

S27-5 Molecular modules and morphology

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Abstract We present a phylogenetic perspective on morphological and molecular characters and character complexes and their properties at different hierarchical levels, using both examples from literature and case studies in carduelid finches and pigeons. Phylogenetic hypotheses inferred from both molecular (cytochrome-*b*) and morphological characters (plumage ornamentations), and combined analyses in carduelids, indicate that in both datasets corresponding patterns occur at particular nodes and systematic levels. A new method is introduced to partition and quantify correlation in cladogenetic, anagenetic and environmental signal, to estimate by their degree of similarity the underlying factors producing that correspondence.

Key words Cladogenesis, Anagenesis, Environmental signal, Matrix similarity, Cytochrome-*b*, Plumage ornamentation

1 Introduction

One approach towards realizing a holistic view of organismal structure and evolution is the concept of morphological integration (Olson and Miller, 1958). Its tools are morphometrics, the quantitative study of changes in the size and shape of the organism during ontogeny and evolution (Crowe, 1994). Patterns of trait correlation are traced to identify the underlying processes that produce corresponding structures. In the modern formulation of the concept of morphological integration (Cheverud, 1982, 1988; Wagner, 1996; Wagner and Altenberg, 1996; Chernoff and Magwene, 1999; Magwene, 2001), the importance of pattern correspondences and of the evolution of modularity are stressed, and principles of quantitative genetics (Steppan et al., 2002) as well as aspects of developmental biology (Nemeschkal, 1999) are included.

Phenotype-genotype mapping are a focus in current research, testing also the involvement of ecology (Marroig and Cheverud, 2001), life history (Roff, 1996) and other environmental factors, as well as physiology (Villani et al., 1992) and behavior (Bloemer and Crowe, 1998). Both the ideas of “morphological integration” and “modularity” are now being used at the molecular genetic level (Raff, 1996; Wagner and Altenberg, 1996) to describe hierarchical levels and interactions within and between biological domains. Most investigation into morphological integration and modularity, however, has focused on morphological or phenotypic (Badyaev et al. 2001) characters at the microevolutionary level, in analyses of character coupling or decoupling within or between populations. At the macroevolutionary level, trans-specific exploration of characters or character

modules are the tools to explore the frameworks of phylogenetic effect.

2 Molecular and morphological modularity in phylogenetics

For phylogenetic inference, the most frequently used exploratory gene is cytochrome-*b*, a protein-coding mitochondrial gene. Analogous to morphological evolution, molecular evolution is affected by its own constraints, such as the genetic code, secondary and tertiary structure, and replication rate or gene or protein function (for an overview, see Mindell and Thacker, 1996). In an analysis of falcons and caracaras, the extent of substitution saturation in cytochrome *b* was correlated with functional modules in its structure (Griffiths, 1997). In several species of carduelid finches, productive change in aminoacids occurs solely (van den Elzen, 2001) or preferredly (Arnaiz-Villena et al., in press) within the transmembrane region of the cytochrome *b* molecule. That region has been found to be hypervariable in mammals too (Irwin et al., 1991).

Results from our investigations add to the phylogenetic perspective. In two disparate bird families (Fringillidae-Carduelinae and Columbidae), infraspecific analyses of skeletal characters exhibited patterns of character correlation that corresponded with developmental requirements, and may be taken as modules of the body plan (Nemeschkal, 1999). And a trans-specific investigation of the same species (van den Elzen et al., 1987; Nemeschkal et al., 1990) identified several units of character complexes that reflect patterns of shared biological roles. These character modules tend to coevolve and are clade specific (Nemeschkal et

al., 1992). Finches and pigeons differ in their levels of morphological integration and in the variance of phenotypic characters, as well as in the numbers of characters integrated within covarying units. Such differences reflect phylogenetic effect, the geological age of pigeons probably doubling that of finches. Yet phylogenetic effect has scarcely been considered in phenotype-genotype matching, even though there is a comprehensive literature dealing indirectly with it.

3 Alternative perspectives on the phenotype-genotype match: the phylogenetic approach

Phylogenetic methods estimate relationships on the basis of change in homologous, novel characters, which in cladistic terminology are synapomorphies, or shared derived characters inherited from a common ancestor. In the cladistic approach to phylogeny, characters are not evaluated by quantity (=measurements) but quality (=occurrence of characters and their states). These are transformed into a numerical distance matrix to link taxa by parsimony (=minimal number of steps in character change) and/or degrees of distance (extent of differences in characters). Cladogenetic (=speciation) events are then mapped on to a dichotomously bifurcating branch-pattern.

This phylogenetic model rests on the assumption that both phenetic and genetic distance matrices include a module of identically or similarly structured cladogenetic information (patterns of speciation, phylogenetic signal), and a module of anagenetic information (number of evolutionary steps within a species or lineage not associated with speciation). Phylogenetic estimates of relationships among taxonomic groups are thought to improve in precision with the combination of different datasets. In the concept of total evidence (Kluge, 1989), all relevant character information is combined in a single phylogenetic analysis. The total evidence approach has been practised successfully in diverse organismal groups at various systematic levels (e. g., Eernisse and Kluge, 1993; Mattern and McLennan, 2000; overview in Chippindale and Wiens, 1994), including birds (e. g., Zink and Blackwell, 1996; Griffiths, 1999; Johnson and Sorensson, 1999).

A restricted, conditional combination approach (Bull et al., 1993; Gatesy et al., 1999) only allows the combination of data sets that are not significantly contradictory when tested for congruence. In cases of incongruence, comparisons of single data sets can elucidate the underlying evolutionary phenomena (McCracken and Sheldon, 1998). If phylogenetic tree-length is accepted as a measure of evolutionary change, incongruence between trees built from different data sets may be interpreted as differences in evolutionary steps manifested in these characters.

In the Carduelinae, we have inferred phylogenetic hypotheses from siskins (van den Elzen et al., 2001) and serins (Arnaiz-Villena et al., in press) using both molecular

(cytochrome-*b*) and morphological characters (plumage features) and a combination of both data sets. Results indicate that corresponding patterns of characters occur at particular nodes and systematic levels in both species-groups. Tempo of diversification in plumage characteristics precedes that of molecular change. In siskins, moreover, phenotypic change is faster in male than female ornamentation, but not in serins.

To quantify similarity patterns between these different taxa and data sets, matrix comparison can be used. This approach has been used successfully in birds in genealogical comparisons of phenotypic and genetic differentiation in the South American antwrens (Hackett and Rosenberg, 1990). Distance matrices for plumage features, morphometrics and genetic distance based on protein electrophoresis were compared by Mantel's (1967) test. General patterns of genetic diversification paralleled those of both sets of morphological traits, and rate of plumage feature divergence again exceeded that for the protein genes screened; rates for morphometric change were slowest. To quantify phylogenetic relationships and effect, pattern correlations may be tested by application of cladistic principles.

4 Separating cladogenetic and anagenetic signal in phylogeny

Accordingly, we outline here a new approach to statistically separate cladogenetic from non-cladogenetic signal in phenotypic and molecular distance matrices. We compared distance patterns between these matrices and their separated portions and to environmental parameters, to estimate the underlying factors producing correspondence. A detailed description of the method and its material is in preparation. Three hierarchical datasets for siskins were built for comparison: one phenetic from plumage ornamentation of the phenotype, another molecular from DNA sequences of the mitochondrial cytochrome *b* gene, and the third environmental from such information such as geography, climate and habitat.

Distance matrices were calculated in the same way for the three datasets by the phylogenetic software package PAUP* (Swofford, 2001). Mean character differences (uncorrected pairwise character distance, mean character difference) as offered by PAUP* were taken as distance measures for all but the single character datasets. Phylogenetic distance (cladogenesis) was estimated from the topology of a cytochrome *b* gene NJ-bootstrap tree based on maximum likelihood, counting branching points between each species-pair. This is here referred to as the cladistic matrix, because it includes cladogenetic information derived from cladistic principles.

Using a modified Mantel's test (Nemeschkal, 1991, 1999), significant correspondence was found between basic molecular and phenotypic distance matrices, and between both of them and the cladistic matrix (ZEISUO-MOLZM, $r=0.77352$, $P<0.0016^{**}$, ZEIPHYLL-ZEISUO, $r=$

0.5153854, $P < 0.02^*$, ZEIPHYLL-MOLZM, $r = 0.4876437$, $P < 0.0001^{***}$). We interpret these findings as indicating that cladogenesis is the underlying factor inducing proportionality of character change in both phenetic and molecular characters. The observed congruence among cladistic, phenetic and genetic distance matrices is not unexpected, being concordant with other observations of pattern congruence between phenotypic and genetic matrices mentioned above. It also agrees with similarities in tree topology among morphological- and molecular- based phylogenies at different systematic levels from species to class (e. g., Smith et al., 1992; Potter and Doyle, 1994; Bridge et al., 1995; Griffiths, 1999; Titus and Frost, 1996; Frost et al., 2001; but see Baker et al., 1998 for conflicting results).

The hypothetical environmental matrix also exhibited significant pattern similarity with the rest. Mantel's test revealed significant congruence between body mass (SIZE-ZEISUO, $r = 0.50283$, $P < 0.0438^*$), the complete environmental matrix (ENVIR-ZEISUO, $r = 0.57275$, $P < 0.0125^*$), and its respective its biogeographic and topographic portions (GEOG-ZEISUO, $r = 0.57227$, $P < 0.0298^*$, AREA-ZEISUO, $r = 0.68307$, $P < 0.0012^{**}$), but not with habitat nor climatic data. Molecular distance (MOLZM) exhibited fewer (two instead of four) significant similarities in pattern, matching phenetic matrix correspondence in biogeographical and topographic attributes (GEOG-MOLZM, $r = 0.52038$, $P < 0.0013^{**}$, AREA-MOLZM, $r = 0.69395$, $P < 0.004^{***}$), but not in body mass (SIZE) nor the complete environmental dataset (ENVIR). The cladistic matrix ZEIPHYLL, as expected, resembled the genetic matrix from which it had been extracted (GEOG-ZEIPHYLL, $r = 0.52938$, $P < 0.02^*$; AREA-ZEIPHYLL, $r = 0.52042$, $P < 0.009^{**}$).

Our results from siskins in general corroborate already well-known interrelationships between geographic distribution and phylogeny, especially in distribution pattern and cladogenetic events. The results also encourage application of Lande's (1979) model for assessing phenotypic variance-covariance to distance measure analysis. This will open the way for integrating cladogenetic, biogeographical and ecological information into the explanations of morphogenesis over the interface of quantitative genetics theory (Steppan et al., 2002).

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