

A TEST FOR PATTERNS OF MODULARITY IN SEQUENCES OF DEVELOPMENTAL EVENTS

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Abstract.—This study presents a statistical test for modularity in the context of relative timing of developmental events. The test assesses whether sets of developmental events show special phylogenetic conservation of rank order. The test statistic is the correlation coefficient of developmental ranks of the N events of the hypothesized module across taxa. The null distribution is obtained by taking correlation coefficients for randomly sampled sets of N events. This test was applied to two datasets, including one where phylogenetic information was taken into account. The events of limb development in two frog species were found to behave as a module.

Key words.—Development, evolution, heterochrony, modularity, statistical test.

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The concept of a developmental module has a long history in studies of the evolution of development (e.g., Needham 1933; Olson and Miller 1958; Gould 1977; Bonner 1988; Raff 1996). Roughly, modules are sets of characters (anatomic or genetic) within an organism that possess some autonomy relative to other characters. Raff (1996; discussed in Bolker 2000) characterized modules as genetically discrete, hierarchically organized units that undergo transformation both developmentally and evolutionarily and that are anatomically localized within development and display varying degrees of connectivity with other modules. Modularity in evolution is an inherently comparative concept. That is, modules are identifiable as comparable subunits in different species (Bolker 2000). In spite of great historical and continued interest in modularity, quantitative treatments of this concept have been few, and statistical significance tests for the modularity versus nonmodularity of particular suites of characters are rarer still. Quantitative approaches that have been or could be applied to modularity include Smith's (1996; see also Mabee and Trendler 1996; Velhagen 1997) event-pairing, Zelditch's (1987) multivariate factor analyses, Von Dassow et al.'s (2000) use of computer simulations to demonstrate resistance to perturbation in *Drosophila* segment polarity genes, Magwene's (2001) multivariate graphical modeling of continuous data, and Schlosser's (2001) bivariate graphical method for discrete event data. One aspect of modularity that is amenable to hypothesis testing is the expected evolutionary conservation of modules. That is, modules are sets of events or processes in development for which evolutionary change within such sets is more difficult than evolutionary change between sets (Von Dassow and Munro 1999). In this note I suggest a statistical test for this aspect of modularity in the context of timing of developmental events.

Sequences of developmental events afford a convenient means to address questions in the evolution of development (Smith 2001). "Events" in this case refer to markable phenomena in development such as the ossification of the premaxilla bone or the first appearance of striations in trunk muscle. Such events may be ranked according to timing of appearance, and these rankings may be compared across species to test hypotheses of heterochrony. For example, Nunn and Smith (1998) demonstrated differences in the relative

developmental timing of elements of the central nervous system in a comparison of marsupial and placental mammals.

Smith (2001) suggested that sets of developmental events that function as modules should be especially constrained in their order. That is, in the developmental sequence of events ABCDEFG, if ADE constitutes a module, these elements should retain their order over evolutionary time more so than other sets of elements such as CFG. Although Smith (2001) proposed that suites of events behaving as modules should show especially high conservation of order—and Nunn and Smith (1998) examined overall development using correlation tests—she did not propose a significance test for this contention. The structure of Smith's (2001) view of modularity suggests a simple statistical test. If the order of occurrence of a set of modular events is especially conserved across taxa over evolutionary time, then the correlation of the ranks of these events between taxa should be especially high. The test statistic is the correlation coefficient (e.g., Kendall's τ) for the N hypothesized modular events. The appropriate null hypothesis is that the order of the N hypothesized modular events is no more evolutionarily conserved across taxa than other sets of N developmental events. Thus, an appropriate null distribution is obtained by sampling sets of N events at random and obtaining correlation coefficients for each of these random selections of N events (e.g., using a computer program such as Mathematica, Wolfram 2003). If the correlation value for the N events in the hypothesized developmental module is higher than some specified number (e.g., 95%) of correlations for sets of N randomly selected events, the tested set of events is behaving as a module.

TWO EXAMPLES

I applied this test to data from two frog species. Schlosser (2001) listed the timing of 149 developmental events for *Discoglossus picta* and *Eleutherodactylus coqui*. He categorized these events according to region of development and type of event. For example, he listed 23 events as pertaining to the development of the central nervous system. Some of these categories may be interpreted as potential developmental modules. For example, Schlosser (2001) concluded from patterns in his "heterochrony plots" (see below) that

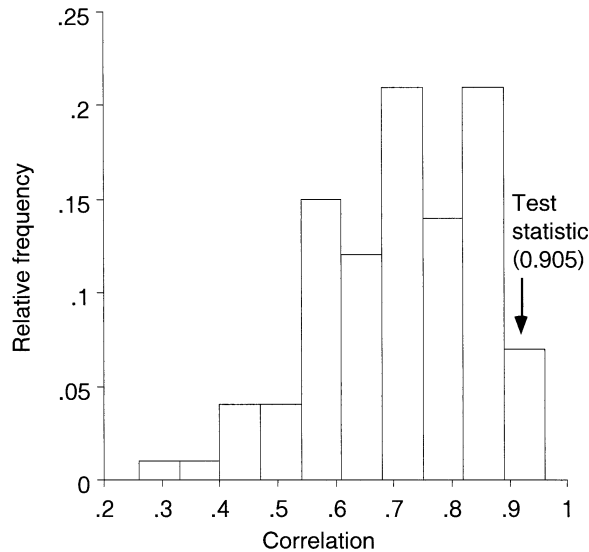


FIG. 1. Null distribution for correlations of random samples of 12 developmental events between two frog species.

the 12 events involved in the development of limb structure and limb innervation underwent “coevolved dissociation” relative to the other developmental events. I tested the hypothesis that the order of events of limb development is especially conserved. I used Kendall’s τ to assess the correlation of events. The correlation of the 12 events of limb development is 0.905 in the two frog species (correlation for the entire set of 149 events is 0.692; $P < 0.0001$). To generate a null distribution, I took 99 random samples of 12 events from the total of 149 and obtained the correlation between *D. picta* and *E. coqui* for each sample. The distribution of these values is shown in Figure 1. The value for the test statistic is greater than all but four of the 99 random samples. Thus, the events of limb development are significantly more correlated than randomly selected developmental events ($P = 0.05$). According to this criterion, the developmental events of the limb behave as a module.

This correlation test is simple to implement if just two taxa are analyzed. However, more general hypotheses will involve multiple species. In such cases, the nonindependence of species necessitates an approach that incorporates information on phylogeny (e.g., Harvey and Pagel 1991). One way to incorporate such information is with a version of Felsenstein’s (1985) independent contrasts. That is, hypothetical ancestral developmental sequences are calculated for each node of the phylogeny, and independent comparisons of sequences are made between pairs of independent nodes of the phylogeny. The test statistic is the average correlation for these node-pairs for the N events of interest. The null distribution is calculated by obtaining average correlation values for random selections of N events.

I applied this approach to data presented by Nunn and Smith (1998; tables 2, 4) on 28 ranked events in craniofacial development of three species of mammals (two placental and one eutherian). The phylogeny for these species is uncontroversial and is shown in Figure 2. Nunn and Smith (1998; see also Smith 1996) categorized the 28 developmental events

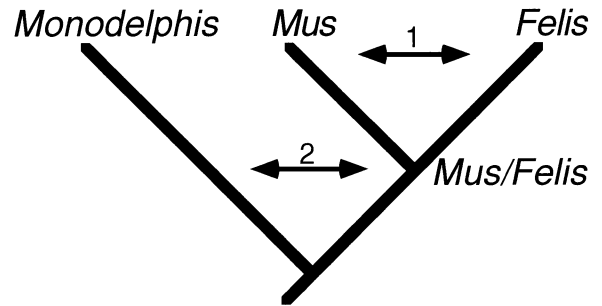


FIG. 2. Phylogenetic tree used in testing for modularity in three mammal species. Independent comparisons are shown with arrows.

as involving formation of the central nervous system (six events), formation of muscular structures (three events), ossification of bones (12 events), and development of other skeletal structures (seven events). I hypothesize that the 12 ossification events form a developmental module. The appropriate phylogenetically independent comparisons are between (1) sister species *Mus* and *Felis*; and (2) *Monodelphis* and the ancestor of *Mus* and *Felis* (Fig. 2; see Felsenstein 1985). Thus, some estimate of the ancestral state for the *Mus-Felis* node is needed. This hypothetical sequence could be estimated in several ways, potentially taking evolutionary branch lengths and models for evolutionary change into account (Pagel 1999). For the purposes of this analysis, I simply take the average rank for each event between *Felis* and *Mus* as an estimate of the developmental sequence of the *Felis-Mus* ancestor. The test statistic is the average Kendall’s τ for the (in this case) two independent comparisons. Kendall’s τ for the correlation of the 12 events in *Mus* and *Felis* is 0.670 (for all 28 events, $\tau = 0.666$, $P < 0.0001$). Between *Monodelphis* and the *Mus-Felis* ancestor Kendall’s τ for the ossification events is 0.803 (for all 28 events, $\tau = 0.675$, $P < 0.0001$). Thus, the test statistic is $(0.670 + 0.803)/2 = 0.737$.

I generated a null distribution for this statistic by randomly sampling sets of 12 events from the total of 28. For each sample of 12 events, Kendall’s τ was calculated between *Mus* and *Felis* sequences and between the *Monodelphis* sequence and the estimated *Mus-Felis* ancestral sequence. The average of these two values was recorded for random samples of 12 events, and the resulting distribution is shown in Figure 3. The test statistic is not significantly greater than the null distribution ($P = 0.24$). Thus, the events of ossification of bones are no more tightly correlated than other events. According to this test, the ossification patterns of facial bones in these three mammals do not behave as a module.

DISCUSSION

The biological conclusions of this study accord well with previous ideas of modularity. The vertebrate limb is perhaps the archetypal example of a module, so it is reassuring that the test presented in this paper recovered the suite of limb characters as significantly correlated between species of frogs. This result offers statistical confirmation for Schlosser’s (2001) finding that these limb characters behave as a “unit of evolution” in these species. Although the finding that the developing limb behaves as a module may be ex-

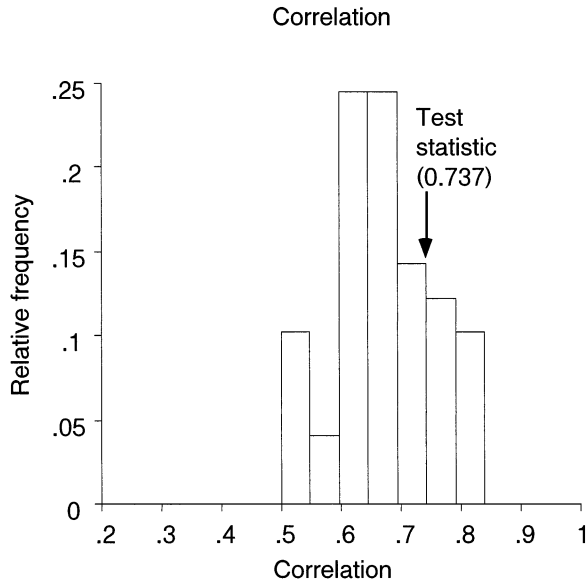


FIG. 3. Null distribution for correlations of random samples of 12 developmental events between three mammal species. Values are averages of correlation between *Mus* and *Felis* and correlation between *Monodelphis* and the ancestor of *Mus* and *Felis*.

pected, the recovery of this module in two frogs with very different modes of development may be surprising. *Discoglossus picta* undergoes typical anuran biphasic development from aquatic tadpole stage to terrestrial adult. *Eleutherodactylus coqui* develops directly into a terrestrial form. Hanken et al. (2001) noted differences in limb development between *E. coqui* and other frogs, such as the absence of an apical ectodermal ridge in *E. coqui* (Richardson et al. 1998), and suggested that these differences were related to *E. coqui*'s direct development. However, these authors also noted several similarities between *E. coqui* and other frogs and concluded that in spite of trenchant differences between direct and biphasic developers the tetrapod limb should retain its status as a module. The results of this paper, in which the events that are shared between species retain a tightly conserved order, support this contention.

The bone ossification events in mammals did not behave as a module. This too is an unsurprising result, as there is little evidence that sequences of ossification exhibit modularity. However, it must be noted that none of the event-type subdivisions of Smith form modules according to this test (e.g., the six events of the central nervous system; results not shown), and the power of this test is likely to be low when the total number of developmental events is small, as in the Smith dataset of just 28 events. The most interesting applications of this test will involve large numbers of developmental events and large numbers of taxa.

The only previous quantitative approach that is similar to the one presented here is Schlosser's (2001) heterochrony plot. Schlosser (2001) plotted the timing of character development in one species against the timing of character development in a second species for developmental event data. Unlike the test of this paper, Schlosser's graphical approach does not incorporate quantitative criteria for evaluating

whether events are significantly coevolved. However, these plots do appear useful for visualizing trends and identifying suites of characters that may be of interest. The test that I present requires a specific a priori hypothesis of modularity. Schlosser's (2001) approach is not so restricted and therefore may be useful in more exploratory studies.

There exist many conceptions and definitions of modularity, most of which are specific to particular contexts (Bolker 2000). The test presented in this paper is an attempt to operationalize one aspect of modularity—evolutionary conservation—in terms of patterns of sequences of developmental events. Other kinds of data (e.g., gene interactions) and other aspects of modularity (e.g., genetic discreteness) will require additional tests.

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