Letter to the Editor

Pancreatic Polypeptide and the Sister Group of Birds[1]

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A recent review of molecular evidence for amniote relationships revealed eight genes for which there was sufficient taxonomic representation to address the question of the relationships of birds, mammals, and crocodilians (Hedges et al. 1990). The surprising result was that three genes (beta hemoglobin, myoglobin, and 18S rRNA) unambiguously identify mammals as the sister group of birds, whereas only one gene (histone H2B) supports (weakly) a bird+crocodilian relationship. The remaining four genes (alpha crystallin A, alpha hemoglobin, insulin, and 28S rRNA) support different groupings, depending on the type of analysis. An additional gene, pancreatic polypeptide (also referred to as pancreatic hormone), now can be added to the genes bearing on the sister group of birds. Although amino acid sequences of this protein from birds, mammals, and a crocodilian have been available previously, the conclusion that this gene supports a bird+crocodilian relationship (Larhammar and Milner 1989) was based on an analysis of only a small subset of available sequences and required a constant-rate assumption. The amino acid sequence of pancreatic polypeptide from an amphibian (bullfrog) recently has been added to the protein data banks and therefore can provide an outgroup for examining, without the constraint of a constant-rate model, amniote relationships.

All 14 available sequences of pancreatic polypeptide were obtained from the National Biomedical Research Foundation Protein Identification Resource data bank and from the Swiss Protein data bank and were aligned (fig. 1). The species are bullfrog (*Rana catesbeiana;* Pollock et al. 1988), alligator (*Alligator mississippiensis;* Lance et al. 1984), ostrich (*Struthio camelus;* Litthauer and Oelofsen 1987), chicken (*Gallus gallus;* Kimmel et al. 1975), goose (*Anser anser;* Xu et al. 1984), human (*Homo sapiens;* Leiter et al. 1985), rat (*Rattus norvegicus;* Yamamoto et al. 1986), mouse (*Mus musculus;* Yonekura et al. 1988), guinea pig (*Cavia porcellus;* Blackstone et al. 1988), pig (*Sus scrofa;* Chance et al. 1979*a*), cow (*Bos taurus;* Chance et al. 1979*b*), sheep (*Ovis orientalis;* Chance et al. 1979*b*), dog (*Canis lupus;* Chance et al. 1979*b*), and cat (*Felis sylvestris;* Nielsen et al. 1986). Two methods of phylogenetic analysis—maximum parsimony [PAUP 3.0 (Swofford 1990)] and neighbor-joining [Saitou and Nei 1987; NJBOOT (Whittam 1991)]—were used with the frog as the outgroup. For both methods, the statistical significance of each node was evaluated by the bootstrap method (Felsenstein 1985), with 2,000 iterations.

Both analyses of the sequence data for pancreatic polypeptide support (significantly) a bird-crocodilian relationship (fig. 2). In the maximum-parsimony analysis, there were 123 most-parsimonious trees, each of length 60 and with a consistency index of 0.92 (with unique sites removed). Of 36 total sites (29 variable), there are six shared-derived sites (4, 13, 14, 16, 18, and 28) supporting birds+crocodilians, one (36) supporting birds+mammals, and (3) one supporting crocodilians+mammals. Amino acid residues are shared by birds and crocodilians (not mammals) at four additional sites (22, 25, 30, and 31); but in these cases the outgroup has a unique

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bullfrog      APSEPHHPGDQATPDQLAQYYSDLYQYITFITRPRF
human         ··L··VY···N···E·M···AA··RR··NML····Y
cow           ··L··EY···N···E·M···AAE·RR··NML····Y
pig           ··L··VY···D···E·M···AAE·RR··NML····Y
rat           ··L··MY···Y··HE·R···ETQ·RR··NTL····Y
mouse         ··L··MY···Y···E·M···ETQ·RR··NTL····Y
dog           ··L··VY···D···E·M···AAE·RR··NML····Y
cat           ··L··VY···N···E·M···AAE·RR··NML····Y
sheep         ·SL··EY···N···E·M···AAE·RR··NML····Y
guineapig     ··L··VY···D···Q·M···AAEMRR··NML····Y
chicken       G···Q·TY···D·PVED·IRF·DN·Q···LNVV···H·Y
ostrich       G·AQ·TY···D·PVED·VRF·DN·Q···LNVV···H·Y
goose         G···Q·TY···ND·PVED·RF···DN·Q···RLNVF·H·Y
alligator     T·LQ·KY···G·PVED·I·F·D···Q···LNVV·····
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FIG. 1.—Aligned sequences of pancreatic polypeptide from one amphibian and 13 species of amniotes. A dot (·) represents identity with the first sequence.

residue, and therefore they do not support a bird+crocodilian grouping under the parsimony criterion. As was found with insulin (Hedges et al. 1990), pancreatic polypeptide supports the clustering of ostrich (Struthioniformes) and chicken (Galliformes) rather than that of goose (Anseriformes) and chicken. However, the relationships between these three orders are not well resolved, in that alpha and beta hemoglobin each support Struthioniformes+Anseriformes (Goodman et al. 1987), whereas albumin immunology (Prager et al. 1974) and DNA-DNA hybridization (Sibley and Ahlquist 1990) support the "classical" grouping of Anseriformes+Galliformes.

These results lend support to the "classical" amniote relationships based on morphology (Romer 1966; Carroll 1988) and weaken the molecular evidence for a bird+mammal relationship. However, the size of each data set is an important factor to consider. For example, the two genes—histone H2B and pancreatic polypeptide—now supporting birds+crocodilians are small and limited in taxonomic scope. No sequences are yet available for the other major amniote lineages—squamates and turtles—at those two genes. As a result, the hypothesis that birds, mammals, and crocodilians form a monophyletic group within the amniotes (Gardiner 1982) cannot be rejected by analyses of histone H2B and pancreatic polypeptide. On the other hand, the three genes supporting a bird+mammal relationship are larger and have broader taxonomic representation. The present finding with pancreatic polypeptide underscores the lack of consensus for amniote relationships that was observed among molecular-data sets (Hedges et al. 1990) as well as among morphological data sets (Gauthier et

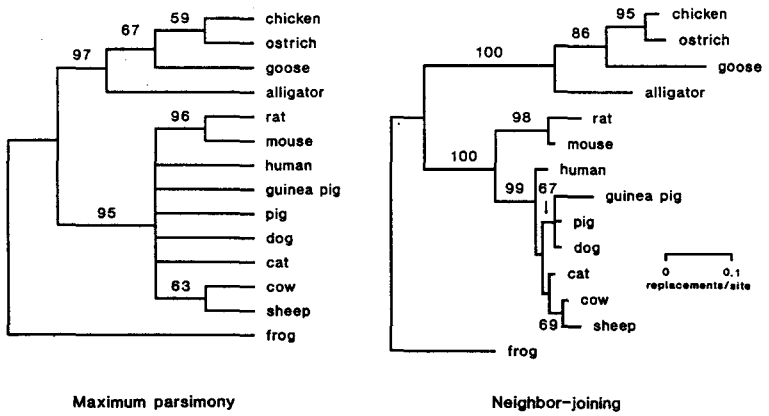

FIG. 2.—Relationships of birds, mammals, and crocodilian by maximum-parsimony and neighbor-joining analyses of pancreatic polypeptide sequences (rooted with frog). Bootstrap confidence limits (>50%) are indicated on trees. Only branching order is implied in the parsimony tree.

al. 1988). Sequences from additional slow-evolving genes are needed before a robust phylogeny for the amniotes can be obtained.

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LITERATURE CITED

BLACKSTONE, C. D., S. SEINO, T. TAKEUCHI, T. YAMADA, and D. F. STEINER. 1988. Novel organization and processing of the guinea pig pancreatic polypeptide precursor. J. Biol. Chem. **263**:2911–2916.

CARROLL, R. L. 1988. Vertebrate paleontology and evolution. W. H. Freeman, New York.

CHANCE, R. E., M. G. JOHNSON, J. A. HOFFMAN, and T.-M. LIN. 1979*a*. Pancreatic polypeptide: a newly recognized hormone. Pp. 419–425 *in* S. BABA, T. KANEKO, and N. YANAIHARA, eds. Proinsulin, insulin, C-peptide. Excerpta Medica, Amsterdam.

CHANCE, R. E., N. E. MOON, and M. G. JOHNSON. 1979*b*. Human pancreatic polypeptide (HPP) and bovine pancreatic polypeptide (BPP). Pp. 657–672 *in* B. M. JAFFE and H. R. BEHRMAN, eds. Methods of hormone radioimmunoassay. Academic Press, New York.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39**:783–791.

GARDINER, B. G. 1982. Tetrapod classification. Zool. J. Linnean Soc. **74**:207–232.

GAUTHIER, J., A. G. KLUGE, and T. ROWE. 1988. Amniote phylogeny and the importance of fossils. Cladistics **4**:105–209.

GOODMAN, M., M. M. MIYAMOTO, and J. CZELUSNIAK. 1987. Pattern and process in vertebrate phylogeny revealed by coevolution of molecules and morphologies. Pp. 141–176 *in* C. Patterson, ed. Molecules and morphology in evolution: conflict or compromise? Cambridge University Press, Cambridge.

HEDGES, S. B., K. D. MOBERG, and L. R. MAXSON. 1990. Tetrapod phylogeny inferred from 18S and 28S ribosomal RNA sequences and a review of the evidence for amniote relationships. Mol. Biol. Evol. **7**:607–633.

KIMMEL, J. R., L. J. HAYDEN, and H. G. POLLOCK. 1975. Isolation and characterization of a new pancreatic polypeptide hormone. J. Biol. Chem. **250**:9369–9376.

LANCE, V., J. W. HAMILTON, J. B. ROUSE, J. R. KIMMEL, and H. G. POLLOCK. 1984. Isolation and characterization of reptilian insulin, glucagon, and pancreatic polypeptide: complete amino acid sequence of alligator (*Alligator mississippiensis*) insulin and pancreatic polypeptide. Gen. Comp. Endocrinol. **55**:112–124.

LARHAMMAR, D., and R. J. MILNER. 1989. Phylogenetic relationship of birds with crocodiles and mammals, as deduced from protein sequences. Mol. Biol. Evol. **6**:693–696.

LEITER, A. B., M. R. MONTMINY, E. JAMIESON, and R. H. GOODMAN. 1985. Exons of the human pancreatic polypeptide gene define functional domains of the precursor. J. Biol. Chem. **260**:13013–13017.

LITTHAUER, D., and W. OELOFSEN. 1987. Purification and primary structure of ostrich pancreatic polypeptide. Int. J. Pept. Protein Res. **29**:739–745.

NIELSEN, H. V., U. GETHER, and T. W. SCHWARTZ. 1986. Cat pancreatic eicosapeptide and its biosynthetic intermediate. Biochem. J. **240**:69–74.

POLLOCK, H. G., J. W. HAMILTON, J. B. ROUSE, K. E. EBNER, and A. B. RAWITCH. 1988. Isolation of peptide hormones from the pancreas of the bullfrog (*Rana catesbeiana*). J. Biol. Chem. **263**:9746–9751.

PRAGER, E. M., A. H. BRUSH, R. A. NOLAN, M. NAKANISHI, and A. C. WILSON. 1974. Slow evolution of transferrin and albumin in birds according to micro-complement fixation analysis. J. Mol. Evol. **3**:243–262.

ROMER, A. S. 1966. Vertebrate paleontology. University of Chicago Press, Chicago.

SAITOU, N., and M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**:406–425.

SIBLEY, C. G., and J. E. AHLQUIST. 1990. Phylogeny and classification of birds. Yale University Press, New Haven, Conn.

SWOFFORD, D. L. 1990. PAUP. Phylogenetic analysis using parsimony, version 3.0. Illinois Natural History Survey, Champaign.
WHITTAM, T. S. 1991. NJBOOT: neighbor-joining bootstrap analysis. Institute of Molecular Evolutionary Genetics, Pennsylvania State University, University Park.
XU, Y., N. LIN, and Y. S. ZHANG. 1984. Isolation and sequence determination of goose pancreatic polypeptide. Sci. Sin. [B] **27**:590–592.
YAMAMOTO, H., K. NATA, and H. OKAMOTO. 1986. Mosaic evolution of prepropancreatic polypeptide. J. Biol. Chem. **261**:6156–6159.
YONEKURA, H., K. NATA, T. WATANABE, Y. KURASHINA, H. YAMAMOTO, and H. OKAMOTO. 1988. Mosaic evolution of prepropancreatic polypeptide. J. Biol. Chem. **263**:2990–2997.

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