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Molecular Relationships among Iberian, Moroccan, and South African Lacertid Lizards (Reptilia: Lacertidae)

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Abstract. Relationships among representatives of five genera of lacertid lizards from Iberia, Morocco, and South Africa were studied using quantitative micro-complement fixation analysis of serum albumin evolution. Using the albumin molecular clock to establish divergence times we suggest (1) South African *Ichnotropis* and North African *Psammodromus* diverged from the lineage representing *Lacerta lepida-L. monticola* during the Oligocene, (2) South African *Pedioplanis* and *Heliobolus* diverged from this lineage during the late Miocene, and (3) ancestral representatives of *L. andreanszkyi, L. perspicillata* and *Podarcis hispanica* diverged from lineages leading to *L. monticola* and *L. lepida* during the mid-Miocene. Radiation within the Palearctic Lacertidae has clearly been extensive, yet fewer than twenty percent of the species in this radiation have been examined biochemically. Until additional data can be gathered, the current classification of the Palearctic Lacertidae cannot be much improved and we recommend adherence to the taxonomy proposed by Arnold (1973).

Introduction

The genus *Lacerta* "is one on which more attention has been bestowed than on any other among the Lacertilia, with astonishing differences as to the delimitation of the species and their classification (Boulenger, 1920: 29)." Böhme (1971) and Arnold (1973) have more recently addressed the evolutionary history of the Lacertidae through an investigation of morphological characters considered to be non-adaptive. Arnold (1973) supplemented morphological data with ecological data and recommended that the Palaearctic Lacertidae be partitioned into five genera, *Algyroides, Gallotia, Lacerta, Podarcis*, and *Psammodromus*. This classification has subsequently achieved general acceptance.

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Morphological and ecological attributes, however, may be subjected to varying selective pressures and reflect the degree of adaptation rather than the degree of phylogenetic relationship among taxa; Arnold himself addressed this issue (1973: 346). Because molecular evolution is primarily a divergent process and not as subject to the confounding effects of convergence and parallelism as morphology and ecology are, several workers in recent years have used biochemical approaches to help elucidate the phylogenetic relationships within the Lacertidae. Horizontal starch-gel electrophoresis has been used to distinguish between morphologically similar species (Guillaume et al., 1976), to suggest or confirm taxonomic status (Mayer, 1981), to identify continental affinities of insular populations (Mayer & Tiedemann, 1980), and to assess genetic variation between insular populations of a single species (Gorman et al., 1975; Mayer & Tiedemann, 1981). Genetic distances generated from electrophoretic data have also been used to assign species to genera and subgenera. Mayer and Tiedemann (1982), for example, examined ten species of the Palearctic Lacertidae and concluded that these species should be reassigned to the subgenera into which they had been placed prior to Arnold's revision. Guillaume and Lanza (1982) also examined five species from this assemblage and recommended that the subgenus *Archaeolacerta* be given generic status.

Immunological investigations of relationships among the Lacertidae, first limited to comparative precipitin tests (Lanza et al., 1977; Lanza & Cei, 1977) and immuno-electrophoresis (Engelmann & Schäffner, 1981), have begun to include quantitative micro-complement fixation analysis (Lutz & Mayer, 1984 & 1985). Micro-complement fixation (MC'F) data provide a direct estimate of the degree of amino-acid sequence differentiation between homologous proteins of related taxa and cladistic relationships may be derived directly from these data (Wilson et al., 1977; Maxson & Maxson, 1986). In addition to providing a phylogeny based on amino acid differences in homologous proteins, the MC'F-measured divergence between albumins of extant species also provides a basis for estimating the time that has elapsed since two lineages diverged from a common ancestor (Maxson & Maxson, 1975; Wilson et al., 1977; Carlson et al., 1978).

We report new data regarding immunological estimates of relationship among taxa inhabiting the Iberian peninsula, and those inhabiting Morocco and more southern Africa. In addition, we present a reinterpretation of the MC'F data gathered by Lutz and Mayer (1984, 1985).

Materials and Methods

Antisera were prepared to albumin purified from plasma samples of *Lacerta lepida* and *L. monticola* by established procedures (Maxson & Szymura, 1979). Plasma albumin samples from *Heliobolus lugubris, Ichnotropis capensis, L. andreanszkyi, L. perspicillata, Pedioplanus lineoocellata, Podarcis hispanica*, and *Psammodromus algirus* were used in one-way comparisons. Collection data, permanent museum numbers, and locations of

Table 1. Unscaled one-way immunological distance data representing comparisons among various representatives of the Lacertidae.

Antigen	Antibody	
	Lacerta lepida	*Lacerta monticola*
Lacerta lepida	0	20
Lacerta monticola	21	0
Lacerta andreanszkyi	24	20
Lacerta perspicillata	25	20
Podarcis hispanica	30	19
Pedioplanis lineoocellata	30	33
Heliobolus lugubris	34	40
Psammodromus algirus	43	49
Ichnotropis capensis	48	60

voucher specimens representing material we examined are provided below (Specimens Examined).

MC'F analyses were performed according to methods described by Champion et al. (1974). Data are reported as immunological distance (ID) units (Table 1) where one unit of ID is roughly equivalent to one amino acid substitution between compared albumins (Maxson & Wilson, 1974; Maxson & Maxson, 1986), and to between 0.55 and 0.60 million years of lineage independence (Wilson et al., 1977).

We used the Maxson and Maxson method (Maxson, 1973 & 1984) and the program FITCH in the phylogeny inference package PHYLIP (Felsenstein, 1984) to reanalyze the data provided by Lutz and Mayer (1985). The reciprocal ID data of Lutz & Mayer (1985: Table 2) were scaled by the Sarich and Cronin (1976) procedure prior to analysis by the Maxson and Maxson method, but we used the scaled data provided by Lutz and Mayer (1985: Table 3) for the FITCH analysis. Branch lengths and the branching sequence presented in Figure 1A were determined by PHYLIP after our specification that negative branch lengths not be allowed.

Results

The 22 h titer was 4000 for *L. monticola* and 2300 for *L. lepida*, and the average slope of both antisera was 400. Both antisera were directed solely to serum albumin, as evidenced by a single precipitin arc in immunoelectrophoresis when tested with whole serum. MC'F tests using either purified albumin or whole plasma were indistinguishable.

Our data are summarized in Table 1; the standard deviation (Maxson & Wilson, 1975) from perfect reciprocity in immunological comparisons between *L. monticola* and *L. lepida* is 0.7%. We used the FITCH program to examine 66 phylogenetic interpretations of the reciprocal data provided by Lutz and Mayer (1984: Table 3). The

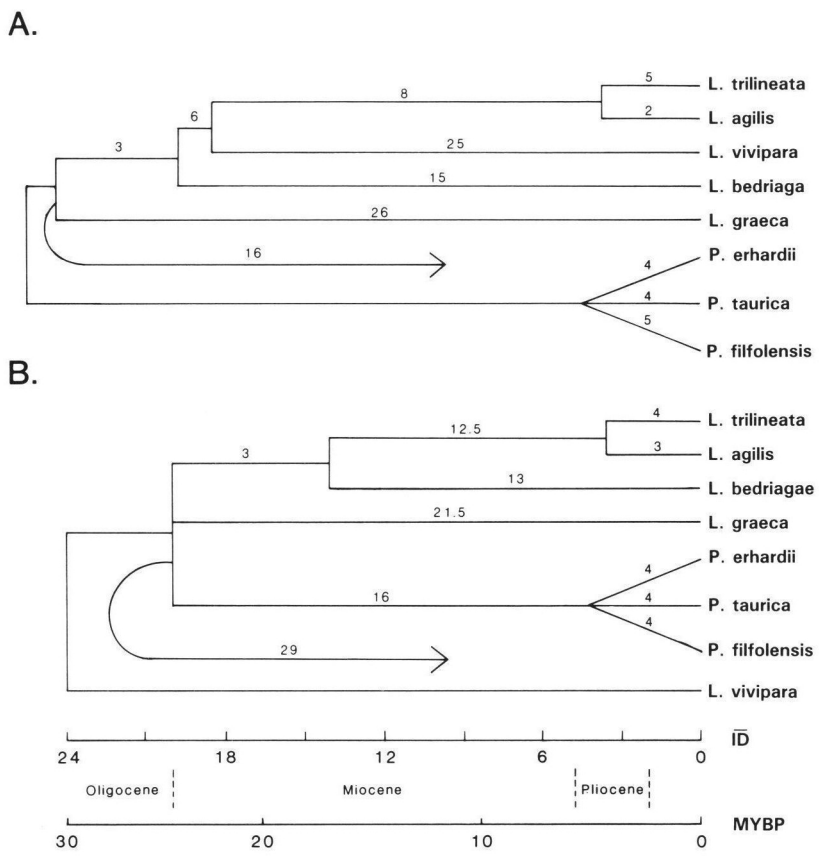

Figure 1. Phylogenetic trees constructed from the reciprocal immunological data of Lutz and Mayer (1985: Table 3). "A" calculated by the method of Fitch and Margoliash, "B" by the method of Maxson and Maxson. See Materials and Methods for details, Discussion for interpretations.

unrooted tree (Figure 1A) with the best statistical fit to these data differs from that presented by Lutz and Mayer (1985: Fig. 2) and has both a lower standard deviation (4.3%; Fitch & Margoliash, 1967) and percentage error (2.8%; Prager & Wilson, 1976). The unrooted tree (Figure 1B) calculated by the Maxson & Maxson method has a standard deviation of 11.6% and a percentage error of 6.6%.

Discussion

Divergence Times

When converted to geological time, the immunological data (Table 1) indicate that South African *Ichnotropis* and North African *Psammodromus* diverged from the lineage representing *L. lepida-L. monticola* during the Oligocene (24-36 million years before the

present [mybp]) and that South African *Pedioplanus* and *Heliobolus* diverged from this lineage during the late Miocene (17-24 mybp). Palearctic representatives of the Lacertidae, however, have apparently achieved lineage independence more recently; one way; immunological comparisons suggest that ancestral representatives of *L. perspicillata, L. andreanszkyi* and *P. hispanica* diverged from lineages leading to *L. monticola* and *L. lepida* during the mid-Miocene, 12-18 mybp.

Generic Allocations of the Palearctic Species

While the genus *Lacerta* appears to be a morphologically heterogeneous assemblage of taxa, Arnold (1973) felt that many of the distinctions noted among taxa within the genus were simply representative of ecologically labile characteristics and he chose not to grant formal taxonomic status to subgeneric designations previously based upon these distinctions. He instead recognized two adaptive groups within the genus. *Lacerta* part I species are medium to very large species that are sexually dimorphic, have an ontogenetically variable dorsal coloration, and have a ventral surface that is never brightly colored. Most forms within this group are the largest lizards occuring in their ranges, tend to feed upon larger prey than other sympatric species, are typically associated with dense bushy vegetation, and do not climb on rock surfaces to any great extent. *Lacerta* part II species, on the other hand, are smaller than *Lacerta* part I species, breeding males generally display brightly colored ventral surfaces, and most members occupy a wide variety of structural niches, including rock faces. In spite of these differences, members of *Lacerta* I and *Lacerta* II are very similar in skeletal and hemipenial features.

Recent workers (Guillaume & Lanza, 1982; Lutz & Mayer, 1985) have suggested that Arnold's (1973) classification of the Palearctic Lacertidae is in need of revision because the generic allocations it recommends are contradicted by biochemical evidence. Citing electrophoretic evidence (Mayer & Tiedemann, 1982: Table 3; Guillaume & Lanza, 1982), Guillaume and Lanza (1982) advocated raising the subgenera *Archaeolacerta, Zootoca, Podarcis*, and *Lacerta* to genera. Lutz and Mayer (1985) were more conservative, stating that their immunological data were consistent with subgeneric partitioning in general acceptance before Arnold's (1973) revised classification.

Of the 23 loci examined in the electrophoretic studies (16 by Mayer & Tiedemann [1982], 17 by Guillaume & Lanza [1982]), only 10 were considered by both pairs of investigators. It has been well established that proteins accumulate electrophoretically detectable substitutions at differing rates and that the consideration of different proteins, even in studies involving the same taxa, may result in differing estimates of divergence (Sarich, 1977; Busack, 1986). Genetic distances (Nei's D) resulting from comparisons between members of the subgenera *Archaeolacerta, Zootoca, Podarcis*, and *Lacerta* range from 1.0 to 1.9 (Mayer & Tiedemann, 1982: Table 3; Guillaume & Lanza, 1982) and, because a maximum of one substitution per locus can be detected by

electrophoresis, Nei's D may be nonlinear much above a value of 1.0 (Maxson & Maxson, 1979). Comparisons involving genetic distances derived from differing protein systems are not directly comparable and genetic distances above 1.0 suffer from a lack of precision. These cautionary notes admonish against using these genetic distances for estimating phylogeny for the Palearctic lacertids.

Immunological distances based upon evolution of the albumin molecule appear to be reliable in the range of values obtained from comparisons among taxa currently considered to represent the genera *Lacerta, Podarcis*, and *Gallotia* (Lutz & Mayer, 1984, 1985; Maxson & Maxson, 1979, 1986: Table 1). When inter- and intrageneric comparisons of IDs resulting from reciprocal MC'F experiments are examined (Lutz & Mayer 1984: Table 2; Lutz & Mayer, 1985: Table 2; Table 1), we do find some support for Arnold's partition of the genus *Lacerta* into two genera (*Podarcis* and *Lacerta*). The average of all immunological distances ($\overline{ID}$) measured among six species of the genera *Gallotia* and *Lacerta* that have been compared ($\overline{ID}$ = 59), and among four species of the genera *Gallotia* and *Podarcis* ($\overline{ID}$ = 64), is large; the average of $\overline{ID}$'s among 20 species of the genera *Lacerta* and *Podarcis* is also moderately large ($\overline{ID}$ = 42). The average ID among three species within *Podarcis* ($\overline{ID}$ = 9), or among seven species within *Lacerta* ($\overline{ID}$ = 34), is less than the average ID between species across genera. Species currently assigned to *Podarcis* or *Lacerta*, for which we have reciprocal immunological data, appear more closely related to each other than they are to members of the other genera. When considered in concert with morphological and ecological characteristics (Arnold, 1973; Böhme, 1971), the immunological data do tend to support the recognition of *Podarcis* and *Lacerta* as independent genera.

There is no objective manner, however, in which to use these limited biochemical data to establish generic or subgeneric limits. Our Fitch-Margoliash tree (Fig. 1A), with a lower percentage error (2.8 vs 4.7%) than the Fitch-Margoliash tree presented by Lutz and Mayer (1985: Fig. 2), was computed from their data. Any attempt to reconcile the placement of *L. bedriagae* and *L. graeca* using these data would necessitate assigning each species to a separate genus or subgenus. The phylogenetic tree constructed from these same data using the Maxson and Maxson algorithm (Maxson, 1973 & 1984) produces another interpretation of the branching sequence but also suggests that *L. bedriagae* and *L. graeca* may be representative of separate lineages (Fig. 1B). While there is but one actual sequence of evolutionary events leading to these extant Palearctic species, there is no objective and defensible means by which to identify which phylogenetic tree most closely represents that sequence. It has not been demonstrated, nor is it to be expected on theoretical grounds, that the phylogenetic tree with the lowest standard deviation is the tree that most closely represents the true phylogeny (Tateno et al., 1982; Maxson, 1984). Both trees point out two major groups, but using the Maxson and Maxson technique forces *L. vivipara* into an outgroup position, a position that draws support from distributional, morphological, and ecological data (Arnold, 1973: 337).

Traditional generic or subgeneric classifications are based upon anatomical

characteristics. Both *L. bedriagae* and *L. graeca* have been previously considered to be representatives of the genus or subgenus *Archaeolacerta*, and both share a number of anatomical characteristics including the possession of simple spines on the hemipenial epithelium (Böhme, 1971). If one utilizes either interpretation of phylogeny presented in Figure 1 to determine generic or subgeneric status, the traditional diagnosis required of generic or subgeneric distinction becomes difficult, if not impossible. While biochemical data remain critical for distinguishing between competing hypotheses concerning the interpretation of morphological and ecological data, we feel that a taxonomy including several monotypic genera or subgenera based solely upon limited data from biochemistry is no substitute for the traditional taxonomy based upon clearly defined anatomical and ecological characteristics.

Radiation within the Palearctic Lacertidae has clearly been extensive, yet fewer than twenty percent of the species in this radiation have been examined by MC'F analyses. Without data from additional reciprocal comparisons with which to enhance the interpretation of relationships reflected in the trees presented in Figure 1, the current classification of the Palearctic Lacertidae cannot be much improved. An immunological study that considers the relationships among all members of the Palearctic *Lacerta* may provide guidance to a final reconciliation of the problem, but until these data are available we recommend adherence to the taxonomy proposed by Arnold (1973).

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Specimens examined. Numbers with an LM prefix refer to the albumin samples housed in the laboratory of L.R. Maxson at the University of Illinois, Urbana-Champaign. Specimens from which these albumin samples were collected are housed in the permanent herpetological collections of the Museum of Vertebrate Zoology, University of California, Berkeley (MVZ), Los Angeles County Museum of Natural History (LACM), and the Facultad de Biología, Departamento de Zoología, Universidad de León, Spain (SDB).

Heliobolus lugubris: (LM 1637) LACM (field number) A10200, Botswana (Kgala Gadi), Leudril (= 14 km NE Twee Rivieren, South Africa).

Ichnotrophis capensis: (LM 1820) LACM (field number) KC 1221-1225, Botswana (Ngamiland), 100 km (air) NE Maun at Khwai.

Lacerta andreanszkyi: (LM 1485) MVZ 178221-178232, Morocco (Marrakech), Oukaïmedene.

Lacerta lepida: (LM 918) SDB 1672, Spain (Cádiz), 9.3 km NNE Benalup de Sidonia; (LM 867) Spain (Zamora), Ribadelago.

Lacerta monticola: (LM 1483) SDB 1699-1704, Spain (Ávila), vicinity of Navalperal de Tormes, Sierra de Gredos.

Lacerta perspicillata: (LM 733) MVZ 186193-186202, Morocco (Rabat-Salé), Rabat (Topotypes).
Pedioplanis lineoocellata: (LM 1638) LACM (field number) A10201, Botswana (Kgala Gadi), Leudril (= 14 km NE Twee Rivieren, South Africa).
Podarcis hispanica: (LM 736) MVZ 186225-186232, Morocco (Tétouan), Asilah.
Psammodromus algirus: (LM 732) MVZ 186208-186209, Morocco (Tétouan), 8.4 km SW of the intersection of road 601 with road 8302 (on 601).

References

Arnold, E.N. (1973): Relationships of the palaearctic lizards assigned to the genera *Lacerta, Algyroides* and *Psammodromus* (Reptilia: Lacertidae). Bull. Br. Mus. nat. Hist. **25**: 291-366.

Böhme, W. (1971): Über das Stachelepithel am Hemipenis Lacertider Eidechsen und seine systematische Bedeutung. Z. f. zool. Syst. Evolutionsforsch. **9**: 187-223.

Boulenger, G.A. (1920): Monograph of the Lacertidae, vol. I. British Museum (Natural History), London.

Busack, S.D. (1986): Biogeographic analysis of the herpetofauna separated by the formation of the Strait of Gibraltar. Natn. geogr. Res. **2**: 17-36.

Carlson, S.S., Wilson, A.C., Maxson, R.D. (1978): Do albumin clocks run on time? Science, N.Y. **200**: 1183-1185.

Champion, A.B., Prager, E.M., Wachter, D., Wilson, A.C. (1974): Micro-complement fixation. In: Biochemical and Immunological Taxonomy of Animals, p. 397-416, Wright, C.A., ed., Academic Press, London.

Engelmann, W.E., Schäffner, H. (1981): Serologisch-immunologische Untersuchungen zu einigen taxonomischen Problemen innerhalb der Sammelgattung *Lacerta* (Sauria, Lacertidae). Zool. Jb. Syst. **108**: 139-161.

Felsenstein, J. (1984): The statistical approach to inferring evolutionary trees and what it tells us about parsimony and compatibility. In: Cladistics: Perspectives in the reconstruction of evolutionary history, p. 169-191, Duncan, T., Stuessy, T.F., eds., Columbia Univ. Press, N.Y.

Fitch, W.M., Margoliash, E. (1967): Construction of phylogenetic trees. Science, N.Y. **155**: 279-284.

Gorman, G.C., Soulé, M., Yang, S.Y., Nevo, E. (1975): Evolutionary genetics of insular Adriatic lizards. Evolution. **29**: 52-71.

Guillaume, C.-P., Lanza, B. (1982): Comparaison electrophorétique de quelques espèces de Lacertidés Mediterranéens, Genera *Podarcis* et "*Archaeolacerta*". Amph.-Rept. **4**: 361-375.

Guillaume, C.-P., Pasteur, N., Bons, J. (1976): Distinction par électrophorèse sur gel d'amidon des espèces de Lézards *Lacerta muralis* Laurenti 1768 et *Lacerta hispanica* Steindachner 1870 dans des populations sympatriques d'Espagne et du Languedoc-Roussillon. C.r. hebd. Séanc. Acad. Sci., Paris. **282D**: 285-288.

Lanza, B., Cei, J.M. (1977): Immunological data on the taxonomy of some Italian lizards (Reptilia Lacertidae). Monitore zool. ital. (N.S.) **11**: 231-236.

Lanza, B., Cei, J.M., Crespo, E.G. (1977): Immunological investigations on the taxonomic status of some Mediterranean lizards (Reptilia Lacertidae). Monitore zool. ital. (N.S.) **11**: 211-221.

Lutz, D., Mayer, W. (1984): Albumin-immunologische und proteinelektrophoretische Untersuchungen zur systematischen Stellung von *Lacerta lepida* Daudin und *Lacerta princeps* Blanford (Sauria, Lacertidae). Zool. Anz. **212**: 95-104.

Lutz, D., Mayer, W. (1985): Albumin evolution and its phylogenetic and taxonomic implications in several lacertid lizards. Amph.-Rept. **6**: 53-61.

Mayer, W. (1981): Elektrophoretische Untersuchungen an europäischen Arten der Gattungen *Lacerta* und *Podarcis* III. *Podarcis tiliguerta*—Art oder Unterart? Zool. Anz. **207**: 151-157.

Mayer, W., Tiedemann, F. (1980): Elektrophoretische Untersuchungen an europäischen Arten der Gattungen *Lacerta* und *Podarcis*. I. Die *Podarcis*-Formen der griechischen Inseln Milos und Skiros. Z.f. zool. Syst. Evolutionsforsch. **18**: 147-152.

Mayer, W., Tiedemann, F. (1981): Elektrophoretische Untersuchungen an europäischen Arten der Gattungen *Lacerta* und *Podarcis* II. Zur systematischen Stellung der Eidechsen auf der Insel Piperi (Nordliche Sporaden, Griechenland). Zool. Anz. **207**: 143-150.

Mayer, W., Tiedemann, F. (1982): Chemotaxonomical investigations in the collective genus *Lacerta* (Lacertidae; Sauria) by means of protein electrophoresis. Amph.-Rept. **2**: 349-355.

Maxson, L.R. (1973): A phylogeny based on the method of Maxson and Maxson. In: A molecular approach to the study of hylid evolution, p. 133-137, Maxson, L.R. Ph.D. Dissertation, Univ. Calif., Berkeley & Calif. State Univ., San Diego.

Maxson, L.R. (1984): Molecular probes of phylogeny and biogeography in toads of the widespread genus *Bufo*. Molec. Biol. Evol. **1**: 345-356.

Maxson, L.R., Maxson, R.D. (1979): Comparative albumin and biochemical evolution in plethodontid salamanders. Evolution **33**: 1057-1062.

Maxson, L.R., Szymura, J.M. (1979): Quantitative immunological studies of the albumins of several species of fire bellied toads, genus *Bombina*. Comp. Biochem. Physiol. **63B**: 517-519.

Maxson, L.R., Wilson, A.C. (1974): Convergent morphological evolution detected by studying proteins of tree frogs in the *Hyla eximia* species group. Science, N.Y. **185**: 66-68.

Maxson, L.R., Wilson, A.C. (1975): Albumin evolution and organismal evolution in tree frogs (Hylidae). Syst. Zool. **24**: 1-15.

Maxson, R.D., Maxson, L.R. (1986): Micro-complement fixation: A quantitative estimator of protein evolution. Molec. Biol. Evol. **3**: 375-388.

Prager, E.M., Wilson, A.C. (1976): Congruency of phylogenies derived from different proteins. J. molec. Evol. **9**: 45-57.

Sarich, V.M. (1977): Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies. Nature, Lond. **265**: 24-28.

Sarich, V.M., Cronin, J.E. (1976): Molecular systematics of the primates. In: Molecular Anthropology, p. 141-170, Goodman, M., Tashian, R.E., eds., Plenum Press, N.Y.

Tateno, Y., Nei, M., Tajima, F. (1982): Accuracy of estimated phylogenetic trees from molecular data. I. Distantly related species. J. molec. Evol. **18**: 387-404.

Wilson, A.C., Carlson, S.S., White, T.J. (1977): Biochemical evolution. A. Rev. Biochem. **46**: 573-639.

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