

PHYLOGENETICALLY BASED STATISTICAL METHODS: A PRIMER AND APPLICATIONS TO VERTEBRATE LOCOMOTOR PERFORMANCE AND MORPHOMETRICS. *Garland, T., Jr. University of Wisconsin, Madison, USA*

This talk will cover three topics: the application of phylogenetically based statistical methods to the analysis of phenotypic evolution; the importance of direct measures of whole-organism performance abilities; and the application of phylogenetic approaches to integrative studies of the evolution of locomotor performance abilities in mammals and squamate reptiles.

The last decade has witnessed a revolution in the way interspecific, comparative data are analysed. Phylogenetic information is required both to allow proper statistical testing with nonindependent data points and to address various evolutionary questions (e.g., origins of traits (Luke, '86), rates of evolution [Garland, '92]). A number of different phylogenetic approaches are available; some are explicitly statistical, others are not (see discussion in Garland and Adolph, '94). Several methods are now used routinely in analyses of phenotypic diversity. The two most widely used statistical methods for continuous-valued characters are Felsenstein's ('85) phylogenetically independent contrasts (e.g., Purvis and Garland, '93, Diaz-Uriarte and Garland, '96) and Monte Carlo computer simulations to derive empirically scaled null distributions for hypothesis testing (Martins and Garland, '91; Garland et al., '93). Both can be applied to a wide range of analytical approaches, including multiple regression, ANOVA, ANCOVA and principal components analysis; they can also be used to compare rates of evolution across clades. Computer programs that implement both methods are available from the author and other workers at no cost. The logic and mechanics of these two methods will be discussed and illustrated with examples from vertebrate locomotion.

Until the early 1980s, relatively few studies of vertebrate locomotion included direct measurements of locomotor performance capacity (some studies of fish are notable exceptions). Studies in ecomorphology, for example, often related morphometric variation to behaviour or ecology without demonstrating that the former actually affected abilities to perform various tasks. Following the publication of three seminal papers (Huey and Stevenson, '79; Bennett, '80; Arnold, '83), many workers in physiological ecology, functional morphology and comparative physiology came to recognize the key role of performance abilities. In particular, natural selection is expected to act much more directly on performance abilities than on lower-level morphological, physiological or biochemical characteristics (e.g., leg length, heart size, haemoglobin properties, enzyme activities). Thus, studies of the adaptive significance of morphological variation will be incomplete and possibly misleading in the absence of empirical information on (1) the relationship between morphology and performance and (2) the relationship between performance and Darwinian fitness in natural

populations. Although both types of information are required for a complete picture, each is also of interest in its own right. The former are usually accomplished in the laboratory (e.g., Garland, '84); the latter require studies in natural or semi-natural situations (e.g., Jayne and Bennett, '90; Garland et al., '90). Both typically begin with observational (correlational) approaches but can be enhanced by experimental manipulations (e.g., Carothers, '86; Sinervo, '93). Some methods for measuring locomotor performance will be outlined (e.g., photocell-timed racetrack, high speed treadmill).

Details on the comparative approach to locomotor performance will be provided for some of our studies on squamate reptiles and mammals. Different clades within the two groups vary in their suitability as subjects for studying different aspects of the correlated evolution of morphology, performance and behaviour/ecology. For example, lizards are generally cooperative subjects for measurement of locomotor performance abilities; however, some are small, nocturnal, and difficult to observe under natural conditions. In both lizards and mammals, analyses with phylogenetically independent contrasts indicate that interspecific variation in locomotor abilities and behaviour is related to limb proportions (e.g., Losos, '90; Garland and Janis, '93; Bauwens et al., '95; Zani, '96; Harris and Steudel, '97). For example, hind limb length is positively correlated with maximal sprint running speed in both groups.

We are emphasizing lizards as a group within which to examine the relationship between speed and stamina, and how this relationship is determined by multiple aspects of morphology and physiology. For example, a first-principles consideration of the effects of variation in muscle fibre composition (e.g., fast glycolytic versus slow oxidative) might suggest a necessary trade-off between speed and stamina. However, across species both body mass and body temperature are positively correlated with both aspects of performance; this effect may obscure a trade-off between speed and stamina. Moreover, in lizards, hind limb length may be negatively correlated with endurance-running capacity as measured on a motorized treadmill. Whether some of these correlations reflect cause-and-effect relationships (e.g., direct Q10 effects of temperature on locomotor abilities) or correlated evolution without causality (e.g., past selection acted independently to alter both body temperature and locomotor performance) is a major outstanding question. Further progress will require multivariate statistical analyses of multiple aspects of the phenotype. Various clades of lizards seem to show clear 'grade shifts' with respect to both limb morphology and locomotor abilities, but demonstrating the statistical significance of some of these apparent differences can be difficult.

## References

- Arnold, S. J. 1983 Morphology, performance and fitness. *Am. Zool.* **23**, 347-361.
- Bauwens, D., Garland, T., Jr., Castilla, A. M. and Van Damme, R. 1995 Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioral covariation. *Evolution* **49**, 848-863.
- Bennett, A. F. 1980 The thermal dependence of lizard behaviour. *Anim. Behav.* **28**, 752-762.
- Carothers, J. H. 1966 An experimental confirmation of morphological adaptation: toe fringes in the sand-dwelling lizard *Uma scoparia*. *Evolution* **40**, 871-874.
- Diaz-Uriarte, R. and Garland, T., Jr. 1996 Testing hypotheses of correlated evolution using phylogenetically independent contrasts: sensitivity to deviations from Brownian motion. *Syst. Biol.* **45**, 27-47.
- Felsenstein, J. 1985 Phylogenies and the comparative method. *Am. Nat.* **125**, 1-15.
- Garland, T., Jr. 1984 Physiological correlates of locomotor performance in a lizard: an allometric approach. *Am. J. Physiol.* **247**, R806-R815.
- Garland, T., Jr. 1992 Rate tests for phenotypic evolution using phylogenetically independent contrasts. *Am. Nat.* **140**, 509-519.
- Garland, T., Jr. and Adolph, S. C. 1994 Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiol. Zool.* **67**, 797-828.
- Garland, T., Jr. and Janis, C. M. 1993 Does metatarsal/femur ratio predict maximal running speed in cursorial mammals? *J. Zool., Lond.* **229**, 133-151.
- Garland, T., Jr., Hankins, E. and Huey, R. B. 1990 Locomotor capacity and social dominance in male lizards. *Funct. Ecol.* **4**, 243-250.
- Garland, T., Jr., Dickerman, A. W., Janis, C. M. and Jones, J. A. 1993 Phylogenetic analysis of covariance by computer simulation. *Syst. Biol.* **42**, 265-292.
- Harris, M. A. and Steudel, K. 1997 Ecological correlates of hind limb length in the Carnivora. *J. Zool., Lond.* in the press.
- Huey, R. B. and Stevenson, R. D. 1979 Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *Am. Zool.* **19**, 357-366.
- Jayne, B. C. and Bennett, A. F. 1990 Selection on locomotor performance capacity in a natural population of garter snakes. *Evolution* **44**, 1204-1229.
- Losos, J. B. 1990 Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecol. Monog.* **60**, 369-398.
- Luke, C. 1986 Convergent evolution of lizard toe fringes. *Biol. J. Linn. Soc.* **27**, 1-16.
- Martins, E. P. and Garland 1991 Phylogenetic analyses of the correlated evolution of continuous characters: a simulation study. *Evolution* **45**, 534-557.
- Purvis, A. and Garland, T., Jr. 1993 Polytomies in comparative analyses of continuous characters. *Syst. Biol.* **42**, 569-575.
- Sinervo, B. 1993 The effect of offspring size on physiology and life history. *BioScience* **43**, 210-218.
- Zani, P. A. 1996 Patterns of caudal-autotomy evolution in lizards. *J. Zool., Lond.* **240**, 201-220.

## CONGRUENCE BETWEEN MOLECULAR AND MORPHOLOGICAL SYSTEMATICS. Meyer, A., State University of New York, Stony Brook, USA

Since the advent of the polymerase chain reaction (PCR), which much facilitated the collection of DNA sequence data, many new molecule-based systematic hypotheses have been proposed for vertebrates. These molecular phylogenies offer several attractive characteristics. For example, by assuming a 'molecular clock' one can suggest not only a topology of a tree, but one can also estimate the ages of lineages, where those might not be available from the fossil record. This provides information on the rate with which phenotypic evolution might have proceeded. While the ease of PCR has brought about a 'democratization of the genetic code' and a virtual explosion of DNA sequence data and molecular phylogenies these molecular phylogenies are sometimes seemingly incongruent with those that are based on phenotypic (mostly morphological) characters. Moreover, the combination of these two types of data sets in a combined phylogenetic analysis sometimes results in yet another topology rather than supporting one or the other. There are several kinds of reasons why molecular and morphological phylogenies sometimes seem to disagree. Most often particular tree topologies are actually not strongly supported and cannot be statistically distinguished from alternative, seemingly conflicting, topologies. If the conflict is