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A COMPARATIVE METHOD

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Evolution is a process of modification through descent. The chief agent of modification is natural selection, as a consequence of which organisms are more or less well adapted to the conditions in which they live. Selection is often capable of producing substantial change in the short term (see Endler 1985), in which case it can be studied by direct observation and experiment. However, many characters have evolved over long periods of time and cannot be studied experimentally because they cannot be appropriately manipulated. Theories that attempt to identify the circumstances in which such characters are adaptive must therefore be evaluated by comparing groups of organisms living in different circumstances. But because evolution is emphatically a process of modification through descent, the similarity of related organisms sharing a common way of life could be held to reflect their common ancestry as well as any common cause of modification. This, in brief, is the dilemma of the comparative method, which has been the subject of much recent discussion (references below). I propose here that this dilemma can be largely resolved through the use of an appropriate statistical technique. The technique itself has been known for many years, but to the best of my knowledge it has not previously been applied to comparative data, and it seemed worthwhile to introduce it to a wider audience.

The problem that we face is as follows. A biologist studying brain size, chromosome number, or some other character that cannot be deliberately modified without the grave risk of destroying the phenomenon under investigation proposes that the variable of interest, Y, evolves in response to changes in another variable, X. Accordingly, it is predicted that Y will correlate with X when both are measured in extant organisms living under natural conditions. When Y and X are measured in a large number of species, a significant correlation is indeed discovered, and to this extent the hypothesis is supported. But the species that have the most extreme values in one direction of both Y and X, thereby making the largest contribution to the correlation, belong to the same genus. Clearly, some part of the similarity between these species may have arisen because they share a recent

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common ancestor, rather than because they have all responded in the same way to similar forces of selection. Any such effect of descent must be removed before a functional hypothesis of modification by natural selection can validly be tested. Two means of removal have been suggested. In both views the problem is to ensure the statistical independence of data points, so that the usual significance tests can be applied to the correlation coefficient.

Clutton-Brock and Harvey (Clutton-Brock and Harvey 1979, 1984; Harvey and Mace 1982) have pointed out that a nested analysis of variance enables one to estimate the variance of a character attributable to a particular taxonomic level, independent of other levels. The level at which the data should be analyzed is the one that contributes most of the variance of the dependent variable. Thus, if there were substantial variance at the level of subfamilies, but not at higher or lower levels, they would recommend testing the functional hypothesis by calculating the correlation of subfamily means. Techniques of essentially this sort have been used in most recent major comparative analyses (especially Stearns 1983; Dunham and Miles 1985). Although this procedure introduces a welcome note of statistical rigor, it has several drawbacks. One is that more or less equal variances may exist at several taxonomic levels. In many cases there may be no true dependent variable, while the two covariates display different taxonomic distributions of variance. Further, although we are interested primarily in covariance, it is not necessarily greatest at the level where the variance of the dependent variable is greatest. A more fundamental objection, involving the decision to measure correlations between rather than within groups at the chosen taxonomic level, is advanced below.

A more radical solution, proposed by Ridley (1983), requires counting the number of times that a character has evolved independently during the history of a group. Cladistic methods are used to sort into groups all taxa having the same value of Y and the same common ancestor. Since there is no variance of Y within groups there can be no covariance, and the test of the functional hypothesis involves the covariance between groups. In effect, Ridley eliminates the effect of ancestry by his grouping procedure, leaving function as the only explanation of the remaining covariance. This method was developed for attribute data, but an essentially similar technique that can be applied to continuously distributed characters has been proposed recently by Felsenstein (1985). In order to apply such techniques, a completely specified phylogeny with respect to the character in question is required, severely limiting the techniques' practical utility. Moreover, the cost of ensuring the independence of data points is that comparisons are inevitably made between taxa of very different rank, with one datum representing a single species and another being the average of a whole family. The method thus assumes that no special explanation is required for all members of a large clade varying in the same direction, implying the very widespread occurrence of phylogenetic constraints on adaptation.

Although both approaches have considerable merit, I suggest that one important goal of comparative biology should be, not to eliminate the effects of common descent, but rather to estimate their relative importance. This can be done through the nested analyses of variance and covariance.



FIG. 1.—Hypothetical lineages of fissiparous worms. Three ancestors A are cultured, giving rise to three families of descendants D. The *y*-axis is time; fission occurs at times marked F. By analogy, this diagram could also represent phylogenetic trees in which F indicated the splitting of species.

NESTED ANALYSIS OF VARIANCE AND INTRACLASS CORRELATION

Imagine a population of worms that reproduce by equal vegetative fission. By keeping track of all descendants of an initial group of ancestors, we can define a series of families, or lineages, as illustrated in figure 1. If the worms differ with respect to some character Y, we can establish whether or not this character is heritable (in the sense of being transmitted from parent to offspring during reproduction by fission) by a simple analysis of variance (ANOVA). The overall variance is made up of two components: the variance of individuals within families, σ_{eY}^2 , and the variance between families, σ_{gY}^2 . These variances are estimated by the nested ANOVA, in which the magnitude of σ_{gY}^2 relative to the total variance σ_Y^2 = $\sigma_{eY}^2 + \sigma_{gY}^2$ is an estimate of the degree of genetic determination of the character. An asexual family tree is directly analogous to a phylogenetic tree. Figure 1 could stand for either, since we could replace fission and genetic families with speciation and clades of taxa. The partition of variance within and between families is now replaced by a precisely similar partition within and between descendant taxa of any rank. Because the ancestral taxa are rarely available for study, we need a means of distinguishing between groups of species with different common ancestors. This is provided by a phylogenetic hypothesis. It is no more possible to study comparative biology without erecting a phylogenetic hypothesis than it is possible to study genetics without some knowledge, direct or inferential, of family structure.

The partition of sums of squares between taxonomic levels in a Linnaean hierarchy is illustrated in table 1 by data on body mass in eutherian mammals. The character was chosen because it can be expressed in the same units for all forms; the infraclass Eutheria was used because large data sets are available from secondary sources and because its taxonomy is relatively uncontroversial. Information on a number of characters including body mass was first collected from a

Level	df	Sum of Squares	Mean Squares	Variance Component	SCV*
Infraclass	0				0
Superorder	4	2165.08	541.27	0.509 (18.8%)	0.188
Order	13	2426.42	186.65	1.195 (44.2%)	0.630
Suborder	8	247.76	30.97	0.404 (14.9%)	0.779
Family	81	610.77	7.540	0.303 (11.2%)	0.891
Subfamily	85	185.11	2.178	0.101 (3.75%)	0.929
Genus	405	257.21	0.635	0.133 (4.91%)	0.978
Species	726	70.50	0.097	0.047 (1.75%)	0.995
Within species	1344	16.89	0.013	0.013 (0.46%)	1.000
Total	2666	5979.74	2.243	2.704	

TABLE 1

NESTED ANALYSIS OF VARIANCE FOR BODY MASS IN EUTHERIAN MAMMALS

Note.—The variable analyzed is \log_{10} (body mass) in kilograms for nonparous adult females; see the text for sources. Sums of squares and coefficients of expected mean squares were obtained from the NESTED procedure in SAS 1986.

* SCV, The cumulative variance standardized by dividing by the total variance; it represents an intraclass correlation coefficient.

number of sources in connection with the covariance analysis to be described below: Eisenberg (1981, all eutherians), Millar (1981, all eutherians), Swihart (1984, lagomorphs), Harvey and Clutton-Brock (1985, primates), and Gittleman (1986, carnivores). These observations were then supplemented by further records of body mass from regional faunas, until the total number of observations exceeded 2500. All identical values reported for the same species were discarded. The classification used was that of Anderson and Jones (1984), referring to Miyamoto and Goodman (1986) for the supraordinal level and neglecting infraordinal and superfamilial groupings: this yielded superorder, order, suborder, family, subfamily, genus, and species as seven strictly nested levels of classification, the lowest level being represented by replicated observations of the same species. In all, the analysis comprised 2667 observations of 1325 species representing all taxa at the level of family or higher; all regions of the world were represented, though South and Central America and central Asia were relatively poorly sampled. Logarithmic values are used in all analyses, since it was found that variances (and variance components) were independent of mean values on a logarithmic scale.

The component of variance associated with each level of classification was estimated in the usual way, by equating observed with expected mean squares. These variance components are the essential elements of the situation. They can be used either directly, to describe the process of evolution, or indirectly, by calculating from them the covariance components, in order to analyze the process of evolution by testing particular adaptive hypotheses. Their use in description is the subject of this section, with analysis discussed in the following section.

The variance component at a given level of classification is an estimate of the variance of the true mean values of taxa at that level around their joint mean. When expressed as a fraction of some overall variance, it is an intraclass correlation coefficient. For example, returning to figure 1 as a representation of the family tree of some fissiparous worms, the heritability $\sigma_{gY}^2/(\sigma_{gY}^2 + \sigma_{eY}^2)$ is an



Fig. 2.—The increase in variance of body mass through time in eutherian mammals. Only data on nonparous adult females were used. SCV, The cumulative variance at a given taxonomic level (sum of variance components up to and including that level) standardized by dividing by the total variance. The scale is logarithmic; the corresponding intraclass correlation coefficients are indicated on the right. The measure of geologic age used is the first record, averaged across all taxa at a given level; these estimates were obtained from the faunal lists by Savage and Russell (1983). Taxa without a fossil record were excluded; their inclusion does not alter the pattern of the results. The values plotted (log millions of years: mean, standard deviation, and sample size) are genus, 0.660, 0.480, 289; subfamily, 0.880, 0.450, 126; family, 1.363, 0.337, 86; suborder, 1.638, 0.108, 26; order, 1.695, 0.109, 19; superorder, 1.755, 0.065, 5.

intraclass correlation coefficient. It expresses the correlation between two worms chosen at random from the same family. The same interpretation applies to taxonomic components of variance; thus, $(\sigma_{superorder}^2 + \sigma_{order}^2 + \sigma_{suborder}^2)/\sigma_{total}^2$ is the expected value of the correlation between two randomly chosen mammals from the same suborder, which for body size is 0.779 according to my data. Such correlations are ordered by the highest and lowest levels of classification adopted, since the correlation between two random eutherian mammals must be zero when only the single infraclass is represented, and the correlation at the lowest, unreplicated level of classification is always unity. The intraclass correlations should therefore be interpreted only relative to a given frame of reference, and they will change if that frame is changed. Thus, the correlation between random forms from the same suborder would be greater than 0.779 if all vertebrates were analyzed, but less than 0.779 if the analysis were restricted to a single mammalian superorder.

At any given taxonomic level, the intraclass correlation coefficient represents the sum of variance components at this and higher levels, standardized by the total variance. The cumulative variance calculated in this way can then be used to describe the evolution of phenotypic variation. I have plotted the cumulative variance for the seven taxonomic levels used in the analysis against a measure of the average age of these levels in figure 2. This diagram can be read either forward



FIG. 3.—The increase in variance of body mass and chromosome number through time in eutherian mammals. For body size and dating, see the text and figure 2. Information on chromosome number from Matthey (1973). Since no meaningful information on variation of chromosome number within species could be obtained, both body mass and chromosome number are analyzed as species means; this is why the body-mass plot differs slightly from that of figure 2.

or backward. When it is read forward, the diagram shows how variance increases through time. This shows a steep increase in variance at the supraordinal and ordinal levels in Paleocene and lower Eocene times, perhaps attributable to the adaptive radiation of the eutherians shortly after their appearance in the upper Cretaceous, followed by a period of much lower rates of diversification in the remainder of the Tertiary. When the diagram is read backward, it shows how the correlation between forms declines as successively more-distant relatives are considered, at first slowly but much more steeply at subordinal and higher levels of classification.

Any curve of this sort refers only to the character under consideration, and may differ between characters. Figure 3 compares the curve for body mass with that for chromosome number. Since the curve for chromosome number lies below that for body mass, we can conclude that the correlation between forms declines more steeply with taxonomic level for chromosome number than it does for body mass. A possible interpretation of this diagram is that speciation is more frequently associated with a change in chromosome number than with a change in body mass in eutherians.

The quantitative description of long-term evolutionary change has focused on the rate of change in mean phenotype through time within a single lineage, following Simpson (1953, especially fig. 4). It seems equally, if not more, valid to



FIG. 4.—The increase in variance of body mass among species within genera of eutherian mammals. Only the 64 genera with a fossil record of at least one million yr and with at least 2 df available for estimating variance within species and at least 2 df available for estimating variance between species were used. Genera with at least 10 df for estimating variance among species are identified on the scatter plot. A, Artiodactyla; B, Chiroptera; C, Carnivora; E, Edentata; I, Insectivora; L, Lagomorpha; M, Pholidota; P, Primates; R, Rodentia; S, Perissodactyla; W, Cetacea. Least-squares regression, weighted by the number of species in a genus, log $\sigma_{species}^2 = 2.866 + 0.566 \log age; SE, 0.180; P = 0.0026.$

picture evolution as causing a change in the variance between lineages over time. Figure 2 is a crudely averaged representation of this sort, which corresponds reasonably well with what we know of the early Tertiary eutherian radiation. A more precise treatment can be attempted by analyzing taxonomic levels separately. I used 64 genera for which two or more degrees of freedom were available for estimating both the between-species and within-species mean squares. These data yielded estimates of $\sigma_{\rm species}^2$ from which the within-species variance had been removed. From figure 2 we anticipate that the variance between species within genera will increase over time and that the rate of increase will fall over time. In figure 4 I have plotted the between-species variance against the age of the genus; since the slope of the log-log regression is 0.57 ± 0.18 , these data appear to support both predictions. It would be of some interest to know how general patterns of this sort are.

Finally, we can use the nested analysis of variance to investigate the relationship between independently estimated components of variance. A speculation dating back to Darwin (1859) is that the number of varieties per species and the number of species per genus are directly related. We might generalize this by

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saying that the variance within species will vary directly with the variance between species within genera. Figure 5a only weakly supports this speculation. Since the within-species variance includes errors of measurement and transcription and possibly differences in age and condition as well as genuine variance between populations, this result need not be too disappointing. However, these objections do not apply to the variance between species (within genera) and the variance between genera (within families), which appear to be entirely unrelated (fig. 5b).

NESTED ANALYSIS OF COVARIANCE AND INTERCLASS CORRELATION

The discussion so far has been restricted to the phenotypic variation of a single character. The second major aspect of comparative analysis is to evaluate the support given to an evolutionary hypothesis that predicts a relationship between two or more different characters, when this relationship may be expressed at different taxonomic levels. If we were to measure a second character, X, in our hypothetical culture of fissiparous worms, we could calculate σ_{eX}^2 and σ_{gX}^2 just as before. Suppose, however, that we were interested in the covariance of Y with X. This covariance also has genetic and environmental components, which can be partitioned by a nested analysis of covariance (ANCOVA). A particularly clear and simple description of the method is given by Kempthorne (1957, pp. 264–267). We first construct a third variable Z = Y + X. Clearly, the variance of Z can be partitioned within and between families in the same way as the variance of Y or X. From first principles,

$$\operatorname{var}(Y + X) = \operatorname{var}(Y) + \operatorname{var}(X) + 2\operatorname{cov}(Y, X);$$

and it follows that

$$\sigma_Z^2 = \sigma_Y^2 + \sigma_X^2 + 2\sigma_{YX},$$

where σ_{YX} is the covariance of Y with X. Further, these arguments hold not only for the overall variance, but also for the variance at any level in the analysis. Thus, we can write

$$\sigma_{gZ}^2 = \sigma_{gY}^2 + \sigma_{gX}^2 + 2\sigma_{gYX}$$

and

$$\sigma_{eZ}^2 = \sigma_{eY}^2 + \sigma_{eX}^2 + 2\sigma_{eYX}$$

(Kempthorne 1957). The genetic (between-family) and environmental (within-family) covariances are then estimated as

$$s_{gYX} = (s_{gZ}^2 - s_{gY}^2 - s_{gX}^2)/2$$

and

$$s_{eYX} = (s_{eZ}^2 - s_{eY}^2 - s_{eX}^2)/2$$

where s^2 is the estimate of the corresponding σ^2 from the nested ANOVA. This partitioning of the covariance enables us to estimate the genetic and environmen-



FIG. 5.—Relation between variance components on adjacent taxonomic levels. *a*, Variance within and between species (within genera). Slope of the least-squares regression, +0.223; SE, 0.156 ($r^2 = 0.03$); P = 0.16. *b*, Variance among species and among genera (within families). Least-squares slope, -0.008; SE, 0.162 ($r^2 = 0.00$); P = 0.96.

tal correlations as

$$r_{\rm gYX} = s_{\rm gYX} / (s_{\rm gY}^2 s_{\rm gX}^2)^{1/2}$$

and

$$r_{\rm eYX} = s_{\rm eYX} / (s_{\rm eY}^2 s_{\rm eX}^2)^{1/2}$$

(Kempthorne 1957, p. 267). The genetic correlation estimated in this way is the correlation of the true family means, which I shall call the *intrinsic correlation*. It is not the same as the observed correlation between the mean values of families, which is estimated as the ratio of the mean product to the square root of the product of the two mean squares and is therefore given by

$$r_{\overline{\mathbf{YX}}} = (s_{e\mathbf{YX}} + ns_{g\mathbf{YX}}) / [(s_{e\mathbf{Y}}^2 + ns_{g\mathbf{Y}}^2)(s_{e\mathbf{X}}^2 + ns_{g\mathbf{X}}^2)]^{1/2},$$

where n is the number of individuals scored in each family. The observed correlation of family means is therefore influenced in a complex way by the variance and covariance within families, which do not contribute to the intrinsic correlation. The steps involved in the calculation of intrinsic and observed correlations are shown in more detail in table 2.

Let us now return to the analogy between an asexual family tree and a phylogenetic tree, representing the relationships of species within genera. Any covariance between Y and X may arise from common ancestry. At the same time, we are led by some argument to believe that Y and X should be correlated as a consequence of natural selection acting independently of ancestry. Suppose that one measurement of each variable is available for a number of species, and that these scores have a certain overall ("phenotypic") covariance, σ_{pYX} . By classifying the species into genera, we can partition this overall covariance into two components, that between species within genera ("environmental") and that between genera ("genetic"): $\sigma_{pYX} = \sigma_{eYX} + \sigma_{gYX}$. If the genera merely represent random samples of the species, then the expected covariance between genera (σ_{gYX}) is 0, and the covariance between species is unaffected, $\sigma_{gYX} = \sigma_{pYX}$. But it is often the case that our classification procedure leads us to group species with similar combinations of scores, in which case σ_{gYX} is nonzero. This component of covariance cannot be attributed to adaptation, or function, since it arises solely from a phylogenetic hypothesis. Thus, σ_{gYX} measures the covariance attributable to ancestry. The remaining covariance (assuming that the error covariance has previously been estimated and removed) cannot be attributed to phylogeny, whose effect has been removed in σ_{gYX} . Thus, σ_{eYX} measures the covariance arising independently of ancestry and tests the functional hypothesis. It is worth emphasizing that a functional hypothesis, specifying the acquisition of adaptation through natural selection independent of ancestry, is evaluated by the covariance within taxa, σ_{eYX} . The common practice of testing a functional hypothesis by the correlation of the mean values of higher taxa while suppressing the covariance within these taxa is surely incorrect, not only for the statistical reasons given above but more importantly because any additional covariance attributable to the grouping of species within higher taxa must be due to ancestry.

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Statistic	Between Clones	Within Clones
Degrees of freedom	(c - 1)	c(n - 1)
Mean squares		
Y	V_{Y}	$E_{\mathbf{Y}}$
X	$V_{\mathbf{X}}$	$E_{\mathbf{X}}$
Z = Y + X	V_{Z}	E_{Z}
Mean product	$V_{\rm YX} = (V_{\rm Z} - V_{\rm Y} - V_{\rm X})/2$	$E_{\rm YX} = (E_{\rm Z} - E_{\rm Y} - E_{\rm X})/2$
Variance components		
Y	$s_{gY}^2 = (V_Y - E_Y)/n$	$s_{eY}^2 = E_Y$
X	$s_{gX}^2 = (V_X - E_X)/n$	$s_{eX}^2 = E_X$
Z = Y + X	$s_{gZ}^2 = (V_Z - E_Z)/n$	$s_{eZ}^2 = E_Z$
Covariance component	$s_{gYX} = (V_{YX} - E_{YX})/n = (s_{gZ}^2 - s_{gY}^2 - s_{gX}^2)/2$	$s_{eYX} = E_{YX}$ = $(s_{eZ}^2 - s_{eY}^2 - s_{eZ}^2)/2$
Correlation		
Intrinsic	$r_{gYX} = s_{gYX} / (s_{gY}^2 s_{gX}^2)^{1/2}$	$r_{\rm eYX} = s_{\rm eYX} / (s_{\rm eY}^2 s_{\rm eX}^2)^{1/2}$
Observed	$r_{\overline{\mathrm{YX}}} = \frac{s_{\mathrm{eYX}} + ns_{\mathrm{gYX}}}{[(s_{\mathrm{eY}}^2 + ns_{\mathrm{gY}}^2)(s_{\mathrm{eX}}^2 + ns_{\mathrm{gX}}^2)]^{1/2}}$	$r_{\mathbf{Y}\mathbf{X}} = \frac{s_{\mathbf{e}\mathbf{Y}\mathbf{X}}}{(s_{\mathbf{e}\mathbf{Y}}^2 s_{\mathbf{e}\mathbf{X}}^2)^{1/2}}$
Regression		
Intrinsic	$b_{gYX} = s_{gYX}/s_{gX}^2$	$b_{\rm eYX} = s_{\rm eYX}/s_{\rm eX}^2$
Observed	$b_{\overline{\text{YX}}} = \frac{s_{\text{eYX}} + ns_{\text{gYX}}}{s_{\text{eX}}^2 + ns_{\text{gX}}^2}$	$b_{\rm YX} = \frac{s_{\rm eYX}}{s_{\rm eX}^2}$

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Note.—The variance and covariance of Y and X are partitioned within and between clones, as for an experiment such as that outlined in figure 1. There are c clones, with n individuals scored in each. V, Between-clone variances and covariances, estimated by mean squares and mean products; E, corresponding within-clone estimates; s^2 , r, and b, the estimates of the corresponding variances σ^2 , correlations ρ , and regressions β (see Kempthorne 1957, pp. 364–367).

ANALYSIS OF TWO CORRELATED CHARACTERS

To illustrate the use of nested ANCOVA, I have collected measurements of reproductive variables in a series of eutherian mammals. This group was chosen because large data sets are available from secondary sources and because its taxonomy is relatively uncontroversial. The literature sources have already been cited. The final data matrix included 574 observations from 370 species, with lagomorphs, rodents, primates, and carnivores well represented and other groups, notably bats and cetaceans, under-sampled.

The primary functional hypothesis chosen for examination is that gestation period increases with litter mass among mammals of the same size. This might be the outcome of a form of reproductive cost: the penalty for producing a large mass of offspring is that it takes longer to do so, given a fixed supply or rate of acquisition of resources. Before we proceed to analyze the data, one difficulty must be addressed. This concerns the use of partial or total correlations as tests of hypotheses, and it is best illustrated by a different example. Suppose that we wish to understand the function of secondary compounds in the leaves of trees, and we propose that they are synthesized as a defense against herbivorous insects. We might reason that trees with low concentrations of secondary compounds are relatively defenseless and therefore suffer severely from insect pests; this argument leads us to expect a negative correlation between the quantity of secondary compounds and the degree of herbivore damage. But we might instead reason that trees that are liable to suffer extensive damage evolve high concentrations of secondary compounds; this argument predicts a positive correlation between the quantity of secondary compounds; the degree of herbivore damage.

Which of these two apparently contradictory approaches is correct? The answer is that either may be correct, depending on how levels of the independent variate (in this case, herbivore damage) are distributed among the units of observation (species or other groupings of trees). If herbivore damage can be regarded as inflicted at random among trees, then the first interpretation is correct, and the hypothesis is tested by the total correlation of secondary compounds with herbivore damage. However, such an assumption is often unjustifiable in comparative work, where it is impossible to ensure that treatments are allocated at random. For example, it may be known that larger trees generally support larger and more diverse communities of herbivores than do smaller trees. We then expect larger trees to evolve higher concentrations of secondary compounds as a response to their greater likelihood of damage. Nevertheless, if the effect of size could be removed, the underlying negative correlation showing the antagonistic effect of the secondary compounds on the insects would be displayed. In other words, when levels of the independent variate cannot be regarded as allocated at random among the units of observation, it is the partial correlation that appropriately tests the hypothesis. Calculating the partial correlation is, of course, equivalent to calculating the total correlation using the residual values of the independent variate after least-squares regression on the confounding variate (in this case, tree size), which is in practice a more useful method. Naturally, this approach requires that the confounding variate be identified, and its effect removed, in advance of testing the hypothesis of interest.

In the present case, we might suspect that litter mass and gestation period are strongly correlated because both are correlated with overall body mass, whose effect must therefore be eliminated in advance. Since the hypothesis does not specify an independent variate, the linear effect of body mass is removed from both variables. To do so, the logarithm of litter mass (LLITM = log_{10} neonate mass + log_{10} litter size, kg) and the logarithm of gestation period (LGEST, days) were regressed separately on the logarithm of adult mass (LADWT, kg), using all data. The predictive equations were

LLITM =
$$-0.9578 + 0.7778$$
 LADWT $(r^2 = 0.925)$;
LGEST = $+1.8310 + 0.2181$ LADWT $(r^2 = 0.576)$.

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These regression slopes are similar to those obtained by previous authors (collected in Peters 1983). The residual values RLLITM and RLGEST are then obtained as deviations from the expected values yielded by the regressions. The hypothesis asserts that RLLITM and RLGEST are positively correlated.

The nested ANOVA and ANCOVA are summarized in table 3. The overall correlation is slight, but it differs significantly from zero at the 1% level in the expected direction. There are moderately large positive correlations within species and between higher taxa at the family and suborder levels, but no appreciable covariance is added by grouping the data into species, genera, or subfamilies. The intrinsic correlations at ordinal and superordinal levels cannot be calculated because one of the two estimates of variance components is negative. There is in this case little disagreement between intrinsic and observed correlations.

The covariance within species is especially interesting because it emphasizes the utility of replicating observations of species. Comparative biologists commonly use only one set of measurements per species (Jarman [1982], for example, discussed the "problem" of obtaining a representative average value for each species). The within-species covariance should instead be regarded as a valuable source of information. In this analysis it confounds two effects: the covariance of traits between populations within species, and the error covariance caused by imprecision in measurement, calculation, and transcription. In principle, the within-species components of variance and covariance could be partitioned into these two effects; for instance, by including "primary authority cited" as the lowest level of analysis, the error from transcription could be separated from other within-species effects. I think it reasonable to suppose in this case that the true error covariance is zero, but the correlation between populations is still affected somewhat because both the variance components and the degrees of freedom for the comparison are reduced. Although I have not attempted such a partition, it is reasonable to interpret the within-species covariance as arising primarily through the covariance between populations of the same species. This effect is illustrated in figure 6a, which is a plot of the deviation of each observation from the mean for the species, for all cases in which this deviation is not zero. Although this provides an easy way of understanding the nature of the covariance, it must be borne in mind that this and the other plots in figure 6 do not precisely correspond to the intrinsic correlations or regressions computed by a nested ANCOVA.

The covariance within species is in one sense fundamentally different from the taxonomic components of covariance. It is only within a species that correlations between characters attributable to ancestry can be broken down by cross-fertilization and crossing-over. At higher levels of classification, taxa behave like asexual genotypes among which ancestral correlations can be eroded only by differential extinction. The higher the level of classification, the more severely this constraint will operate. This is why comparative biologists should make use of the within-species covariance and also why comparative hypotheses should be tested within rather than between higher taxa. In parthenogenetic groups, of course, the within-species covariance has no special significance.

			Mean	SQUARES			VARIANCE COI	MPONENTS		CORREI	ATIONS
LEVEL	df	RLLITM	RLGEST	SUMR	Mean Product	RLLITM	RLGEST	SUMR	COV	Intrinsic	Observed
Superorder	4	118908	87248	364145	+ 78994	727	(-2544)	2215	+2016	NE	+0.7755
Order	12	35277	151795	93648	-46712	(-2155)	4302	(-5201)	- 3674	NE	-0.6383
Suborder	9	100760	64920	263373	+ 48846	3028	2411	9159	+ 1860	+0.6883	+0.6039
Family	52	28247	8852	47798	+ 5349	2757	772	5052	+ 762	+0.5219	+0.3383
Subfamily	47	8892	3326	12631	+206	911	426	1320	%	-0.0130	+0.0379
Genus	120	5107	1533	6602	+230	512	336	1023	+ 88	+0.2112	+0.0821
Species	128	3871	750	4696	+38	1310	427	1586	- 76	-0.1009	+0.0220
Within spp.	204	1903	108	2313	+ 151	1903	108	2313	+ 151	+0.3327	+0.3327
Total	573	8528	6071	15994	+ 697	11148	8783	22670	+ 1369	+0.1384	+0.0969
NoteTI	le two v	ariates are R	LLITM, the re	esidual valu	e of log ₁₀ litter ma	uss after linear	r regression of	n log ₁₀ adult	body weigh	nt (kg), and F	LGEST, the

NESTED ANALYSES OF VARIANCE AND COVARIANCE FOR LITTER MASS AND GESTATION PERIOD AMONG EUTHERIAN MAMMALS

TABLE 3

corresponding residual value of gestation period (days); SUMR = RLLITM + RLGEST. COV, Covariance component; NE, not estimable. All quantities (except the correlation coefficients) have been multiplied by 10^5 for convenience of presentation.

The residual data are the species means, which are shown in figure 6b. They are only weakly correlated; moreover, what covariance does exist is not attributable to grouping into genera or subfamilies. Instead, it reappears at the level of families and suborders. These levels also happen to have elevated components of variance and probably would be chosen for analysis by Harvey's criterion, but this is not always the case. A plot of family means, figure 6c, suggests that most of the covariance at this level is attributable to a single family, Ursidae, which scores low for both variables. This hypothesis can be examined by repeating the analysis with all data for Ursidae removed. The percentage of variance contributed by families then drops from 27.2% to 9.2% for RLLITM and from 27.5% to 8.2% for RLGEST, and the intrinsic correlation between families goes down from 0.522 to 0.124. As a consequence, the overall correlation is reduced to an insignificant value, but the intrinsic correlations at other taxonomic levels are scarcely affected; in particular, the correlations within species and between suborders are little changed.

When discussing a single variable, I interpret the variance component for, say, families as representing the variance generated during the divergence of families within suborders. This process can be illustrated by calculating the correlation between forms within successively lower taxonomic groupings, using a cumulative sum of variance components standardized by the total variance, and plotting this intraclass correlation coefficient against geologic time. Over the same period of time, any two characters will diverge from their value in the common ancestor of all extant families, and the covariance component for families expresses the extent to which they diverged independently of one another. Covariance components can thus be interpreted as representing the covariance generated, possibly through selection, at different periods in the past. To illustrate this process, we can calculate the cumulative covariance at successively lower taxonomic groupings, standardized by the geometric mean of the cumulative variances. This yields an interclass correlation coefficient, which can be plotted against geologic time. Plots for RLLITM and RLGEST are given in figure 7. This shows how the standardized variances of both characters taken separately decrease with time before the present, while the standardized covariance between the characters increases: correlation is greatest at the subordinal level and falls steadily at successively lower levels of classification. The algebraic reason is that, although the covariance is tending to increase, it is increasing more slowly than the variances. A substantial correlation at the level of the suborder is thus gradually eroded through time.

Components of variance or covariance at a given taxonomic level represent phylogenetic effects attributable to differences among ancestors. These differences may themselves have arisen through selection acting in the past, however, and the mere existence of phylogenetic effects is not evidence for phylogenetic constraint. A somewhat more complex interpretation is required if correlation or regression coefficients vary substantially among taxa at a given level, and particularly if they vary in sign. This amounts to an interaction between phylogeny and function, with selection acting differently in different taxa. It can be identified by a conventional ANCOVA at each level, a significant mean square for the interaction



FIG. 6.—Scatter diagrams illustrating covariance of gestation period with litter mass at different taxonomic levels among mammals. Both variables are expressed as residuals from log-log regressions on body size (see the text). a, Within species. Each point is the deviation of an observation from the mean of the species. Points are too numerous to plot within the box around 0,0. b, Between species. Each point is the mean value of a species. c (facing page), Between families. Each point is the mean value of a family. The outlying point for Ursidae is identified. Solid lines, Least-squares regressions of the plotted points.



FIG. 6 (continued)

between the taxon and the independent variate showing that the regression slopes are heterogeneous. Phylogenetic constraints of this sort may arise from the effect of an unidentified third variate that changes the way in which selection acts. The problem is then the same as deciding whether to use absolute or residual values of either or both of the original variates.

SUMMARY

The main methodological problem of comparative biology is to distinguish between effects attributable to ancestry and those attributable to function. The nested analysis of variance addresses this problem by partitioning variance and covariance between taxonomic levels. The estimation of variance components and the derived intraclass correlation coefficient can be used to describe the overall increase in variation through time and the relation between variances at different taxonomic levels. Applying this procedure to data about the body mass of eutherian mammals showed a steep increase in variance associated with the adaptive radiation of the group in early Tertiary times, with much lower rates of increase thereafter; chromosome number showed a similar but shallower increase, implying that chromosome number varies more between taxa at lower levels of classification than does body mass. The variance of species within genera increased with the geologic age of the genus, permitting a quantitative description of the rate of evolutionary divergence. A straightforward extension of the technique yields estimates of taxonomic components of covariance and the associated interclass correlation coefficients. It is argued that covariance between taxa is attributable to ancestry and covariance within taxa to function, and that adaptive



FIG. 7.—Evolution of variance and covariance of litter mass and gestation period in eutherian mammals. *Open circles*, Standardized cumulative variances (intraclass correlation coefficients) of (residual logarithmic values of) litter mass RLLITM and gestation period RLGEST for successively lower levels of classification. *Solid circles*, Standardized cumulative covariances (interclass correlation coefficients) of RLLITM with RLGEST at the same levels. Only data from the subordinal level downward are used because of the difficulty of interpreting the negative variance components obtained in some cases at higher levels (see table 3): SO, suborder; F, family; SF, subfamily; G, genus; S, species; unlabeled point at top, within species.

hypotheses should be tested by the within-taxon correlation. This technique is illustrated by analyzing the covariance of litter mass with gestation period, independent of body mass, among eutherians. This reveals substantial correlation within species, attributed to function, and between suborders and orders, where it was attributed to ancestry, with little or no correlation at intermediate taxonomic levels. The components of variance and covariance can be used to describe how the correlation changes through time, in this case declining continuously from the time of divergence of suborders onward. Two sources of phylogenetic constraint are identified: the taxonomic components of covariance themselves, which may reflect the past operation of selection; and differences among correlation or regression coefficients among taxa at a given level of classification, representing an interaction between phylogeny and function, which may arise when a third variable alters the pattern of selection.

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