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Congruency of Phylogenies Derived from Different Proteins

A Molecular Analysis of the Phylogenetic Position of Cracid Birds*

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Summary. This communication examines the question of phylogenetic congruency – i.e., whether or not the branching order of evolutionary trees is independent of the protein studied. It was found that trees constructed for birds on the basis of immunological comparison of their transferrins, albumins, and ovalbumins agree approximately with a published tree based on the amino acid sequences of their lysozymes *c*.

This congruency is especially noteworthy with respect to the phylogenetic position of the chachalaca, a Mexican bird classified on morphological grounds in the family Cracidae of the order Galliformes. At the protein level, this species differs as much from non-cracid galliform birds as does the duck, which belongs to another order. Despite the organismal similarity between cracid and non-cracid galliform birds, the molecular relationship is remote. If this contrast between organismal and molecular results had been based on comparative studies with only lysozyme, one could have ascribed the contrast to the possibility that chachalaca lysozyme was paralogous, rather than orthologous, to the other bird lysozymes *c*. Examination of several proteins is thus desirable in cases of possible paralogy.

Key words: Transferrin – Albumin – Ovalbumin – Lysozyme – Micro-complement fixation – Bird evolution – Chachalaca – Duck – Chicken – Phasianoid birds.

Introduction

To test the reliability of the use of proteins for working out phylogenetic relationships, it is essential to determine whether the branching order of the evolutionary tree one obtains depends on the protein studied. This question is investigated here through

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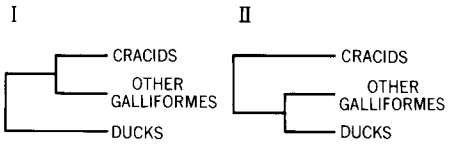

Fig. 1. Alternative models for the phylogenetic position of the Cracidae. Model I is derived from morphological criteria (Wetmore, 1960; Delacour and Amadon, 1973), while Model II is suggested by protein evidence obtained with lysozyme *c* (Jollès et al., 1976). This figure indicates branching order only; distances along the horizontal axis are purely schematic

the assessment of the phylogenetic position of the family Cracidae among the major groups of birds. This family, comprising the chachalacas, guans, and curassows of the New World, is classified by morphological criteria in the order Galliformes along with the phasianoid birds and megapodes (Wetmore, 1960; Delacour and Amadon, 1973). Recent protein evidence, however, did not confirm this classification. Comparison of the amino acid sequence and immunological properties of chachalaca (*Ortalis vetula*) lysozyme *c* with those of other birds and of mammals and subjecting the data to phylogenetic analysis (Jollès et al., 1976) yielded a surprising result: the lineage leading to chachalaca lysozyme *c* was no closer phylogenetically to that of the other galliform birds than was that leading to duck lysozymes *c*, though the duck is classified in the order Anseriformes (Wetmore, 1960). To determine whether at the molecular level the chachalaca is indeed quite different from most other gallinaceous birds, we have now compared immunologically by quantitative micro-complement fixation 3 additional chachalaca proteins, transferrin, albumin, and ovalbumin, with the homologous proteins from several members of the order Galliformes and from representatives of other avian orders. With the data obtained we have constructed evolutionary trees for these 3 proteins in order to try to distinguish between the 2 alternative models (Fig. 1) for the phylogenetic position of the Cracidae.

Materials and Methods

Materials. Bird egg whites, sera, and tissue extracts were obtained, prepared, and stored as described (Prager et al., 1974a).

Protein Purification. Ovotransferrin or serum transferrin was purified from 20 avian species, serum albumin from 10 species, and ovalbumin from 6 species. The species represent, respectively, 14, 6, and 3 of the 27 bird orders. Table 1 lists these species, their scientific names, and the references describing the protein purification procedures used. Details of the purification of most of these 36 proteins have been given previously (Prager et al., 1974a; Prager and Wilson, 1975; Nolan et al., 1975; Ho et al., 1976), as indicated in Table 1. Those not described in prior works were prepared as follows: chachalaca ovotransferrin was isolated from egg white by preparative 8 % polyacrylamide gel electrophoresis at pH 8.9 according to Prager and Wilson (1975) and bobwhite quail and megapode ovotransferrin by the same method as carried out by Ho et al. (1976). Chachalaca, loon, gull, and cowbird serum albumin were purified by Rivanol precipitation and 8 % polyacrylamide gel electrophoresis at pH 8.9