Flightless birds—ratites—have become a testing ground for ways to establish evolutionary relationships.

**THE SIGNIFICANCE OF FLIGHTLESS BIRDS**

by Edward Edelson

As the last land bridges among the segments of Gondwanaland were severed, the ancestors of today's ratites were isolated on drifting continents, each to take its own evolutionary course.

Dates from Joel Cracraft (1972); L. R. Maxson, Vincent Sarich, Allan Wilson (1975).
Ever since they were discovered, the flightless birds—the ratites—have fascinated ornithologists and other scientists. Visually, these birds are spectacular: the ostrich of Africa, at eight feet tall and two-toed, the world's largest living bird (but nonetheless smaller than two extinct groups of rats, the moas and the elephant birds); the two-toed, ostrich-like South American rheas; the emu of the Australian plains; the aggressive, forest-dwelling cassowary of northern Australia and New Guinea with its two-inch, razor sharp claw; the three species of kiwi (or apteryx) of New Zealand—nocturnal birds whose five-inch egg weighs up to a quarter of the bird's body weight.

But another reason for fascination is the series of scientific questions presented by the existence of the ratites. For well over a century, the debates have gone on: Are the ratites living fossils, descendants of ancient species that never evolved flight? Or did they lose their wings because there was no selection in their environment? Or did they develop from different stocks, growing to look alike through individual with reduced powers of flight? Did these birds, scattered across more than half the planet, have a common ancestor and somehow disperse despite their lack of useful wings? Or did they develop from different stocks, growing to look alike through the evolutionary phenomenon called convergence, in which unlike species evolve common characteristics in response to similar spectra of environmental conditions?

Are the ratites related to the tinamous, the ground-dwelling Latin American birds that share many ratite characteristics but still can fly? And finally, if the ratites did have a common ancestor, what are their interrelationships?

Today, the common ancestry of the ratites has been established with a high degree of certainty. But, with a twist that is worthy of a Hitchcock movie, the last question has become the focus of the kind of keen controversy that often develops at the cutting edge of a science. ("It's almost the flailing edge of this science at the moment," comments one observer.)

The attempt to construct a phylogenetic tree that would establish the relationships among the ratites has become a major testing ground for an emerging science, with lively arguments about both which data to use for such an effort and which mathematical techniques are best for analysis of the body of data and its representation in a phylogenetic tree or branching diagram.

The debate about the phylogenetic branching order of the ratites thus has greater significance than might ordinarily be attached to a discussion of whether the ostrich is related more closely to the kiwi or to the cassowary. It is a gripping case history of the demanding effort being made to change the process of species classification from a subjective, ill-defined discipline to a modern, quantitative science. "This is one of the first good cases where different kinds of data can be applied to the same group," says Walter M. Fitch of the University of Wisconsin about the ratites. "That's a plus because it's an extremely interesting group."

Those interested in permutations and combinations should know that there are at least three different techniques being used to gather data about the relationships among ratites: two that examine molecules from the birds and the third, a modern version of the classical method of studying morphological differences. Additionally, several different methods can be used on the different sets of data to construct phylogenetic trees. As a result, despite a plethora of often conflicting conclusions, each proposed phylogenetic tree (and its supporting data) is thus assured the kind of close scrutiny that, ultimately, helps hone a research strategy to perfection.

**Clearing the underbrush**

To get to where they are, the scientists studying phylogeny via the ratites first had to clear away the underbrush by answering some basic questions. It is now generally agreed that the ratites are not primitive birds left over from an earlier evolutionary stage. Instead, they appear to be highly evolved descendants of an ancestor that had the ability to fly.

Although the ratites share many anatomical characteristics, there are some notable differences. The rhea and the ostrich have feathers that have one central shaft, for instance, while the feathers of the emu, kiwi and cassowary have two: a major one, called a rachis and a secondary "aftershaft." And, while most ratites are large birds, the kiwi is small. Further, and most obviously, the ratites are not geographic neighbors.

To those who once held to the belief that the ratites had to be an example of convergent evolution—the development of like characteristics by divergent species subjected to similar evolutionary opportunities—the biogeographic question was the clincher: How could nonflying birds spread across the vast oceanic distances between continents, virtually in all directions?

Anatomical study of the ratites, however, finally convinced scientists of the flightless birds' common ancestry. (That the ratites must have had a common ancestry, biogeography notwithstanding, had been concluded as long ago as 1867, by Thomas Henry Huxley, from comparison of physical features. And parasitologists, who consider the fact that ratites share parasites that do not plague other birds as clear evidence of common ancestry, have argued it for many years; parasites and their hosts are an example of coevolution (see "Feedback Produces a Theory of Ecology," *Mosaic*, Volume 10, Number 6). But it took geology, and the evolution of the theory of plate tectonics (see "What Drives the Earth's..."
Sustained Fliers

Non-sustained Fliers

Non-fliers

Plates, "Mosaic, Volume 10, Number 5) to solve the biogeographic riddle: The birds had merely to stand still while the proto- continent called Gondwanaland broke into raftlike fragments that drifted apart over tens of millions of years, carrying all kinds of life forms that shared common ancestries along with them.

With that question answered, the question of phytogeny—how the ratites relate to their common ancestor and to each other—came into sharper focus. And getting a correct answer to this question is surprisingly important, since the methods used to develop an accurate phylogenetic tree for the ratites can also be used to place the ratites in their proper relationship to other birds, to develop an overall phylogenetic tree for all the orders of birds, which has not yet been done to everyone's satisfaction and, indeed, to perfect techniques for the development of quantitative phylogenies in general.

The approaches

There are three major efforts to gather data on the ratites, all three being carried out in the wider context of a study of all birds. At Yale University, Charles G. Sibley and Jon E. Ahlquist are studying the quantitative differences, between the genetic material of the various ratites. Allan C. Wilson of the University of California at Berkeley has made a similar biomolecular study of one Mr. Edelson is preparing a Mosaic article on artificial intelligence.

Ratite relationships. The higher the temperature at which a hybrid duplex DNA strand dissociates, the closer the phylogenetic relationship between the two species. With a natural emu duplex for a standard, Charles Sibley's molecular technique (below) makes the cassowary the emu's closest relative among the ratites and the rhea and ostrich most distant. A conjectural phylogenetic model for the origin of flight (above) suggests that the loss of all flying ability among ratites occurred only once, in a common ancestor.

blood protein, transferrin, from the different ratite species. And at the University of Illinois, ornithologist Joel Cracraft, taking a comparative approach to the study of morphological characteristics shared by different avian species, is employing a method designed to bring more testable procedures into a process long ruled by subjective judgments.

The two molecular techniques are based on the same principle: that evolution goes on not only among physical features but also in molecules (see "Molecular Evolution: A Quantifiable Contribution," Mosaic, Volume 10, Number 2). The DNA molecule in which genetic information is coded is hypothesized to experience mutations at a measurable rate. The proteins produced from the

**Phylogenetic trees.** Results of three different ways of determining the relationships among the ratites: through nucleotide replacement in DNA (above), amino acid substitution in a protein (below, left) and modern comparative morphology (below, right).

Charles Sibley, Jon Ahlquist; Allan Wilson; Joel Cracraft, all by permission.
DNA instructions also show the change. By comparing either DNA or proteins from two species, an investigator can determine the extent of the difference between them and thus how far, if not when, the two species have evolved from their common ancestor. By comparing DNA and proteins from several species, data for a phylogenetic tree can be gathered.

Sibley’s method is to look directly at the DNA. If purified DNA is dissolved and heated, the two strands of the DNA molecule can be separated. If the solution is then allowed to cool, the strands will reunit as complementary bases on each strand, “recognize” each other and join together. Sibley puts single strands of DNA from different species into the same solution and measures the extent to which they join. The closer the match, the closer the genetic relationship between the two species.

It is not nearly as simple as it sounds. Early work gave confusing results which were not cleared up until the discovery that the genetic material of higher species includes long stretches of repetitive DNA sequences whose function is still unclear. Sibley and Ahlquist now fractionate the DNA to remove the repetitive sequences, leaving behind the single-copy sequences of DNA that give meaningful results. The single-copy DNA is labeled with radioactive iodine and added to a solution of nonradioactive DNA from another species. (Most of the analysis is now done automatically, by an ingenious machine designed by the Yale investigators.)

The mixture first is incubated to allow the DNA from the two different species to form hybrid duplex molecules, composed of one strand from each of the two species. Then the mixture is heated again in a series of carefully controlled steps. The temperature is raised a degree or two, and the DNA which has dissociated is removed for measurement; the amount of radioactivity in the sample indicates the degree of dissociation. By comparing the difference between the temperature required to dissociate the duplex DNA of a single species used as a control and the temperature that dissociates a hybrid duplex, Sibley and Ahlquist get a numerical reading on the similarity of the DNA strands of the two species.

A great virtue of the technique, Sibley says, is that it looks at the entire genome of a species, all the genes that count. Individual genes are known to evolve at vastly different rates; there is a 400-fold difference between the slowest-changing and fastest-changing genes, Sibley explains. By studying the whole genome, his method gives an average, overall, quantified rate of change, from which he derives estimates of genetic distance between species.

Sibley describes the relationship as rather straightforward: a difference of one degree Celsius in melting point equaling approximately a difference of one percent in DNA pairing. The results are expressed in a mathematical matrix of what Sibley calls “delta modes,” each delta mode expressing the genetic difference between two species. Though his colleagues are far from unanimous in agreement, Sibley maintains that the analysis of such a matrix to produce a phylogenetic tree is relatively straightforward. "The readout we get out of the computer requires very little interpretation," he declares. "The data speak for themselves."

Protein comparisons

A major biochemical role for DNA is the synthesis of proteins, and nucleotide replacement in DNA should be reflected as amino acid substitution in a protein. So the comparison of proteins from species to species, as Allan Wilson, working with Ellen Prager at the University of California at Berkeley, is doing, should also reveal phylogenetic links and distances.

Wilson’s method is to purify the chosen protein—transferrin in the case of the ratites—inject it into a rabbit and harvest the antibodies produced by the rabbit. Those antibodies then are added to a mixture containing transferrin from another species and a substance called complement, which helps the antibody to join with the protein. By measuring the amount of free complement left after the antibody-protein reaction has occurred, Wilson and Prager get a measure of the differences between the two proteins.

While the method measures mutations in only a single protein, Wilson points out, “this protein is a large one, containing 700 amino acids. The number of substitutions by which the birds under consideration differ from each other is...about 100. So we really are looking at a large number of traits at once.”

The method is not quite precise, Wilson acknowledges; it detects no more than about 85 percent of the changes between proteins of two closely related species. The advantage of the method, he says, is that the validity of the tree obtained with one protein can be tested by investigating additional proteins.

The technique proved itself, says Wilson, with work on a Mexican bird, the chachalaca, which had been classified as one of the galliforms, an order including such game birds as the quail and the pheasant. The study of one protein, lysozyme, indicated that the chachalaca was distant from the galliforms, a result so startling that Wilson decided to check it further. “We looked at five different proteins. All show that the chachalaca forms a group as distinctive from gallinaceous birds as ducks are.” Wilson reports.

Nevertheless, Sibley disagrees. He says that his analysis of DNA shows that the chachalaca is indeed a galliform—although he agrees with Wilson that it is distant from the other gallinaceous birds.

Wilson says, nonetheless, that his conclusions about the ratites are not very different from those of Sibley. Both have the tinamous as the closest relatives of the ratites, and both have the ostrich rather closely related to the kiwi and the rhea. The differences are reconcilable, Wilson says, because imprecision that still exists in any available molecular method leaves room for changes in the resulting phylogenetic tree; “the two molecular methods agree on the whole.”

A new morphology

The Wilson and Sibley constructions, however, in addition to differing in some ways from each other, differ in apparently small but nonetheless significant ways from the phylogenetic tree drawn by Cracraft. The Illinois ornithologist is using a technique of comparing morphological differences—variations in the shapes of bones and other physical traits—that is little more than a decade old in North America. The historic method of assigning relationships between species
by looking at physical traits was once hope­lessly subjective, Cracraft notes. But help for the anatomists, he says, came with the development some 25 years ago of a more sophisticated technique, called the cladistic method, often credited to a German entomologist named Willi Hennig.

The essence of the cladistic method, Cra­craft says, is that the anatomist looks for derived characteristics the group under study might share rather than for primitive characteristics—for features that are evolutionary novelties rather than those that many species have in common. To give a most basic example, a primitive feature of birds is the presence of feathers; a derived characteristic would be their absence or a modification of the feather shared with one group of birds but not another.

There are two ways to do such an analysis, Cracraft explains. One is “outgroup comparison,” a study of how a group that is under study compares with a presumably closely related group. For horses, outgroup comparison could focus on the foot structure of the order of ungulates that includes the horse (perissodactyls) or of mammals in general. The modern horse has just one toe, and outgroup comparison indicates that it descended from primitive ancestors having five toes. A phylogenetic tree might be constructed by studying the character transformations presented by many such morphological features.

A second method is to study ontogenetic transformation, the development of an individual from fetus to adult. In the flounder, for example, the eyes are on either side of the head at first, but migrate until both are on the same side of the head. This ontogenetic transformation is evidence that the primitive ancestor of the flounder had symmetrically placed eyes and that asymmetry is a derived characteristic.

The important point is that the cladistic method can make comparative morphology more rigorous and testable. The further importance of the ratites to Cracraft is that his study of them was the first application in birds of Hennig’s methods.

Cracraft has found evolutionary trends in a number of morphological characteristics of the ratites. For example, studying the tibiotarsus, one of the bones in the lower leg, he finds that the base of the cnemial crest, a feature at the upper end, is broad in the tinamous, narrower in the cassowaries and the emu and extremely narrow in the rheas and ostriches. As do Wilson and Sibley, Cracraft believes the tinamous to be the closest relatives of the ratites. He concludes from his study that the ostrich is closest to the rhea among the ratites—that they are more closely related than either Wilson or Sibley would agree. Further, Cracraft and Sibley both have the ostrich and rhea more closely related to each other, so far, than does Wilson.

**Which analysis?**

The differences are important because they involve a critical issue in the discipline: analysis of the data. Drawing up a table of differences, molecular or anatomical, between species is the only first step in constructing a phylogenetic tree. The next step is to apply to the data one method or another of comparative analysis to construct the tree.

Several methods of analysis can be applied to the different sets of data. One method, developed by Fitch at the University of Wisconsin, can be used on the DNA hybridization data of Sibley or the protein differences of Wilson. It starts with a matrix that lists, in tabular form, the differences between species. Constructing a phylogenetic tree can be regarded as building another matrix, read directly from the tree, which also gives the distances between species. The basic idea of this analysis is to minimize the difference between the first matrix and the second.

A different technique has been used by James S. Farris of the State University of New York at Stony Brook. Using the same table of differences between characteristics Farris’s system produces a phylogenetic tree that meets what are called parsimony criteria by having branches that are as short as possible. That the application of one system or another is far from straightforward at this stage, however, is illustrated by what happened when Sibley distributed some of his unpublished data to other investigators for comment. Farris, at the request of Cracraft, did an analysis of the Sibley data, using the method developed by Fitch. Farris and Cracraft then announced their results: Sibley’s data fit Cracraft’s tree better than they did Sibley’s.

Sibley is unable to understand how a rigorous analysis could have produced such a result. His own subsequent analysis, he says, employing both Fitch’s and Farris’s techniques as well as others, resolves the distance between the emu and cassowary as well as quantifies distances from the nearby kiwi and distant tinamou. He has been unable to examine Farris’s procedure, he says, but his own analyses produce not only distances between species but quantitative measures of the reliability of the distance estimates as well.

To compound the difficulties, Wilson argues further that the Fitch type of analy­sis “doesn’t give a unique solution. You try a whole bunch of trees and estimate the goodness of the fit. You get several solutions that are almost equivalent. Our tree is a blend of the best ones.”

The reason these points are important, Wilson says, is that “we are seeing a big revolution in the way people are looking at evolutionary biology. Biology is being uni­fied in a way it never was before, and tax­onomists now have to know molecular biology, as do paleontologists.”

For the first time, Wilson continues, ornithologists can talk about constructing a quan­titative phylogenetic tree for all the 27 orders of birds. “Despite more than a century of research,” he says, “morphologists did not succeed in producing such a tree. But the main outlines of the avian family tree are now evident....” Such a biochemical tree for birds was included in an article Wilson and Ellen Prager have in the proceedings of the 1978 International Ornithological Congress.

The controversies are probably just beginning as systematic biology enters a new era of numerical measurement and analysis. “We’re going to fight about methodology for a long time,” Fitch says. “The truth is that our methods are just not good enough right now.”

As a consequence, different actors in this drama can and do point out the shortcomings of the various approaches. Critics of Sibley’s DNA hybridization technique contend that, by looking at the entire genome of a species—the method’s strong point, according to Sibley—the approach smooths out the distinctive differences that could be important. Cracraft’s technique draws the same criticism: “Morphology is the conse­quence of many genes acting on structure,” Fitch says. “So the morphological data could be subject to a smoothing caveat similar to that raised against Sibley’s data.” Wilson himself points out the imprecision of his own data. And, against both protein and morphological analysis is raised the epigram: the farther from the gene, the farther from the truth.

The debate about ratite phylogeny thus can be seen as the forerunner of future controversies that will arise as the new methods spread through systematic biology and pro­duce new information. “We’re going to have information coming out of our ears,” says Fitch. “It will be marvelous, tantalizing informa­tion. The data are going to tell us a lot. But we don’t know what.”

**The National Science Foundation contributes to the support of the research discussed in this article through its Systematic Biology Program.**