THE RELATIONSHIPS OF THE FLAMINGOS AS INDICATED BY THE EGG-WHITE PROTEINS AND HEMOGLOBINS

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The living species of flamingos form a small, easily defined group of three genera, *Phoenicopterus, Phoeniconaias,* and *Phoenicoparrus. Phoenicopterus* is usually divided into three forms, considered to be species by some authors while others treat them as races of the Greater Flamingo, *Phoenicopterus ruber.* These are *P. r. ruber* of the New World tropics and subtropics, *P. r. chilensis* of temperate South America, and *P. r. roseus* (=*antiquorum*) of the Old World. The Lesser Flamingo, *Phoeniconaias minor,* occurs in Africa and India and *Phoenicoparrus andinus* and *P. jamesi* are confined to the Andes in Perú, Argentina, and Chile.

These four (or six) living species are the survivors of a long and well-documented lineage extending at least to the early Tertiary and possibly into the Cretaceous. In spite of this unusually good fossil record the relationships of the flamingos to other groups of birds have been difficult to determine beyond doubt. A large body of evidence, mainly anatomical, suggests an alliance to the Ciconiiformes (herons, storks, ibises), but the bill and feet, development of the young, the voice, and the mallophagan parasites have been interpreted as indicating an origin from the Anseriformes (ducks, geese).

The question is, are the flamingos most closely related to the herons and storks and merely convergent to the anseriform birds in certain characters or were they derived from the ducks and geese and later converged toward the ciconiiform birds? A third possibility is that they were derived from some other group and are similar to both geese and herons only by convergence.

In this paper we will review various opinions about the classification of the flamingos, the fossil history, and the anatomical, behavioral, and other evidence which has been presented. We will then present some new data from our studies of the egg-white proteins and hemoglobins of birds which bear upon this problem.

THE CLASSIFICATION OF FLAMINGOS

The disposition of the flamingos in 15 classification systems, from 1867 to 1961, is summarized in table 1. This table does not include all of the classifications in the literature, only some of the major ones. Most workers have considered the flamingos to be either an order apart or allied to what we presently call the Ciconiiformes; and these two trends are rather evenly distributed chronologically. Furthermore, whether workers have used a few characters or a variety of evidence has not seemed to influence the trends to one decision or the other. Although almost every author has noted resemblances between flamingos and waterfowl, only a few have actually placed them in the same order in formal classifications. However, a number of ornithologists in their general writings have considered the flamingos to be anseriform, and this possibility has been more and more seriously considered in recent decades. In general, systematists placing the flamingos with the Ciconiiformes have based their conclusions on anatomical evidence of various kinds, while those considering them to be independent or anseriform have relied on characters such as the bill and feet, development of the young, and voice.

The flamingos have always been given at least family status, with three well-established genera. The living species are closely related, and no attempt will be made to distinguish "primitive" from "advanced" members of the family for comparison with other groups. Except for slight differences in size, coloration, feeding structures and associated habits, and the absence of the hallux in *Phoenicoparrus,*

TABLE 1. Position of the Phoenicopteridae in some major classifications.

Author	Year	Arrangement	Characters used
Huxley	1867	Order: Chenomorphae (= Anseriformes) Amphimorphae (= Phoenicopteriformes) Pelargomorphae (= Ciconiiformes)	Skull, muscles
Gadow	1877	Superorder: Grallae Order: Gressores (Ciconiiformes) Family: Ciconiidae—including: *Platalea* *Phoenicopterus* *Tantalus*	40 characters, anatomical and biological
Gadow	1892	Order: Ardeiformes Suborder: Steganopodes (Pelecaniformes) Herodii (Ardeidae) Pelargii (Ciconiidae, Phoenicopteridae) Order: Falconiformes Anseriformes	As above
Weldon	1893	Order: Chenomorphae Amphimorphae Pelargomorphae	Anatomy
Sharpe	1895	Order: Chenomorphae Suborder: Anhimae Phoenicopteri Anseres	Various characters
Beddard	1898	Order: Herodiones Suborder: Phoenicopteri	Anatomy
Reichenow	1913	*Reihe*: Grallatores Order: Cursores (Charadriiformes) Pelopatides (Phoenicopteriformes) Gressores (Ciconiiformes) *Reihe*: Natatores Order: Lamellirostores (Anseriformes)	Anatomical and biological characters
Hartert	1921	Order: Phoenicopteri Gressores Anseres	Anatomical and biological characters
Peters	1931	Order: Ciconiiformes Suborder: Ardeae Balaenicipites Ciconiae Phoenicopteri	Not stated
Mayr and Amadon	1951	Order: Ciconiiformes Phoenicopteriformes Anseriformes	Anatomy, life history, behavior
Stresemann	1959	Order: Anseres Anhimae Steganopodes Phoenicopteri Gressores	Anatomy, mallophaga, life history
Verheyen	1959	Subclass: Limnornithes (all shore and wading birds) Order: Ardeiformes Ciconiiformes Phoenicopteriformes Anseriformes Anhimiformes	Osteology
Delacour	1959	Order: Ciconiiformes Phoenicopteriformes Anseriformes	Morphology, posture, life history, behavior, distribution
Wetmore	1960	Order: Ciconiiformes Suborder: Ardeae Balaenicipites Ciconiae Phoenicopteri	Fossils and various other characters

TABLE 1. (Continued)

Author	Year	Arrangement	Characters used
		Order: Anseriformes Suborder: Anhimae Anseres	
Delacour	1961	Order: Anseriformes Anatidae Phoenicopteridae Anhimidae	As above

little has been noted in the literature on species differences or speciation within the family.

The classification used here for the Anseriformes will follow Johnsgard's (1961b) which agrees largely with that of Delacour and Mayr (1945). The Anseriformes, although closely related to one another, do include groups with different evolutionary trends. The Anhimidae may be an offshoot of ancient anseriform stock and possibly illustrate the primitive characters of the order, but because they are so aberrant from typical waterfowl of today and because information on so much of their biology is lacking, we will rely mainly on comparisons of the flamingos with swans, geese (Anserinae), and Magpie Geese (Anseranatinae). The Anserinae are generally agreed to be more representative than the Anatinae of basic anseriform characters (Lorenz 1941; Delacour and Mayr 1945; Delacour 1954–59), while Johnsgard (1961a) has suggested that the Magpie Goose (*Anseranas*) represents the most generalized evolutionary condition of the Anatidae and may constitute a direct link between them and the Anhimidae.

The Ciconiiformes present a different problem. This is a large, loosely allied assemblage of at least three heterogeneous groups: the Ardeidae (herons, egrets, and bitterns), the Ciconiidae (storks, Jabirus, and Wood Ibis), and the Threskiornithidae (ibises and spoonbills). In addition there are two small, aberrant families (Balaenicipitidae and Scopidae) which will not be included in comparisons here. The degrees of relationship among these groups are uncertain and doubts that the order is monophyletic have often been expressed. For example, Ligon (1967:24) has proposed "A taxonomic arrangement that . . . would place the herons and storks in separate orders . . . and the storks and [cathartid] vultures in the same order . . . ." Verheyen (1959) presented the only recent revision of the order based almost entirely on osteological characters. In his scheme, as in most others, the Ardeidae are placed first and the Threskiornithidae last, but with such diverse groups it is difficult to say that one is more "primitive" or nearer to a generalized ciconiiform type than is another. If, as Storer (1960) suggests, the amount of adaptive radiation is indicative of a more highly evolved group, the Ardeidae could not be considered especially primitive Ciconiiformes. If it is the degree of specialization which indicates evolutionary development, the bitterns may be the most primitive and the ibises and spoonbills the most advanced. However, in view of our uncertainties about the relationships within the Ciconiiformes, and because some workers relate the flamingos to storks, others to herons, and still others to ibises, comparisons will be made interchangeably with all three of these families.

FOSSIL HISTORY

Howard (1950, 1955) and Wetmore (1955) have summarized the fossil history of the flamingos. Miller (1963) and Brodkorb (1963a) have described additional forms, and Brodkorb (1963b, 1964) has provided a catalogue of fossil birds, including the flamingos and their relatives. Table 2 is a summary of the Suborder Phoenicopteri primarily according to Brodkorb (1963b, 1964).

The Cretaceous and early Tertiary are the most critical periods in the fossil history of the flamingos in relation to their ancestry. The Cretaceous birds *Gallornis*, *Parascaniornis*, and *Torotix* and the early Tertiary genera *Scaniornis* and *Telmabates* are the only known fossils which seem to qualify as possible ancestors. Howard (1950) thought that *Gallornis* may have been a primitive anatid but Brodkorb (1963a) disputed this allocation and assigned it to the Phoenicopteri. If this is correct the ancestry of the flamingos goes back to the Lower Cretaceous which makes them an ancient group indeed, for, as Brodkorb (1963a:63) notes, this horizon is "only slightly younger than *Archaeopteryx*." *Gallornis* is based upon the proximal portion of a femur and a piece of a humerus. The latter, as noted by Brodkorb, is "of no comparative value." The head of a femur is thus the only basis for

TABLE 2. The Suborder Phoenicopteri (fossil portion after Brodkorb 1963b, 1964).

Geologic age	Species	Family	Locality
Recent	*Phoenicopterus r. ruber*	Phoenicopteridae	New World
	Phoenicopterus r. roseus	Phoenicopteridae	Old World
	Phoenicopterus r. chilensis	Phoenicopteridae	South America
	Phoeniconaias minor	Phoenicopteridae	Africa, India
	Phoenicoparrus andinus	Phoenicopteridae	South America
	Phoenicoparrus jamesi	Phoenicopteridae	South America
Upper Pleistocene	*Phoenicopterus minutus*	Phoenicopteridae	Europe
Middle Pleistocene	*Phoenicopterus copei*	Phoenicopteridae	North America
Lower Pleistocene	*Phoeniconaias gracilis*	Phoenicopteridae	Australia
	Phoenicopterus ruber	Phoenicopteridae	Australia (Miller 1963)
Middle Pliocene	*Phoenicopterus stocki*	Phoenicopteridae	North America
Lower Pliocene	*Phoenicopterus floridanus*	Phoenicopteridae	North America
	Megapaloelodus opsigonus	Palaelodidae	North America
Upper Miocene	*Palaelodus steinheimensis*	Palaelodidae	Europe
Lower Miocene	*Phoeniconotius eyrensis*	Phoenicopteridae	Australia
	Phoenicopterus novaehollandiae	Phoenicopteridae	Australia
	Phoenicopterus croizeti	Phoenicopteridae	Europe
	Megapaloelodus connectens	Palaelodidae	North America
	Palaelodus gracilipes	Palaelodidae	Europe
	Palaelodus minutus	Palaelodidae	Europe
	Palaelodus ambiguus	Palaelodidae	Europe
	Palaelodus crassipes	Palaelodidae	Europe
	Palaelodus goliath	Palaelodidae	Europe
Upper Oligocene	*Agnopterus turgaiensis*	Agnopteridae	Europe
Lower Oligocene	*Tiliornis senex*	Phoenicopteridae	South America
	Elornis grandis	Phoenicopteridae	Europe
	Elornis littoralis	Phoenicopteridae	Europe
Upper Eocene	*Elornis anglicus*	Phoenicopteridae	Europe
	Agnopterus laurillardi	Agnopteridae	Europe
	Agnopterus hantoniensis	Agnopteridae	Europe
Lower Eocene	*Telmabates antiquus*	Telmabatidae	South America
Lower Paleocene	*Scaniornis lundgreni*	Scaniornithidae	Europe
Upper Cretaceous	*Torotix clemensi*	Torotigidae	North America
	Parascaniornis stensioi	Torotigidae	Europe
Lower Cretaceous	*Gallornis straeleni*	Torotigidae	Europe

the suggestion that the ancestry of the flamingos may extend to the Lower Cretaceous. However, the paucity of Cretaceous avian fossils, the possibilities for convergent similarities, and the limitations of the femur as a source of characters combine to make this a highly tentative assignment. *Gallornis* has not been "proved" to be a flamingo although it could be. The uncertainty about it simply renders *Gallornis* neutral as evidence of phoenicopteran ancestry.

According to Howard (1950) *Parascaniornis* and *Scaniornis* show evidence of relationships to both herons and flamingos. However, the material is fragmentary and difficult to interpret and it cannot be considered as proved that these genera represent common ancestors of flamingos and herons.

Brodkorb (1963a) described *Torotix clemensi* from the Upper Cretaceous of Wyoming on the basis of the head of a humerus. He assigned this fossil to the Phoenicopteri and made it the type of a new family to which (1963b) he also allocated *Gallornis* and *Parascaniornis.*

In the Eocene there are four known species which seem to be flamingos. Three of these, *Elornis anglicus, Agnopterus laurillardi,* and *A. hantoniensis,* are from Europe. The fourth, *Telmabates antiquus* from Patagonia, was described by Howard (1955). *Telmabates* was a primitive wading bird with resemblances to the flamingo genus *Palaelodus* and also to the anseriform birds. Howard considered *Telmabates* to be a flamingo ancestor but not a ciconiiform. Rather, she suggested that *Telmabates* supports the allocation of the flamingos to a separate order which should include three families, Telmabatidae, Palaelodidae, and Phoenicopteridae. *Elornis* has been referred to the Phoenicopteridae and thus becomes the earliest known representative of the modern flamingos.

In the Oligocene the Phoenicopteridae are

TABLE 3. Summary of anatomical characters of flamingos.

Characters shared with Ciconiiformes	Characters shared with Anseriformes	Characters shared with both orders	Characters shared with neither order
Developmental: (partly) nidifugous two coats of down	Developmental: thick down on young nidifugous	Integumental: tufted oil gland present 11 primaries diastataxic	Integumental: reduced hallux inverted bill filter apparatus
Integumental: down structure pterylosis aftershaft present	Integumental: feather structure waterproof plumage webbed feet lamellate bill	Skeletal: carinate desmognathous holorhinal pervious nares no ectocondylar process 16–25 cervical vertebrae	Muscular: flexor tendons type IV 1 pair syrinx muscles small femoral-caudal BXY+ muscle formula
Skeletal: basipterygoid process present palatine and vomer rostrum pelvis number ribs	Skeletal: nasal aperture supraorbital depression lachrymals quadrate mandibular angle pectoral girdle	Muscular: ambiens present	Other: type of air cells in lung
Muscular: flight muscle attachment gastrocnemius	Others: caeca tongue shape		
Others: carotid artery arrangement cervical air sacs divided intestinal convolutions penis rudimentary abdominal air sacs large			

represented by two species of *Elornis* and *Phoenicopterus croizeti* from Europe and *Tiliornis* from Argentina.

Milne-Edwards (1867–71) described several species of the genus *Palaelodus* from the Lower Miocene of Europe. *Palaelodus* had shorter legs, longer toes, and a straighter bill than *Phoenicopterus*. The more heavily-built palaelodids also probably lacked the filter feeding bill structures of modern flamingos (Jenkin 1957). Miller (1963) described *Phoeniconotius eyrensis* and *Phoenicopterus novaehollandiae* from the late Oligocene or early Miocene of Australia and placed *Phoeniconotius* in the Phoenicopteridae.

In the Miocene *Palaelodus* is found in Europe and *Megapaloelodus connectens* (A. Miller 1944; L. Miller 1950) is known from North America. Pliocene and Pleistocene flamingos include several species of *Phoenicopterus* and *Phoeniconaias gracilis* described by Miller (1963) from the early Pleistocene of Australia.

The early history of the Ciconiiformes is unknown unless *Scaniornis* is accepted as the Cretaceous ancestor of both flamingos and Ciconiiformes. By the Eocene the present families of herons and ibises are found in Europe and the storks are known from the Lower Oligocene of Africa (Howard 1950).

The fossil record is thus of uncertain value in understanding the origins of the flamingos. Clearly they represent an ancient group which extended to all parts of the world early in its history and which evolved several adaptive types. The question of relationship to the Anseriformes and Ciconiiformes rests primarily upon the interpretation of the fossil remains of *Gallornis*, *Scaniornis*, and *Telmabates*. As noted above, the relationships of *Gallornis* cannot be considered as proved. The material representing *Scaniornis* is fragmentary and perhaps questionable but it seems to ally the flamingos to the Ciconiiformes. That of *Telmabates* is considerably better and, if Howard (1955) is correct in her interpretation, suggests an alliance between the flamingos and the anseriforms. Possibly the only valid conclusion to be drawn from the fossil evidence is that the flamingos, the ciconiiforms, and the anseriforms were derived from a common ancestor but that the degrees of relationship among them cannot be determined from the material available at this time.

ANATOMICAL EVIDENCE

Table 3 summarizes the principal anatomical characters which have been used to define the Phoenicopteridae. This summary is based upon the publications of the following authors: Owen 1832; Huxley 1867; Reichenow 1877; Gadow 1877, 1892; Goodchild 1891; Weldon 1893; Sharpe 1895; Beddard 1898; Shufeldt 1901; Hartert 1912; Chandler 1916;

Gardner 1926; Stresemann 1927; Hudson 1937; Glenny 1953; and Jenkin 1957. Of the many aspects of anatomy described for the flamingos, we have included only those which are in general use in systematics and which usually show consistency at the ordinal, or at least family, level. Characters obviously susceptible to convergence but traditionally used by taxonomists also have been included. We have not attempted to evaluate characters shared by all three taxa, as it is impossible to know what degree of relatedness they signify without knowing how many other orders share the same traits.

Unfortunately many of the anatomical traits often cited as evidence for flamingo relationships are characters which seem highly susceptible to convergence. However, one of the most striking of these, the two coats of nestling down in the flamingos, is a character of unknown functional significance. The same may be said for pterylosis and the presence of an aftershaft, but feather structure itself is another matter. Chandler (1916) felt that the minute structure of feathers would have little adaptive value and could therefore be used as a trustworthy taxonomic character. But Rutschke (1960), although not primarily interested in classification, has shown by quantitative measurements of feather structure that water birds in different orders are more alike in feather structure than non-aquatic birds even within the same order. Unfortunately Rutschke did not examine feathers from flamingos. Although Chandler considered their feather structure to be very similar to that of geese, Reichenow (1877) pointed out that the down feathers of young flamingos are simple and unbranched, as in storks, and not at all like the "true" down of ducks and geese. A thorough study of the feather structure of flamingos, such as Rutschke has made for other water birds, would be of great interest here, especially if it supported Reichenow's opinion.

As for other external morphological characters, the close, hard, waterproof nature of the plumage as a whole, shared by flamingos and geese, could easily be the result of convergence. The same must be said of the webbing of the toes (absent in the Anhimidae, reduced in *Anseranas* and other terrestrial geese, present to a slight extent in some Threskiornithidae) and the lamellate structure of the bill. Although bill structure is so obviously related to feeding habits, it is a character cited again and again to show anserine similarities for flamingos. However, Reichenow (1877) considered the bill of flamingos to be more similar to those of ibises and spoonbills than to that of ducks in a variety of characters and, even in the lamellae, to be at least as reminiscent of *Anastomus* as of ducks. Jenkin (1957), in a study of the feeding mechanisms of flamingos, has pointed out that many characters of the bill, such as shape, size of jaws, and joints of mandibles, are correlated with the pumping and filter mechanisms of feeding. In her opinion the filtering apparatus of flamingos is far more specialized than that of ducks (or *Anastomus*), although they probably both received it from a common ancestor. The bend in the bill developed later in evolution, as it does in ontogeny. The condition of the bill in the hatchling flamingo, straight and goose-like, lends weight to these opinions, but it is important to note that the young of many Ciconiiformes with specialized bills, such as spoonbills, are also hatched with straight bills, very like flamingo chicks.

The absence of the hallux, sometimes used to emphasize the distinctness of flamingos from either geese or storks, together with the relatively short toes of flamingos, is often found as a convergent condition in cursorial and nonperching birds.

Characters of skeletal anatomy are unfortunately difficult to evaluate for the flamingos because of differences of opinion among the anatomists themselves. For instance, Shufeldt (1901) considered the skull of flamingos, in general, to be most like that of the ibises, while Weldon (1893) considered its overall condition to be anserine. In contrast to Shufeldt, Weldon believed the forelimb to be stork-like. If we discard these characters, there still seems to be an approximately equal number of skeletal characters which flamingos share with the Anseriformes and Ciconiiformes.

The most widely used muscle characters in non-passerine taxonomy, those of the thigh and the flexor tendons, do not seem particularly useful here because they are different in all three groups. The thigh muscle formula is so variable, even within the Ciconiiformes, that it is probably not even justifiable to use it as evidence of the independent position of the flamingos.

The carotid arteries, used by Glenny (1953, 1955) as the basis for a revision of avian classification, show the bi-carotid condition in the adult stage in all Anseriformes as well as most Ardeidae and Ciconiidae. Some Ciconiiformes and flamingos are conjuncto-carotid. In most birds (including *Balaeniceps*) where reduction occurs it is in the right carotid, but in the flamingos and in those Ciconiiformes which show reduction, it is in the left. Glenny believes that this tendency indicates the affinity

of the flamingos to the Ciconiiformes. However, the variety of conditions of the carotids within the Ciconiiformes, plus the fact that the flamingos, on the basis of artery condition alone, would have to be a fairly recent group within that order, make this character of questionable value in this situation.

Among the organ systems used in avian taxonomy, the cervical air sacs divided by septa have often been cited as a character shared by flamingos and storks. Beddard (1898), however, has pointed out that this condition also occurs in *Chauna,* of the Anhimidae. The intestinal convolutions are also stork-like, but may not be a reliable character, as Ridley (1954) has shown that the great length of the intestine in flamingos is probably an adaptation to their feeding niche. The condition of the caeca, well developed in flamingos and waterfowl, both vegetarians, is probably convergent. The absence of a well-developed penis cannot be considered a taxonomically significant character for flamingos since the copulatory organ is rudimentary in the Anhimidae as well as in the Ciconiiformes.

Fox (1962a, b, c), Fox et al. (1965), Fox and Hopkins (1966a, b), and Fox et al. (1967) found the carotenoid pigment canthaxanthin in the feathers of the Scarlet Ibis (*Eudocimus ruber*), all species of flamingos, and the Roseate Spoonbill (*Ajaia ajaja*). These were the only birds known to possess this pigment, and for a time it seemed to be a reliable character allying the flamingos to the Ciconiiformes. However, Brush (1967) has found canthaxanthin in the Scarlet Tanager (*Piranga olivacea*) and other species of *Piranga,* thus showing it to be subject to convergence. It is highly probable that the synthesis of canthaxanthin from its precursor, β-carotene, is mediated by identical or extremely similar enzymes in flamingos and ciconiiforms and that the homologous enzymes in *Piranga* differ significantly. Until this is demonstrated, however, this character must be regarded as unreliable.

In summary, the anatomical evidence for the relationships of the flamingos would seem to lie in the following characters: (1) Shared with the Ciconiiformes are the two coats of down in the young, pterylosis, presence of an aftershaft, rostrum in general, including condition of palatine and vomer, presence of a basi-pterygoid process, number of ribs, pelvic structure, flight muscles in two layers, leg muscles, and large abdominal air sacs. (2) Shared with the Anseriformes are the condition of the nasal apertures, supraorbital depression, lachrymals, quadrate, and pectoral girdle. The type of air cells in the lungs and the Type IV flexor tendons are characters which flamingos share with neither the Ciconiiformes or Anseriformes.

Two main conclusions may be drawn from this analysis. The first is that the majority of anatomical characters are shared with the Ciconiiformes. The second is that there seem to be very few anatomical characters in which flamingos are unique. It may be that the taxonomists who place the flamingos in a separate order on the basis of the purported large number of characters in which they seem "different," are either simply considering them different from the Ciconiiformes on the basis of characters shared with geese (cf. Hartert 1912; Reichenow 1877), or are using characters, such as bill structure, which are specific to the flamingos, but which are not taxonomically stable in other groups and so invalid for comparisons.

EVIDENCE FROM PARASITES

Mallophaga or feather lice are host-specific obligate parasites which probably became associated with birds well before the Eocene when most of the living orders of birds evolved (Clay 1957). The main selective factors affecting the evolution of the Mallophaga derive from interspecific competition resulting in the adaptation of different species to the variety of ecological niches on each host, and from predation by the bird, resulting in a high degree of competition for head and neck niches. Speciation in the parasites probably occurred most often through isolation of the host population for a sufficient length of time to allow the parasite subpopulations to develop ecological or sexual isolating mechanisms before they were rejoined (Clay 1949, 1957). Opportunities for speciation by interspecific transfer must be rare, the main ones being from brood parasites, predators, dust baths, re-use of old nests, or from body contact between colonial birds in mixed colonies. In addition, the high degree of specialization of many Mallophaga to a particular niche on a particular host species makes them poorly adapted both for transfer to a new host environment and for competition with established parasites already there.

The overall effect of these various factors on the evolution of the Mallophaga has been to maintain a fairly close association between host and parasite so that the taxonomic indications of mallophagan distribution are frequently in accordance with accepted avian classification. The findings relative to the flamingos are among the most interesting. Of

the 10 genera of Mallophaga on the Ciconiiformes, except for *Colpocephalum*, an extremely generalized genus found on many bird orders, none is present on the flamingos. The Ardeae and Ciconiae have two common genera (disregarding *Colpocephalum*), the Threskiornithidae have two genera in common with rails and one with swans, and *Scopus* has two genera common to waders. The Phoenicopteridae have four genera of Mallophaga: *Colpocephalum* and three (*Anateocus, Anaticola,* and *Trinoton*) found elsewhere only on the Anatidae. The Anseriformes in turn possess a distinct group of five genera unrelated except to the flamingo lice.

In evaluating the bearing of the Mallophaga on avian relationships it must be remembered that several factors may obscure the initial relationship between host and parasite. Clay (1950) and Hopkins (1949) have enumerated these possible causes of apparently anomalous distributions of Mallophaga. "Secondary absence" may occur when a genus of lice, once widespread, becomes extinct on some orders, thus showing nothing by its present distribution. This is not likely the case with the three genera of flamingo lice shared with the Anatidae, since they occur on no other group. Convergent evolution and the resulting confusion in the taxonomy of the Mallophaga must certainly be another factor, although Clay (1957) feels that probably most cases of convergence among the Mallophaga have now been recognized.

In the case of the flamingos, probably the main cause of the discrepancy in the distribution of the Mallophaga lies either in an error in the classification of the Phoenicopteridae with the Ciconiiformes, or in the occurrence of a secondary transfer of Mallophaga from the waterfowl to the flamingos. Mayr (1957) considers host transfer of Mallophaga to be very frequent, and von Keler (1957) gives examples of how this could have occurred, via water plants, between geese and flamingos. He believes, as does Stresemann (1959), that the flamingos have acquired their feather lice from the Anatidae since they have lived in the same environment and have similar feather structure. Otherwise, they argue, if the flamingo lice have really been derived from ancestral anatid lice, it is strange they have not diverged further in all this time, since flamingos must have separated from geese at a very early time. Clay agrees that secondary transfers could have occurred at a time in the evolution of the flamingos and waterfowl when they offered a relatively uniform environment, and when the lice were not yet highly specialized, but this would have had to occur not once, but three times with the flamingos. She thinks it is more likely that the flamingos are anseriform, as is also suggested by some of the fossil evidence. Hopkins (1942) considers cases of host transfer to be so rare that the evidence from Mallophaga is practically conclusive for bird taxonomy, provided that the taxonomy of the Mallophaga themselves is accurate and that the groups of Mallophaga in question are "representative" of one another on the two different hosts. Of the three genera of Mallophaga shared by ducks and flamingos, two are representative, but not the same as the ciconiiform genera and so can be used as good evidence. Hopkins thus considers the correspondence of duck and flamingo lice as conclusive proof that flamingos not only are anseriform, but have only recently diverged from anatid stock.

In view of this great divergence of opinion, it is difficult for a non-specialist to reach a decision about the reliability of the mallophagan evidence for the flamingos. Unfortunately, even to parasitologists the biology and systematics of the Mallophaga are poorly known, according to Ash (1960). Although the distribution of the Mallophaga may be among the strongest evidence we have in support of anseriform affinities for the flamingos, it is far from conclusive in the present state of our knowledge.

Although the mallophaga of flamingos are most like those of ducks, Baer (1957) has found that the tapeworms of flamingos are apparently related to those of the Charadriiformes. Here again, the same questions must be raised. Are the parasites from the two bird groups actually closely related or only convergently similar? If related, are they shared by flamingos and shorebirds because the birds are closely related or because the two groups of birds happen to provide the same ecological niche? In the absence of supporting evidence from other sources it seems certain that the similar tapeworms do not indicate that flamingos and shorebirds are closely related.

In summary, the evidence from parasites is as conflicting and difficult to interpret as is that from morphology. Depending upon the bias of the observer it is possible to use the same data to support essentially opposite taxonomic opinions.

EVIDENCE FROM LIFE HISTORY

Information on the life history and general biology of flamingos may be found in observations by McCann (1940), Yeates (1950), Lomont (1954), Brown (1957), Johnson et al.

(1958), and Rooth (1965) as well as in various sources mentioned above. Flamingos, as Allen (1956) has pointed out, are a good example of a relict group (Amadon 1953). Although they may once have been dominant in many parts of the world they now exhibit an extremely discontinuous distribution and are confined to shallow alkaline lakes and salt lagoons, usually barren of vegetation. Their unique manner of feeding is obviously a specialization which has evolved as a closer and closer adaptation to a narrow ecological niche with a minimum of competition and predation from other animals. Also correlated with this adaptive retreat to barren, isolated areas was the evolution of a high degree of sociality, exemplified in breeding colonies so compact that individual distance does not even allow for taking off into flight. This, according to Swift (1960), may in turn have been correlated with the fact that flight from predators was not important in the evolution of flamingos because of the open nature of the habitat they frequent. It must certainly be correlated with the evolution of a reproductive physiology requiring a high degree of social stimulation for initiation and synchronization of the breeding cycle.

Thus these two factors, feeding ecology and sociality, represent such primary specializations in the biology of flamingos, with which so much else is correlated, that it is practically impossible to find biological characters which are taxonomically valid. The affinity for water, ability to swim, feeding structures including long legs and neck, large fat deposits under the skin, pigmentation of plumage, and the parental feeding of the young are examples of characters directly related to the feeding niche, while pattern of molt, type of nest, number of eggs, and state of development of young at hatching are indirect correlates of adaptations to habitat. Such characteristics as size of nesting territory, communal displays, noisiness, low level of aggression, absence of predator response, irregularity of breeding, crèche system for young, and, again, the alleged simultaneous wing molt, are all probably related to the extreme gregariousness of flamingos.

EVIDENCE FROM BEHAVIOR

Most attempts to utilize behavioral data in systematics have concerned the relationships of species and genera and have been based largely on courtship displays and other ritualized behavior patterns. Hinde and Tinbergen (1958), Mayr (1958), Amadon (1959), Tinbergen (1959), Cullen (1959), Wickler (1961a, b), and Ficken and Ficken (1966) have reviewed the problems associated with the application of behavioral evidence to taxonomy. Most of these authors have concluded that, given certain restraints, behavioral evidence is as valid as morphological characters, at least at the level of species and possibly genera. Like morphological evidence the stability of behavioral characters varies from group to group, and whether or not they are useful as clues to relationship can be determined only by observing their correlations with other characters. In short, since we still lack valid measurements of the degrees of genetic relatedness among organisms, all genetically controlled characters must be given consideration.

Although there may be little basis for optimism about the value of behavioral characters in higher category systematics it is necessary to compare the behaviors of the groups of birds involved in the present problem because various authors have cited behavioral evidence in support of arguments about the relationships of the flamingos.

In the following, except where otherwise noted, data for the Anatidae are drawn mainly from the studies of Heinroth (1911), Lorenz (1941), and Johnsgard (1961b; 1965); for the flamingos from Allen (1956), Brown (1958), and Rooth (1965); for the Ardeidae from Verwey (1930) and Meyerriecks (1958, 1959); and for the Ciconiidae, from Siewert (1955), Haverschmidt (1949), and Kahl (1966).

LOCOMOTION

The use of intention movements for flight, such as head-shaking or pumping and mutual calling, as social signals within the flock is a widespread character in the Anatidae including *Anseranas*. Although Meyerriecks (1958) records flight intention movements for herons and Kahl (1966) for the Marabou Stork (*Leptotilos crumeniferus*), these movements seem to have no specific signal function, and it is possible that this is true in other Ciconiiformes. No similar intention movements of flamingos have been recorded, although Chapman (1905) mentions an increased "gabbling" among the flock before it takes off, and Rooth (1965) describes "walking in stretched attitude alternated with wing preening, proceeding into flying" during the pair formation display.

The fact that flamingos do not soar as do the storks and ibises has been considered an anserine character, but Johnsgard (1961a) has pointed out that soaring is found in the Anhimidae and *Anseranas*. The same argument has been used for the tendency of flamingos to fly in a V or line formation, but storks,

ibises, and spoonbills often take up formation on longer flights (Bent 1926; Mackworth-Praed and Grant 1952). The habit of constantly vocalizing in flight seems to be a consistent character of the Anatidae and Phoenicopteridae, which is rare in the Ciconiiformes. The function of this is not certain but seems to be a correlate of flock behavior; certainly in the Ciconiiformes it is most pronounced in highly social species, such as the night herons (Noble et al. 1938; Allen and Mangels 1940).

The tendency to walk from a predator rather than to take flight immediately and the ability to run are most pronounced in the flamingos. This is of course a correlate of their terrestrial mode of life, just as bitterns, the most terrestrial of the Ciconiiformes, try to escape from danger by skulking or "climbing" away among the reeds.

Members of all three groups can swim. The fact that the young of flamingos, herons, and ibises swim with far more ease and grace than they walk and seem preferentially to seek the water when in danger (Weston 1913) seems suggestive of an aquatic ancestry for all of them. However, although they can swim, adult flamingos do not voluntarily do so, in contrast to the Anatidae. It is difficult to know which is the crucial fact here: that flamingos can swim, or that they do not.

MAINTENANCE BEHAVIOR

Since flamingos are such specialized feeders it is difficult to find comparable feeding behavior patterns in other birds. When feeding in deep water they characteristically tip up like ducks and geese, but when occasionally they find a larger food item plentiful, such as small crabs, they give up their typical filter-feeding and stalk the prey in a manner reminiscent of the feeding behavior of herons and storks (Macnae 1960): the neck is extended forward and down, the head held at body level or lower, the bird strides along at a rapid pace. Crabs are picked up with the tip of the bill and tossed into the gape with a jerk of the raised head, the same treatment given live prey by storks and herons. Although none of the Ciconiiformes can perform the filter-feeding of flamingos, the gross feeding comportment of many, especially wood ibises, ibises, and spoonbills, is often very flamingo-like. Roseate Spoonbills wade along in shallow water sweeping the bill from side to side, "munching" microscopic forms with the papillae of the bill (Allen 1942). Various kinds of foot-trampling and stirring movements are found in many Ciconiiformes as well as in flamingos (Rand 1956; Meyerriecks 1959). Bent (1926) even reports a Wood Ibis feeding by walking back and forth, sweeping the head in a wide arc with the bill pointed downward and backward, rapidly opening and closing the mandibles "in the manner of flamingos."

The motor patterns of preening and bathing might be among the more conservative behavioral characters, but adequate descriptions of these activities seem to be lacking for flamingos. Scratching in all three groups is direct, or under the wing.

GENERAL SOCIAL BEHAVIOR

Flamingos are highly gregarious at all seasons of the year with birds in a flock maintaining an individual distance too small even to allow for taking off into flight. The extreme reduction of territory, the absence of a complex peck order, and the tendency of the colony to respond as a whole to intruders are correlates of this sociality (Lorenz 1938). Most waterfowl are also highly social except for nesting. However, in the Anserini the basic social unit within the flock is the family group rather than the individual as in flamingos. Among the Ciconiiformes, all degrees of sociality are found, including colonial breeding, even in a single family, as Bock (1956) and Meyerriecks (1959) have shown for the Ardeidae. They believe the primitive condition was one of solitary existence, the more highly evolved of present-day forms showing the greatest degree of social organization, but it is hard to say whether this generalization holds for the order as a whole. Since there is no evidence of family grouping among Ciconiiformes, the gregariousness as such of flamingos might be an anserine trait. However, the retention of social behavior during breeding, breeding in mixed colonies, and the organization within the flock based on the individual rather than the family are all more reminiscent of the Ciconiiformes.

Territorial behavior in flamingos is minimal and differs from that of either Anseriformes or Ciconiiformes. Feeding flamingos maintain "individual distance" from one another and the male of a mated pair defends an area around his mate (Rooth 1965). The nesting territory extends as far as the bill will reach, on all sides of the nest. Geese defend large and definite nesting territories. In most socially nesting Ciconiiformes, a large nesting territory is initially staked out by the male, and is the site of pair formation and copulation, but this often dwindles as the breeding cycle progresses, until it includes only the area which can be defended from the nest with the bill (Witherby et al. 1939; Allen 1942).

Threat displays in flamingos seem less elaborate than those of the Anseriformes and Ciconiiformes. Rooth (1965) describes several variations of aggressive behavior in flamingos all of which involve the bill as the weapon. The scapular and back feathers are usually raised, the neck may be held at different angles, and sometimes a "growling" noise is given. Neck posturing is also prominent in the threat displays of geese and swans, but attack is with the wings, except in *Anseranas* where it is with the bill.

The aggressive behavior of storks and herons tends to be highly ritualized with specialized components such as bill-snapping, crest-raising, and tail-flipping, although other components such as hissing are shared with geese. For example, the nest defense of the White Stork (*Ciconia ciconia*) consists of bill-clattering coupled with a threat posture in which the tail is fanned up, the body held forward, scapulars raised, the bill pointed downwards, and the wings spread out and down. The "Forward Clattering Threat" in the Marabou Stork involves a downward movement of the head between the legs, a rapid upward return with a loud clap of the bill, and a downward sweep of the head accompanied by rapid bill-clattering. Thus flamingos share neck posturing with geese, but their use of scapulars as releasers both in hostile and courtship behavior is a trait most highly evolved in the ciconiiform families.

One of the most conspicuous and widespread social displays of geese is the so-called "triumph display" (Delacour and Mayr 1945). No comparable behavior has been described for any of the Ciconiiformes or flamingos.

Also of special interest is the display, practically universal among the Anatidae (Lorenz 1941), which consists of bill-dipping or displacement drinking. This behavior pattern does not occur among the Ciconiiformes, but a similar movement is performed by both sexes in flamingos during pre-copulatory activities.

COURTSHIP AND COPULATORY BEHAVIOR

In evaluating the taxonomic usefulness of courtship displays and other sexual behavior, it must be kept in mind that these are tied up to a great extent with general aspects of breeding biology, such as pair formation, duration of the pair bond, and the effects of sexual selection. Sibley (1957) has formulated a general statement of these correlations and Johnsgard (1960) has shown how it applies in the Anseriformes.

In the Anseranatinae and Anserinae, where the pair bond is formed once and is permanent and there is no sexual dimorphism in plumage, courtship tends to involve simple, mutual displays which are similar throughout the group. In the Anserini the pair bond is established and maintained by the triumph ceremony and centers around the nest territory although copulation does not occur there. (It does in *Anseranas*.) In the Anatinae, where the pair bond is seasonal and there are many sympatric species, there are more complex displays differing between male and female and from species to species, those of the male being enhanced by strikingly developed nuptial plumage.

The storks, which may also mate for life, show the same general courtship patterns as the geese. But the situation in most Ciconiiformes and in the flamingos, where there are short pair bonds and heterosexual displays but mostly monomorphic plumages, seems to be intermediate. In most Ciconiiformes the nest and nest site is the area of male advertising display, courtship, pair bond formation, and copulation. The pair bond is established by the exchange of nesting material between mates or by the entering of the nest by the female. Within the Ardeidae, Meyerriecks (1959) has outlined two main trends in the evolution of courtship, which are characteristic of other Ciconiiformes as well. In the first a stationary male, on territory, displays to a mobile female, while in the second a mobile male displays before several other birds of undetermined sex. This latter type of pair bond formation most nearly approximates that of flamingos. It is of interest that this is also the type found among the most highly social species of herons. In flamingos the formation of the pair bond apparently occurs when the male first leaves the communal display group and singles out a female for copulation. As mentioned above there seems to be no particular courtship territory, nor is the nest site, staked out after pair bond formation, the scene of courtship display or copulation.

Courtship displays themselves, in the Anserini, include the triumph ceremony as well as simple, mutual pre-copulatory displays involving bill-dipping, and rising in the water, probably derived from bathing movements. Displays in typical pond ducks involve such components as ritualized preening behind the wing, ritualized feeding and drinking movements, head-pumping, laying-the-head-on-the-back, etc. Female displays are more uniform and typically involve inciting by bill-dipping and calling. None of these components except the bill-dipping of the female and possibly the *Kopfzurücklegen* of the male seems homolog-

ous to anything found in the courtship of the flamingos.

Flamingos, in early courtship, display in groups in shallow water, standing with necks stretched high, flagging heads from side to side, and flashing open wings momentarily, revealing contrasting axillaries and primaries. A bird may also bow forward, opening the wings slightly to reveal the scarlet upper wing coverts after which the neck is stretched sharply backward so that the head touches the back. Finally the group takes off, flies for several minutes, and returns to the display area. Bill-dipping may also occur, as well as "hooking," which is primarily a threat display in which birds rush toward one another with aggressive cries, neck crooked and bill down, in the same manner as described for Great Blue Herons (*Ardea herodias*) on the breeding grounds (Bent 1926). Many birds may participate in display activities at one time, and there is usually a group of "spectators" as well. During the display the birds give a goose-like cackling call, a "chogógo" call, and a number of grunting notes (Rooth 1965).

A practically universal characteristic of courtship behavior in the Ciconiiformes is the offering and passing of twigs and other nest material on the nest itself. Along with this are several highly ritualized displays with varying components of hostile and sexual tendencies. Typical examples are the Clattering Strophe of storks, and the Stretch Display of herons. In the display of the White Stork, the bird throws its head and neck backwards so the crown touches the back feathers, then brings the head forward and down, "clappering" all the while with the bill. The display may be performed by one or both members of the pair, and it is of interest that this ceremony is important not only in courtship but in any situation of "excitement" and in meetings of the pair. In this way it may have a function similar to that of the triumph ceremonies of the Anserinae. The Stretch Display seen in most Ardeini is performed mainly by the male on the nest and consists of first stretching the head, neck, and bill upwards and raising the plumes of the back and neck to their fullest, then lowering the head backwards, almost touching the back. In different species head-tossing or pumping may also occur and in many there is also a bowing component at the end. In the highly social Snowy Egret (*Leucophoyx thula*) the display often attracts a group of "onlooker" egrets. Meyerreicks (1959) also describes a male Reddish Egret (*Dichromanassa rufescens*) performing the Stretch Display which included components of head-tossing, bill-dipping, and wing-flashing. Similar movements have been described in the Marabou Stork (Kahl 1966).

Thus, there are similarities, such as a *Kopfzurücklegen* component, which occur in the courtship displays of all three groups. Daanje (1950) has pointed out that many such components may be expected to show convergence, since they derive from basic motor activities. However, it may be significant that the displays of flamingos and herons seem especially similar in this and a number of other elements, such as head-tossing, bowing, and wing-flashing, as well as the attraction of the display to other, non-breeding individuals. On the other hand, bill-dipping, according to Lorenz one of the most universal displays among the Anserini, is also an important component of courtship in the flamingos (Wackernagel 1959; Suchtantke 1959).

Copulation in the Ciconiiformes and *Anseranas* occurs at the nest, while in most other Anatidae and in the flamingos it takes place in deep or shallow water some distance from the nest site. Copulatory behavior as recorded for the three groups does not afford much evidence for comparisons except that pre-copulatory activities in the Anserini commonly contain head- and tail-raising and bill-dipping components, the latter of which is a main component of copulatory behavior in flamingos, while pre-copulatory display in Ciconiiformes typically contain "billing," feather nibbling, and bill-snapping components. During copulation in the waterfowl (except *Anseranas*) the male grasps the nape of the female. Likewise, in storks and herons there is nibbling of the neck feathers during copulation. The observations of flamingo copulation rarely include a feather-nibbling or grasping component, although Suchtantke (1959) reports that the male stretches his neck forward with that of the female, and strokes his bill on her neck. Rooth (1965) observed one case in which the male pecked the neck and head of the female. There are various post-copulatory displays in Anseriformes and Ciconiiformes. In the flamingos there is sometimes a "turning away" movement of the head but it is not always given.

NEST-BUILDING, PARENTAL CARE, AND BEHAVIOR OF THE YOUNG

Nest building is typically performed by both the male and female in all three groups except in the Anatini where, according to Kendeigh (1952), building and incubation by the female alone is probably a secondarily derived pattern correlated with polygamy. In none of the Anatidae is nest material carried; it is

simply passed over the shoulder with the bill. There has been much debate in the literature as to whether or not flamingos carry mud for building (Chapman 1905; Ali 1945; Allen 1956; Brown 1958), but most recent evidence indicates that they at least can. However, there is one report of passing mud from mate to mate (Allen op. cit.), and, in any case, flamingos certainly tend, like geese and unlike ground-nesting Ardeidae (Weller 1961), to use material within reach of the nest rather than to transport it.

Incubation duties in all three groups, according to Kendeigh (1952), are, or were originally, shared by both sexes. In those Anseriformes which still share in incubation, nest relief ceremonies occur but are not elaborate or highly ritualized. The two birds call to each other and may indulge in mutual preening (Anhimidae and *Anseranas*) or the triumph ceremony (swans and geese). In flamingos there is apparently no nest relief ritual, except possibly wing-waving (Allen 1956). In contrast, in all families of the Ciconiiformes nest relief is accompanied by elaborate mutual ceremonies, often consisting of the highly ritualized displays used in courtship. Other incubation behavior which could be of taxonomic value includes retrieval of lost eggs and turning of the eggs in the nest. Contrary to expectations for a true ground-nesting bird, flamingos apparently do not retrieve eggs rolled from the nest, even where these are low (Gallet 1950). The fixed behavior patterns involved in shifting the eggs within the nest have been described in detail for geese, herons, and storks but not for flamingos.

Patterns of parental care are difficult to compare in these groups because of the difference in development of the young. Among the Anseriformes only *Anseranas* is known to feed its young. Although the newly hatched young peck about for themselves, the parents also fetch water plants and "dribble" this food into the bills of the young. This is the same method used by flamingos, except in their case the food is a regurgitated liquid and is the only food the young get. The parent may feed the young bird as it stands in the nest or as it pokes its head out from under the parent's wing during brooding. This method of feeding while brooding has also been described for the Black-crowned Night Heron, but, in general, feeding of the young in the Ciconiiformes differs from that of the flamingos and *Anseranas* in that ciconiiform young either pick up food from the floor of the nest, or beg by grasping the bill of the parent (Ardeidae and Ciconiidae) or by reaching into the parent's bill and taking food from the throat (Threskiornithidae).

Although considered altricial, the young of several Ciconiiformes, especially ibises and egrets (Bent 1926; Witherby et al. 1939) and particularly where the nest site is low, often leave the nest some days or weeks before fully fledged. In such cases they climb in and out of the nest and about the nest tree using bill, wings, and feet in a manner characteristic of young flamingos, who also wander to and from the nest before they leave for good. As mentioned above, the young of all three groups take to water under stress, and swim with ease. Young flamingos, although still fed by the parents for many weeks, wander about in herds, pecking at all sorts of objects the way young Anatidae do. The massing of young flamingos in groups, or crèches, is probably a protection against aerial predators (Rooth 1965). Occasional flocking of the young has also been observed in ibises and spoonbills (Bent 1926; Witherby et al. 1939).

SUMMARY

The behavior of flamingos contains several elements which differ more or less from those of either Anseriformes or Ciconiiformes; among these are virtual absence of territory, emphasis on communal displays, stroking of female's neck by male during copulation, virtual absence of post-copulatory display, and crèche behavior of the young. Elements shared with Anseriformes include: vocalization during flight, ability of adults to swim easily, tip-up method of feeding, use of neck postures in threat, displacement feeding of female as appeasement, bill-dipping in courtship activities, occurrence of copulation in water, tendency not to carry nest material, simple nest relief ceremonies, method of feeding young, and pecking behavior of young. Behavior elements shared with the Ciconiiformes include; tendency of adults not to swim though able; stalk, "filter," and foot-trampling methods of feeding; individual rather than family as basic unit of flock; gregariousness during breeding; use of scapulars as releasers in hostile behavior; seasonal pair bonds; head-turning, wing-flashing, and bowing components of courtship displays; attraction function of courtship display to other members of the species; ability to carry nest material; and wandering of young about the nest. There are also behavior patterns shared by all three groups: ability of young to swim, flight in formation, method of scratching the head, general gregariousness, and *Kopfzurücklegen* component in courtship display.

It seems unlikely that any valid conclusions about relationships can be drawn from these data. Approximately equal numbers of behavioral elements are shared by flamingos with the Anseriformes and with the Ciconiiformes, but there are also many traits which all three groups have in common.

It should not surprise us that it proves to be difficult or impossible to assess the value of behavior as the basis for speculation about the relationships of the higher categories. As taxa diverge and adapt to diverse ecological niches they will modify behavioral elements accordingly, but there will be no evidence of the changes except observations of the movements of the living animals. To recognize homologous elements and to separate them from those similar by convergence alone will become increasingly difficult in proportion to the degree of ecological divergence between the forms being compared.

In the present case the three groups have been separate since the Cretaceous and each has evolved along different adaptive pathways. The result is simply that each group has become so different from the others in behavior that even those movements that are most similar are not necessarily homologous. In such cases it is not possible to assess the significance of differences and similarities until the degrees of genetic relatedness among the species being compared are known.

EVIDENCE FROM PREVIOUS STUDIES OF PROTEINS

The rationale underlying the use of data from comparisons of the properties of homologous proteins in systematics has been discussed by Sibley (1960, 1962, 1964, 1965, 1967). The flamingo problem has been considered in various degrees of detail by Sibley (1960, 1967), Mainardi (1962, 1963), and Haavie (1962). Sibley (1960) used paper electrophoresis to compare the egg-white proteins of 359 species of non-passerine birds, including 23 species of ciconiiforms, one flamingo, and 59 species of anseriforms. The flamingo pattern, under all conditions, showed more resemblances to those of the Ciconiiformes than to those of the Anseriformes.

Haavie (1962) used acylamide gel ("disc") electrophoresis to compare *Phoenicopterus* and *Phoeniconaias* egg white with five ciconiiforms and five anseriforms. She too found the greater similarities between the flamingos and the Ciconiiformes. Haavie also prepared antisera against the egg-white proteins of a flamingo, a heron, and a swan and compared them using the Ouchterlony technique. Although the reactions were not always consistent, the overwhelming majority of tests showed that the flamingos share more antigens with the Ciconiiformes than with the Anseriformes.

Mainardi (1962, 1963) compared the red-cell antigens of *Phoenicopterus* with those of anseriform and ciconiiform birds. The immunological data showed that the flamingos are related both to Anseriformes and Ciconiiformes, "proving that their resemblance to these two groups is not due to convergence" (Mainardi 1963:111). Mainardi concluded that the three groups show about equal degrees of immunological relationship but that the fossil evidence suggests that the waterfowl branch diverged first, and later the flamingos and herons diverged from one another.

THE PRESENT STUDY

MATERIALS AND METHODS

Materials. The egg-white proteins of five species of Ciconiiformes, one flamingo, and four species of Anseriformes were compared using starch-gel electrophoresis. Figure 1 indicates the species studied. The hemoglobins of six species of Ciconiiformes, two flamingos, and six species of Anseriformes were compared, using starch-gel electrophoresis, as indicated in figure 2. The tryptic peptides of the hemoglobins of two species of herons, a flamingo, three Anseriformes, the domestic fowl, a gull, and a loon were analyzed by ion-exchange column chromatography. The species are indicated in figures 3 and 4.

The tryptic peptides of the hemoglobins of a heron, a flamingo, and a duck were compared using one-dimensional, thin-layer electrophoresis, as indicated in figure 5. The tryptic peptides of the ovalbumins of four Ciconiiformes, a flamingo, and three ducks were compared by one-dimensional thin-layer electrophoresis as indicated in figure 6.

Preparation of samples for electrophoresis. Egg-white proteins were obtained from the thin egg white of fresh or slightly incubated eggs and stored at 4.0°C prior to use. For starch-gel electrophoresis, a sample was diluted with buffer immediately before placing it in a sample slot of the starch-gel.

For the preparation of hemoglobin, blood samples were collected using 10 per cent (w/v) ethylenediamine tetraacetate disodium salt (EDTA) as an anticoagulant. The whole blood was centrifuged at 500–1000 rpm for 5 min to separate the plasma from the cells. The plasma and the "buffy coat" of white cells were removed and the red cells were suspended in 5–10 times their volume of 1 per cent (w/v) NaCl and centrifuged at 1000 rpm for 5 min at room temperature. The supernatant was discarded and the washing procedure repeated five more times. After the sixth wash, a volume of distilled water equal to twice that of the red cell pad was added to the cells and stirred thoroughly to cause lysis. The cellular debris was removed from the lysate by centrifugation at 4000 rpm. After carbon monoxide was bubbled through the supernatant for several seconds, the hemoglobin solution was frozen and stored at –75°C. Immediately prior to analysis by starch-gel electrophoresis, a hemoglobin sample was thawed, diluted with starch-gel buffer, and placed in the application slot of the gel.

Starch-gel electrophoresis. The egg-white proteins were analyzed in 16-sample-slot gels and the hemoglobins in 10-slot gels. A discontinuous buffer system was used for both sets of comparisons (Ashton and Braden 1961; Ferguson and Wallace 1961) with a starch-gel buffer, *p*H 7.95, composed of 0.046 M tris(hydroxymethyl) aminomethane, 0.007 M citric acid-H_2O, 0.005 M LiOH, and 0.019 M boric acid. The bridge buffer, *p*H 7.98, was composed of 0.05 M LiOH and 0.19 M boric acid. Electrophoresis was effected by a variable voltage of 400–600 v and a constant current of 35 ma for 3.5 hr. The gels were positioned vertically with the anode uppermost. Bromphenol blue was placed in the cathodal buffer chamber at the start of an electrophoretic analysis, and the power was turned off when the bromphenol blue, which migrated at the anodal buffer front, had moved 8 cm from the sample slots. One-half of the starch-gel was stained for total protein using amido black 10B. Gels were destained in 2.5 per cent acetic acid.

Tryptic digestion of hemoglobin. Hemoglobin samples to be digested were shaken with an equal volume of toluene and then centrifuged at 10,000 rpm for 20 min at 4°C. This was repeated until the toluene layer was clear. The hemoglobin was then lyophilized. Heme-free globin was prepared using a modification of the method described by Hill et al. (1962). Fifty mg of the lyophilized hemoglobin were dissolved in 5 ml of distilled water and placed in a 50 ml separatory funnel fitted with a rubber stopper. Two syringe needles (20 gauge) and a glass tube (3 mm o.d.) were passed through the stopper. The glass tube reached to the bottom of the funnel. Carbon monoxide was bubbled through the hemoglobin solution for 5 min followed by nitrogen for the remainder of the time required to remove the heme. One ml of 0.1 N HCl was added through one of the syringe needles; the other served as a vent. The solution was mixed by shaking the funnel gently and then cooled in a bath of crushed ice and water. When the solution was cold, 10 ml of chilled methylethylketone saturated with water was added through a syringe needle. The rubber stopper was replaced with a glass stopper and the contents of the funnel were shaken. After the phases were allowed to separate, the lower aqueous phase containing the globin was drained into a dialysis bag and dialyzed against water and Amberlite MB-3 at 4°C until the odor of methylethylketone could not be detected. This required 4–5 changes of distilled water, one liter each. The dialyzed globin sample was placed in the digestion chamber of a *p*H-stat (Radiometer Automatic Titrator). The sample was warmed to 37°C under a nitrogen atmosphere and 0.02 M ammonium hydroxide was added until the *p*H held constant at 8.5. One-half ml of distilled water containing 0.2 mg of 2× crystallized trypsin (Worthington Biochemical Corp.) was added to the sample and digestion was allowed to continue until the *p*H remained constant without the addition of base. The time required to achieve this varied from species to species. Seventeen hours was sufficient time for the *Phoenicopterus, Ardea,* and *Anas* samples, but the *Branta bernicla* sample was digested for 24 hr, the *Gavia stellata* sample for 40 hr, and the *Larus argentatus* sample for 44 hr. At the end of digestion, each sample was acidified to *p*H 3.2 by the addition of 0.1 N HCl and lyophilized.

Purification of ovalbumin. Equal volumes of thin egg white and saturated ammonium sulfate were combined and gently mixed. The precipitate that formed was removed by centrifugation at 10,000 rpm for 10 min at 4°C. Additional saturated ammonium sulfate was added until a second precipitate (ovalbumin) became dense. A second centrifugation sedimented the ovalbumin which was redissolved in 5 ml of distilled water and the precipitation steps were then repeated two more times. The final ovalbumin precipitate was dissolved in a minimal quantity of distilled water and dialyzed against two liters of distilled water and Amberlite MB-3 for 24 hr at 4°C and then for 24 hr against one liter of a buffer composed of 0.004 M KH_2PO_4, and 0.02 M glycine adjusted to *p*H 3.0 with 0.1 N HCl. This sample was placed on a carboxymethyl cellulose column equilibrated with the same buffer. The ovalbumin was eluted from the column by a *p*H and salt gradient that changed from the conditions of the starting buffer to *p*H 7.0 and 0.1 M KH_2PO_4, 0.1 M K_2HPO_4, and 0.02 M glycine over a volume of 300 ml. The eluate was monitored at 280 mμ and 10-ml fractions were collected. Fractions containing ovalbumin were pooled, dialyzed against one liter of distilled water and Amberlite MB-3 for 24 hr at 4°C, and then lyophilized.

Digestion of ovalbumin with trypsin. Five mg of lyophilized ovalbumin were dissolved in 5 ml of distilled water and placed in the digestion vessel of the Radiometer *p*H-stat. The temperature of the vessel was raised to 90°C and the *pH* of the ovalbumin solution adjusted to 4.6 using 0.24 N HCl. When the ovalbumin appeared to be completely denatured as indicated by the turbidity in the *p*H-stat vessel, the temperature was lowered to 37°C and the *p*H raised to 8.0. The remainder of the digestion procedure was identical to that used for hemoglobin.

Ion-exchange column chromatography of tryptic peptides of hemoglobin. A Technicon "Auto-Analyzer" set up for peptide analysis was used to characterize the tryptic peptides of the hemoglobins of nine species. The column was packed to 100 mm with Technicon "Chromobead" type "P" resin and equilibrated with the starting buffer. Thirteen mg of each hemoglobin digest were dissolved in 0.5 ml of the starting buffer and applied to the column. Peptides were eluted by a gradient of increasing *p*H and ionic strength using a Buchler Varigrad with the following buffers in the respective chambers: chambers 1 and 2 (90 ml each), 0.10 M citric acid-H_2O, 0.212 M NaOH, adjusted to *p*H 3.2 with 6.0 N HCl; chambers 3 and 4 (90 ml each), 0.132 M citric acid-H_2O, 0.297 M NaOH, 0.143 M sodium acetate-$3H_2O$, adjusted to *p*H 4.60 with 6.0 N HCl; chamber 5 (88 ml), 0.224 M citric acid-H_2O, 0.527 M NaOH, 0.436 M sodium acetate-$3H_2O$ adjusted to *p*H 5.0 with 6.0 N HCl; chamber 6 (87 ml), 0.428 M citric acid-H_2O, 1.0 M NaOH, 0.855 M sodium acetate-$3H_2O$ adjusted to *p*H 5.1 with 0.87 M acetic acid; chamber 7 (87 ml), 0.320 M citric acid-H_2O, 0.75 M NaOH, 1.145 M sodium acetate-$3H_2O$, adjusted to *p*H 5.25 with 0.87 M acetic acid; chambers 8 and 9 (87 ml each), 2.0 M sodium acetate-$3H_2O$ adjusted to *p*H 6.8 with 0.87 M acetic acid. The column was heated to 55°C during the elution of the peptides and the flow rate was 35 ml/hr. The Technicon peptide manifold was modified in several ways. The eluate stream was split as it emerged from the column and 75 per cent of the eluate was combined with a stream containing 4.87 M NaOH. This was passed through the Teflon coil of a Technicon hydrolysis bath and heated to 95°C for approximately 2 hr. After alkaline hydrolysis the stream was split in half and each resulting stream was buffered to *p*H 5.5 with water, glacial acetic acid, and 4.0 N sodium acetate-$3H_2O$ *p*H 5.1 (10:10:3). Each stream then was combined with ninhydrin reagent and passed through the standard length glass coil of a Technicon heating bath. The color developed during the reaction of the ninhydrin with the peptides was monitored continu-

ously at 570 mμ in a Technicon colorimeter equipped with a cuvet of standard path length (Technicon tubular flow cell).

The remaining 25 per cent of the eluate stream from the column was shunted to a manifold designed to assay for the amino acid histidine (Pauly reaction). The sample stream was sequentially combined with streams containing 2.0 M Na_2CO_3, 0.029 M sulfanilic acid in 0.5 N HCl, and 0.057 M $NaNO_2$. The extent of this reaction was monitored at 505 mμ in a Technicon colorimeter equipped with a tubular flow cell of standard path length.

Thin-layer electrophoresis (TLE) of tryptic peptides. A portion of each digest (0.5 mg) was dissolved in 0.01 ml of 0.1 N $NaHCO_3$ or distilled water and applied 3 cm from the edge of a glass plate precoated with 250 μ of Silica Gel H without UV indicator (E. Merck, Darmstadt). After heating to 110°C for 10 min, the plate was sprayed with pyridine (redistilled Mallinckrodt AR grade), acetic acid, and water (25:1:225) and placed on a Savant FP-18 flat plate electrophoresis apparatus in a cold room. Water cooled to 2°C was circulated through the cooling block. The tryptic peptides of hemoglobin were separated for 2 hr at 30 v/cm. The tryptic peptides of ovalbumin were separated for 90 min at 40 v/cm. The TLE plates were dried in a forced draft oven heated to 105°C and then sprayed with 0.4 per cent ninhydrin in n-butanol. The rate of the reaction of the peptides and the ninhydrin was increased by heating the plates in the oven at 105°C for 5 min.

RESULTS

Starch-gel electrophoresis of egg-white proteins. On the basis of starch-gel electrophoresis, the egg whites of ibises, spoonbills, storks, herons, ducks, geese, screamers, and flamingos contain fewer protein components than does the egg white of the chicken (Rhodes et al. 1958; Feeney et al. 1960; Lush 1961). The electrophoretic pattern of the egg-white proteins of the Green Heron (*Butorides virescens*) contains 12 fractions (fig. 1), the largest number detected in any of the patterns examined, whereas in that of the screamer (*Chauna torquata*) only six fractions can be seen although more may be present.

In addition to the variation observed in the total number of egg-white fractions, there also are differences in the mobilities of the major proteins in different species. In the herons (e.g. *Ardea herodias*) and the flamingos the ovotransferrins are either loosely clustered around the ovomacroglobulin (component 18) or to the cathodal side of the ovomacroglobulin (e.g. *Butorides virescens*). The ovotransferrins of ducks apparently are isoelectric at a lower *p*H and migrate in a tight grouping of 4–5 components on the anodal side of ovomacroglobulin. As expected, the lysozyme of the duck egg whites migrates cathodally at *p*H 8.0, but no lysozyme can be distinguished in the patterns of *Threskiornis aethiopica, Platalea alba, Butorides virescens, Ciconia ciconia, Ardea herodias, Phoenicopterus ruber,* or *Chauna torquata.* The heron, ibis, stork, and flamingo patterns have a protein fraction that migrates midway between ovomacroglobulin and ovomucoid. This may or may not be homologous to a fraction that migrates close to or coincident with the ovomacroglobulin of the screamer and the ducks.

The mobility of ovomucoid varies somewhat among the species and no ovomucoid fraction is detectable in the pattern of *Chauna torquata.* Likewise the mobility of ovalbumin varies from species to species, although less so than does that of ovomucoid.

It seems clear that the pattern of the flamingo in figure 1 has more aspects in common with those of the ciconiiforms than with those of the anseriforms. The apparent absence of lysozyme and the relative positions of the ovotransferrins constitute similarities shared by the ciconiiforms and the flamingo in which they differ from the anseriforms. The patterns most similar to that of the flamingo are those of *Ardea* and *Ciconia,* but this does not necessarily indicate that flamingos are closer to herons and storks than to ibises since the pattern of *Butorides,* a heron, seems more like that of *Threskiornis* than like that of *Ardea.*

Thus, as in the previous studies by Sibley (1960) and Haavie (1962), the starch-gel electrophoretic comparisons indicate that the egg-white proteins of the flamingos have more in common with those of the Ciconiiformes than with those of the Anseriformes.

Starch-gel electrophoresis of hemoglobin. The hemoglobins of 14 species were examined by starch-gel electrophoresis and these patterns are shown in figure 2. Represented are an ibis, a stork, four herons, two flamingos, two geese, two ducks, and two screamers. The hemoglobin mobilities at *p*H 8.0 vary somewhat but the overall patterns of all species are fairly similar and it is not possible to come to any conclusions about which are the most similar. It is of interest to note that the patterns of the most closely related groups of species are much alike. Thus the three anatids (*Anas, Anser, Branta*) are essentially identical to one another as are the two anhimids (*Anhima, Chauna*), the two flamingos, and the five ciconiiforms. *Guara alba* differs in mobility but resembles the storks and herons in the overall pattern.

Tryptic peptides of hemoglobins. The tryptic peptides of the hemoglobins of nine species (figures 3 and 4) were compared using ion-exchange column chromatography. The hemoglobins of a chicken, a gull, and a loon were included in figure 4 to demonstrate the mag-

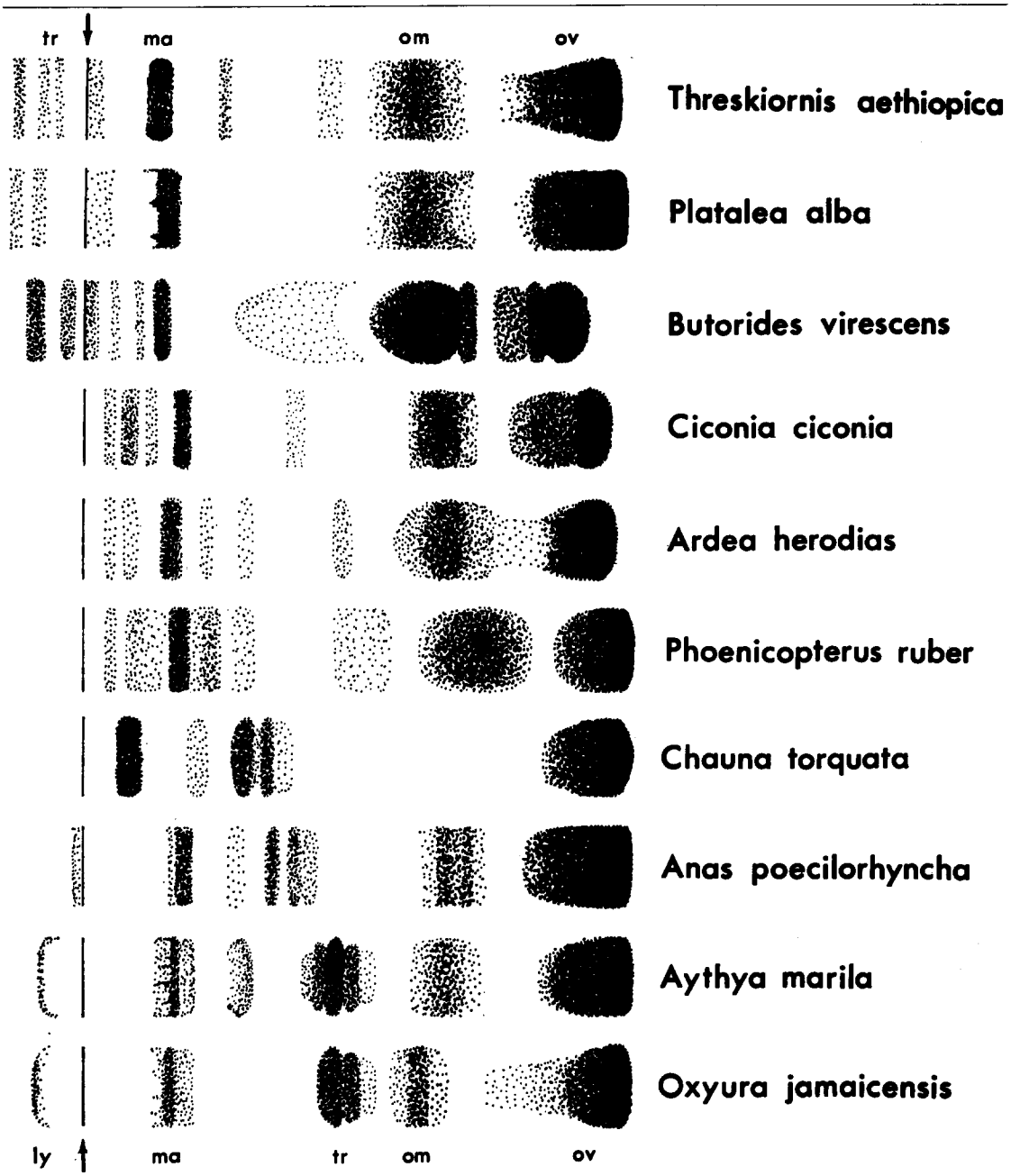

FIGURE 1. Starch-gel electrophoretic patterns of some avian egg-white proteins. Lysozyme (ly) is present only in the anatids (*Anas, Aythya,* and *Oxyura*). Ovomacroglobulin, also known as "component 18," is the dark band indicated by ma. The ovotransferrins (tr) are cathodal in *Threskiornis, Platalea,* and *Butorides,* close to the ovomacroglobulin in *Ciconia, Ardea,* and *Phoenicopterus* and more anodal in the anseriforms. Ovomucoid (om) and ovalbumin (ov) are multiple in some species. The arrows indicate the sample application point with the cathode to the left, anode to the right.

nitude of the differences among several orders of birds.

Comparisons between the chromatograms are based upon the positions of peaks, not upon the heights of peaks. Each peak or "shoulder" indicates the presence of a tryptic peptide and it is assumed that peaks at identical positions are produced by identical peptides. This assumption is reasonable for purposes of comparison but it is probably not valid unless the two species being compared are known to be related. However, if two chromatograms are similar enough so that most of the peaks in one can be seen to have counterparts in the other, it is reasonable to assume that the counterparts are homologus

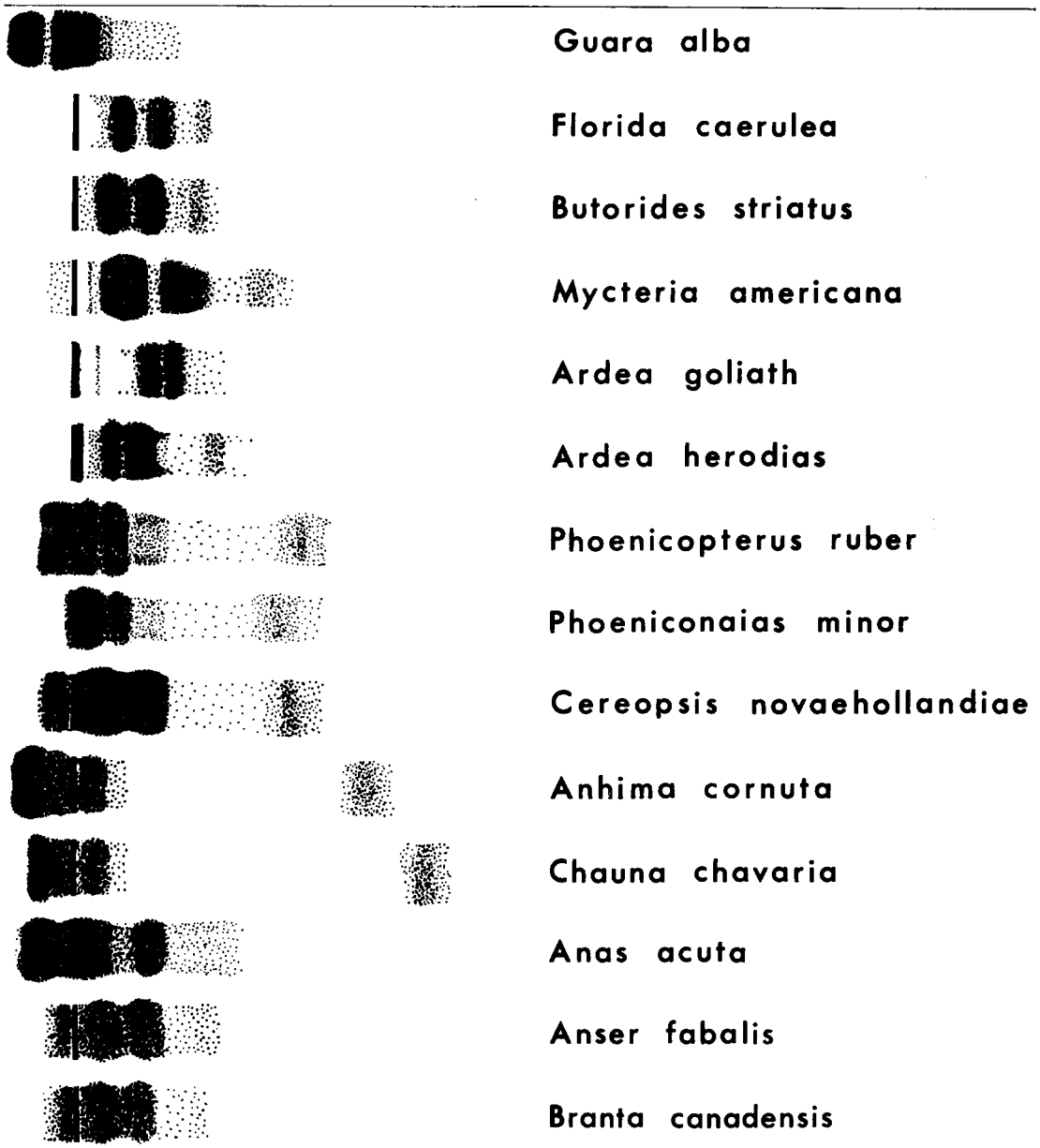

FIGURE 2. Starch-gel electrophoretic patterns of some avian hemoglobins. The sharp, solid vertical line indicates the application point. From the top, *Guara* to *Ardea* are Ciconiiformes, *Phoenicopterus* and *Phoeniconaias* are flamingos, *Anhima* to *Branta* are Anseriformes.

peptides. The original chromatograms are nearly 6 ft long and contain more detail than can be incorporated in the curves as re-drawn for publication. For this reason it may be difficult to make precise comparisons among the curves in figures 3 and 4. However, the following discussion is based upon the original curves and we have been conservative in estimating differences and similarities.

First, it is important to establish that closely related species have similar peptide patterns. This is demonstrated by comparisons between the two herons and among the three anseriforms in figure 3. The chromatogram of *Ardea purpurea* differs from that of *A. goliath* by only one or possibly two peptides at positions corresponding to eluate volumes of 425 ml and 575 ml (fig. 3). They are thus virtually identical and the differences could be due to a single amino acid substitution.

Similarly, the chromatograms of *Anas erythrorhyncha, A. platyrhynchos,* and *Branta bernicla* differ from one another by very few peptides. In contrast to these similar patterns in closely related species it is impossible to identify homologous peptides when the chro-

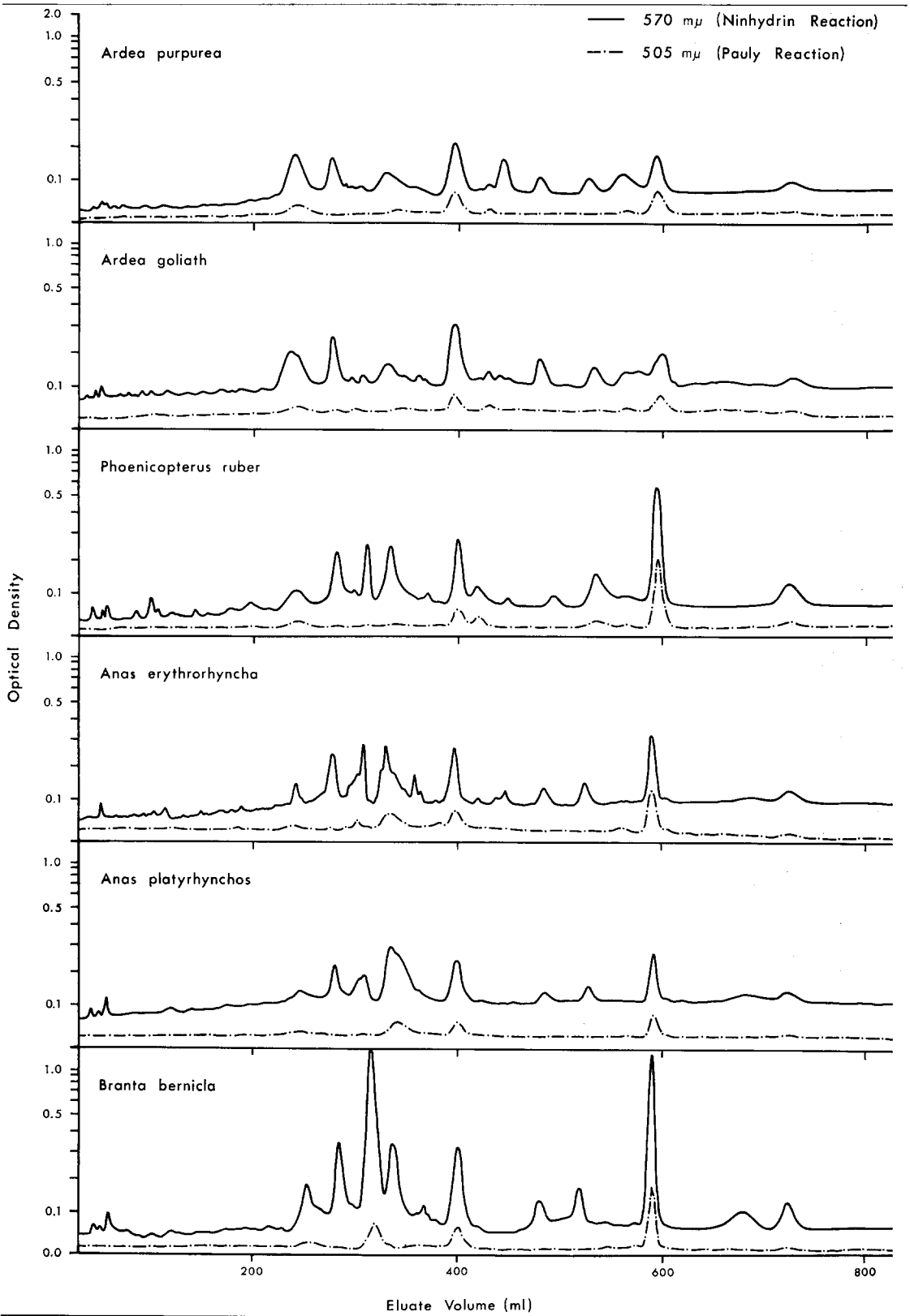

FIGURE 3. Chromatograms of the tryptic peptides of some avian hemoglobins. The ninhydrin reaction (solid line) detects all peptides, the Pauly reaction (broken line) detects only those peptides containing histidine.

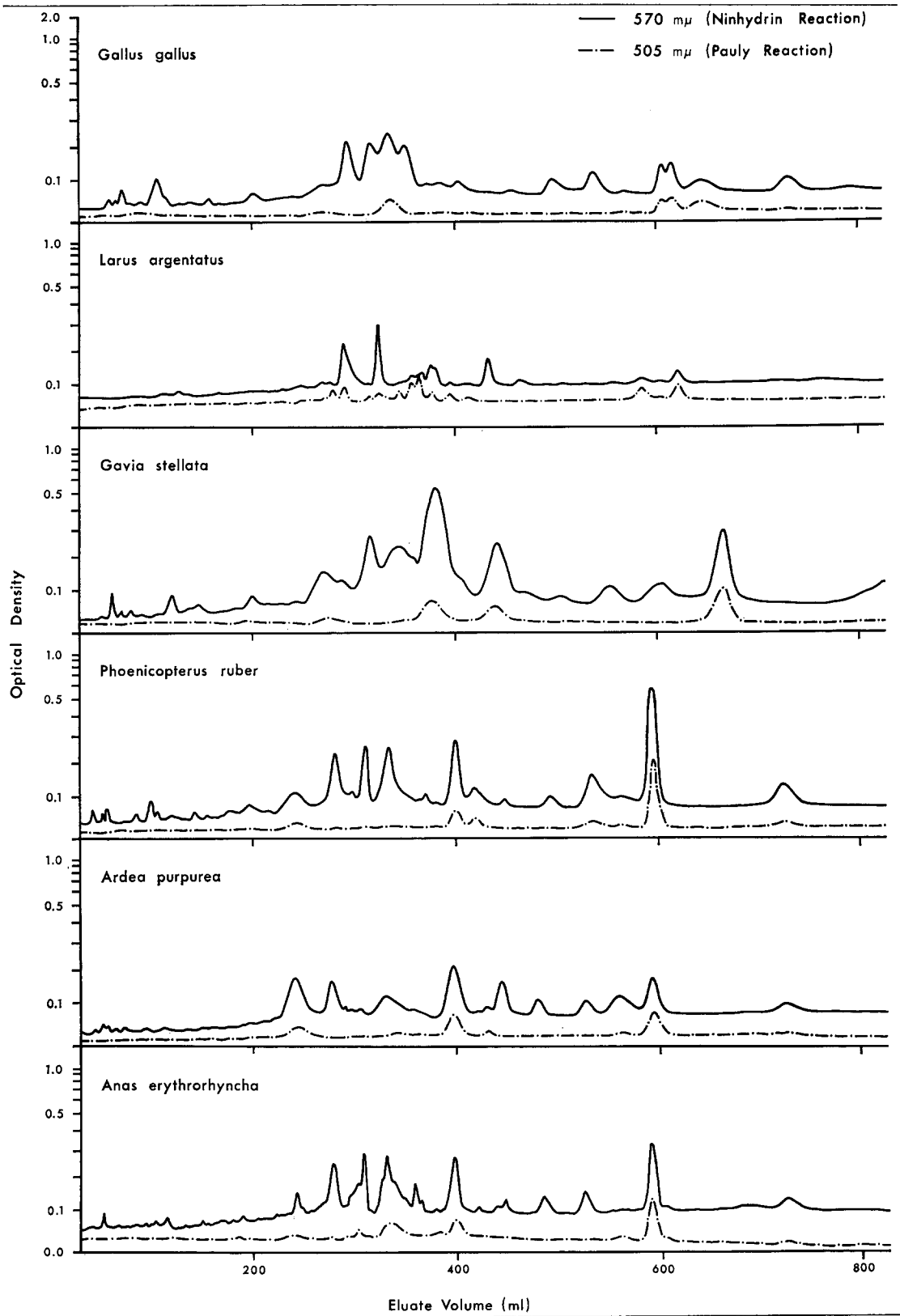

FIGURE 4. Chromatograms of the tryptic peptides of some avian hemoglobins. See legend of figure 3.

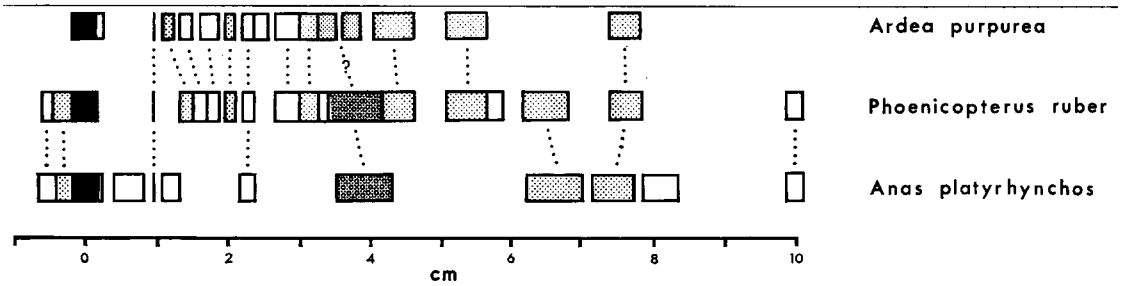

FIGURE 5. Thin-layer electrophoretic patterns of the tryptic peptides of some avian hemoglobins. The application point is the black rectangle at 0 cm. The cathode is to the right. The dotted lines connect presumably homologous peptides. The different densities are approximately proportional to the intensity of the ninhydrin staining reaction in the original analysis.

matograms of unrelated species are compared. Thus, in figure 4, the patterns of *Gallus, Larus,* and *Gavia* differ greatly from one another and from those of *Phoenicopterus, Ardea,* and *Anas.* Figures 3 and 4 also show clearly that the hemoglobins of *Ardea, Phoenicopterus,* and the anatids are quite similar to one another. A detailed study of the original chromatograms has shown that the hemoglobins of *Ardea* and *Phoenicopterus* have at least 17 tryptic peptides in common and differ by a minimum of six peptides. The hemoglobins of *Anas* and *Phoenicopterus* have at least 14 tryptic peptides in common and differ by at least eight.

From these data we conclude that herons, flamingos, and ducks have hemoglobins more similar to one another than to galliform, charadriiform, or gaviiform birds and that the hemoglobins of flamingos are more similar to those of herons than to those of ducks and geese.

Thin-layer electrophoresis of the tryptic peptides of hemoglobin. Tryptic digests of the hemoglobins of a heron, a flamingo, and a duck were compared, and a diagram of the patterns is shown in figure 5. The pattern of *Phoenicopterus* contains at least 19 peptides, that of *Ardea* contains 15, and that of *Anas,* 12. It seems clear that some peptides in *Ardea* and *Anas* were not detected since the ion-exchange chromatography data (figs. 3 and 4) show fewer differences. Thus a detailed comparison of the thin-layer electrophoretic patterns is not trustworthy. However, the pattern

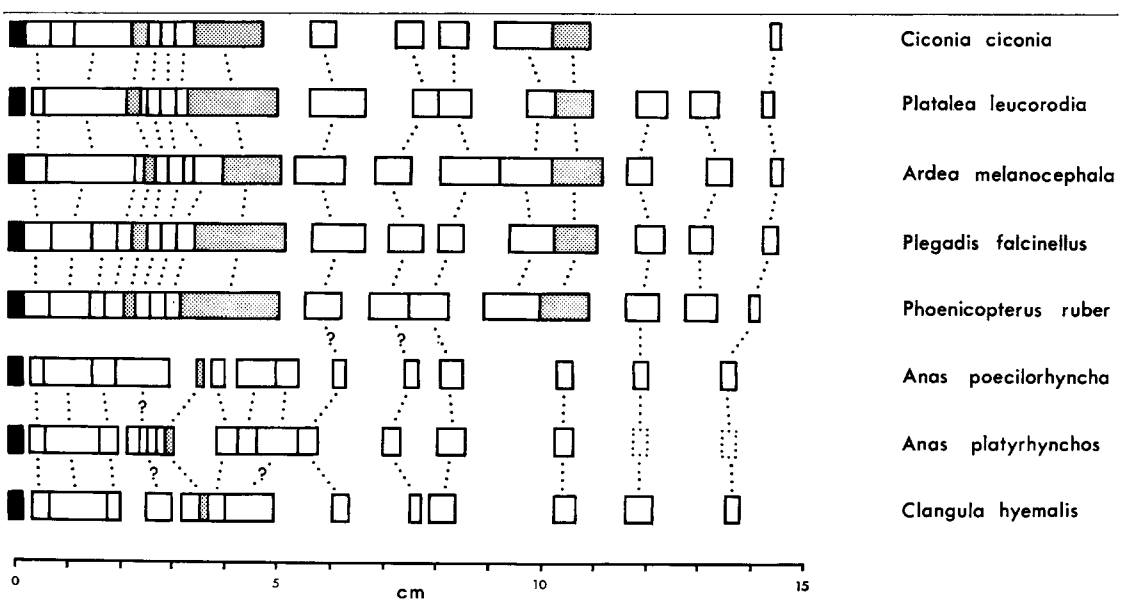

FIGURE 6. Thin-layer electrophoretic patterns of the tryptic peptides of some avian ovalbumins. The application point is at 0 cm and the cathode is to the right. The dotted lines connect presumably homologous peptides. The different densities are approximately proportional to the intensity of the ninhydrin staining reaction in the original analysis.

of the flamingo does have 12 peptides in common with that of the heron and only eight in common with that of the duck.

Thin-layer electrophoresis of the tryptic peptides of ovalbumin. Tryptic digests of the purified ovalbumin fraction from the egg whites of four ciconiiforms, a flamingo, and three ducks were compared and a diagram of the patterns is shown in figure 6. These patterns reval a high degree of similarity among the ciconiiforms and *Phoenicopterus* but many differences between *Phoenicopterus* and the ducks. The patterns of *Phoenicopterus, Plegadis,* and *Ardea* are nearly identical and those of *Platalea* and *Ciconia* only slightly less so. Between *Phoenicopterus* and *Plegadis* it is possible to be confident that there are at least 17 homologous peptides or groups of peptides, while between *Phoenicopterus* and *Anas* only three, or possibly five, peptides can be considered to be homologous.

These data from the tryptic peptides of the ovalbumins offer some of the strongest evidence available of a closer relationship between the flamingos and the Ciconiiformes than between flamingos and the Anseriformes.

SUMMARY AND CONCLUSIONS

A review of the literature concerning the classification, fossil history, morphology, parasites, life history, behavior, and comparative studies of certain proteins leads to the conclusion that the flamingos, Ciconiiformes, and Anseriformes are related to one another. Furthermore, the weight of evidence from several sources indicates that the flamingos are closer to the Ciconiiformes than to the Anseriformes.

The fossil evidence is frequently difficult to interpret, but it shows that the divergence among the three groups occurred in the Cretaceous and that they probably shared a common ancestor.

The anatomical evidence is also difficult to interpret, but the flamingos share more characters with the Ciconiiformes than with the Anseriformes.

The evidence from parasites is conflicting, and firm conclusions about the relationships of the hosts based upon the relationships of the parasites may well be impossible. Flamingos share more Mallophaga with Anseriformes than with any other group, but whether this is due to close host relationship or a result of convergent similarities in the plumages of the two groups of birds is uncertain. The tapeworms of flamingos are related to those of Charadriiformes, thus presenting further problems of interpretation.

Comparisons of the life histories and behavior of the three groups of birds presents a bewildering array of observations which do not consistently support any one of the possible patterns of relationship. It seems possible to conclude only that as each group has evolved along divergent adaptive pathways they have modified their behaviors and life histories until it is now impossible to be confident that similar movements are homologous, and even difficult to identify similar patterns of behavior.

Comparative studies of proteins using a variety of techniques have been consistent in indicating (1) that flamingos, Ciconiiformes, and Anseriformes are related to one another and (2) that the flamingos and Ciconiiformes are closer to one another than either is to the Anseriformes.

Although the degree of relationship remains to be expressed in more precise terms, we suggest that the flamingos be treated as a suborder, Phoenicopteri, in the Order Ciconiiformes and that, in a linear list, the Anseriformes and Ciconiiformes be placed adjacent to one another as in the classification of Wetmore (1960).

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