plane shown in Fig. 2, we could not detect any positive cell staining, but a few fibers appeared in the indusium griseum which covers the superior surface of the corpus callosum. Some of these fibers pass parallel with the dorsal corpus callosum; others radiate, rapidly taper off, and fade away into the cingulate cortex.

We also examined the CAT-positive neurons of the guinea pig and found their distribution and morphological features to be similar to those of the rat.

Our results indicate that CAT, a reliable marker for cholinergic neurons, occurs in certain neurons of the rostral forebrain as well as the spinal cord. Cell bodies, fibers, and probably nerve terminals were stained. These findings in the rostral forebrain provide the morphological basis for the view that ACh may be the transmitter substance in some of the neuronal pathways postulated as a result of biochemical, neurophysiological, and histochemical studies (*2*). CAT-reactive cells in the medial septum and a part of the diagonal band seem to be the source of the cholinergic septo-hippocampal pathway. Some of the positive neurons in the diagonal band may be the origin of part of the presumed cholinergic pathway to the interpeduncular nucleus via the fasciculus retroflexus of Meynert. The morphological evidence for these pathways was obtained by testing histochemically for the presence of AChE, but that method is not a sufficient criterion for the identification of cholinergic neurons. Our results strongly indicate that these pathways are truly cholinergic. Cholinergic pathways originating from cells in the area of the olfactory tubercle and medial forebrain bundle have not yet been reported, but may be part of the sources for the cholinergic input to the olfactory bulb. It is noteworthy that those areas that contain a rich distribution of CAT terminals are also well known to receive a dense dopaminergic innervation.

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M-Statistics and Morphometric Divergence

Cherry, Case, and Wilson (*1*) compared morphological divergence between humans and chimpanzees to that of various taxa of frogs. They suggested that morphological differences between humans and chimpanzees were greater than between suborders of frogs although the divergence in structural genes in humans and chimpanzees is known to be small (*2*). Cherry *et al.* concluded that morphological evolution and biochemical evolution in structural genes can proceed at independent rates. Their work warrants scrutiny in view of its importance to current dialogue in evolutionary biology.

The distance statistic used by Cherry *et al.* to estimate the degree of morphological divergence may give erroneous results. Humans and chimpanzees may be so different morphologically that the results reported by Cherry *et al.* would also be obtained if the widely recommended Mahalanobis (*3*) distance were used. In view of the problems described below these data should be reanalyzed.

Cherry *et al.* used nine seemingly comparable continuous characters measured on frogs, humans, and chimpanzees. Each measure was expressed as a fraction of the combined length of all nine measurements; differences between means of the scaled variables were divided by the standard error of the difference between the means and the results for all characters were summed. Cherry *et al.* defined this *M* statistic as the average number of standard deviations by which two taxa differ.

An important deficiency of the *M* statistic is that it ignores correlations between characters. Multidimensional "divergence" statistics such as the *M* statistic must incorporate information not only about the means and variances of the characters but also about the intercorrelations between characters. In biologically divergent taxa, one would expect not only differences in means and variances but also in correlations as well. Serious statistical mistakes as well as biological misinterpretations may result from ignoring intercorrelations in morphometric data.

To understand the effect of character correlations, consider the Pythagorean distance (D_P^2) for characters X_1 and X_2 and taxa A and B

$$D_P^2 = d_1^2 + d_2^2 \qquad (1)$$

where

$$d_1^2 = (\bar{X}_{1A} - \bar{X}_{1B})^2 \text{ and } d_2^2 = (\bar{X}_{2A} - \bar{X}_{2B})^2 \qquad (2)$$

Equation 1 can be rearranged to produce

$$D_P^2 = d_1^2 + d_1^2 f^2 \qquad (3)$$

where $f = d_2/d_1$. The Pythagorean distance does not take into account intercorrelations between characters. A distance measure that accounts for intercharacter correlations is the Mahalanobis distance (*3*). If r is the nonzero within taxon correlation between X_1 and X_2, then the Mahalanobis distance for the two-dimensional case can be written as

$$D_M^2 = d_1^2 + \frac{d_1^2(f - r)^2}{1 - r^2} \qquad (4)$$

Figure 1 shows the change in D_M^2 as r varies from -0.9 to 0.9 for various positive values of f. Figure 1 indicates that, when d_1 and d_2 are positive, negative correlation always increases the distance; however, positive correlations can have a manifold effect in that they augment the distance in some instances and decrease it in others.

Distance statistics that do not account for intercorrelated characters have been proposed in the past for morphometric data; for example, the coefficient of racial likeness of Pearson (*4*) which is very similar to the *M* statistic. However, these methods have also been heavily criticized by statisticians since the 1930's (*3, 5–8*). Fisher (*5*, p. 62) stated that "the effect [of not accounting for intercorrela-

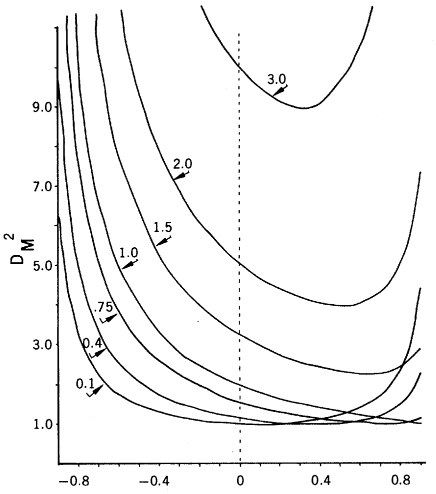

Fig. 1. The effect of intercharacter correlation (r) on the Mahalanobis distance (D_M^2) for values of f ranging from 0.1 to 3.0. All curves are defined with $d_1^2 = d_1 = 1.0$. The value of D_M^2 when $r = 0$ is also the Pythagorean distance with no correlation while values to the left and right of zero for each curve demonstrate the effect of correlated characters.

tions] is to cause very high or very low values of the coefficient to occur more frequently by chance than they should. This effect increases rapidly, both for statistical and anatomical reasons, as the number of different measurements used is increased." Talbot and Mulhall (*7*, p. 82) conclude that ignoring the existence of intercorrelated characters alters the magnitude of the distance significantly and can result in invalid conclusions being drawn regarding taxa affinity. Blackith and Reyment (*8*, pp. 36–38), studying sexual dimorphism in wasps, examined the effect of character correlation and concluded that "the main influence of the correlation has been greatly to exaggerate the distances between groups which are already well separated (that is, queens and workers) . . . whereas those between males and workers are uniformly reduced." These conclusions, together with the results from Fig. 1, indicate that ignoring intercorrelations may have the effect of exaggerating both taxa affinity and divergence.

I have been unable to obtain a copy of their data from Cherry *et al.* for further analysis. However, Wilson (*9*) provided me with both the *M* statistic and Mahalanobis distance for three separate pairwise examples from their data. In all three instances, there is a disparity between numerical values for the *M* statistic and the Mahalanobis distance and the relative distances between taxa are greater based on *M* rather than the Mahalanobis distance. Such deviations would be expected when the character-intercorrelations are ignored.

A second point requiring emphasis is that the *M* statistic uses the ratio of each measurement divided by the sum of all measurements of the individual specimen. This procedure can significantly alter the correlation structure of the data (*10*). One can conclude that Cherry *et al.* may have induced a complex change in the existing correlation structure of their data and then selected a measure of morphological divergence that fails to account the correlations between characters.

An accepted distance statistic for continuous, intercorrelated variables is the generalized Mahalanobis distance (*3*). This statistic was introduced in 1936 as a response to Pearson's coefficient of racial likeness which, like the *M* statistic, does not correct for correlated characters. The Mahalanobis distance is widely used and is a by-product of many computer programs for discriminant analysis. Reference to the use of Mahalanobis distance is found in most modern textbooks on multivariate statistics (*11*).

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12. I am indebted to N. A. Campbell, J. J. Rutledge, and E. V. Nordheim for their criticisms of a draft of this comment. This research was supported by the College of Agriculture and Life Sciences, University of Wisconsin, Madison.

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Atchley (*1*) may be correct in suggesting that the Mahalanobis method (*2*) is superior, in theory, to the method of Cherry *et al.* (*3*) for the study of anatomical evolution (*4*). In practice, however, the Mahalanobis method has a major shortcoming in that it provides a stable estimate of morphological distance only if one examines a large number of individuals in each population.

Table 1. The instability of metric values based on covariance. The relative lengths of the nine morphological traits described by Cherry *et al.* (*3*) were used to compute Mahalanobis *D* and the *M* statistic for two pairs of species. The primate species, 1 and 2, were humans ($N = 16$) and chimpanzees ($N = 13$), respectively. The frog species, 1 and 2, were *Rana boylei* ($N = 14$) and *Rana muscosa* ($N = 23$), respectively. For the rows labeled species 1, the distance between species 1 and 2 was calculated from the variances (and covariances in the case of *D*) for species 1 alone. An analogous procedure was used for the rows labeled species 2. For the rows labeled both species, the variances (and covariances) were calculated in the standard way, that is, by pooling the variances and covariances for both species.

Metric	Variability calculated from	Distance between species	
		Frogs 1 versus 2	Primates 1 versus 2
D	Species 1	11.2	39.3
	Species 2	5.0	23.5
	Both species	4.6	23.5
M	Species 1	0.85	4.8
	Species 2	0.87	4.5
	Both species	0.83	4.4

The requirement for a large sample size stems from the fact that the Mahalanobis method is designed to correct for correlations between traits. To do that, one must obtain an accurate estimate, r, of the actual correlation, ρ, between traits. The difficulty of this task can be gauged by looking at the graph of the confidence limits of r in an elementary statistical text (*5*). Sample sizes greater than 50 are needed before r begins to behave consistently, especially when ρ is zero. The problem of accurate estimation of ρ is compounded when the covariance structures of the two populations being compared differ greatly (*6*).

As a consequence of these considerations about covariance, the Mahalanobis generalized distance *D* would be expected to be unstable when small numbers of individuals per population are used. This same criticism does not apply to the *M* statistic of Cherry *et al.* (*3*, *7*), which takes variance but not covariance into account. Empirical examples demonstrating the instability of Mahalanobis *D* are given in Table 1. For each pair of species compared, the values of *D* span a wide range (about twofold) while those for *M* span a narrow range (about 1.1-fold).

The simple metric, *M*, used by Cherry *et al.* (*3*) does not correct for covariance mathematically. If two traits are correlated and both contribute to differences between populations, the double dose of difference is accepted by M. The problem of correlation of traits has not been ignored, however. Rather, it has been minimized by working with a small number of traits (*8*) and ensuring that they represent all major parts of the body. Because the M statistic eschews correction for covariance, it facilitates the study of those many species for which few specimens are available in museum collections (*9*).

Responding to Atchley's comment on ratios, we recognize that the use of ratios alters the correlation structure of the data. As our goal was to compare the shapes of organisms, it was essential to remove trait correlations due to variation in body size. The ratio method achieves this. According to the criteria of Anderson and Lydic, our use of ratios is warranted (*10*).

While M may not be a perfect distance metric, it seems more valuable in practice than Mahalanobis D for broad comparative studies of evolution at the organismal level. This impression is reinforced by the observation that M is correlated more highly with rank in the taxonomic hierarchy than is D (*11*). We assume that rank in the hierarchy summarizes judgments made by earlier generations of taxonomists about the degree of anatomical difference between organisms. The weaker correlation of D with taxonomic rank is ascribed to the instability of D values calculated from comparisons involving small numbers of individuals per population.

Although we consider Mahalanobis D to be less useful than the M statistic for our type of research, the Mahalanobis distance between humans and chimpanzees (Table 1) is large compared to that between species of frogs. The conclusion reached by use of the M statistic (*3*) is thus bolstered by the results obtained with the Mahalanobis method.

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4. The broad studies of anatomical evolution in which we are engaged require the comparison of extremely dissimilar creatures as well as very similar ones. The Mahalanobis method was designed to compare very similar organisms, such as those from different populations within a species (*2*). We are not convinced that it is justifiable, in theory, to use the Mahalanobis method for estimating the morphological distance between such dissimilar species as humans and chimpanzees (where $D^2 = 552$). Others have expressed similar caution [R. D. Reyment, *Biometrics* **18**, 1 (1962); R. S. Corruccini, *Am. J. Phys. Anthropol.* **40**, 425 (1974)].
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6. In the case of humans and chimpanzees, for example, the covariance structures are very different. The differences in r value for pairs of traits range from -0.68 to $+0.62$. In contrast, the variances of each trait are similar in the two species (*3*).
7. In defining the M statistic Atchley substitutes "standard error of the difference between the means" for "pooled standard deviation of relative trait length." For the original definition of M, see (*3*).
8. We do not recommend the use of more than nine traits in computing M (*3*). More traits will magnify the problem of correlated traits to the point where M values become unreliable estimates of distance.
9. For a majority of species in museum collections, complete specimens of only a few representatives are available (*3*, footnote 8).
10. D. E. Anderson and R. Lydic, *Biobehav. Rev.* **1**, 225 (1977).
11. The high correlation of M with taxonomic rank ($r = 0.88$) was illustrated by Cherry *et al.* (*3*) for a series of frog species. A detailed presentation of the results of empirical tests involving Mahalanobis D ($r = 0.74$) and other metrics is in preparation.
12. We thank R. C. Lewontin for valuable criticism of an earlier draft.

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Anomalous Water in the Deep Ocean Suggests Lateral Advection-Stirring

Although the report of Amos and Gerard (*1*) is tantalizing, the temperature and density data are incorrectly interpreted. These investigators state that bottom water at 40°26.2′N, 56°55.8′W (station *Lynch* 47-186) in 5200 m of water has the properties of water found about 1000 m higher up in the same water column and suggests turbidity current activity. Their figure 2b (*1*) of near-bottom vertical profiles includes a break in the depth scale to show how the values of salinity S, temperature T, density σ_t, and dissolved O_2 content at the ocean bottom are also found from 4000 to 4300 m. The problem with this interpretation is that neither T nor σ_t are conservative properties in the deep ocean (*2*, chap. 3, p. 1087).

The adiabatic gradient is indicated in their figure 2b for the deepest level; the value shown (0.096°C per kilometer) is, however, incorrect (*2*, p. 63; *3*). In fact, the adiabatic temperature change associated with the downslope advection advocated for the 2.32°C water found at ~ 4200 m amounts to a warming of 0.13°C. A problem associated with using the nonconservative density function σ_t in the deep ocean is that profiles of σ_t appear unstable. This is also illustrated in their figure 2b; apparently less dense water is found beneath denser water. This artifact of the equation of state of seawater can be circumvented if one uses a conservative density function referenced to a nearby pressure surface; usually the 4000-dbar surface is used as a reference for the density function, σ_4 (*4*). If potential temperature θ and a properly refer-

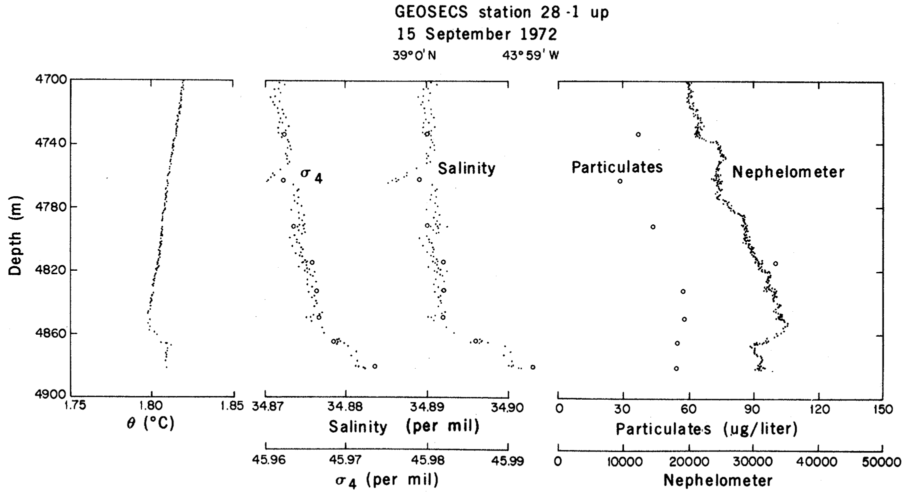

Fig. 1. Profiles of potential temperature θ, salinity S, density σ_4, light-scattering, and particulate matter content at GEOSECS station 28 (*6*).

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