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# Evolution of Flightless Land Birds on Southern Continents: Transferrin Comparison Shows Monophyletic Origin of Ratites*

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*Summary.* A biochemical approach was used to study the evolution of ratite birds, i.e., the ostriches, rheas, cassowaries, emus, and kiwis. Quantitative immunological comparison of transferrin from ratites, tinamous, and other flying birds indicates that all the ratites and tinamous are allied phylogenetically and that they are of monophyletic origin relative to other birds. To explain the current geographic distribution of ratites and the magnitude of the transferrin distances, it is supposed that the ancestors of these flightless birds walked across land bridges between the southern continents during Cretaceous times.

*Key words:* Protein Evolution/Transferrin/Immunology/Micro-Complement Fixation/Phylogeny/Flightlessness/Ostrich/Rhea/Tinamou/Continental Drift

The large flightless birds of the southern continents have intrigued evolutionary biologists for over a century. These birds, known as ratites, include the living ostriches, rheas, cassowaries, emus, and kiwis, as well as the recently extinct moas and elephant birds. Their geographic distribution is indicated in Figure 1. Aside from being completely flightless, the ratites share a few anatomical features that distinguish them

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The following *abbreviation* is used in this work: MC'F = micro-complement fixation.

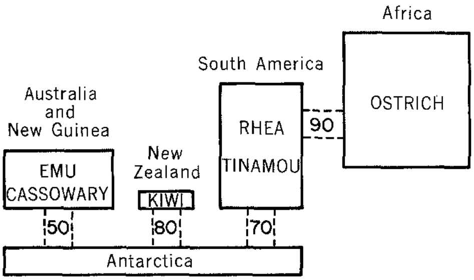

Fig. 1
Geographic distribution of ratites and tinamous. The numbers within the dashed lines indicate, in millions of years ago, the times of disappearance of land bridges between the continents and islands shown (Cracraft, 1972, 1973; Maxson et al., 1975). The ostriches, rheas, emus, and cassowaries, weighing 80-140 kg, are often known collectively as the "large ratites", in contrast to the 2-3 kg kiwis. The tinamous, unlike the ratites, are able to fly; they are included in this figure since their phylogenetic relationship to the ratites is demonstrated. The extinct flightless moas and elephant birds (see text) existed in New Zealand and Madagascar, respectively

from all other birds except tinamous (Parkes & Clark, 1966; Sibley & Ahlquist, 1972; Cracraft, 1974).[1]

Until the theory of continental drift became widely accepted there was much uncertainty regarding the origin of ratites. Their flightlessness and anatomical similarities could have arisen by either convergent or divergent evolution. According to the convergent evolution hypothesis, the ratites on a given land mass arose from ancestors that flew there and later became flightless and convergently similar in anatomy and way of life to the ratites on other continents; thus ratites could be of polyphyletic origin. Alternatively, according to the divergent evolution hypothesis, the common ancestor of ratites was flightless and walked across land bridges to the various land masses now inhabited by ratites; thus ratites could be of monophyletic origin. As soon as geologists became convinced that land bridges existed between all the southern land masses during the Cretaceous period (see Figure 1), i.e., during the early stages of bird evolution, it became easy to accept the hypothesis of monophyletic origin. Although scarcely any new morphological evidence has been uncovered in the last 2 decades and although the available morphological evidence is ambiguous, ornithologists now tend to favor the monophyly hypothesis.

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[1] The ratites all lack a keel on the sternum; on this basis the 4 ratite orders (Struthioniformes, Rheiformes, Casuariiformes, Apterygiformes) are grouped as the Ratitae in contrast to the remaining 23 avian orders (Wetmore, 1960), comprising the Carinatae, all of whose members have a keel. The most salient features shared by ratites and tinamous but absent in all other carinate birds are the palaeognathous palate and the unique structure of the bill (Parkes & Clark, 1966; Sibley & Ahlquist, 1972; Cracraft, 1974).

Much effort has been devoted to the purification and characterization of various proteins, particularly egg white proteins, from ratites and tinamous (Osuga & Feeney, 1968) and to the comparison of ratite and tinamou proteins with those of other birds and reptiles by chemical, electrophoretic, and qualitative immunological methods (Miller & Feeney, 1964; Osuga & Feeney, 1968; Feeney & Allison, 1969; Sibley & Ahlquist, 1972; Sibley & Frelin, 1972; Sibley et al., 1974; Wilson et al., 1964). While those biochemical results[2] are generally consistent with the monophyly hypothesis, they do not suffice to demonstrate ratite monophyly rigorously.

The quantitative immunological comparisons of transferrin reported below are consistent both with the monophyly hypothesis and with the idea that the ancestors of ratites walked across land bridges in late Cretaceous times. These comparisons also demonstrate a probable phylogenetic relationship between ratites and the South American tinamous.

As discussed in an accompanying article (Ho et al., 1976, and references therein), comparison of the amino acid sequences of homologous proteins among living species permits the construction of molecular phylogenies and thereby the determination

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[2] Electrophoretic methods, though useful for comparing proteins from closely related species, particularly members of the same genus, generally cannot yield quantitative measures of the amount of difference between proteins from phylogenetically more distantly related species such as members of different orders. On the basis of such electrophoretic comparisons of the egg white proteins of representatives of all avian orders (Sibley, 1970; Sibley & Ahlquist, 1972; Sibley & Frelin, 1972; Sibley et al., 1974) as well as comparison of the electrophoretic mobility of the tryptic peptides of ovalbumin from ratites, tinamous, and a few other carinate birds (Sibley & Frelin, 1972), Sibley and coworkers concluded that the large ratites were closely related, with the cassowary and emu particularly close; they could not place the kiwi especially close to any other avian group and suggested a possible kiwi-tinamou alliance as well as a possible tinamou-galliform alliance.

Though immunoelectrophoresis and immunodiffusion can give a more realistic appraisal of the similarities between proteins, the immunological studies of Feeney's group (Miller & Feeney, 1964; Osuga & Feeney, 1968) were somewhat limited in scope, involving, principally, antisera to whole egg white and purified ovotransferrin from the chicken and the cassowary and including species from only a few avian orders besides the ratites and tinamous. The overall conclusions of Feeney and coworkers were that all the ratites formed one assemblage, with the emu most closely related to the cassowary, and that the tinamou was related to the ratites.

Further qualitative evidence (Wilson et al., 1964) in favor of a single assemblage of ratites and tinamous came from comparative electrophoretic and thermostability studies with the heart form of lactate dehydrogenase.

of evolutionary relationships among extant species despite inadequate fossil evidence and ambiguous interpretation of morphological data. The degree of amino acid sequence difference among proteins is most readily assessed on a large scale by the quantitative immunological technique of microcomplement fixation (MC'F) (Champion et al., 1974, 1975; Ho et al., 1976), and we now report MC'F comparisons of transferrin from ratites with the homologous protein from representative species of all orders of birds.

## MATERIALS AND METHODS

Antisera were elicited in rabbits to transferrins purified from egg white or serum of 5 ratites (ostrich, rhea, cassowary, emu, kiwi), one tinamou, and 10 additional species (Adelie and Emperor penguins, arctic loon, great crested grebe, owl, Wester gull, pigeon, blackbird, chicken, and duck). Details of protein purification, antiserum production, and immunological methods are given in an accompanying article (Ho et al., 1976), as are the scientific names of these 16 species representing 14 of the 27 avian orders.

## RESULTS

In Table 1 we show the results of MC'F tests carried out with the 16 different antisera. Each antiserum was tested with samples derived from representatives of each of the 27 bird orders. By inspection of Table 1 we see in the upper right-hand corner a clustering of low values among the ratites and tinamou relative to the other birds. Abstracted from Table 1 and presented in Table 2 are the averages of reciprocal immunological distance measurements obtained with the antisera to the 16 transferrins. The clustering of low values among the ratites and tinamou appears in the lower right-hand corner of this table.

We have used the data in Table 2 to construct the transferri phylogeny shown in Figure 2. Salient features to note are 1. th close association of the cassowary and emu, consistent with previous morphological and biochemical evidence; 2. the almost simultaneous divergence into 3 lineages at 2 points (tinamou, ostrich, other ratites and, later, kiwi, rhea, cassowary + emu) and, most important, 3. the monophyletic assemblage of the ratites and tinamou relative to all other birds.

Our conclusion, based on transferrin immunological distance that the ratite-tinamou assemblage is monophyletic is supporte by MC'F tests with serum albumin and ovalbumin. With antisera to rhea albumin the average immunological distance between the

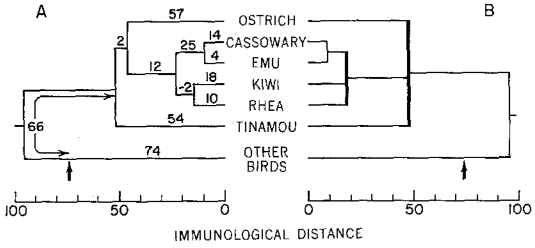

Fig.2
Phylogenetic relationships of ratites and tinamous based on transferrin immunological distances in Table 2. The heavy vertical arrows indicate the start of the divergence of the reference species among themselves; this section of the phylogeny is presented in detail elsewhere (Ho et al., 1976). Phylogeny A, constructed according to the method of Fitch & Margoliash (1967), shows the branching order of the lineages leading to the present-day species as well as the immunological distance units of transferrin change calculated to have occurred along each lineage. The reconstructed distances for the entire tree appear in Table 2. The percent standard deviation, a measure of the goodness of fit of the output versus input data, is 12.1% for the complete phylogenetic tree. Phylogeny B, which shows only branching order, takes into account alternative phylogenies (Ho et al., 1976) having similar percent standard deviations and does not give credence to common ancestral lineages along which only a few units of transferrin change may have occurred. The thick lines on the cladogram indicate those sections which have been collapsed.

We have also constructed a phylogeny based on the data in Table 2 according to the Farris (1972) method. The resulting tree was far less satisfactory, having a percent standard deviation of 29.6%. While the Fitch-Margoliash (1967) and similar (Sarich, 1969a,b) methods are essentially iterative averaging procedures, the Farris technique is based on the assumption that each input datum is either a true estimate or an underestimate, but never an overestimate, of the actual distance between 2 taxonomic units. Since, however, immunological data are as likely to include overestimates as underestimates of distances between species, Farris trees based on immunological data are often inferior to Fitch-Margoliash trees constructed from the same data (Prager et al., 1976; Prager, unpub. calculations)

rhea and other ratites and tinamous is 37 units (Prager et al., 1974), while the average distance between ratites and tinamous and all other orders of birds is 57 units (Prager et al., 1974).[3] Using antisera to rhea ovalbumin, Brush[4] found that the ostrich and tinamou both were 48 immunological distance units from the rhea, while representatives of 20 orders outside the ratite-tinamou assemblage were an average of 75 units from the rhea.

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[3] Prager, unpublished observations, using antisera to albumin from loon (*Gavia arctica*), herring gull (*Larus argentatus*), cowbird (*Molothrus ater*), and duck (*Anas platyrhynchos*).

[4] A.H. Brush, personal communication.

Table 1
Immunological distances among bird transferrins[a]

| Species | Antiserum | | | | |
|---|---|---|---|---|---|
| | AP | EP | Loon | G | Owl |
| Struthioniformes--Ostrich, *Struthio camelus* | 224 | 195 | 246 | 220 | 143 |
| Rheiformes--Rhea, *Rhea americana*[b] | 139 | 124 | 143 | 163 | 201 |
| Casuariiformes--Cassowary, *Casuarius aruensis* | 137 | 152 | 166 | 169 | 240 |
| --Emu, *Dromiceius novae-hollandiae* | 141 | 154 | 143 | 161 | 232 |
| Apterygiformes--Kiwi, *Apteryx australis* | 146 | 154 | 193 | 161 | 146 |
| Tinamiformes--Tinamou, *Eudromia elegans*[c] | 170 | 128 | 191 | 132 | 198 |
| Gaviiformes--Common loon, *Gavia immer* | 35 | 17 | 1 | 40 | 48 |
| --Arctic loon, *Gavia arctica* | 31 | 17 | 0 | ND | ND |
| Podicipediformes--Western grebe, *Aechmophorus occidentalis* | 51 | 47 | 55 | 4 | 76 |
| --Great crested grebe, *Podiceps cristatus* | ND | ND | ND | 0 | ND |
| Sphenisciformes--Adelie penguin, *Pygoscelis adeliae* | 0 | 12 | 47 | 50 | 82 |
| --Emperor penguin, *Aptenodytes forsteri* | 13 | 0 | 27 | 37 | 68 |
| Procellariiformes--Albatross, *Diomedea immutabilis* | 31 | 32 | 36 | 49 | 78 |
| Pelecaniformes--Snake bird, *Anhinga anhinga* | 61 | 63 | 92 | 62 | 98 |
| Ciconiiformes--Heron, *Ardea cocai* | 35 | 32 | 55 | 61 | 84 |
| Anseriformes--Duck, *Anas platyrhynchos* | 103 | 112 | 152 | 128 | 174 |
| Falconiformes--Falcon, *Falco sparveria* | 56 | 36 | 50 | 61 | 94 |
| Galliformes--Chicken, *Gallus gallus* | 115 | 118 | 129 | 129 | 186 |
| Gruiformes--Rail, *Rallus limicola* | 71 | 52 | 62 | 60 | 71 |
| Charadriiformes--Western gull, *Larus occidentalis* | 67 | 49 | ND | 58 | 89 |
| --Herring gull, *Larus argentatus* | 65 | ND | 60 | ND | ND |
| Columbiformes--Pigeon, *Columba livia* | 59 | 59 | 83 | 68 | 110 |
| Psittaciformes--Parrot, *Psittacus erithacus* | 60 | 45 | 52 | 57 | 91 |
| Cuculiformes--Cuckoo, *Coccyzus americanus* | 88 | 64 | 96 | 99 | 107 |
| Strigiformes--Owl, *Asio otus* | 71 | 67 | 82 | 86 | 0 |
| Caprimulgiformes--Goatsucker, *Caprimulgus parvulus* | 69 | 56 | 58 | 54 | 77 |
| Apodiformes--Swift, *Chaetura pelagica* | 74 | 59 | 68 | 59 | 86 |
| Coliiformes--Mousebird, *Colius sp.*[e] | 77 | ND | 80 | ND | ND |
| Trogoniformes--Trogon, *Aphaloderma narina* | 99 | 92 | 103 | 96 | 136 |
| Coraciiformes--Kingfisher, *Megaceryle alcyon* | 58 | 52 | 61 | 60 | 93 |
| Piciformes--Woodpecker, *Colaptes auratus* | 81 | 93 | 111 | 87 | 142 |
| Passeriformes--Blackbird, *Agelaius phoeniceus* | 119 | 111 | 92 | 94 | 110 |

[a] The data were obtained by quantitative micro-complement fixation using antisera to 16 purified transferrins. The degree of antigenic difference in the MC'F test is expressed in immunological distance units. Species are listed by orders in the first column; the orders are arranged according to traditional ornithological classification (Wetmore, 1960). The abbreviations used for some of the antisera are AP, Adelie penguin; EP, Emperor penguin; G, great crested grebe; P, pigeon; B, blackbird; C, chicken; T, tinamou; Ca, cassowary; Os, ostrich (cf. Table 2). The heavy lines in the upper right-hand corner of the table outline the data among the ratites and tinamous.

[b] The immunological distance between Darwin's rhea (*Pterocnemia pennata*) and *Rhea americana* was 1 unit with anti-rhea ovotransferrin, demonstrating, as d

| Gull | P | B | C | Duck | T | Rhea | Kiwi | Emu | Ca | Os |
|---|---|---|---|---|---|---|---|---|---|---|
| 253 | 268 | 224 | 234 | 163 | 126 | 69 | 99 | 142 | 110 | 0 |
| 205 | 124 | 115 | 117 | 247 | 59 | 0 | 19 | 61 | 46 | 86 |
| 245 | 143 | 151 | 152 | 211 | 88 | 42 | 33 | 21 | 0 | 73 |
| 246 | 121 | 136 | 151 | 192 | 87 | 35 | 40 | 0 | 16 | 85 |
| 243 | 145 | 137 | 139 | 248 | 69 | 34 | 0 | 68 | 44 | 67 |
| 241 | 164 | 180 | 126 | 262 | 0 | 74 | 85 | 109 | 120 | 108 |
| 66 | 81 | 93 | 111 | 168 | 158 | 141 | 175 | ND[d] | 196 | 185 |
| ND | ND | ND | ND | ND | ND | ND | ND | 180 | ND | ND |
| 58 | 82 | 85 | 97 | 174 | 134 | 145 | 166 | 210 | 196 | 171 |
| | | | | | | | | | | |
| ND | ND | 87 | 99 | ND | ND | ND | ND | ND | ND | ND |
| 88 | 73 | 77 | 132 | 169 | 232 | 158 | 179 | 206 | 238 | 229 |
| 65 | 74 | 110 | 116 | 167 | 217 | 174 | 184 | 198 | 244 | 222 |
| 82 | 49 | 93 | 137 | 168 | 227 | 146 | 155 | 199 | 232 | 226 |
| 113 | 79 | 118 | 124 | 182 | 146 | 135 | 144 | 216 | 241 | 224 |
| 89 | 65 | 116 | 136 | 180 | 222 | 175 | 178 | 162 | 188 | 173 |
| 176 | 116 | 110 | 97 | 0 | 235 | 154 | 157 | 163 | 185 | 190 |
| 87 | 86 | 94 | 122 | 167 | 248 | 153 | 163 | 185 | 239 | 187 |
| 155 | 114 | 138 | 0 | 163 | 145 | 148 | 143 | 160 | 183 | 175 |
| 89 | 83 | 84 | 138 | 168 | 235 | 186 | 175 | 204 | 235 | 188 |
| 0 | ND | ND | ND | ND | ND | 206 | 186 | ND | 269 | 207 |
| 1 | 78 | 93 | 112 | 192 | 170 | ND | ND | 225 | ND | ND |
| 77 | 0 | 76 | 112 | 179 | 170 | 196 | 164 | 185 | 234 | 190 |
| 79 | 86 | 88 | 84 | 166 | 165 | 120 | 154 | 167 | 183 | 171 |
| 93 | 82 | 99 | 193 | 163 | 250 | 210 | 203 | 206 | 221 | 223 |
| 93 | 105 | 98 | 135 | 204 | 255 | 209 | 192 | 212 | 248 | 193 |
| 73 | 92 | 94 | 111 | 184 | 221 | 171 | 184 | 208 | 217 | 190 |
| 92 | 93 | 94 | 100 | 171 | 134 | 124 | 140 | 210 | 250 | 185 |
| ND | 112 | 83 | 116 | 176 | 183 | ND | ND | ND | ND | ND |
| 162 | 140 | 177 | 163 | 204 | 250 | 231 | 162 | 234 | 243 | 259 |
| 79 | 94 | 110 | 133 | 191 | 153 | 149 | 142 | 175 | 217 | 208 |
| 129 | 119 | 122 | 148 | 174 | 188 | 204 | 215 | 190 | 250 | 197 |
| 116 | 80 | 0 | 138 | 178 | 211 | 160 | 215 | 206 | 240 | 205 |

the result obtained with serum albumin (Prager et al., 1974; Prager & Wilson, 1975), minor divergence at the molecular level within the Rheiformes.

[c] The tinamous form a relatively close-knit group. Seven species in 4 genera tested with anti-*Eudromia* ovotransferrin gave immunological distances of 5-23 as follows: *Nothoprocta ambigua* 5, *Crypturellus tataupa* 8, *Crypturellus soui* 11, *Crypturellus cinereus* 16, *Nothura maculosa* 18, *Tinamus major* 19, and *Tinamus tao* 23.

[d] ND, not done.

[e] The representative of the Coliiformes was tested by MC'F with only 7 of the 16 antisera in order to conserve material, since only tissue extracts, having a relatively low transferrin content, were available. A positive result in the Ouchterlony double diffusion test was obtained with the remaining antisera.

Table 2

Immunological distances among bird transferrins[a]

| Species compared | AP | EP | L | G | O | Gu | P | B | C | D | T | R | K | E | Ca | Os |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Adelie penguin (AP) | - | 13 | 40 | 51 | 77 | 77 | 66 | 98 | 124 | 136 | 201 | 149 | 163 | 174 | 188 | 227 |
| Emperor penguin (EP) | *13* | - | 22 | 42 | 68 | 57 | 67 | 111 | 117 | 140 | 173 | 149 | 169 | 176 | 198 | 209 |
| Loon (L) | *35* | *30* | - | 48 | 65 | 63 | 82 | 93 | 120 | 160 | 175 | 142 | 184 | 162 | 181 | 216 |
| Grebe (G) | *51* | *46* | *48* | - | 81 | 58 | 75 | 90 | 114 | 151 | 133 | 154 | 164 | 186 | 183 | 196 |
| Owl (O) | *76* | *71* | *73* | *65* | - | 91 | 108 | 104 | 161 | 189 | 227 | 205 | 169 | 222 | 244 | 168 |
| Gull (Gu) | *69* | *64* | *66* | *58* | *95* | - | 78 | 105 | 134 | 184 | 206 | 206 | 215 | 236 | 257 | 230 |
| Pigeon (P) | *79* | *74* | *76* | *68* | *99* | *98* | - | 78 | 113 | 148 | 167 | 160 | 155 | 153 | 189 | 229 |
| Blackbird (B) | *89* | *84* | *86* | *78* | *109* | *108* | *78* | - | 138 | 144 | 196 | 138 | 176 | 171 | 196 | 215 |
| Chicken (C) | *123* | *118* | *120* | *112* | *143* | *142* | *112* | *122* | - | 130 | 136 | 133 | 141 | 156 | 168 | 205 |
| Duck (D) | *167* | *162* | *164* | *156* | *187* | *186* | *156* | *166* | *130* | - | 249 | 201 | 203 | 178 | 198 | 177 |
| Tinamou (T) | *194* | *189* | *191* | *183* | *214* | *213* | *183* | *193* | *169* | *213* | - | 67 | 77 | 98 | 104 | 117 |
| Rhea (R) | *162* | *157* | *159* | *151* | *182* | *181* | *151* | *161* | *137* | *181* | *76* | - | 27 | 48 | 44 | 78 |
| Kiwi (K) | *170* | *165* | *167* | *159* | *190* | *189* | *159* | *169* | *145* | *189* | *84* | *28* | - | 54 | 39 | 83 |
| Emu (E) | *183* | *178* | *180* | *172* | *203* | *202* | *172* | *182* | *158* | *202* | *97* | *37* | *45* | - | 19 | 114 |
| Cassowary (Ca) | *193* | *188* | *190* | *182* | *213* | *212* | *182* | *192* | *168* | *212* | *107* | *47* | *55* | *18* | - | 92 |
| Ostrich (Os) | *199* | *194* | *196* | *188* | *219* | *218* | *188* | *198* | *174* | *218* | *113* | *77* | *85* | *98* | *108* | - |

[a] Reciprocal MC'F values obtained using antisera to 16 transferrins were averaged to derive the immunological distances shown in the upper right-hand section of the table. The original data matrix from which the average values were calculated is presented elsewhere (Ho et al., 1976); the data are also contained within Table 1. In *italics* in the lower left-hand section of the table are given the reconstructed immunological distances computed from the complete phylogeny shown in part in Figure 2A and in part in Figure 1 of Ho et al. (1976). The species abbreviations in the horizontal row are indicated in the vertical column at left. As in Table 1, the heavy lines outline the intra-ratite-tinamou values.

## DISCUSSION

### Continental Drift

The results we report here may be considered in relation to current theories of continental drift (Cracraft, 1972, 1973; Maxson et al., 1975). In Figure 1 we have summarized the drift of the southern continents, once all connected via Antarctica. In a previous study (Maxson et al., 1975) substantial agreement was obtained on the times of divergence of the Australian and New World marsupials and hyline frogs between immunological estimates using the albumin molecule as an evoltionary clock and estimates from continental drift. Similarly, if one assumes a rate of avian transferrin evolution of 1-1.2 units of immunological distance per million years (Prager et al., 1974), the immunological distances shown in Figure 2 from the ostrich and tinamou to species on other continents are consistent with the times of disappearance of land bridges shown in Figure 1. However, the distances between the kiwi and rhea and from these 2 species to the emu and cassowary are, on the average, roughly half the values predicted from continental drift and the rate of transferrin evolution. These discrepancies are probably due to the statistical limitations (Maxson et al., 1975) of the transferrin clock, particularly since avian transferrin evolves more slowly and irregularly than mammalian albumin or transferrin (Prager et al., 1974; Ho et al., 1976), and to possible convergence and conservatism (Cronin & Sarich, 1975) at the transferrin locus in the kiwi and rhea lineages.

### Morphological Phylogeny

Cracraft's cladogram (Cracraft, 1973, 1974), based on analysis of derived morphological character states, agrees with our transferrin phylogeny (Figure 2) in showing the ratite-tinamou assemblage to be monophyletic. However, his cladogram differs from ours rather conspicuously in placing the divergence of the ostrich and rhea at the last branching point along the entire cladogram. Since according to the previously available evidence and that presented here the ratites form a monophyletic group and since (cf. Figure 3 below) the common ancestor of the ostrich and rhea could not have flown between Africa and South America, Cracraft's cladogram along with considerations of continental drift (Figure 1) implies that the ostrich and rhea diverged from a common ancestor at least 80-90 million years ago. His cladogram further implies that the other ratites and the tinamou lineage diverged off much earlier, 100 million or more years ago. Given our transferrin results as well as the

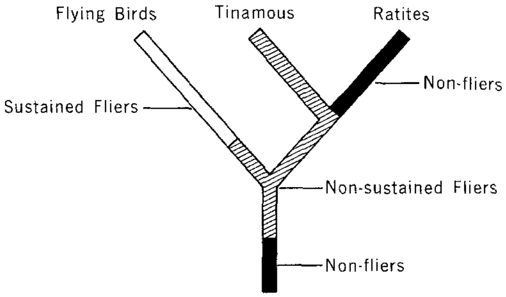

Fig.3
Conjectural phylogenetic model for the origin of flight in 2 stages. The dark, striped, and clear areas represent flightlessness, non-sustained flight, and sustained flight, respectively. The number of species in the ratite-tinamou assemblage is less than 1% of the nearly 9000 living bird species.

Though Figure 3 suggests that the loss of all flying ability among the ratites occurred only once, in an ancestor common to all the ratites, Figure 2 is consistent with the possibility that flightlessness among these birds may have evolved more than once - for instance, once along the lineage leading to the ostrich and again along that leading to the remaining ratites Clearly it would be most parsimonious for flightlessness to have developed only once, prior to the beginning of the break-up of the southern continents and prior to any intra-ratite divergence, as Cracraft (1972, 1973, 1974) suggests. Molecular phylogenies constructed on the basis of comparisons of additional proteins such as ovalbumin and albumin permit further testing of this hypothesis. As discussed elsewhere (Ho et al., 1976) and illustrated here in Figure 2, flightlessness in ratites has arisen entirely independently from its development in penguins, the only other flightless avian order. (There have of course been occasional instances of flightlessness developing in selected species or subspecies *within* a number of bird orders, due primarily to selective pressures on islands)

available data obtained with albumin and ovalbumin, we submit that such ancient times for the start of the intra-ratite divergence are improbable. Were the position of the ostrich shifted on Cracraft's cladogram, his branching order could otherwise in broad outline be reconciled with our transferrin phylogeny as well as with current theories concerning the drift of the southern continents.

## Origin of Flight

Our transferrin phylogeny may have a bearing on theories as to how flight originated. The oldest known fossil bird, *Archaeopter* was probably able to fly (Ostrom, 1974). However, it is uncertain whether this animal was capable of sustained flight (Ostrc 1974). The transferrin results suggest that there are, phyloger etically speaking, 2 major groups of living birds, namely the ratites and tinamous on the one hand and the remaining birds o the other hand. Consideration should therefore be given to the scheme shown in Figure 3 for the origin of flight. Tinamous, though able to fly, appear incapable of sustained flight (Hudson, 1920; Wagner, 1949; Pearson & Pearson, 1955; Van Tyne

Berger, 1961; Lancaster, 1964; Johnson, 1965; Vigil, 1973). This finding is accounted for by the phylogenetic model in Figure 3. Ratites and tinamous, according to the model, represent a lineage which branched off from that leading to other birds before the acquisition of the capacity for sustained flight.[5] The model further assumes that ratites then lost the ability to fly at all, while tinamous retained the ability to fly in a non-sustained manner. Thus the ancestor of ratites and tinamous may never have been capable of sustained flight. This hypothesis is easily reconciled with Ostrom's suggestion (Ostrom, 1974) that the common ancestor of all birds was terrestrial rather than arboreal and was able to fly only in non-sustained bursts.

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[5] Most members of the order Galliformes (chickens and other fowl-like birds) are also incapable of sustained flight (Welty, 1955; Wilson et al., 1963). Figure 3 allows for the possibility that the Galliformes diverged off after the 2 major groups of birds (ratites + tinamous versus all others) split but before sustained flight developed. Some transferrin molecular evidence (Ho et al., 1976; Prager & Wilson, manuscript subm.) exists which is compatible with an early divergence of the gallinaceous birds from other flying birds.

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