

A REVIEW OF THE NUMERICAL METHODS FOR RECOGNISING AND ANALYSING RACIAL
DIFFERENTIATION

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INTRODUCTION

Racial differentiation includes not only differentiation between spatially segregated populations (geographic variation) but also differentiation between temporally segregated populations (paleontological, annual and seasonal studies) as well as sympatric populations (e.g. karyotypic races, chronoraces, monophagous and parasitic races). Racial differentiation is investigated to deduce the causative factors, nature and mode of evolution and speciation, for taxonomic purposes e.g. erection of subspecies and also to indicate the nature of species and evaluate species concepts.

Having relatively recently attempted a review of the use of biometric methods to analyse geographic variation in phenotypic characters (Thorpe, 1976) this review will selectively emphasize facets such as the separation of ontogenetic (growth) variation from racial variation and the comparison of multivariate techniques to indicate phenetic patterns. The use of techniques to directly hypothesize phylogenetic patterns (e.g. Thorpe, this volume) are not considered. Whilst techniques that depend on the geographic location of the populations are dealt with separately (Sokal, this volume) it must be stressed that this division is arbitrary and it is frequently necessary to use both types of techniques together

These biometric techniques can generally be used with both allozyme data and phenotypic characters although the emphasis is on the latter. These two sources of information both have their advantages and disadvantages. Allozyme frequencies have the advantage that they are insusceptible to external environmental induction but have the disadvantage that they may be subject to post-transcriptional modification (i.e. internal environmental induction), the level of electrophoretic coincidence confusing an assessment of similarity may be quite high (Johnson, 1977), they can only be obtained from live specimens which can restrict their use to readily available extant forms from a limited geographic range, and a few allozyme frequencies may not be an adequate or unbiased representation of the status of the total genome. Allozyme frequencies are not the total 'genetic picture' as other factors such as the karyotype can be very important.

Phenotypic characters have the disadvantage of being influenced by

both genetic and environmental variation. However, they have the advantage of generally being readily recordable and polygenically controlled so they can reflect the condition of a large number of loci. This can outweigh the disadvantage of environmentally induced random noise. For example, comparison (Thorpe, 1981a) of various researches on genetic strains and sublimes of mice shows that multivariate morphometrics (Festing, 1972) is distinctly superior at discriminating between these genetic stocks than multivariate analysis of gene frequencies (Taylor, 1972). Genetic stocks of mice are now routinely validated by multivariate morphometric analysis of mandible shape (Festing, 1974). This practical superiority of multivariate morphometric analysis can be extended to natural populations where it can be used to hypothesize the phylogeny of, and discriminate between, electrophoretically indistinguishable karyotypic races of mice (Thorpe et al., 1982).

There can be little doubt that when the phenotype reveals broad racial patterns in time and space these generally have a genetic basis particularly since many patterns, e.g. progressively higher levels of anagenesis near the out-group root of phylogenetic trees, are inexplicable in terms of environmental induction. Recent work (Atchley et al., In Press) suggests that between group patterns of affinity based on multivariate morphometry are heritable and that there is a very high correlation (.9) between the 'genetical' and phenetical D^2 between populations. I interpret this as being supportive of the use of phenotypic characters to investigate race.

NEGATING NON-RACIAL VARIATION

Intraspecific variation is generally composed not only of racial variation but also sexual and ontogenetic variation etc. It is, or at least should be, intuitively obvious that one does not want one's assessment of the racial variation to be perturbed by a sample bias in the sexual or ontogenetic variation; i.e. one wishes to compare like with like. When studying geographic variation in characters also showing sexual and ontogenetic variation one would not wish to compare a sample composed of juvenile females to a sample composed of adult males. Sexual variation is easily dealt with by analysing the variation within the sexes separately but ontogenetic variation, e.g. growth and ageing, is more difficult to deal with.

What this section considers is the definition and negation of this ontogenetic component of the variation so that sample bias in growth stage does not perturb the assessment of racial variation. It must be stressed that this can be logically distinct from the analysis of size

and shape variation for its own sake (Fleischer and Johnston, 1982), although several recent papers have ignored or confused this distinction (Atchley, this volume; Humphries et al., 1981). Features other than linear variables are influenced by ontogenetic variation, for example, tooth number in lizards (Thorpe, In Prep.a), colour pattern fading in snakes (Thorpe, 1975a). More importantly whilst size generally varies ontogenetically it can also vary racially. When the latter is the case one may wish to characterize size and shape separately to investigate natural selection for those components (Fleischer and Johnston, 1982) or include a measure of size in ones assessment of the general racial differentiation. This can be quite valid so the implication in Humphries et al. (1981) that I believe that size should be discarded is misleading as it is taken out of its ontogenetic context (Thorpe, 1976).

When size varies both racially and ontogenetically the problem is how to measure racial size so that it is not perturbed by a sample bias in the growth stage. When organisms have terminal growth, e.g. birds, then we can take the adult size but when they have indeterminate growth and one does not know the age, e.g. reptiles, it can be impracticable to separate out the racial and ontogenetic components in size. In this situation size may be best discarded particularly if there is little difficulty finding other features that indicate the general pattern of racial affinities. When size is racial and one is assessing the general pattern of racial differentiation then it can be treated as any other phenotypic character, i.e. one does not want to repeatedly measure the same information so canonical analysis or a character selection procedure based on the intra-locality correlation (Thorpe, 1976) can be used.

The relationship between two phenotypic characters (x , y) in the growth of an individual may follow the power curve $y = ax^b$. This curve can be converted to a straight line by taking the logarithms of y and x ($y = a + bx$ in a regression model). The growth of the individual can then be plotted along this axis. In general one cannot follow the growth of an individual so the growth axis is approximated by taking a sample of individuals at different growth stages. If the log-linear model is correct then growth is measured along the axis and deviation from the axis is due to random phenotypic variation between individuals. One attempts to define this growth axis by the slope in a regression model and generally by the principal axis in a principal component model.

Ratios and related methods. Ratios such as y/x , where x is a 'standard' measure of growth stage and y is a growth dependent character, are still widely employed. Ratios are only an acceptable method of adjusting for growth if the relationship between the numerator and de-

nominator is linear and passes through the origin. Logarithmic conversion, to obtain the ratio $\log y/\log x$, as suggested by Hills (1978) may satisfy the former but will not satisfy the latter criteria. Consequently unless ratios satisfy these fundamental geometric criteria there is little point in seriously considering their use, nor is it worthwhile concerning oneself about the more subtle difficulties introduced by their correlation properties (Atchley et al., 1976).

Mosimann and James' (1979) approach to size and shape variation in Blackbirds is also ratio based although they vary the denominator. Since they are dealing with adults with terminal growth they are considering only a very short section on the 'growth' axis and the section farthest from the origin where the ratio changes along the axis are at their lowest. Tests for anisometry in these circumstances are unlikely to reveal a significant association between growth stage and ratio value. Apart from the observation that in these biological circumstances one would not generally wish to exclude 'size' it should be noted that Mosimann and James' procedure for studying size and shape is not designed for, or suitable for, negating growth effects.

Corruccini (1977) has attempted to overcome the problems of ratios by subtracting the intercept 'a' from the numerator so that the axis passes through the origin i.e. $\log y-a/\log x$. This is still not appropriate for comparing racial groups as the groups will have different intercept values if their growth-free variation differs. Subtracting different intercept values for each group will result in different values for the same specimen depending upon which group it is allocated to, and pooling irrespective of group implies an axis which is not parallel to the growth axis and so cannot produce growth-free variables.

Bivariate regression. One can attempt to take out the effects of growth by regressing the growth dependent character (y) against an 'independent' character x, expressing "growth stage" e.g. age or snout-vent length. The characters are generally log transformed to produce a linear relationship. The deviation from the slope is then taken as expressing growth-free variation in character y.

When there are several groups to be compared as in racial studies one uses the pooled within-group slope and not the slope pooled irrespective of group as this will not generally be parallel to the within-group growth axis (Thorpe, 1976). This assumes that the angle of the slopes within the various groups are comparable. Bivariate regression is a straightforward and useful method for attempting to adjust for growth effects when studying racial differentiation (Thorpe, 1975a; Iverson, 1977).

Although there are some relatively trivial geometric limitations to

bivariate regression (Thorpe, In Prep.a) the main limitation is that it assumes growth can be adequately defined by a single independent character. In reality most ontogenetic components, particularly growth in linear proportions, are multivariate in nature. Even age is not entirely satisfactory as, apart from the practical difficulties in estimating age, nutritional and other factors can influence growth rate so that animals of the same age are at different growth stages.

Principal component analysis (PCA). PCA can be used to give a multivariate generalization of growth in a suite of interrelated growth dependent characters. The biological interpretation of the components, i.e. growth or growth-free, is via the magnitude and sign of the eigenvector coefficients. Note that the aim is not to stare in wonder at these coefficients for their own sake but to attempt to define ontogenetic variation so that it can be partitioned, as well as possible, from racial variation. This can be a relatively minor part of the overall process of assessing the racial affinities. Once one has decided to use phenotypic characters to study racial variation in natural populations the fact that these coefficients will be influenced by both genetical and environmental factors is self-evident and of little direct relevance to the specific problem in hand.

When correlation coefficients are used growth vectors should have eigenvector coefficients of the same sign and similar magnitude, the coefficients approximating the square root of the reciprocal of the number of characters. When covariances are used the magnitude of the coefficients does not necessarily indicate contribution of the character to a component as this is influenced by the variance and the character. Although one takes logarithms to render the relationship between the characters linear the often quoted comment by Jolicouer (1963) that this standardizes the variance is erroneous insofar as the variance of the characters can remain very different after log transformation and one still needs to take this into account when interpreting the magnitude of the coefficients (Thorpe, In Prep.a). When covariances are used the contribution of a character to a component is indicated by their correlation which is found by multiplying the eigenvector coefficient by the square root of the eigenroot and then dividing by the standard deviation of the character (Thorpe, In Prep.a). Given that one is working with normalized vectors dividing the eigenvector coefficient by the character standard deviation as recommended in Neff and Marcus (1980) gives an unconstrained value.

PCA is a valuable technique for investigating ontogenetic variation in the phenotype (e.g. growth) within a population since it recognises the multivariate nature of most ontogenetic variation, it takes into account

the interrelationships between characters possibly enabling growth and growth-free components to be recognised, and it has geometric advantages over bivariate regression analysis (Thorpe, In Prep.a). However, selecting a narrow growth phase (Riska, 1981; Thorpe and Leamy, In Press) can make it difficult or impossible to clearly define a growth component; one cannot always mix different types of character together as ontogenetic variation in different character suites e.g. osteometric and weight, may be independent (Thorpe and Leamy, In Press) and one should not mix growth dependent and growth independent characters. It is not generally appropriate to include a 'known growth' character but it can be useful when there are only a few characters or when the ontogenetic variation is small (Thorpe, In Prep.a).

Multiple group principal component analysis (MGPCA) and related methods. If there are several groups (where a group equals a sample of a local population) the data should not be pooled irrespective of group but the eigenvectors should be extracted from the pooled within-group covariance or correlation matrix (Thorpe, 1981a, p 105; Campbell and Dearn, 1980; Thorpe et al., 1982; Thorpe, In Prep.a; Riska, 1981). This is referred to as multiple group principal component analysis (MGPCA). In a multiple group situation ordinary PCA is unlikely to be suitable as the vectors from a matrix pooled irrespective of group are unlikely to be parallel to the within-group growth axes as exemplified in Thorpe (In Prep.a) and explained geometrically for the bivariate case in Thorpe (1976).

A study (Thorpe, In Prep.a) of three growth dependent characters (maxillary and mandibular tooth count and SVL) from seven island populations of lizards, with indeterminate growth and no estimate of age, illustrates this procedure. Importantly, there is a bias between groups in the growth stage sampled, some groups are represented by a full range of specimens whilst others are represented only by grown individuals. The largest component of the pooled within-group covariance matrix of log-transformed data shows a high correlation with all characters and can be interpreted as a component best expressing phenotypic growth. The second largest component is only highly correlated with the tooth characters and can be considered as the component best expressing tooth number independent of growth effects. This interpretation of the vectors is of little interest on its own but it enables one to minimize the effect of having a between group bias in the growth stage of the samples by negating the component that best expresses growth variation.

The F ratio of the between and within-group variance indicates the magnitude of the racial variation for that component given unbiased samples. Whilst the components are orthogonal within-group they may or may

not be correlated between-group depending on whether they have congruent patterns of racial variation. The technique assumes that the within-group growth axes are parallel but does not require the range of growth within-groups to be similar or unbiased. Given this assumption the vectors are robust to the inclusion or exclusion of groups and may in some circumstances reflect biologically meaningful phenotypic components e.g. general tooth number in Thorpe (In Prep.a). The component best expressing growth can be ignored and the remaining, largely growth-free, components can be treated as orthogonal variables and considered independently (Thorpe, In Prep.) or simultaneously in cluster analysis, Steiner graphs (Wagner trees) PCA, and canonical analysis etc. When these 'growth-free' components are input into a canonical analysis (Thorpe et al., 1982; Thorpe and Leamy, In Press; Campbell and Dearn, 1980) then this is equivalent to some forms of growth-free discriminant function analysis (Gower, 1976; Reyment and Banfield, 1976).

The exclusion of the growth component from canonical analysis can have little effect (Thorpe, In Prep.a; Riska, 1980; Thorpe and Leamy, In Press) although the exclusion of the growth component did influence Reyment and Banfield's (1976) study of fossil forams. The inclusion of the growth component will have little effect if its F value is low compared to the sum of the F values of the other components or if its pattern of between group variation is congruent to the overall pattern expressed by the other components. This is discussed by Thorpe and Leamy (In Press).

Humphries et al. (1981) have recently suggested a method for analysing size and shape that is also pertinent to the negation of growth variation. Whilst the above techniques generally require the a priori recognition of groups their procedure does not. They first run a PCA and then recognise the groups. The size vector is extracted from the pooled within-group covariance matrix as in MGPCA and some growth-free discriminant functions. The shape components of the original PCA are then regressed so that they are independent of this size vector. The regressed shape components can then be plotted against other variables or components from other character sets (Chernoff et al., 1982). This is a valid but rather complicated and tortuous procedure. Since they use the pooled within-group principal axis their procedure has the same basis as the more straightforward precedents MGPCA and some growth-free discriminant functions (Campbell and Dearn, 1980; Gower, 1976; Reyment and Banfield, 1976).

In conclusion, there is a need to differentiate between the negation of ontogenetic effects so that sample bias in growth stage does not perturb an assessment of the racial affinities and the study of size and

shape variation for its own sake. A range of techniques are suitable for negating growth effects; I prefer those based on the pooled within-group character relationships whether bivariate or multivariate. Ratios and related techniques are generally inappropriate for this task as are any size/shape coefficients e.g. Penrose coefficients or correlation coefficients (mistakenly described as shape coefficients).

PHENETIC PATTERNS OF RACIAL VARIATION

Types of Pattern. There are a wide variety of patterns of geographic variation amongst contemporaneous populations some of which can be 'overlaid' to give a very complex picture. This can be further complicated by geographic variation in the extent of the local, sexual and ontogenetic variation as in the grass snake (Thorpe, 1973). The variety of patterns include the divergence of peripheral populations as in Rightmire's (1976) study of human populations in Africa; the divergence of island isolates as in studies of the grass snake (Thorpe, 1979); ecogeographic races as in Prentice's (1979) study of campion; mosaic variation as in Sokal et al. (1980) study of aphids; microgeographic variation as in Payne's (1978) study of the song dialects of sunbirds; clinal variation (gradual change over distance) as in the tendency for endotherms to gradually increase in size in colder regions; stepped clines (gradual change over a distance but with sharp alterations in the rate of change); categories of island populations as in Clover's (1979) study of Adriatic wall lizards, and categories of mainland populations and the contact (transition) zones between them as in the grass snake (Thorpe, 1979, 1980a).

Patterns of temporal variation can be studied in the short term (Berry and Jakobson, 1975; Fleischer and Johnston, 1982) or in the long term where the variation may be 'clinal', i.e. gradual, or 'categorical', i.e. punctuational, as in Williamson's (1981a) study of Turkana basin molluscs. There are a range of phenetic techniques that can be used to describe these patterns, e.g. cluster analysis and ordination. Once the pattern has been summarized one can then relate it to the geographic position of the populations in geographic variation (e.g. Thorpe, 1980a) or to chronological sequence in temporal variation.

Cluster analysis. Cluster analysis is widely used to investigate patterns of geographic variation. Earlier references are in Thorpe (1976) and later studies include Gold's (1981) and Loudenslager and Gall's (1980) investigation of racial categories in trout, Jensen and Eshbaugh's (1976) study of potential hybrids in oaks, Timm and Price's (1980) study of parapatric 'species' of parasitic lice, Clover's study of Adriatic lizards (1979), and William and Genoways' studies (1978, 1980) of racial categories in pocket gophers. Schennum and Willey's (1979)

analysis of racial categories in grasshoppers, Benton's (1980) test of the validity of garter snake subspecies, Schmidley and Hendricks' (1976) study of racial categories in the kangaroo rat, Straney and Patton's (1980) study of karyotypic races of pocket mouse, and studies of micro-geographic variation in song dialects of birds (Payne, 1978). With some exceptions these studies are generally orientated around the concept of racial categories.

Cluster analysis imposes a hierarchical/categorical nature on the racial variation which can be misleading. Smith (1979) used cluster analysis on Peromyscus californicus populations and concluded that there were two distinct racial categories, one in the north and one in the south. However, take the canonical variate discriminating between these groups (i.e. function 1 in Fig. 4a) and plot it against latitude. One can then see that this morphological variation is actually clinal and not categorical. Similar conclusions can be drawn from a comparable re-analysis of Havera and Nixon's (1978) study of geographic variation in Illinois gray squirrels. Even when the geographic variation is categorical as in some Adriatic wall lizards (Clover, 1979) cluster analysis can fail to clearly separate the racial categories. Moreover, one cannot assess the relative contribution of the characters in cluster analysis. I conclude that in many instances the use of cluster analysis is mistaken as it is usually less suitable than other techniques for studying patterns of racial variation.

Non-metric multidimensional scaling (NMDS). This is a psychometric technique that aims only to satisfy the rank order of the OTU's by monotonic regression. NMDS is not widely used to study racial variation although recently Clover (1979) has used it to show that there are two racial categories in two species of wall lizard and that their numerous conventional subspecies are valueless, whilst Prentice (1979) has used it to show clinal variation in the white campion and intergrading eco-geographic races in the red campion. Rightmire's (1976) previously mentioned study of peripheral divergence in human populations used NMDS, as did Hartmann's (1980) study of geographic variation in non-metrical traits in kangaroo rats, and Skeel and Carbyn's (1977) study of variation between relict populations of wolves.

In a comparison of NMDS and metric ordination Rohlf (1972) computed the correlation between the distances in the original matrix and the distances in the n-dimensional ordination for both techniques using ten data sets. Rohlf concluded that NMDS was superior because it generally produced ordinations with distances more highly correlated to the original matrix than did metric ordination. However, close inspection of Fig. 5 in Rohlf's paper reveals that this is only so for the higher dimensional

ordinations which are largely irrelevant to the application under consideration. If one compares the one-dimensional solution there appears to be no substantial difference between NMDS and metric technique using the criteria.

I attempted to compare NMDS with principal component/coordinate analysis and canonical analysis using two major racial categories and two divergent island outliers as a known model. The results showed clearly that the two major categories were less clear in NMDS and the divergence of the outliers was obscured. I concluded from this study that NMDS was inferior to metric ordination and this view was reinforced by a reanalysis of Clover's (1979) data. This showed that PCA indicated clearer differentiation of the northern and southern races for Podarcis melisellensis than Clover's NMDS (Thorpe, 1980a). For an alternative view and comment on some of the above work see Pimentel (1981).

Apart from these comparative studies which raise doubts about the suitability of NMDS I consider the non-metric model to be generally less suitable than the metric model for analysing racial variation. Imagine OTU's arranged evenly down a spiral in three dimensional character space. NMDS can represent this in one dimension (whilst PCA will take 3 dimensions) but in doing so it loses the information that the OTU's are arranged in this spiral fashion. Studies of racial affinities may well wish for this non-linearity to be revealed. Moreover, being a monotonic fit, the NMDS axis will follow the spiral of the OTU's and changes direction in character space. Consequently, NMDS axes have no meaning in relation to the original characters, the contribution of the characters cannot be assessed and they cannot either indicate the rate of racial change or the phenetic direction of this change. This is important as plots of the NMDS score in geographic space or against time therefore have little meaning.

In conclusion I can see no advantages but many possible disadvantages in using NMDS to study patterns of racial variation.

Metric ordination. These techniques are based on the extraction of eigenroots and eigenvectors from Q mode (between OTU) and R mode (between character) matrices. They include principal component (PCA), principal coordinate (PCOA), multiple group principal component (MGPCA) and canonical variate analysis (CVA). These techniques are all interrelated to a greater or lesser extent. R mode PCA on a correlation or covariance matrix is dual, i.e. identical, to Q mode PCOA on a taxonomic distance matrix under certain conditions (Gower, 1966a; Thorpe, 1980b). However, PCOA can be carried out on a wider range of Q mode distance/similarity matrices than can be equated by dual R mode covariance or correlation matrices. Canonical variates analysis is conventionally an R mode tech-

nique but the canonical variates can be extracted from a Q mode D^2 matrix by PCOA (Gower, 1966b). MGPCA is of course a PCA on the pooled within-group covariance or correlation matrix. CVA on the components scores from an MGPCA gives dual results to a CVA on the original data (Thorpe et al., 1982; Thorpe and Leamy, In Press; Thorpe, In Prep.a) and CV can be thought of as a MGPCA followed by PCA given certain constraints (Campbell and Atchley, 1981).

When the OTU's are populations or populations means and the aim is to summarize the pattern of racial affinities by PCA, PCOA, or CVA then the importance of the vector is generally related to the magnitude of the associated eigenroot. The pattern is generally portrayed by a two or three dimensional scatter diagram of the vectors with the largest roots. In this way the maximum between group variation is visualized, the residual between group vectors being ignored. This contrasts directly with MGPCA of within-group (population) character interrelationships where the magnitude of the variation expressed by the within-group vectors does not necessarily bear any relation to their between-group (i.e. racial) differences. Consequently, residual within-group vectors can be more influential than vectors with larger eigenroots in terms of their contribution to the pattern of racial affinities.

In the former case where the vectors with the largest eigenroots (from PCA, PCOA or CV) are used to summarize the pattern of racial affinity these vectors may show the primary pattern of differentiation e.g. two large mainland racial categories in grass snakes (Thorpe, 1979). There may still be a considerable amount of geographic variation within these primary categories 'unexplained' but investigation of the subsequent axes will not optimally portray this variation because these vectors must be a compromise between the variation within each of the categories. To overcome this resolution effect one must reanalyse the variation within each racial category separately. This resolution effect is also the reason why one should not attempt to investigate the racial variation in several species simultaneously. After the first vectors have separated the species the subsequent vectors will only be a compromise between the racial variation within the various species. Scatter diagrams are not the only way of portraying racial patterns as the individual between-group (population) vectors from CVA, PCA and PCOA can also be studied separately. Scores of individuals or population means along individual axes can be mapped in geographic space or plotted against time to reveal the pattern of racial variation such as clines in human gene frequency (Menozzi et al., 1978), distinct categories and the exact nature and position of transition zones in grass snakes (Thorpe, 1979) and mosaic variation in aphids (Sokal et al., 1980). The direction of a between-

group vector is dependent upon the inclusion or exclusion of groups and so is potentially labile when the number of groups is limited as when scores from a single discriminant function analysis are used to map a transition (hybrid) zone.

It is doubtful whether individual axes from between-group analyses should be interpreted in terms of character constellations such as size and shape. Between-group axes have no meaning in terms of the interdependence of phenotypic characters within a population. This is illustrated by the fact that they are dependent on inclusion or exclusion of groups and that even if character constellations are independent within-groups they may have similar patterns of racial differentiation between-groups. Consequently, 'size' between-groups may have a component of size but must also include any other factor, e.g. shape, with a congruent pattern of racial variation. In this situation, where one wishes to understand the pattern of racial (between-group) variation in a within-group character constellation MGPCA is the appropriate technique. For example, in Thorpe (In Prep.a) the geographic variation in the within-group character constellation of "growth-free tooth number" is portrayed by MGPCA on the pooled within-group covariance matrix. Pooling specimens irrespective of group as in Fleischer and Johnston (1982) will compromise the within and between group variation.

Analysis of within-group character interrelationships using metric ordination requires a linear relationship (or transformation to give a linear relationship) between the characters. However, it is not true that the between-group, racial, relationships have to be linear or normal for PCA, PCOA or CV when they are used to portray the pattern of racial variation.

Metric ordination techniques can be useful for indicating most patterns of geographic variation but they are not always sufficient. Other techniques, particularly maps or transects, may be necessary to understand the nature of the variation.

Principal component and coordinate analysis. The popularity of PCA/PCOA for analysing patterns of racial variation is second only to CVA. PCA/PCOA can be used with a wide range of types of characters measured on different scales but the characters must first be normalized. PCA/PCOA is a robust and reliable technique. I find the suggestion (Neff and Marcus, 1980) that it is unduly influenced by the inclusion of binomial/multinomial characters to be generally unfounded; for example, in Garrison's (1976) study of dragonfly morphs using multinomial and continuous variables the former do not dominate the latter. Except in the special case of a PCOA on a D^2 matrix these techniques have the disadvantage that they cannot take into account information redundancy

in phenotypic characters by considering their within-locality (group) correlation (Thorpe, 1976).

PCA/PCOA can utilize either individuals or population means as OTU's when investigating the pattern of racial variation. When there are a large number of groups and individuals as in Crome et al.'s (1980) study of spinifex pigeons, and to a lesser extent Nagorsen and Tamsitt's (1981) study of clinal variation in bats, it is best to use the group means as otherwise the scatter diagrams of individuals can be confusing. When one has insufficient populations or specimens to make grouping worthwhile, or when one wishes to test the validity of proposed natural groups, then PCA/PCOA on individuals can be useful. Recent examples of such usage include Chernoff et al.'s (1982) study of fish populations and their potential hybrids, Loch's (1977) study of karyotypic forms of shrew, Thorpe and McCarthy's (1978) study of sympatric house snake 'morphs', and Payne's (1978) study of microgeographic variation in song dialect of sunbirds. Recent examples of the ordination of population (group) means by PCA/PCOA include Birk's (1978) study of pollen variation, studies of racial categories and island divergence in grass snakes (Thorpe, 1979, 1980a, 1980b), William and Genoway's (1978, 1980) studies showing the presence of racial categories in the desert pocket gopher and their absence in the south-eastern pocket gopher, and Dowler and Genoway's (1979) study of clinal, interdemic variation in the yellow-checked pocket gopher, as well as many other studies.

In conclusion PCA and PCOA are extremely useful and reliable techniques for portraying the pattern of racial variation but MGPCA should be more widely used to investigate racial variation in (within-group) character constellations such as size and shape.

Usage and Abuse of Canonical Variates Analysis. CVA or discriminant function analysis is the most widely used technique for investigating multivariate patterns of racial variation. It is twice as popular as PCA, the next most frequently used technique. CVA can be an excellent technique for studying racial variation because when a group represents a local population CVA can maximize the between population variation in relation to the local variation. Moreover when morphometric characters are used it can have the desirable property of negating the effect of information redundancy in the character by taking into account their within-locality correlation (Thorpe, 1976). The disadvantage of CVA is that it requires the data to conform to a certain model, i.e. the within-group covariance matrices should be homoscedastic. This is likely for similar local populations but less likely for divergent populations or species when reorganisation of the developmental pathways has occurred. Whilst CVA is generally robust to the inclusion or exclusion of charac-

ters, a single heteroscedastic character (i.e. invariable in most but variable in a few groups) can perturb the results considerably.

When the heteroscedasticity is more subtle it may be instructive to carry out a MGPCA first and then do a CVA using the resultant component scores. This gives identical results to CVA on the original characters but enables the contribution of the within-group components to be assessed. The contribution of these components to the inter-group variation is indicated by their F score (between/within group variance) which should not necessarily be related to the magnitude of the pooled within-group eigenroot (variance). This is observed in Thorpe and Leamy's (In Press, Table 2b) study of variation in laboratory mice. However, in a study of natural populations of lizards based on 29 linear proportions the F values and eigenroot magnitudes are unrelated except for the smallest three eigenroots which have pathologically enormous F values (up to 4116 for the smallest root so that the smallest within-group component has more influence on the between group difference than all the other components together). When these last three components are excluded the results are congruent to those obtained from other character sets but when they are included one group becomes incongruently more divergent.

Using CVA to discriminate between predefined taxa or widespread races (Campbell and Saunders, 1976; Garrison, 1976; Diersing, 1981) is far less meaningful than using CVA to ordinate local populations to see if they aggregate into racial groups. When entire taxa or widespread races are used as groups the within-group covariance matrices are more likely to be heteroscedastic because they will be influenced by racial variation within the groups. More importantly, just because CVA can discriminate between these predefined categories does not mean that the variation is categorical, i.e. one could be discriminating between two sections of a cline, and if there are natural racial categories discrimination will be achieved even when the predefined groups do not exactly match these natural categories.

Suggestions that CVA is unsuitable stem in part from the above 'mis-use' but are essentially mistaken since the use of homogeneous a priori defined groups based on local populations or even compound localities does not prejudge the subsequent ordination of these groups (Thorpe, 1980b). Consequently, CVA can be validly used to portray a wide variety of patterns of racial variation. Earlier examples of such valid usage can be found in a review (Thorpe, 1976) and more recent examples include Benton's (1980) study of garter snakes, Baker and Mooed's (1979) search for geographic variation in birds recently introduced into New Zealand, Campbell and Dearn's (1980) elucidation of altitudinal differentiation and character displacement in grasshoppers, Goldstein's (1978) search

for geographic variation in the song dialect of bobwhites, Johnston and Sharman's (1976) investigation of Potorous showing the invalidity of conventional subspecies, the study of Lupins showing annual and clinal variation (Riggins et al.'s, 1977), Riska's (1981) study of clinal variation in Limulus, Iverson's (1977) study of musk turtles showing distinct racial categories, and Thorpe et al.'s (1982) study of sequential differentiation in karyotypic races of mice and studies of racial categories, clines, island divergence and transition zones in grass snakes (Thorpe, 1973, 1975a, 1979, 1980a, b, In prep.b).

Whilst careful interpretation of CVA scatter diagrams and the associated D^2 matrix can elucidate a wide range of patterns of racial variation some patterns are better investigated by mapping individual canonical variate scores. The presence of intermediates or 'hybrids' can be indicated by scatter diagrams but the exact position of the transition zone is best indicated by plotting the individual scores on a map. A transect across the mapped scores, as in Thorpe (1980a), can then indicate the nature of the zone i.e. absence of 'parental' forms in the transition area indicating that the intermediates are not F_1 hybrids, increased variation, rate of phenetic change and alteration in this rate of change with geographic distance. Clines can also be seen to advantage by taking transects of mapped scores (Thorpe, In Prep.b) and mosaic variation can be effectively portrayed by illustrative techniques based on mapped canonical variate scores (Sokal et al., 1980).

Since only about 20% of the studies using CVA also use PCA/PCOA there is some indication that these techniques are treated as alternatives rather than complementary. Whilst CVA has many virtues it can give misleading results if the data does not conform to the assumptions. It is therefore often worthwhile using PCA/PCOA to check the ordination of population by CVA as in Benton's (1980) study of garter snakes, Dowler and Genoways' (1979) and Williams and Genoways' (1978, 1980) studies of Gophers, studies of grass snake racial variation (Thorpe, 1979, 1980b, In Prep.b) and Schmidley and Hendrick's (1976) study of kangaroo rats. It is even more important to use PCA/PCOA to check on the ordination of individuals when CVA is used to discriminate between a few predefined groups as in Thorpe and McCarthy (1978) investigation of sympatric 'morphs' in house snakes, Crawford and Thorpe's (1981) preliminary study of green geckos, and Loch's (1977) study of karyotypic forms in shrews.

Additional techniques. Ordination techniques usually only summarize the pattern of racial variation, leaving a proportion of the between-population variation unrepresented. It can be useful to use additional techniques to take this into account. The most straightforward way is to plot the phenetic distance between populations on a map in the form of an

interconnecting network, or plot a minimum spanning tree on the scatter diagram or simply consider the critical values in the original distance matrix (Thorpe, 1976, 1979).

Birk (1978) has used multiplotting to attempt to overcome this problem in an analysis of the geographic variation in Picea pollen. The character or PCA scores of an OTU are represented by a wave function such that similar OTUs have similar wave forms. A diagram results with a wave form for each OTU, but this is virtually impossible to interpret so that one can visualize the pattern of racial variation between a series of populations. Another possible approach is the multivariate generalization of the simultaneous test procedure as used by Baker and Mooed (1979) to study geographic variation in New Zealand mynas. The maximally acceptable sets thereby defined are geographically coherent groups indicating that the between population variation is not random. However, this technique does not indicate the pattern of racial variation and a simple plot of geographic against phenetic distance might be equally or even more useful.

Concluding remarks. The multivariate analysis of patterns of racial variation has shown in many instances that there is no basis for the use of subspecies in the organism being studied, or if trinomials are useful then the conventional subspecies are erroneous (Thorpe, 1975, 1979, 1980a, 1981a, 1981b, In Prep.b; Clover, 1979; Rightmire, 1976; Riggins et al., 1977; Smith, 1979). Species limits have also been redefined on the basis of these multivariate studies (Gould and Woodruff, 1978; Nargossen and Tamsitt, 1981; Williams and Genoways, 1980).

The tendency of conventional taxonomists to section clines into artificial subspecies and arbitrarily delimit subspecies by physiogeographic features has been revealed by multivariate analysis of geographic variation (Thorpe, 1980a, 1981b, In Prep.b). This has shown that the neo-darwinian view of the importance of gene flow has been wrongly supported by the existence of 'subspecies' on either side of physiographic barriers 'restricting gene flow'. In the grass snake, for example, multivariate analysis has shown that the specimens either side of the Danube, Dniepr and Rhine are in fact racially undifferentiated even though these rivers delimit conventional subspecies (Thorpe, 1973, 1980a). Multivariate analysis can be used to derive operational criteria for the recognition of phenetic subspecies (Thorpe, 1979).

When phylogenesis has been the causative factor in differentiation multivariate analysis has revealed phenetic patterns of geographic variation that can be related to patterns of variation in other species and to geological events (Thorpe, 1975, 1979; Clover, 1979). These can be useful in indicating modes of speciation, for example allopatric speciation

due to Pleistocene events (Thorpe, 1975b; White, 1978). When selection for current environmental conditions has been the causative factor multivariate analysis can reveal phenetic changes in time and space (Fleischer and Johnston, 1982 and other references therein).

Multivariate analysis is also useful for investigating the nature of species insofar as it shows that the amount of racial differentiation within a species/semispecies can be greater than or equal to the amount of phenotypic differentiation between species/semispecies (Lessios, 1981; Thorpe, 1979, In Prep.b). This should be of relevance to those that emphasise the punctuation mode of macroevolution. Whilst Williamson (1981a) uses multivariate methods to study phenotypic variation in time he seems unaware of the fact that these same methods have previously shown considerable differentiation between geographically segregated populations as he claims that there is "relative morphological uniformity of most widely distributed modern species" (Williamson, 1981b). Similarly it can be seen that if a time series was selected using populations from different geographic regions one might well obtain a spurious punctuational pattern yet Lewin (1980) quoted Stanley as saying that "you can construct long sections (of a time series) by combining data from several areas". It is clear that the existence of substantial geographic variation should be assimilated into modern concepts of macroevolution.

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