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PHYLOGENETIC ANALYSIS OF PHENOTYPIC COVARIANCE STRUCTURE. II. RECONSTRUCTING MATRIX EVOLUTION

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Abstract.—A modified minimum evolution approach is used to estimate covariance matrices for hypothetical ancestors. Branch lengths are calculated as the mean disparity in corresponding ancestor-descendent covariances. Branches are longest leading to terminal populations and subspecies, while interspecific branches are relatively short, indicating a general conservation of covariance structure among species despite a high degree of intraspecific variability. Absolute deviations in covariance structure are not correlated with phenotypic divergence. Interpreted in light of other studies, the analyses suggest that deviations in covariance structure are most strongly associated with the formation of diagnosably distinct taxa and stochastic sampling of genotypes at the population level. There is no evidence for restructuring of phenotypic covariance structure in association with reproductive isolation. The results suggest that phenotypic covariances are dynamic over short time scales and do not support attempts to extrapolate genetic covariance structure to explain or predict macroevolutionary change. This study further demonstrates that branch lengths, which are not usually analyzed in detail, contain valuable evolutionary information complementary to that residing in the branching pattern.

Key words.—Comparative method, cranial morphology, macroevolution, minimum evolution, phenotypic covariance structure, *Phyllotis*, quantitative genetics.

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Evolutionary constraints, whether due to developmental programs or genetic covariance structure, make the evolution of certain phenotypes more likely than others. Biases toward certain morphologies can control rates of evolution, both facilitate and constrain phenotypic divergence, generate macroevolutionary trends, and promote the differential success of lineages in invading niches. The most important level at which to examine constraints and covariance structure is across the speciation transition. It is with this transition that the cumulative effects of microevolutionary processes can fashion macroevolutionary pattern. The transition from populations to divergent species has been fundamental to evolutionary theory since its beginning (Darwin 1859; Mayr 1963, 1970; Barton and Charlesworth 1984; Carson and Templeton 1984). This is also the transition between population genetics and systematics, where the independent evolutionary trajectories of geographic populations becomes effectively permanent through their reproductive isolation as species. If the diverse fields of evolutionary biology are to be effectively unified, then it is across this transition that they will be bridged. Furthermore, in order to unite the hierarchy of processes, multiple levels in the phylogenetic hierarchy must be encompassed so that the higher-level consequences of lower-level processes can be accurately defined. In this paper, I examine the evolution of covariance structure across the species transition and bracketing phylogenetic levels.

The focus of this research is the evolution of covariance structure and an examination of the assumption from Lande's (1979) model that genetic covariance structure is effectively constant (covariance structure is used here to mean the patterns of variation and covariation among traits). The approach I have taken is to maximize the amount of evolutionary information about covariance structure as it has evolved during speciation and the diversification of clades. In other words, I have tried to sample finely the branches of a phylogeny to estimate ancestral covariance structure or at least the degree of divergence in covariance structure during different stages of a phylogeny. Sampling extends to geographic variation, to test the significance of interspecific differences against intraspecific variation. Previous tests of covariance evolution have typically employed only two taxa (Arnold 1981; Atchley et al. 1981, 1992; Kohn and Atchley 1988; Brodie 1993; Paulsen 1996). Even in those studies with a large number of populations, there has been little or no phylogenetic structure (Riska 1985; James et al. 1990; Voss et al. 1990). Either way, the internal branches of a phylogeny were not sampled. The one exception is Lofsvold (1986, 1988), who studied three taxa. In the two extreme sampling designs, single populations from each of two species or multiple populations of a single species, only one internal node is sampled.

The approach I have adopted expands phylogenetic sampling. As a consequence, phenotypic covariation is explored rather than genetic covariation, which would require impractically large breeding programs established from wild populations. Phenotypic covariances may be substituted for genetic covariances in some instances (Cheverud 1988), but the equivalence of genetic and phenotypic correlation or covariance matrices has not been generally established (Willis et al. 1991). Nonetheless, the evolution of phenotypic covariances provides a first approximation for the evolution of genetic covariances, and provides direct information about the constraints on variation available to selection and the consequences of developmental processes. The effects of developmental constraints are seen in the phenotype, and thus phenotypic covariances are their appropriate estimators (Zelditch et al. 1990).

Analytical approach.—The correlation analyses and com-

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mon principal component (CPC) analyses from Steppan (1997) each incorporate phylogenetic information, but they do so indirectly by defining the grouping of extant operational taxonomic units (OTUs). Statistical tests can be misleading because OTUs share to varying degrees a common evolutionary history, and so the character values for OTUs are not independent (Felsenstein 1985). An alternative that directly incorporates phylogeny is the minimum evolution approach of Huey and Bennett (1987). In this approach, character values are estimated for hypothetical ancestors, and character evolution is calculated for each branch as the difference between ancestor and descendent values. Data points are phylogenetically independent (although not computationally independent, because hypothetical ancestors are estimated with reference to neighboring ancestors and extant populations). Additionally, the evolutionary history of the traits during early branching events can be isolated from subsequent and confounding evolutionary events. The minimum evolution model and other phylogeny-based comparative techniques (Cheverud et al. 1985; Felsenstein 1985; Grafen 1989) were all designed for univariate data, or comparisons between independently estimated univariate traits, but with certain assumptions they can be extended to the multivariate case. One assumption applied in this study is that each element in a matrix evolves independently, and thus matrices were reassembled for each hypothetical ancestor after independent optimization of the matrix elements. This is most likely an oversimplification, because evolution in one trait will generally affect its covariances with all other traits (Lande 1980).

Objectives

The principal set of questions to be addressed are these. Is phenotypic covariance structure conserved throughout the phylogeny? If variation in covariance structure exists, is that variation phylogenetically structured? If there is phylogenetic structure, at what level in the phylogeny does significant divergence occur? The answers to these questions will illuminate the evolution of phenotypic patterns of variation and provide insights into the conservation of genetic covariance structure and the evolutionary stability of developmental or genetic constraints.

METHODS

Materials and Measurements

Thirteen cranial distances were measured for 1606 skulls of six species of *Phyllotis* (leaf-eared mice). Details of how measurements were selected and age criteria are given in Steppan (1997). These 13 measurements are the same subset used in that companion paper for the common principal component (CPC) analyses, and were chosen to most evenly sample different regions of the skull. The measurements are diastema length, molar toothrow length, pterygoid region, basioccipital length, frontal length, nasal length, nasal breadth, interorbital breadth, anterior cranial breadth, posterior cranial breadth, incisor depth, bullar length, and cranial depth. The same phylogenetic hypothesis is used as well, based on the preferred hypothesis from weighted parsimony analysis of DNA sequence data from the cytochrome *b* gene (Steppan 1995a, in press). The two alternative hypotheses within three steps of the shortest trees were also examined to test robustness to the most likely source of error in phylogenetic estimation. A total of 28 populations are analyzed, with sample sizes ranging from 24 to 139. Examined specimens are listed in the accompanying paper (Steppan 1997), and variance/covariance matrices for each population are presented in Steppan (1995a). These populations represent the following species (and subspecies) of *Phyllotis*: *andium*, *osilae* (*osilae*, *phaeus*, *tucumanus*), *magister*, *darwini* (*darwini*, *fulvescens*), *limatus* (northern and southern groups), and *xanthopygus* (*posticalis*, *chilensis*, *vaccarum*, *xanthopygus*). Northern *limatus* is congruent with the traditional definition of *P. x. limatus*, while the southern group is a currently unnamed taxon that represents populations previously assigned to *P. x. rupestris*, but with the relatively deep and narrow incisors diagnostic of the newly elevated species *limatus* (Steppan 1995a, in press).

Analyses: Evolution of Covariance Matrices

Covariance matrices for hypothetical ancestors were estimated using a modification of the minimum evolution method (Huey and Bennett 1987). First, for the three terminal subspecies for which the CPC model (Flury 1987; Flury 1988) was the best fit and in which all the larger components were accepted (*osilae, posticalis, xanthopygus*) (Steppan 1997), a maximum-likelihood estimate of the best pooled covariance matrix was used for each of their ancestral subspecies nodes. CPC analyses were conducted using the program CPC (Phillips 1994). Given the possibly high rates of gene flow among populations, a model of common structure was considered to be biologically more appropriate than an arithmetic mean of the population matrices. All other ancestral nodes (for which a CPC model was rejected and therefore inappropriate) were estimated as the mean of the nearest nodes in the phylogeny; that is, the mean of all nodes connected to it by one branch segment. This set of taxa equates to the direct ancestor and all immediate descendant taxa. This procedure was iterated until estimated matrices converged, with estimates for each element varying by less than 0.01% between iterations. The procedure is iterative because initially, there are no values for the deeper ancestral nodes that can be averaged along with the terminal taxa.

The minimum evolution approach was used rather than other available comparative methods (e.g., independent contrasts, phylogenetic autocorrelation) because it allows the more explicit partitioning of character evolution to specific regions of the tree. These other methods apply to univariate or correlations between univariate data. For bivariate comparisons, minimum evolution is more accurate than independent contrasts methods at estimating realized evolutionary correlations (character changes between speciation events) (Martins and Garland 1991). The method used here assumes a punctuational model of evolution, with all character changes at speciation events, because ancestral reconstructions are not weighted by branch lengths (Martins and Garland 1991). Branch length estimates are not available for most branches. As explained by Martins and Garland (1991), this method is

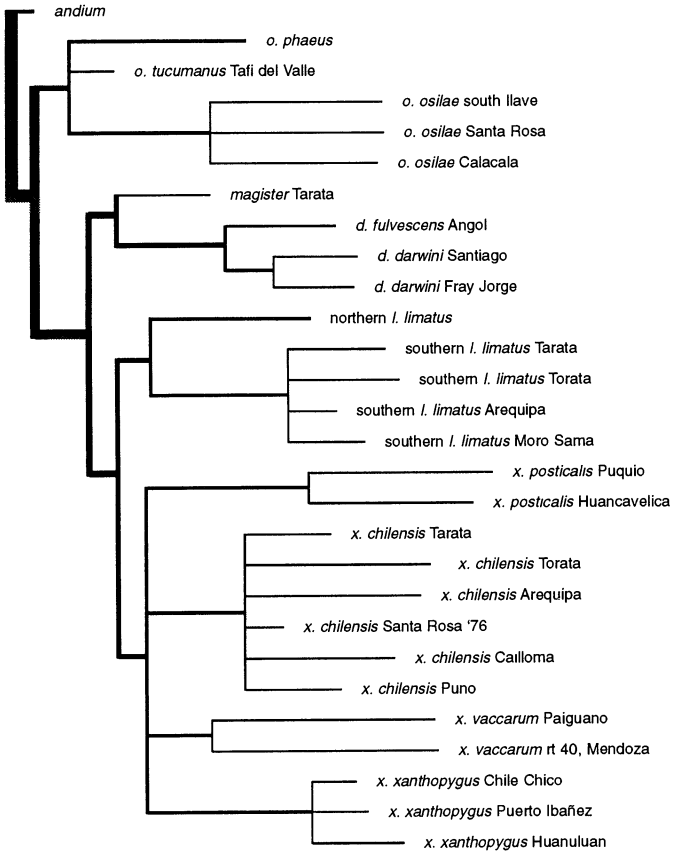

FIG. 1. Phylogram for analyzed species of *Phyllotis* with estimated branch lengths scaled by the matrix disparity between ancestor-descendent pairs. Branch thickness indicates the phylogenetic level of the branch: the thinnest branches lead to the local populations.

both different from and more powerful than the one originally used by Huey and Bennett (1987).

Evolution of covariance structure along each branch of the phylogeny was estimated using matrix disparity. Matrix disparity was calculated as the mean of the absolute value of the pairwise differences between the off-diagonal elements of two matrices, 1 and 2:

$$D_{m_{12}} = \left(\frac{2}{p(p-1)}\right) \sum_{i=1}^{p-1} \sum_{j=i+1}^{p} |(\mathrm{cov}_{ij})_1 - (\mathrm{cov}_{ij})_2|. \quad (1)$$

A wide variety of metrics are possible for the distance between matrices. Matrix disparity was chosen because it is a distance metric, whereas matrix correlation is not. Mahalanobis distance is a multivariate distance metric, but it measures the scaled difference between means of sample dispersions, not between the patterns of association in matrices. Several methods of scaling were considered inappropriate. For example, dividing the differences between elements by the mean of the absolute value of the element pairs,

$$\frac{2|(\mathrm{cov}_{ij})_1 - (\mathrm{cov}_{ij})_2|}{|(\mathrm{cov}_{ij})_1 + (\mathrm{cov}_{ij})_2|} \quad (2)$$

(analogous to the coefficient of variation), produced significant distortions in the metric when negative covariances were present. Negative distances do not exist in standard morphometric applications, and thus are not a problem when normally calculating coefficients of variation. Dividing the element differences by the product of the variances merely transforms the metric to disparities between correlation matrices, correlation being the covariance divided by the product of the standard deviations. From a quantitative genetic perspective, the question of interest is changes in the covariance matrices (Willis et al. 1991), and thus for this analysis, the phenotypic covariances matrices are preferred over the phenotypic correlation matrices.

TABLE 1. Minimum evolution estimates of phenotypic evolution. Matrix disparity and Euclidean distance are each standardized to a mean of 1.00.

Phylogenetic level	n	Matrix disparity, mean	Euclidean distance, mean
Population	24	1.14	0.92
Subspecies	10	1.12	1.06
Species	5	0.48	1.23
Species-group	2	0.25	0.82
2nd species-group	1	0.49	1.33
Branch type			
Intraspecific	34	1.14	0.97
Interspecific	8	0.43	1.14

The same 13 variables used in the estimation of ancestral covariance matrices were used to estimate ancestral phenotypes. The minimum evolution model was used in an iterative procedure to calculate hypothetical ancestor values for each of the 13 traits. Euclidean distances were calculated between each ancestor-descendant pair. Euclidean distance between populations 1 and 2 is calculated as

$$\Delta_{12} = \sqrt{\sum_{i=1}^{p} (\bar{x}_{i1} - \bar{x}_{i2})^2}, \quad (3)$$

where $\bar{x}i$ is the population mean for trait i. Phylograms were drawn with branch lengths proportional to both matrix disparities, D_m, and euclidean distances, Δ. Branch lengths in standardized units of D_m and Δ were regressed against each other to test for a significant association between the evolution of gross phenotype and covariance structure.

RESULTS

Evolution of Covariance Matrices

Covariance matrices were estimated for the hypothetical ancestors. Branch lengths of the phylogeny were scaled equal to the disparity between ancestor-descendent pairs (Fig. 1). The principal result is that the terminal branches leading to the populations and the branches leading to the subspecies are long relative to the internal branches. In other words, intraspecific branches are significantly longer than interspecific branches (Student's $t = 3.82$, $P = 0.0005$). Mean branch lengths leading to each phylogenetic level (e.g., population, species, species group) are listed in Table 1. The branch leading to the second-level species group (*osilae* plus the *darwini* species group) was not included because partitioning the relative amount of disparity leading to *andium* or to the second

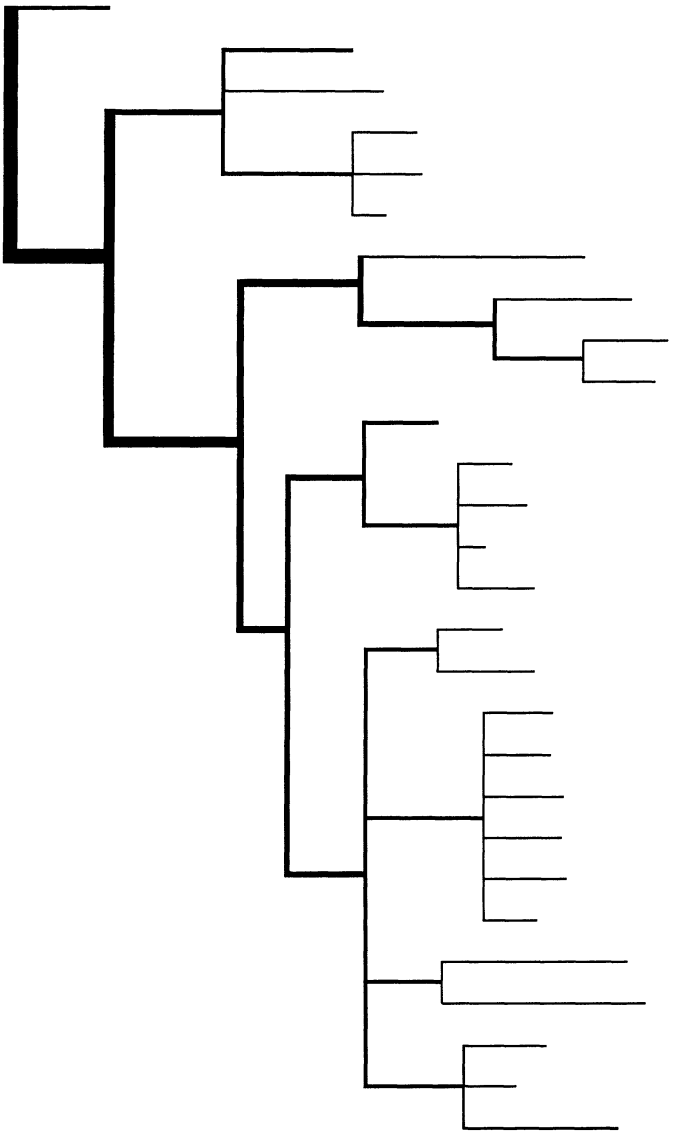

FIG. 2. Phylogram with estimated branch lengths scaled by the Euclidean distance between the means of ancestor-descendent pairs. Thirteen variables included in the analysis. Taxon names removed for clarity, but are the same as in Figure 1.

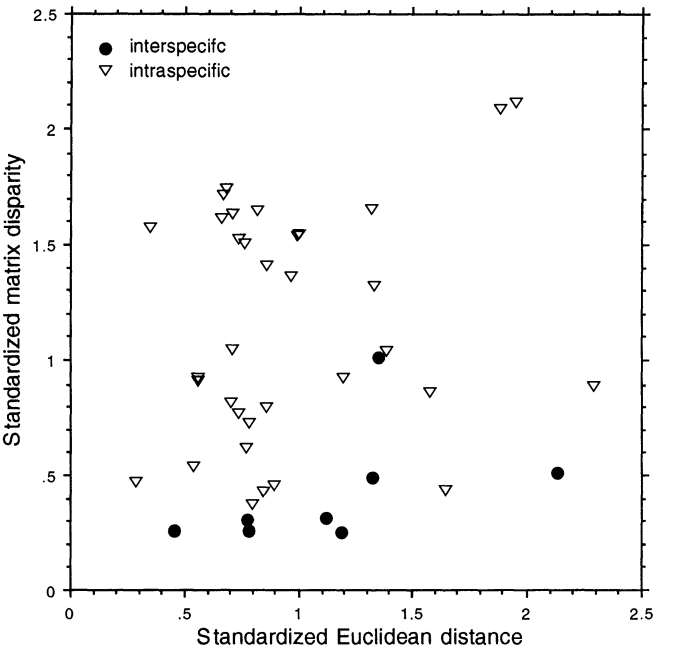

FIG. 3. Plot of estimated branch lengths for matrix disparity against Euclidean distance. Distances are standardized to a mean of 1.0.

species group is arbitrary. The root of the tree is placed for graphical presentation midway along the branch connecting these two taxa, but there is no basis for quantitatively partitioning this disparity.

The Euclidean distances between centroids of ancestor-descendent pairs were calculated and branch lengths scaled accordingly. A different pattern appears than with matrix disparities. The evolutionary change in gross phenotype is more evenly distributed around the phylogeny, with the terminal branches no longer than the internal ones (Fig. 2, Table 1). Intraspecific branches are slightly, though not significantly, shorter than interspecific branches ($P = 0.34$).

Evolution of covariance structure shows no direct association with evolution of the gross phenotype. Branch lengths for the two aspects of phenotype are not correlated (Fig. 3). Intraspecific and interspecific subsets both show regression slopes of 0.21–0.24 respectively, but these slopes are not significantly different from zero ($P > 0.1$).

Analyzing two alternative topologies, (magister (limatus (darwini, xanthopygus))) and (((magister, darwini) limatus) xanthopygus), produced qualitatively identical results. Intraspecific disparities are significantly greater than interspecific disparities and there is no significant correlation between matrix disparity and Euclidean distance. The results are thus robust to some possible error in phylogeny estimation.

DISCUSSION

Synthesis of Results

Two of the three sets of analyses presented here and in the accompanying article suggest similar conclusions. The correlation analyses and the minimum evolution mapping indicate that interspecific divergence in correlation/covariance structure is small relative to population-level variation. Whatever causes the divergence in covariance structure among populations, whether founder effects, genetic drift, or local environmental effects, the accumulation of these effects appears to balance out and does not result in divergence for the whole taxon. The compensatory factors can not be determined from this data, but may be gene flow, the impermanence of environmental fluctuations, or stabilizing selection.

The CPC analysis presents a different perspective on the problem. Common principal component structure is rejected for nearly all clades in the phylogeny (Steppan 1995a, 1997). The primary picture is one of overall divergence in structure, but not necessarily divergence that is hierarchically structured due to phylogeny.

Two other studies that examined population-level patterns

of phenotypic covariance structure found very similar patterns to those seen here. Local populations of aphids showed significant heterogeneity of covariance patterns, but little or no higher-level patterning in geographic variation (Riska 1985). Riska interpreted this pattern as indicating that covariance structure reflected random genetic variations due to small local sample size and frequent population bottlenecks. Regional patterns of geographic variation in phenotypic means resulted from regionally homogenous selection pressures, although the exact genetic mechanisms of short-term responses may differ from population to population. Riska suggested that long-term selection would result in convergence of genetic mechanisms, diminishing interpopulational divergence. He suggested two additional factors that would limit higher level divergence; gene flow among populations, and the maintenance of a "developmental network" (Sander 1983). The developmental network may constrain covariance structures over the long term, while allowing different modifications of specific elements in the network in response to selection. In both Riska (1985) and in this study, sample sizes may introduce estimation noise that obscures the detection of more subtle, higher-level patterns. That puts even more restrictive demands on any model system employed by evolutionary biologists to examine the evolution of covariance structure.

The phylogenetic pattern of covariance similarity seen in *Phyllotis* using matrix correlations (Steppan 1995a, 1997) was also apparent in the land snail *Partula* (Goodin and Johnson 1992). The pairwise similarity of covariance patterns was not significantly affected by the phylogenetic level of the comparison. For both studies, the greatest similarities were among populations of the same subspecies, while higher levels did not show any pattern. Both studies also showed that subspecies were relatively more divergent from each other than are higher categories. In this study, matrix disparity branch lengths leading to subspecies are long, and matrix correlations are slightly lower in comparisons among subspecies; in Goodin and Johnson (1992) multidimensional scaling ordinations showed species dispersions to overlap strongly while subspecies dispersions were distinct.

Goodin and Johnson (1992) estimated similarity in covariance structure differently from the method used here. I used matrix correlations, while Goodin and Johnson calculated an index of factor loading similarity based on the number of traits in each population that share their highest loadings on the same factors derived from independent factor analyses. The statistical and biological properties of such an index are unclear. The index may also be sensitive to oblique factor rotations and unequal numbers of factors extracted (Goodin and Johnson 1992). As a metric for covariance similarity, this index seems to be excessively derived.

The relatively high degree of population-level differentiation in covariance structure has at least four possible causes. These causes include two reflecting real biological differences in covariance structure and two reflecting sampling issues. (1) The genetic covariance structure actually varies, perhaps due to genetic sampling in populations of finite size, that is, genetic drift. That would mean environmental covariances are small enough that phenotypic matrices are accurate reflections of the genetic covariance matrices. In this case, differences would be evolutionarily significant and heritable. (2) Phenotypic covariances may also differ despite constant genetic covariances if environmental covariances are large and geographically variable. In that case, differences would be real and would modify the variation available for selection, but would not be persistent beyond the temporal constancy of environmental effects. Some variation in environmental covariance structure is to be expected, but the magnitude of that variation is uncertain. It is not the magnitude of environmental covariances that is important for this study as much as the magnitudes of the differences in environmental covariance structure. (3) A nonbiological cause may be uneven age distribution of populations, or differentially biased sampling of phenotypes by trapping techniques. These are difficult factors to account for with limited sample sizes. (4) Finally, the long terminal branches illustrated in Figure 1 may be due to sampling related error in estimation of covariances. In that case, even constant phenotypic covariance structures may be obscured. However, CPC analyses demonstrate that sampling alone can not account for the differences among populations. All pairwise comparisons of populations, even among those with larger sample sizes, result in rejecting the hypotheses of matrix equality and proportionality. While sampling error is a greater problem with covariance matrices and thus may increase the terminal branch lengths of matrix disparities to a greater degree than for differences in phenotypic means or DNA sequences, differences in covariance structure among populations are statistically significant and appear to be real. An appropriate nonparametric test of the null hypothesis that the population covariances could be drawn from a single population would be pooling the data for taxa, then repeatedly resampling into random, but nonoverlapping, subsets of the same sizes as the populations. The null hypothesis would be rejected if resampled branch lengths were significantly shorter than those actually observed.

Biology and Systematics of the P. darwini Species Group

Several aspects of *Phyllotis* biology suggest that the demographic explanation of high population variability in covariance structure seen in the aphid *Pemphigus* (Riska 1985) may apply here as well. Local populations of aphids are loosely linked together by gene flow and have a history of repeated extinction and recolonizations (Riska 1985). The result is relatively small effective population sizes resulting from bottlenecks and limited mixing of gene pools. Members of the *darwini* species group live in the rugged and deeply dissected Andes. Populations can be isolated on opposite sides of the cordillera, or in deep river valleys, particularly along the western slopes. The Atacama, one of the world's driest deserts, occupies the lower elevations along the Pacific slope, and latitudinal gene flow is probably quite limited at the current time (Pearson 1958; Caviedes and Iriarte 1989), although less limited in the past (Marquet 1989). The geography of *Phyllotis* promotes isolation of populations as does the largely parthenogenic reproduction of *Pemphigus*.

Small effective population size in some *Phyllotis* is suggested by several lines of evidence. The Moro Sama population sample of southern *limatus* represents a single popu-

lation eruption after heavy rains in an area that may not receive measurable precipitation for years (Pearson 1975). Population sizes during dry years are probably small, as vegetation is sparse. Moro Sama, like several other localities where southern *limatus* has been collected, is a coastal bench (Pearson and Ralph 1978) not far from a river mouth. The coastal zone supports a *loma* vegetation moistened by advection fog from the Pacific. Vegetation decreases away from the coast, and becomes virtually "lifeless" until about 2500 m, where precipitation and vegetation increase (Pearson and Ralph 1978). There are almost no records of *Phyllotis* in this intermediate zone.

Although the coastal populations may not go extinct, they clearly receive colonists from higher elevations. The presence of *limatus,* which lives at low to middle elevations, at Moro Sama is not surprising, but *magister* and *xanthopygus chilensis* have also been collected from there, two taxa that normally are not found below 2400 m and 3500 m, respectively. Isolated populations of *magister* and either southern *limatus* or *xanthopygus rupestris* have also been found near the mouth of the Rio Loa in northern Chile (L. Contreras and J. C. Torres-Mura, pers. comm.). In the case of *magister*, the Rio Loa site is 300 km from any other known locality. These examples from coastal populations probably represent the extreme in *Phyllotis*, as eruptions do not occur at high elevations (Pearson 1975), but topographic complexity should still promote isolation.

Phylogenetic structure in covariance patterns in *Phyllotis* may be difficult to detect because there is not a high degree of hierarchical structure to other aspects of the genome and phenotype. Morphologically, the members of *Phyllotis* sensu stricto are not well differentiated from each other, which has led to frequent misidentifications of specimens in museum collections. Phylogenetic hypotheses for internal nodes have not been robustly resolved using morphological traits (Steppan 1993, 1995b) or karyotype (Pearson and Patton 1976; Spotorno 1986). DNA sequence data has provided improved confidence in phylogenetic relationships, but it required nearly 1000 base pairs of the rapidly evolving cytochrome *b* gene and careful character weighting to achieve that limited level of confidence (Steppan 1995a, in press). Taken as a whole, the phylogenetic data suggests that *Phyllotis* species diverged from each other over a short time relative to the age of the group, thus preventing the accumulation of shared patterns of covariance. A different set of species that are more highly differentiated and exhibit greater phylogenetic structure in morphology and genetics might also exhibit greater phylogenetic structure to covariance patterns (J. Flynn, pers. comm.).

However, the data suggest that the preceding explanation for limited phylogenetic structure (i.e., short internal branches) can be only partially correct. Branch lengths estimated from cytochrome *b* do not show the same phylogenetic pattern as morphology and covariance structure (a sequence phylogram is not presented because the sequenced individuals and populations in covariance analyses are not congruent). Sequence-based phylograms show terminal branches leading to geographically dispersed individuals (i.e., not from same population) to be distinctly shorter than interspecific branches, approximately one-half as long (Steppan 1995a). This is the opposite pattern from covariance structure (Fig. 1), with relatively short interspecific branches. Differences in algorithms for estimating branch lengths (squared-change parsimony for morphometric data, Wagner parsimony for nucleotide data) should not be significant enough to account for this large difference in pattern (Martins and Garland 1991). The pattern exhibited by gross phenotype is intermediate, with intraspecific and interspecific branches of similar length (Fig. 2). Taken together, these results suggest that if sequence evolution is approximately neutral, then there has either been stabilizing selection on the phenotype or genetic/developmental constraints. Furthermore, phenotypic covariance structure is even more conservative, suggesting stabilizing selection on covariance patterns, or more likely, maintenance of underlying genetic organization and developmental programs. While there is *statistically* significant differentiation in covariance structure, that structure appears to evolve more slowly than the overall phenotype. Such a model is consistent with the suggestion by Riska (1985) that although populations may acquire different mutations that modify covariance structure in various ways, mutations accumulated during speciation may be those that conform to a given developmental pathway. The dynamic pattern expected under this model would be that covariance structure would be perturbed in differentiated populations, but would return to an equilibrium state over longer time frames.

While the cause, or even reality, of an equilibrium covariance structure is speculative, its suggestion touches on a number of important issues in evolutionary biology, including genetic revolutions or founder effect speciation (Mayr 1954; Carson 1968; Templeton 1980), canalization (Waddington 1957), and constraints (Maynard Smith et al. 1985; Arnold 1992). Stabilizing selection at this phylogenetic scale would have to be reconciled with the evidence for dietary and habitat partitioning among at least some of the species: *andium*, *magister*, *limatus*, and *x. chilensis* (Pearson 1958; Pearson and Ralph 1978; Pizzimenti and de Salle 1980); *osilae* and *xanthopygus* (Pearson 1958; Hershkovitz 1962). The data suggests that there may be different degrees of constraint at different phylogenetic levels. That is, populations may be relatively unconstrained and subject to random fluctuations within the limits imposed by the underlying constraint observed for the genus. The next stage of investigation might be to test if large and persistent shifts in covariance structure are associated with large changes in morphology only at deeper and more divergent branches of the phylogeny.

Even if there is an equilibrium covariance structure, comparative studies that do not sample intraspecific variation or sister-species will fail to detect perturbations in covariance structure. If these perturbations are associated with periods of rapid morphological evolution, then reconstructions of historical selection will be in error. The long-term dynamics and stability of covariance structure may be less important to understanding morphological evolution than its response to selection and population fluctuations. Only detailed phylogenetic or temporal sampling will detect short-term dynamics. Even if covariance structure does not return to an equilibrium, but instead drifts randomly, then undersampling the phylogeny will result in significantly underestimating the degree of homoplasy in covariance evolution.

Conclusions

Is phenotypic covariance structure conserved throughout the phylogeny?—The generally high matrix correlations, even at more inclusive phylogenetic levels, and the small matrix disparities among basal branches demonstrate that a *similar* covariance structure characterizes the entire clade. The CPC analyses show that although matrices may be similar, there is statistically significant divergence in all levels of covariance structure among populations. Even closely related populations of the same subspecies show deviation from equality and proportionality of matrices. Whether this heterogeneity indicates biologically significant divergence in developmental constraints is unclear, but criteria for defining a common constraint need to be refined.

If variation in covariance structure exists, is that variation phylogenetically structured?—Detectable phylogenetic structure is limited by the among-species variation in covariance structure being less than the variation among subspecies and populations. Two results suggest some phylogenetic structure. First, estimated matrix disparities for interspecific branches are significantly less than for intraspecific branches ($P = 0.005$). Branches leading to populations and branches leading to subspecies are of equivalent disparities (Table 1). Second, there is weak evidence from matrix correlations that populations of different subspecies are less similar to each other than are populations of the same subspecies. The CPC analyses also indicate increasing diversity of covariance structure in more inclusive clades, but may be due simply to the accumulation of randomly varying populations without phylogenetic structure.

If there is phylogenetic structure, at what level in the phylogeny does significant divergence occur?—The CPC analyses provide a simple statistical answer: significant divergence occurs between geographic populations. However, some CPC structure is shared among many populations of individual subspecies. As discussed above, the greatest magnitude of divergence in covariance structure occurs within biological species. Minimum evolution analyses indicate that divergence in structure leading to phylogenetic species (subspecies) is similar in magnitude to that seen among local populations. Because some of the disparity between populations is likely to be due to sampling error (whose effects on subspecies divergence would be dampened by the averaging algorithm), the largest true divergences may be those leading to phylogenetic species (subspecies). The correlation analyses also suggest that this is the more significant transition. This pattern would suggest that with the formation of diagnosable, evolutionary units, phenotypic covariance structure is also modified. The smaller magnitudes of divergence between putative biological species and their sister-species or species-groups suggest that within *Phyllotis*, the formation of reproductive barriers is not associated with similar modifications of covariance structure. The ability to interbreed among phylogenetic species (subspecies) may be considered a plesiomorphic trait (Cracraft 1989), and the limited gene flow may be insufficient to prevent divergence in covariance structure.

Is divergence in covariance structure associated with divergence in overall phenotype, as described by trait means?—Despite the suggestion that covariance structure diverges in association with the evolution of diagnosably distinct taxa, there is no evidence that the magnitude of covariance evolution is correlated with phenotypic evolution. There are several complicating factors. The distribution of estimated branch lengths may be an artifact of the minimum evolution algorithm. If the distribution of branch lengths is an artifact of the algorithm, then both matrix disparity and Euclidean distances should show similar distributions. They do not. The observation that terminal branches leading to populations are slightly longer in matrix disparities than Euclidean distances may be due in part to the greater error in accurately estimating correlations than means.

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