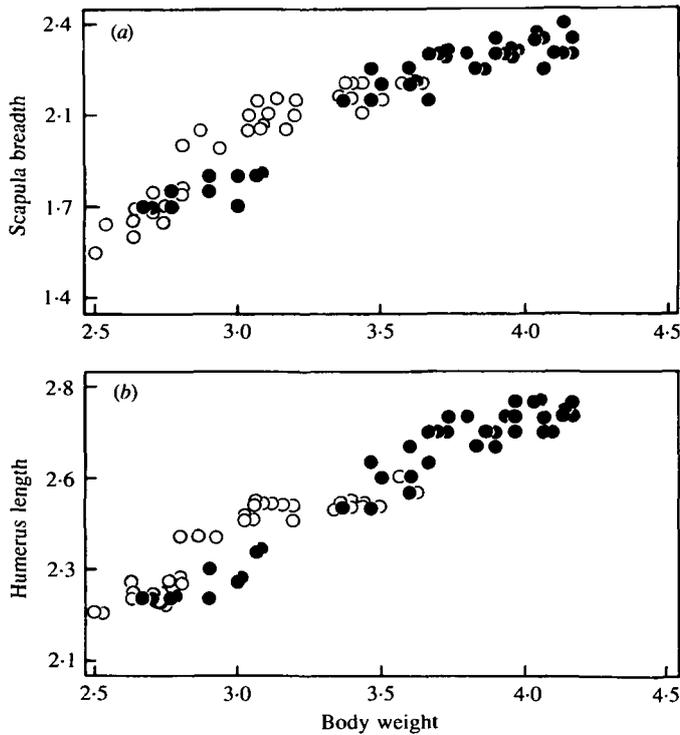
Fig. 2. Logarithmic plots of scapular breadth (a) and humerus length (b) against body weight. Symbols as in Fig. 1. Note the discordance of the two trajectories, particularly between x values of 2·75–3·25. See Table 3 and the text for additional discussion.
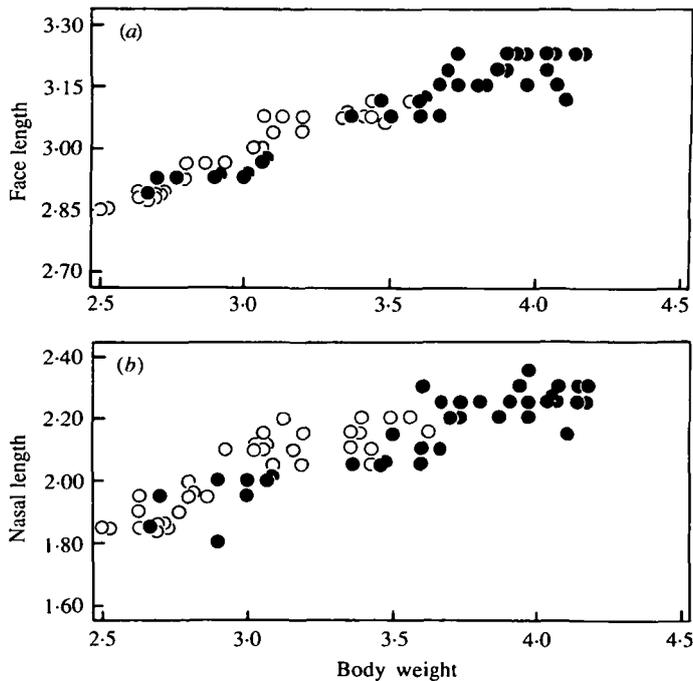

Fig. 3. A logarithmic plot of face length (a) and nasal length (b) against body weight. Symbols as in Figure 1.

biologically, since this is well within the observed scatter for component I and II scores. A t test for differences between the groups on component II scores also produced a significant value (P < 0·008). The variables loading most strongly (and oppositely) on this axis are body weight and tail length.

A similar principal components analysis run on the cranial dimensions plus body weight yields comparable results. First component loadings vary, with body weight having the highest value (as expected when comparing a volumetric with linear dimension). Dimensions of the palate and mandible increase more

Table 4. *Results of logarithmic bivariate regression analyses for selected cranial and postcranial comparisons (y*x). Sample sizes, slopes, standard errors of the slope and correlation coefficients are given. Results of ANOCOVA's comparing the two groups are given as in Table 3 (see Table 1 for measurement abbreviations)*

	Transgenic				Normal				ANOCOVA	
Comparison	*n*	Slope	S.E.	*r*	*n*	Slope	S.E.	*r*	Slope	*y* int.
Postcranial										
ill*isl	39	1·18	0·05	0·97	36	1·22	0·10	0·90	0·717	0·188
pbl*ill	40	0·60	0·04	0·94	36	0·58	0·05	0·90	0·715	0·230
ilw*ill	40	0·51	0·05	0·86	36	0·77	0·08	0·85	0·006	<u>0·001</u>
sw*ill	40	0·73	0·03	0·97	36	0·65	0·05	0·93	0·184	<u>0·000</u>
scb*scl	40	1·21	0·04	0·98	35	1·31	0·06	0·97	0·168	0·024
scl*ill	40	0·70	0·03	0·97	35	0·73	0·04	0·96	0·600	0·932
scb*ilw	40	1·36	0·10	0·90	35	1·01	0·10	0·88	0·016	0·445
ul*hl	40	0·59	0·02	0·98	35	0·70	0·06	0·91	0·032	0·003
tl*fl	40	0·74	0·02	0·99	36	0·70	0·02	0·99	0·197	0·013
hl*fl	40	0·90	0·02	0·99	35	0·80	0·02	0·99	<u>0·007</u>	<u>0·000</u>
ul*tl	40	0·71	0·03	0·97	35	0·82	0·06	0·93	0·074	<u>0·001</u>
Cranial										
bspr*bsns	31	1·03	0·06	0·96	26	1·15	0·06	0·97	0·184	0·433
bz*bsns	31	0·63	0·08	0·82	26	0·73	0·06	0·93	0·423	0·091
bt*bsns	28	0·38	0·07	0·73	24	0·37	0·11	0·59	0·930	0·474
apw*bsns	31	0·54	0·05	0·90	26	0·55	0·06	0·90	0·908	0·792
ppw*bsns	31	0·49	0·05	0·88	26	0·58	0·09	0·81	0·394	0·103
bc*bsns	30	0·35	0·06	0·73	26	0·37	0·06	0·80	0·845	<u>0·001</u>
mdh*bsns	31	1·03	0·08	0·93	26	0·89	0·10	0·87	0·282	0·497
tpf*bsns	31	1·11	0·06	0·96	26	0·97	0·08	0·93	0·187	0·072
nb*bsns	31	0·70	0·08	0·85	26	0·71	0·14	0·72	0·957	0·351
ba*bsns	31	0·55	0·07	0·84	26	0·58	0·06	0·89	0·730	0·161
pl*bsns	31	0·98	0·07	0·93	26	1·06	0·08	0·93	0·519	0·822
nl*bsns	31	1·26	0·10	0·92	26	1·45	0·17	0·87	0·323	0·366
mdl*bsns	31	0·94	0·06	0·95	26	0·89	0·07	0·93	0·561	0·951
apw*pl	40	0·52	0·04	0·92	36	0·46	0·04	0·89	0·253	0·531
nb*nl	36	0·53	0·06	0·83	36	0·41	0·08	0·68	0·215	0·017
mdh*mdl	40	1·04	0·06	0·94	36	0·95	0·09	0·89	0·374	0·327
bc*mdl	39	0·38	0·05	0·81	36	0·37	0·05	0·80	0·892	<u>0·000</u>
bz*nl	36	0·45	0·06	0·81	36	0·40	0·04	0·85	0·514	<u>0·000</u>
bz*pl	40	0·65	0·05	0·89	36	0·59	0·06	0·86	0·479	<u>0·007</u>

quickly than do those of the skull base or vault. Component I accounts for 95·2%, and component II accounts for 1·7%, of the total variance. A plot of component scores (not illustrated) suggest no discernable separation by group along the second component, although analysis of covariance for regression of component II on I scores yields a significant ($P < 0.003$) position difference, as in the previous case. A *t* test for differences in component II scores between the groups was also significant ($P < 0.014$).

Figure 7 illustrates results from a principal components analysis on the covariance matrix for all cranial measurements (including tooth rows), external dimensions, and all postcranial measurements, with body weight not included. Components I and II account for 88·7 and 2·8% of the total variance, respectively. First component loadings reflect changing proportions (e.g. postcranial values are generally higher than the skull values, and viscerocranial values are higher than neurocranial). Unlike all those analyses which included body weight, however, the second component produces no significant separation between the groups in either analyses of covariance for regression of component II on component I scores, or in a *t* test for differences by group on component II scores. There is also no separation between the groups on component II or subsequent components reflecting dissociation of cranial *vs.* postcranial patterns of growth allometry.

Additional principal components analyses were run on subsets of the cranial and postcranial dimensions, also excluding body weight. These yielded results comparable to those just discussed above, in that no significant separation of groups was produced on the second or subsequent components. In such cases, all of the shape variation may be ascribed to extrapolated allometric growth patterns within the skeleton as a unit.

4. Discussion

Results from the bivariate and multivariate comparisons of growth allometry suggest that skeletal
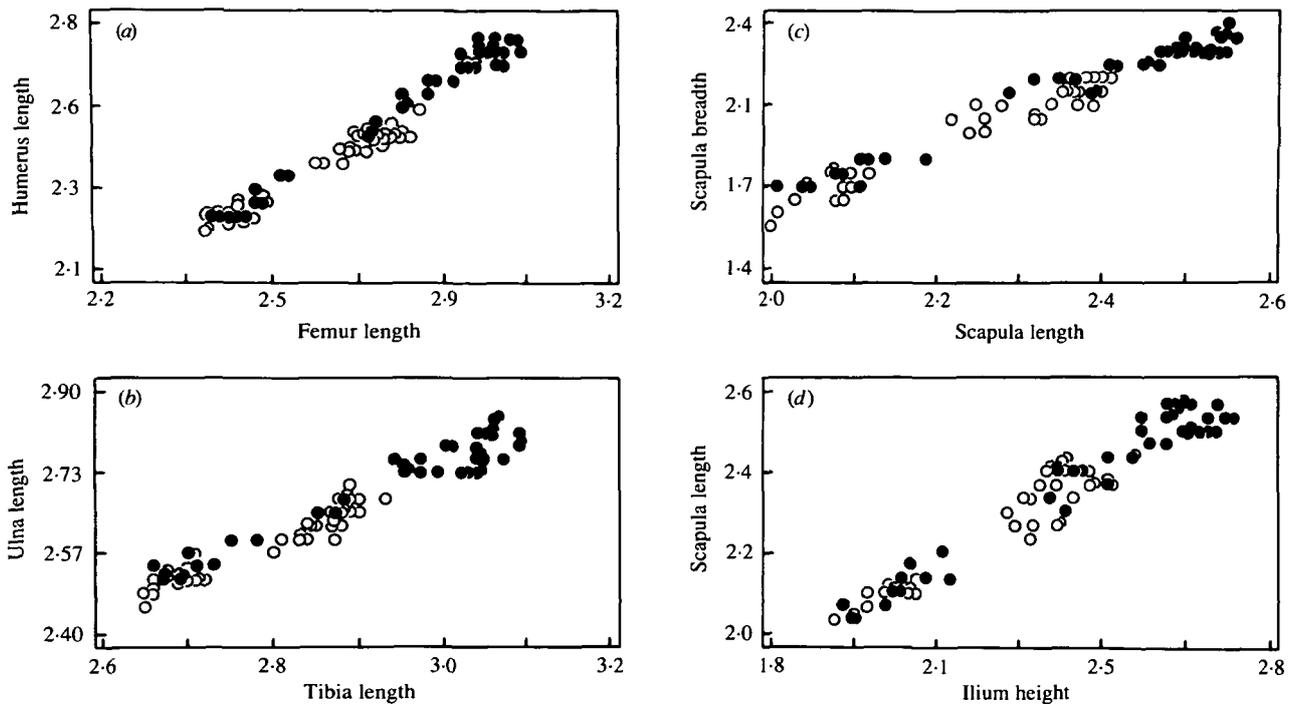
Fig. 4. Selected logarithmic plots among the postcranial skeletal dimensions. (a) Humerus length against femur length; (b) ulna length against tibia length; (c) scapula breadth against scapula length; (d) scapula length against ilium height. Symbols as in Figure 1. Note that the two groups share common cross-sectional trajectories for these comparisons. See Table 4 and the text for discussion.
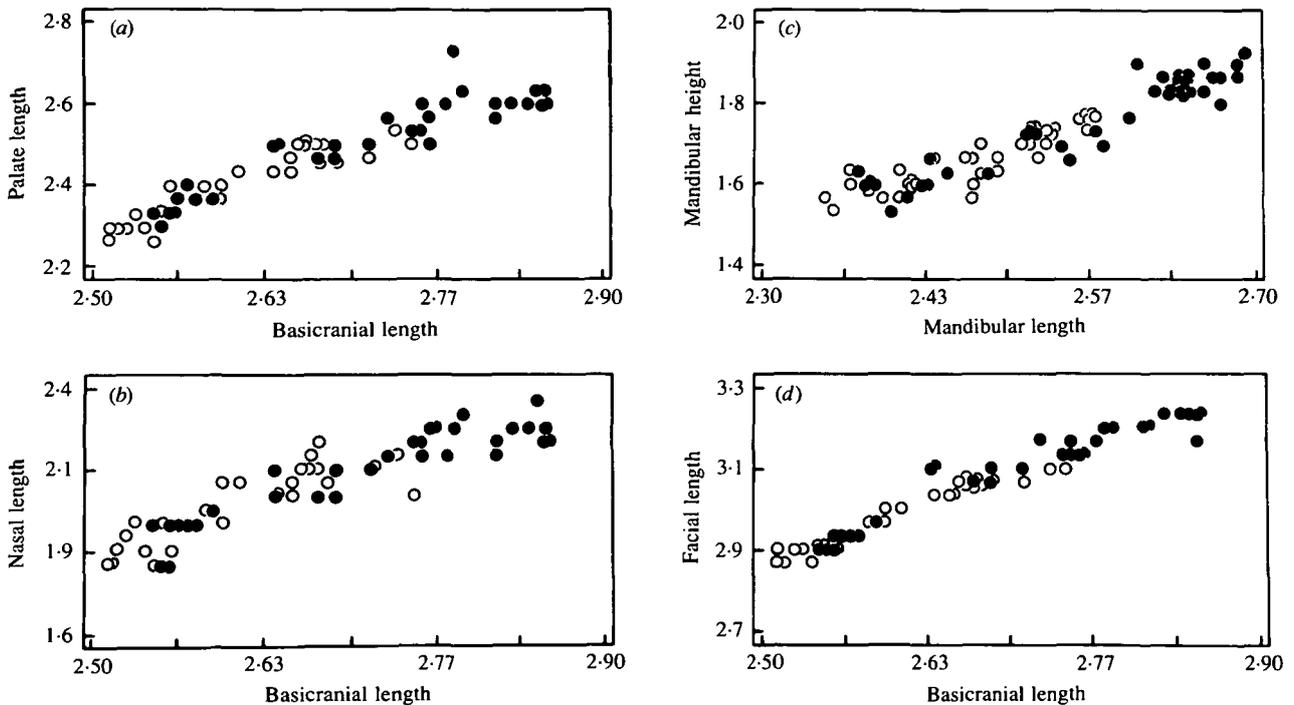

Fig. 5. Selected logarithmic plots among the cranial dimensions. (a) Palate length against basicranial length; (b) nasal length against basicranial; (c) mandibular height against mandibular length; (d) facial length against basicranial length. See Table 4 and the text for discussion.

proportion differences between the transgenic and control mice result predominantly from general and group size factors. In particular, the principal component analyses run on the entire or partial sets of skeletal measurements effectively demonstrate the close similarity of the patterns of growth allometry in each group, as well as how the extrapolation of this complex pattern of proportion changes can yield multiple shape differences in the adult state in the transgenic mice. Neither the bivariate nor the multi-
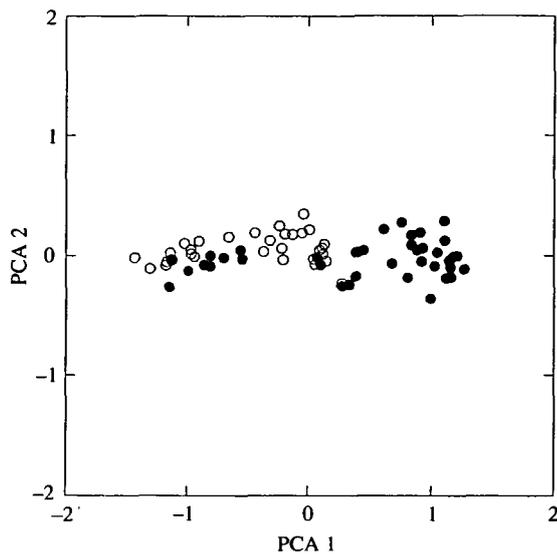
Fig. 6. A plot of individual scores of the control and transgenic mice on the first and second principal components derived from an analysis of postcranial skeletal measurements, external body dimensions, plus body weight. See text for additional discussion. Symbols as in previous figures.
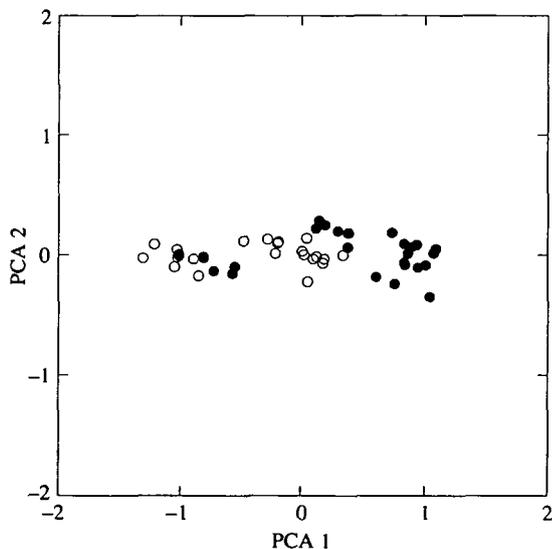

Fig. 7. A plot of individual scores of the control and transgenic mice on the first and second principal components derived from an analysis of all cranial and postcranial skeletal measurements plus external body dimensions. See text for additional discussion.

variate analyses provide any evidence of the effect of special size factors, or isolated dissociations and departures from underlying allometric growth patterns.

When body weights are included in the analyses, statistical results again support the existence of pervasive allometric extrapolations, although the partial group separations seen in the bivariate scatterplots and on principal component II in the multivariate analyses indicate some dissociation of the growth trajectories. There are several possible explanations for these results. One is the existence of various timing (as opposed to simple size) factors

influencing overall growth in body weight. The composition of a given weight increase can vary considerably during ontogeny in terms of both the tissues predominating and the relevant hormones (Eisen, 1976, 1986, 1987; Butterfield *et al.* 1983). For example, early weight gain is predominantly due to musculoskeletal and organ growth, while later weight gain involves larger percentages of fat (Eisen, 1987). The reasons for the divergence of the skeletal *vs.* total weight trajectories in the two groups are undoubtedly complex, and the greater weight of the transgenics at a common value of a given skeletal dimension may reflect the fact that the transgenics are both younger and exhibiting different patterns of growth in the various components that comprise total weight at this point in ontogeny. The fact that for all comparisons examined the controls complete growth at x/y ratios comparable to those of transgenics at the same total size may or may not be particularly significant. This problem deserves additional investigation, preferably using longitudinal approaches.

A second and more likely possibility for the partial dissociation of some of the allometric patterns relates to the complexity and curvature of the underlying trajectories themselves. It was noted previously that many of the bivariate plots against body weight evidence considerable curvilinearity (e.g. Fig. 2). Figure 8*a* illustrates this pattern more clearly in a plot of humerus length against body weight, using the distance weighted least squares (dwls) smoothing algorithm in the Sygraph Graphics component of the Systat Statistical Package. This algorithm allows the fitted curve to flex locally and better fit the data. Both the transgenic and control mice share a pattern of early moderate slope followed by a marked increase in slope, then trailing off into a considerable flattening of the curve. These changes simply reflect shifts in the relative amounts of increase in variable y compared to variable x; in allometric terminology, a departure from linearity in a log–log plot indicates changing patterns in the ratio of specific (logarithmic) growth rates of the two variables.

But what would be an appropriate direct extrapolation of such a complex trajectory? If the 'bends' in the trajectory are in a real sense totally determined by the value of the x variable, or overall body weight, then an appropriate extrapolation would be a simple extension of the late phase of lowered slope into even larger terminal size ranges. In such a case, the early phases of the trajectories would lie on top of one another. If, however, the 'bends' in the trajectory are in some way time-related, the appropriate extrapolation of such a curve would involve extensions of each component (here taken to be three for the sake of simplicity), producing a pattern something like that seen in Fig. 8*b*. In this case, the early phases of the trajectories would not precisely coincide. Yet what appears as a dissociation of allometric patterns (Fig. 8*a*) might actually result from simple extensions of the
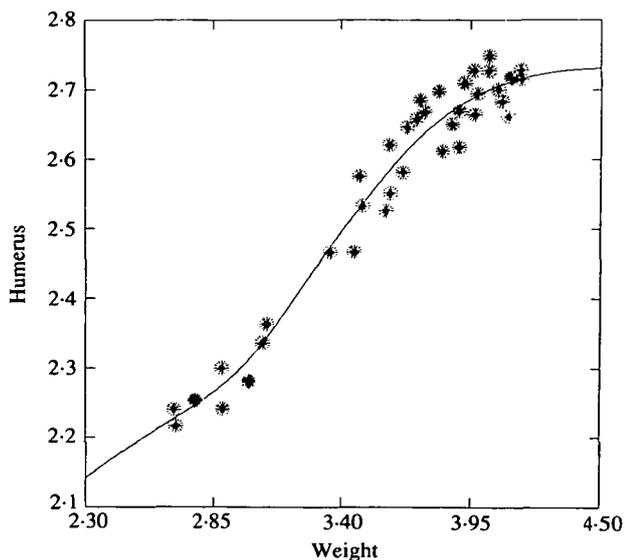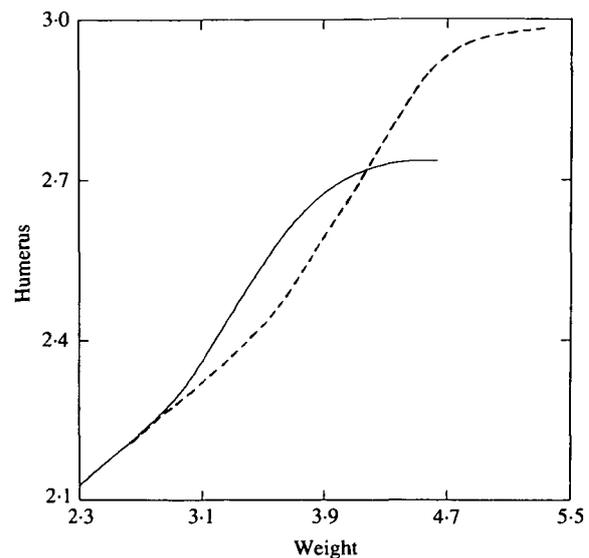
Fig. 8. (a) A logarithmic plot of humerus length against body weight in the transgenic mice. The line through the scatter is fit using Sygraph's (Wilkinson, 1988 b) distance weighted least squares (dwls) smoothing algorithm. (b) A schematic diagram illustrating the extrapolation (dashed line) of a complex growth trajectory such as that illustrated in (a). Each of the three segments of the trajectory is extended to larger size ranges – this results in a clear disjunction of the two curves, particularly in the middle size ranges. See text for additional discussion.

curve's various segments (Fig. 8 b). In joint plots of various dimensions against age, it does indeed appear that the two groups closely correspond in terms of the timing of shifts in their slope values. A firmer test of this hypothesis requires larger samples sizes, better sampling of certain time periods of the growth trajectory, and longitudinal data. Results of such tests will be important, but at this point careful examination of the growth allometric patterns suggests funda-mental similarities and extrapolations in a comparison of the transgenic and control groups. Similar arguments may apply to the differences in allometric patterns between size-selected and control mice presented by Shimizu et al. (1985) and Eisen (1986), especially since they note evidence for changes in slope values during different stages of development. Huxley (1932) presented other such examples.

Previously published studies of organ weight scaling in the transgenic and control mice provide an interesting comparison with the linear skeletal dimensions analysed in this paper. Shea, Hammer & Brinster (1987) found that almost all proportion differences in organ sizes between adult transgenic and control mice resulted from general size effects and simple allometric extrapolation. No partial dissociations during certain phases of the growth trajectories were observed, in contradistinction to some of the linear skeletal comparisons analysed here. The fact that the organ scaling patterns against body weight were strongly linear supports the hypothesis that it is the complex trajectories which produce the partial dissociations, however.

The results of this study clearly suggest that the skeletal shape differences between adults of the two groups are the direct result of overall differences in skeletal size (regardless of how the skeleton as a whole relates to total body weight). The altered hormonal environment of the transgenic mice does not therefore result in any fundamental dissociations of normal patterns of skeletal growth allometry. The extension of these patterns into new size ranges automatically produces significant shape changes any time slopes are significantly different from isometric values. The adult transgenic mice do not exhibit the same body proportions as the smaller adult control mice, although the tendency of many of the postcranial growth allometries to cluster around isometric values means that there is no particularly dramatic shape divergence. This is probably why previous visual examination of the transgenic mice resulted in the conclusion that they are 'normally proportioned' (Palmiter et al. 1983), although this assessment was also a reference to the fact that the transgenic mice do not appear to develop any of the characteristic features of acromegaly, such as enlarged and distorted jaws and distal extremities. To my knowledge, no rigorous assessment of general allometric shape changes in the skeleton of human giants or acromegalics has been made, apart from the focus on the isolated changes of particular interest in these clinical conditions. But clearly these results on the transgenic mice would lead us to expect some shape divergence, resulting from extensions of the underlying ontogenetic patterns of allometry and isometry. It is of considerable interest in this regard that differences in skeletal and anthro-pometric proportions in human pygmies as compared to neighbouring groups from Africa and Asia appear to result from simple truncations of common growth allometries (Shea, 1988, in press: Shea & Pagezy, 1988; Shea & Bailey, 1989).

The preponderance of general allometric effects and the absence of special size factors in the transgenic/normal contrast (and other contrasts involving systemic endocrine shifts) is understandable in light of what we know about the intrinsic (e.g., size of stem cell populations) and extrinsic (e.g. hormones) controls of skeletal growth (see Bryant & Simpson, 1984, for an important review). The elevated level of circulating growth hormone (GH) and insulin-like growth factor I (IGF-I) increases the rate of overall growth for all the skeletal components, but apparently in the relative proportions set by intrinsic growth controls, so that complex allometric relations of shape change are maintained and simply extended to larger terminal sizes (cf. Katz, 1980). Additional work on other mouse strains and various transgenic taxa is required in order to ascertain the generality of these results in the presence of genetically engineered GH overproduction. This hypothesis is currently being investigated in greater detail in a series of primate and other mammalian groups. More detailed information on the developmental bases of different patterns of growth allometry will also further enhance our understanding of the evolutionary significance of allometric patterns.

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