

comprised mainly of juveniles. Such a bias could perturb one's assessment of the interrelationship of the groups. Moreover, if growth is influencing the characters, this can result in a high within-group correlation between the characters indicating that each character is repeatedly measuring a similar facet of the phenotype.

Unfortunately the negation of growth effects is often discussed solely in terms of size and shape. This can lead to some confusion (Atchley, 1983; Humphries *et al.*, 1981) as the rationale for wishing to separate size and shape can be substantially different to the rationale for negating growth effects (Thorpe, 1983; Thorpe & Leamy, 1983).

Separating size and shape to understand the selective forces acting on them and their variation in time and space (Fleischer & Johnston, 1982) stems from an entirely different rationale to that for negating growth-dependent size in samples subject to growth bias. Similarly, size and shape effects may be separated in adult organisms in which growth has clearly terminated e.g. birds, or organisms at the same growth stage. In this situation "size" may be a valid, unbiased and highly heritable (Atchley, 1983) racial characteristic that one would not wish to ignore. The indication in Humphries *et al.* (1981) that I suggest size should be deleted is therefore misleading when taken out of the ontogenetic context in which it was originally discussed (Thorpe, 1976). Evidence that unbiased estimates of size in laboratory animals are heritable (Atchley, 1983; Leamy & Thorpe, in press) is of no significance for the need to consider negating growth effects in natural populations. Similarly, it is self-evident that phenotypic estimates of growth and growth-free variation are going to be influenced by both genetic and environmental factors. This is of no significance to the problem in hand unless one believes that all phenotypic studies on natural populations are pointless because of these environmental influences. This is an obviously facile viewpoint.

Characters and specimens

The growth-dependent characters selected for study are maxillary and mandibular tooth counts and snout-vent length. The specimens are Green geckos (*Phelsuma*) from the central (granitic) Seychelles, i.e. Silhouette, Mahe, La Digue, Praslin, Marianne, Felicite and Denis (coralline). These island populations belong to a species complex the taxonomy and evolution of which is under comprehensive review. Traditionally the populations from these islands are ascribed to *P. abbotti*, *P. longinsulae*, *P. madagascariensis* or *P. sundbergi* but they belong to a single species complex and in this paper they are referred to by island names without implication as to their nomenclatural status. Juveniles from this species complex (with a snout-vent length of circa 25 mm) tend to have few teeth; as low as 21 on the mandible and 23 on the maxilla. Adults on the other hand tend to have more teeth; up to 33 on the mandible and 35 on the maxilla. The clear tendency for tooth number to increase with growth within a population is illustrated by Fig. 1. Since log mandibular tooth count is significantly correlated with log snout-vent length within an island, e.g. Denis ($r=0.94$, $P<0.01$) it is evident that the estimate of the typical (mean, median, mode) tooth number of a population will depend upon the distribution of growth stages in the sample.

Consequently when mandibular tooth number for the Silhouette and Praslin populations are compared (Table I, Fig. 2) it is evident that they do not significantly differ. This is in spite of the fact that when specimens of similar size (circa 45 to 60 mm) are compared (Fig. 2) the specimens from Silhouette obviously tend to have more mandibular teeth than those from Praslin. Similarly, specimens from Praslin clearly tend to have more mandibular teeth

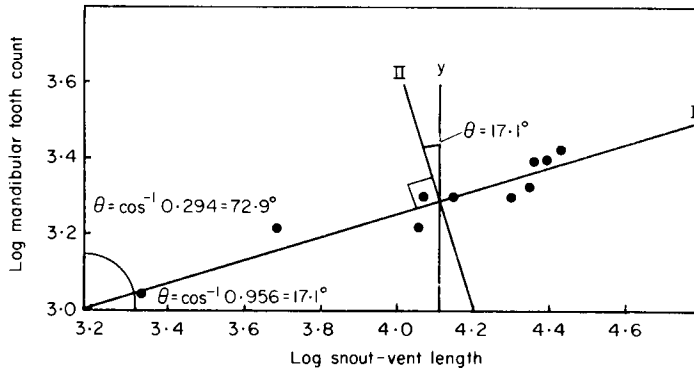


FIG. 1. Bivariate scatter of log mandibular tooth count against log snout-vent length (mm) for the Denis sample. Eigenvectors are given for the largest (I) and residual (II) eigenroot.

TABLE I
Group means

Island	Log transformed characters			Component score (Trivariate multiple group PCA)			n
	Maxillary teeth	Mandibular teeth	Snout-vent length	(1) Growth	(2) Tooth number in independent of growth	(3) Difference between maxillary and mandibular counts	
Silhouette	3.502	3.407	3.942	5.490	3.005	0.321	5
Marianne	3.371	3.266	3.874	5.355	2.852	0.321	8
Denis	3.417	3.294	4.111	5.593	2.809	0.343	10
Felicite	3.415	3.332	4.002	5.503	2.877	0.312	10
La Digue	3.423	3.290	3.977	5.469	2.862	0.346	10
Praslin	3.472	3.381	4.239	5.750	2.855	0.327	8
Mahe	3.499	3.403	4.073	5.610	2.950	0.326	10
<i>F</i> ratio	*3.00	*3.03	1.86	1.31	*21.09	*2.31	

*Indicates $P < 0.05$ significance for the F ratio from a one way analysis of variance of untransformed characters and component scores.

than those from Marianne (Table I, Fig. 2) but this is almost entirely due to their tendency to be larger (Fig. 1).

There may be two factors operating here. First, the average Praslin size is larger due to sample bias as juvenile specimens are absent. Second, whilst the Marianne specimens may be smaller due to sample bias (i.e. adults may not be represented) they may also be smaller due to racial variation in the extent of growth. It is evident from observing these animals in the field that there is racial differentiation in the extent of growth. However, in species

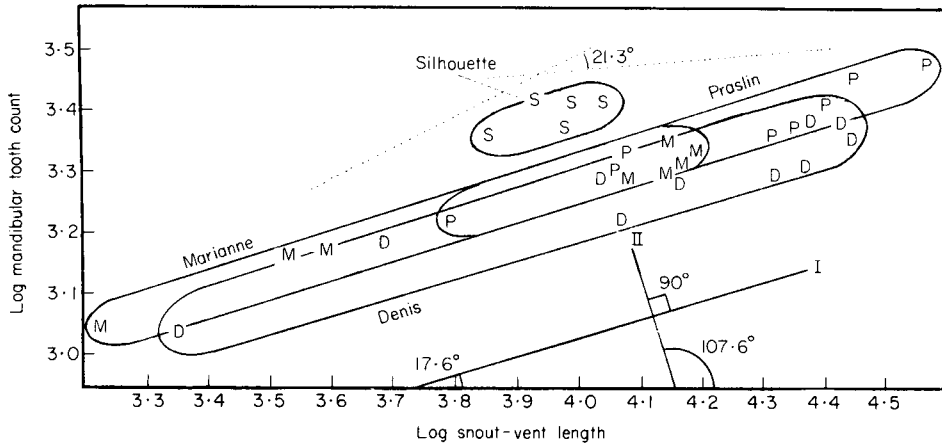


FIG. 2. Bivariate scatter of log mandibular tooth count against log snout-vent length for Silhouette, Praslin and Marianne samples. Eigenvectors of the pooled within-group (all groups) covariance matrix are given for the largest (I) and residual (II) eigenroot. The largest" eigenvector for juvenile Marianne and Silhouette pooled irrespective of group and adult Praslin and Silhouette pooled irrespective of group are given as broken lines. Note that they are not parallel to one another or to the within-group vectors.

that have continuous growth and that cannot be aged one cannot separate out the racial and ontogenetic components. Consequently when ontogenetic growth effects are negated then any information on racial difference in the extent of growth is also lost. This is true of other ontogenetic factors such as colour pattern fading due to ageing. However, this loss of information on the overall pattern of racial differentiation is generally trivial.

Even if the extent of growth (or colour pattern fading) is a valid heritable racial characteristic one may still be unable to use it to assess inter-group relationship if one cannot overcome the problem of sample bias and the problem of measuring the same feature repeatedly. The latter is generally easy to overcome if one can assess the pooled within-group covariance/correlation between characters. The former (bias), can be overcome by negating growth effects in various ways.

Negating growth

In geographic variation studies one cannot generally assess the growth of an individual at different stages. One usually has to attempt to define the growth variation by sampling different individuals at a range of growth stages from an otherwise homogeneous population.

A variety of biometric methods are used to adjust for growth independence in geographic variation studies, some being entirely erroneous whilst others are suboptimal. These methods are reviewed in Thorpe (in press) and just the methods based on bivariate regression, principal component analysis (PCA) and multiple-group principal component analysis (MGPCA) are considered here.

The log-transformed relationship between tooth number and SVL are linear but do not intercept the origin so a ratio of tooth number/SVL cannot be used (Thorpe, in press).

Bivariate regression can be used to adjust for growth independence where the growth "dependent" character (y) is regressed against an "independent" growth variable, e.g. snout-vent length (x). The growth independent variation is then measured along the Y axis at an

acute angle to the regression slope. This widely used technique is explained in most statistics textbooks, e.g. Snedecor & Cochran (1967) and examples are given in Thorpe (1975) and Iverson (1977). When there are several characters to be adjusted, e.g. mandibular teeth and maxillary teeth this has to be done separately for each character.

The linear regression of log mandibular tooth (dependent variable, Y axis) against log snout-vent length (independent variable, X axis) for the Denis sample (Fig. 1) gives the slope, b of 0.303. The angle between the regression slope and snout vent is therefore $\tan^{-1}b = 16.9^\circ$ and the angle between the regression slope and mandibular tooth count is 73.1° .

When there are several groups, as in geographic variation analysis, then one uses the pooled within-group regression slope and not the slope from the data pooled irrespective of group (Thorpe, 1976) as this will be at an angle to the "growth" axis.

The pooled within-group slope of log maxillary tooth count against log SVL is 0.253 whilst the pooled within group slope of log mandibular tooth count against log SVL is 0.314.

One group, two character principal component analysis

Geometric, bivariate explanations of principal component analysis can be found in most elementary texts on multivariate analysis and will not be dealt with in detail here. Principal component analysis has been used to study growth within single groups since at least Teissier's (1960) study.

Unlike straightforward regression, principal component analysis does not recognize "dependent" and "independent" variables. The eigenvector with the largest eigenroot expresses the most variance in the bivariate cluster and so is the "long" axis (Fig. 1) found so that the least square fit of the points at *right angles* to this axis is at a minimum. The vector associated with the remaining smaller eigenroot is at right angles to the first vector and expresses the remaining variance in this bivariate example. The number of roots and vectors being equal to the number of characters or the sample size minus one, whichever is the smaller. The component score for an individual is obtained for each vector by finding the sum of the product of the eigenvector elements and character states. The variance of the component scores gives the appropriate eigenroot.

Taking the Denis sample (Table II(a)) the variables are converted to logs to render the relationship between the variables linear. The variance-covariance matrix between the characters is then computed and the eigenroots and eigenvectors are extracted from this matrix (Table II(a)). The eigenvectors coefficients are the cosine of the angle between the character and the vector. These coefficients are frequently referred to as correlations or covariances but this is erroneous when the vectors are normalized. In this analysis the vector expressing the most variance has coefficients with the same sign. Both characters contribute to this vector which is often termed a "size vector" and expresses, in this example, within-group growth. The eigenvector coefficient for this vector has a value of 0.294 for mandibular tooth so the angle between mandibular tooth and the first principal axis is $\cos^{-1} 0.294 = 72.9^\circ$ whilst the coefficient for snout-vent length is 0.956 so the angle between the first principal axis and snout-vent length is $\cos^{-1} 0.956 = 17.1^\circ$ (note $17.1^\circ + 72.9^\circ = 90^\circ$). This is illustrated in Fig. 1.

The second vector, at right angles to the first, has contrasting signs for its coefficients and expresses mandibular tooth number independent of growth. The component score for tooth number independent of growth is found for each individual by (log mandibular tooth $\times 0.956$) plus (log snout-vent length $\times -0.294$).

TABLE II(a)
Bivariate PCA Denis Sample (n=10)

Variance-covariance matrix			
	Mand. count	SV length	
Mand. count	0.013		
SV length	0.038	0.125	
	Eigenroots & Vectors (character-component correlations in brackets)		
	(1)	(2)	
Root	0.137	0.001	
% Var.	98.98	1.02	
Vectors	0.294 (0.96)	0.956 (0.32)	Mand. count
	0.956 (1.00)	-0.294 (-0.03)	SV length

When covariance matrices are used these eigenvector coefficients are dependent upon the magnitude of the variance of the original characters. The geometric reason for this is easy to see when the axes are shortened or stretched. All things being equal as the magnitude of the variance increases then the magnitude of the eigenvector coefficient increases and the angle between the vector and the character decreases.

If the contribution of the characters is equal and their variances are equal (i.e. as in a correlation matrix) then the eigenvector coefficients should be the square root of the reciprocal of the number of characters.

When the variances are not equal the contribution of the characters to a component can be compared by computing the correlation between the *i*th character and *j*th component as:

$$r_{ij} = \frac{a_{ij} \sqrt{\lambda_j}}{s_i}$$

where a_{ij} is the coefficient of the *i*th character for the *j*th eigenvector, λ_j is the associated eigenroot and s_i is the standard deviation of the *i*th character. Since one is dealing with normalized eigenvectors (Thorpe, 1980) dividing the coefficient by the standard deviation of the character (Neff & Marcus, 1980) is not recommended.

In this bivariate example r_{ij} for log mandibular tooth and the first component is:

$$r_{ij} = \frac{0.294 \sqrt{0.137}}{0.114} = 0.96,$$

r_{ij} for snout vent length and the first component is:

$$r_{ij} = \frac{0.956 \sqrt{0.137}}{0.354} = 1.00.$$

Therefore the difference in the magnitude of the coefficients of the first eigenvector (0.294,

0.946) were largely due to difference in character variances as both characters are highly correlated to the first component. It is evident that, in contradiction to Jolicoeur (1963) and many authors since, log transformation does not adequately standardize the variance of the characters for interpretive purposes.

On comparing principal component analysis to ordinary bivariate regression the latter can be seen to have some slight geometric advantages. When the correlation between the characters becomes weaker the regression slope departs from the principal growth axis to a maximum of almost 45° more or almost 45° less depending on which character is selected as the "independent" variable (of course if the correlation is too low then one would not be carrying out these analyses). In the Denis sample (Fig. 1) the correlation is high ($r=0.94$) so the regression slope and principal axis are largely coincident, i.e. they differ by only 0.2° .

In principal component analysis growth-free variation is measured at right angles to the growth axis whereas in ordinary bivariate regression it is measured along the y axis at an acute angle to the growth axis. When the variances of the two characters are equal the angle between the y axis and the growth-free axis can reach a maximum of 45° . In the mandibular tooth example (Fig. 1) this angle is 17.1° .

These geometric limitations are rather trivial and can to some extent be reduced by using a regression model where both variables are subject to "error". However, the essential point of adjusting a range of growth-dependent characters is that they express growth not just the chosen "independent" growth character. In other words growth is essentially a multivariate rather than a bivariate concept.

One limitation of the multivariate approach that is not widely appreciated is the effect of log transformation when the character interrelationships are not all log-linear. In bivariate regression one can convert to logs (or otherwise) if the relationship between the two variables requires it. In multivariate analysis two characters may have a log-linear relationship with a third character, e.g. body length but a linear relationship with one another. Converting to logs in multivariate analysis may therefore render some relationships linear but at the same time render others curvilinear (since log transformation gives a curvilinear relationship if the original relationship was already linear).

Converting to logs in multivariate analysis of within-group growth is therefore done with the expectation of a general improvement in linearity. Since curvilinearity will result in a lower inter-character correlation an improvement in linearity should generally be apparent by a proportionally larger first eigenroot in the correlation (not covariance) matrix. In this data log transformation results in a slight overall improvement in linearity.

Multiple group principal component analysis (MGPCA)—two characters

Earlier studies on the multivariate analysis of growth (Jolicoeur, 1963) did not tackle the problem of inter-group comparison of growth-free variation. As with bivariate analysis when several populations (groups) are to be compared it is better to use the pooled within-group character relationships rather than pooling irrespective of group (Thorpe, 1976, 1981; Campbell & Dearn, 1980; Gower, 1976; Reyment & Banfield, 1976; Humphries *et al.*, 1981). This can be illustrated using the log mandibular tooth count and by SVL from several islands (Fig. 2).

The pooled within-group covariance matrix between log mandibular tooth count and log snout vent length (Table II(b)) was computed using samples from all seven islands. The eigen

TABLE II(b)
Bivariate MGPCA. All 7 samples

Pooled within-group variance-covariance matrix			
	Mand. count	SV length	
Mand. count	0.0096		
SV length	0.0267	0.0849	
Eigenroots & Vectors (character-component correlations in parentheses)			
	(1)	(2)	
Root	0.093	0.001	
% Var.	98.82	1.18	
Vectors	0.303 (0.94)	0.953 (0.31)	Mand. count
	0.953 (0.997)	-0.303 (0.03)	SV length

vector loading for mandible and length for the first vector were 0.303 and 0.953, respectively giving an angle between the first vector and character of 72.4° and 17.6° respectively (Fig. 2). In closely related populations one would expect the orientation of the within-group growth axis to be parallel for each sample so it is not surprising to find that the principal axis of the pooled within-group matrix is largely coincident (only 00.5° different) with that of the Denis sample on its own (Fig. 2). The direction of the vectors of a pooled within-group matrix should be robust to the inclusion or exclusion of groups since the technique assumes that the vectors of one group are parallel to those of another. The technique does not assume that the relative magnitude of the growth component (i.e. the % variation expressed by the associated eigenroot) is equal from one group to another. The more elongate the distribution, i.e. the broader the sample of growth stages sampled, the greater the difference in magnitude between the two eigenroots, i.e. the eigenroots for Silhouette sample (86.5%, 13.5%) are more similar than those of the Denis sample (98.9% 1.1%). This does not influence the separation of growth and growth-free variation and is of no significance unless one wishes to attribute percentage variation to the biological components which in this case is rather pointless.

If one wishes to compare growth-free variation between samples the first component in this analysis can be ignored as it expresses growth. The second component expresses tooth number independent of growth and the component scores of the individuals can be used directly for inter-group comparison (Fig. 2). The Silhouette sample can be seen to be clearly distinguished from the Praslin/Marianne samples along this second principal axis. The pooled within-group variance of these scores is of course the associated eigenroot.

If we were to pool the specimens irrespective of group the growth and growth-free components of tooth number would be confused. This can be illustrated by comparing the Silhouette and Praslin samples. If these samples are pooled irrespective of group the first principal component is six degrees divergent from the within-group growth axis. The fact that these vectors are highly sample dependent (unlike the pooled within-group vectors) is illustrated when the vectors are computed from the Silhouette and juvenile Marianne samples and then from the Silhouette and adult Praslin specimens. These two sets of vectors are widely divergent, the angle between them being over 21° (Fig. 2).

TABLE II(c)
Irvivariate MGPCA. All 7 samples

Pooled within-group variance-covariance matrix				
	Max count	Mand count	SV length	
Max count	0.0066			
Mand count	0.0073	0.0096		
SV length	0.0215	0.0267	0.0849	
Eigenroots & Vectors (character-component correlations in parentheses)				
	(1)	(2)	(3)	
Root	0.099	0.001	0.001	
% Var	97.89	1.48	0.62	
Vectors	0.239 (0.93)	-0.645 (-0.31)	0.725 (0.22)	Max count
	0.296 (0.95)	-0.662 (-0.32)	-0.688 (-0.98)	Mand count
	0.925 (0.999)	0.379 (0.05)	0.033 (0.00)	SV length

Consequently, it can be seen that vectors from samples pooled irrespective of group are labile in direction and can be at an angle to the growth and growth independent vectors found within a group. This is why it is generally better to use the pooled within-group vectors when there are several groups. Note that in multiple group principal components analysis the pooled within-group correlation between the component and a character is found as above except that the denominator is the pooled within-group standard deviation of the character.

Multiple group principal component analysis (MGPCA)—three characters

The primary values of principal components analysis is that one can consider many characters simultaneously and investigate their inter-relationships. In this example we add only a third character, log maxillary tooth count, but in most applications, e.g. analysis of linear dimensions the number of actual and potential variables is much greater.

The analysis is carried out in the same manner as the two characters, multiple group case above. That is, the three variables (maxillary tooth count, mandibular tooth count and snout vent length (mm)), are log transformed to render their relationships linear, the pooled within-group covariance matrix between characters is computed using samples from all seven populations; the eigenvectors and eigenroots are extracted and the principal component scores for each individual and group means are computed.

The eigenvectors coefficients for the eigenroot of the greatest magnitude (Table II(c)) have the same sign. The difference in magnitude of the eigenvector coefficients reflect the difference in the character variance as the pooled within-group correlation between the characters and this component are very high ($r=0.93, 0.95, 0.999$) for the three characters in Table II(c). This vector expresses within-group growth and we can see that the number of both maxillary and mandibular teeth increase with growth as does snout vent length.

Investigation of the component/character correlations (Fig. 2(c)) shows that the second vector contrasts tooth number with snout vent length and expresses tooth number independent of growth effects. Note that maxillary and mandibular teeth contribute equally to this vector.

The third vector is independent of snout vent length and contrasts maxillary tooth number with mandibular tooth number.

Once again we can ignore the component scores for the first vector in inter-group comparisons as it will be dependent on the size distribution of the sample of these continually growing organisms. The component scores for the second vector can be used to indicate general tooth number independent of growth.

Although the component scores represent linear combinations of the original characters they can be treated as any other continuous variable and used singularly or simultaneously in phenetic, evolutionary and cladistic studies as well as morphometric studies of natural (Campbell & Dearn, 1980; Thorpe, Corti *et al.*, 1982) or laboratory (Thorpe & Leamy, 1983) populations.

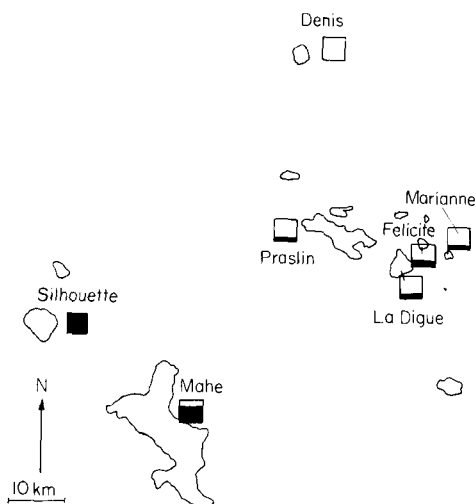


FIG. 3. Map of the Central Seychelles showing graphically inter-island variation in growth-independent tooth number (second component of trivariate, multiple group PCA). The boxes represent the proportional difference between the two extremes of Silhouette with the most teeth and Denis with the least teeth. The north-eastern populations (Praslin, Marianne, Denis, Felicite and La Digue) tend to have fewer teeth than the south-western populations (Mahe, Silhouette).

When the islands populations are compared using these growth-free scores (component 2) (Fig. 3) it can be seen that Silhouette has a greater growth-free tooth number than Praslin. Similarly one can see that there is no difference between growth-free tooth number in the Marianne and Praslin populations. Reference back to the discussion of Fig. 1 will show that the inter-island differences in tooth number were initially confused by growth effects.

The pattern of geographic variation in dentition

The growth-free pattern of geographic variation in tooth counts (Fig. 4(b), (c); Fig. 5(b)) shows that the south-western islands (Mahe, Silhouette) are distinct from the north-eastern islands (Praslin, La Digue, Denis, Marianne, Felicite). In studies currently in progress this is related to geologically determined phylogenetic events and is supported by congruent variation in the colour pattern. Variation in the scalation and shape emphasize differences between the north eastern populations (Gardner, pers. comm.).

The two growth-free principal components analyses (Fig. 4(b), (c)) and the growth-free canonical analysis (Fig. 5(c)) gave practically identical ordinations. Once growth is taken out the results are robust and do not depend on either the method of negating growth (except that its based on the pooled-within group relationships) or the final method of between-group ordination.

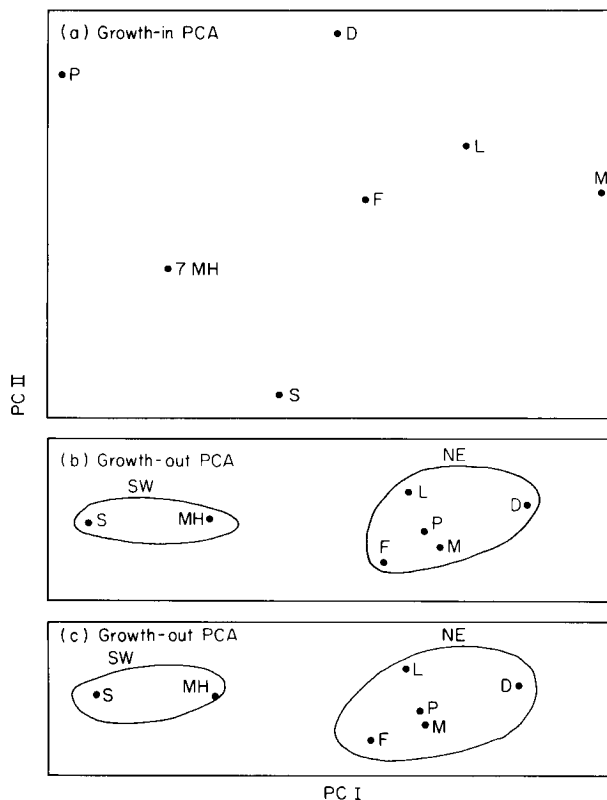


FIG. 4. Principal component analysis of population means. Populations coded as S=Silhouette, M=Marianne, D=Denis, F=Felicite, L=La Digue, P=Praslin, MH=Mahe, NE=north-eastern group and SW=south-western group. (a) Unadjusted for growth. PCA on mandibular and maxillary mean counts unadjusted for growth. (b) Adjusted for growth. PCA on mandibular and maxillary mean counts adjusted for growth by using the pooled within-group bivariate regression slope against snout-vent length. (c) Adjusted for growth. PCA on component means derived from the growth-free multiple-group principal components. The growth component of the analysis is ignored. The MGPCA was run on mandibular and maxillary tooth counts and SVL.

Since canonical analysis can be thought of as a PCA on group means derived from MGPCA scores (Campbell & Atchley, 1981; Thorpe, In press) it is not surprising that Figs 4(c) and 5(b) are so similar (the cophenetic correlation being 1.00, between the growth free D matrix and the taxonomic distance matrix, d , derived from MGPCA-PCA procedure). However, the technique of taking our 'growth' by bivariate regression and then running a PCA on group means (Fig. 4(b)) is technically independent to the above procedures (Fig. 4(c), 5(b)) yet gives extremely similar results (cophenetic correlation = 0.99).

The principal component analysis on the group means for tooth number *unadjusted* for

growth (Fig. 4(a)) gives a substantially different portrayal of the group inter-relationships to PCA ordinations on tooth number where growth has been negated by bivariate regression against SVL using the pooled within-group slope (Fig. 4(b)). The cophenetic correlation between the two inter-groups distance matrices being extremely low, i.e. $r=0.17$. Obviously the PCA ordination based on a multivariate adjustment for growth (Fig. 4(c)) is also very different to this unadjusted PCA (Fig. 4(a)) with their distance matrices being correlated at only $r=0.16$.

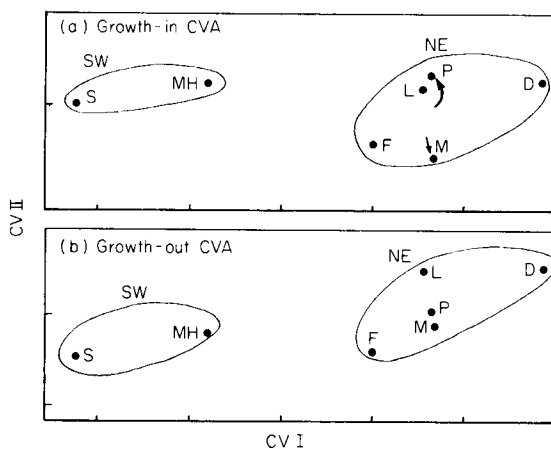


FIG. 5. Canonical analysis of population. Canonical variates in units of within-group standard deviations. Codes as for Fig. 4. (a) Unadjusted for growth. Canonical analysis on mandibular and maxillary counts and SVL. Note the displacement of the Prasin sample compared to the growth-free analyses (Fig. 4(b), 4(c), 5(b)). (b) Adjusted for growth. Canonical analysis on the growth-free multiple-group principal component scores (growth component ignored). The MGPCA was run as for Fig. 4(c).

When canonical analysis is run on MGPCA scores it gives identical results to using the raw data. Excluding the scores of the MGPCA growth component from a canonical analysis (Thorpe, Corti *et al.*, 1982; Thorpe & Leamy, 1983) is equivalent to some forms of growth-free discriminant function analysis (Gower, 1976; Reyment & Banfield, 1976). *Growth influenced* (Fig. 5(a)) and *growth-free* (Fig. 5(b)) canonical analysis give similar but not identical ordinations. As previously mentioned the Prasin sample is biased by the lack of juveniles. When Prasin is excluded the Mahalanobis D matrices for the growth-in and growth-out studies are correlated at $r=1.00$. However, the distances between Prasin and the other north eastern populations for the growth-in and growth-out are not similar, $r=0.39$. Consequently although the growth-in and growth-out canonical analyses were overall very similar their difference was largely due to displacement of the Prasin sample due to its growth bias.

Conclusion

Two factors operate when growth perturbs assessment of population affinities in geographic variation analysis. First, the pervasive influence of growth results in high (within-group) correlations between all characters so that each character repeatedly measures a similar facet of the phenotype. Second, in natural populations which exhibit continuous growth and cannot be aged there may be a bias in the growth stage of the sample.

One can see that the former (correlations) can be far more perturbing than the latter (bias) by comparing the extent of perturbation in growth-in PCA to growth-in CVA. When the within-group covariance is not negated (growth-in PCA) then both factors operate and the resultant inter-group "affinities" as so disrupted that they are of little or no value in indicating the pattern of geographic variation. When the within-group covariance is taken into account (as in canonical analysis and D^2) then only the latter factor (i.e. bias) operates. This does perturb the geographic variation, and could give very misleading details, but in both this study and other studies (Thorpe & Leamy, 1983; Riska, 1981) its effects on the overall pattern are relatively small.

Consequently if growth is not negated then the choice of techniques is important as the group interrelationships are going to be more suspect if assessed by principal component/coordinate analysis on group means than if canonical variates, and related analyses are used. If growth has been negated then the choice of technique is not so important.

The contribution of the within-group growth vector (from MGPCA) to canonical analysis is not assessed by the proportion of within-group variation it represents (this can be very high and is circa 98% in this example) but by the magnitude of the F value of its component scores compared to the sum of the F values of the other components (Thorne & Leamy, 1983). In this example the growth vectors F value (between group variance/within group variance) is a small proportion (Table I) of the total F value (i.e. 5%). Consequently the growth component can have little overall influence on the canonical variate scores even though it shows a different pattern of between group variation to the other component. When one takes out any ontogenetic effect such as growth one loses information on the racial variation in the extent of that ontogenetic effect. However, as shown above, if one is interested in the general pattern of racial affinities this loss of between-group information is relatively trivial. But even if it were not trivial one could not use it unless one was confident that it were unbiased. In organisms like lizards where one has continuous growth and no method of ageing then is unlikely to be sure that there is no bias in the ontogenetic stage of a sample.

Whilst such "bias" effects are not relevant to studying geographic variation in the linear dimensions of organisms with a determinable age or terminal growth one may still need to use canonical variates or related techniques to overcome the "correlation" effects of general size.

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