TAXONOMIC RELATIONSHIPS OF SOME *COLUMBA* SPECIES

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The taxonomic relationships within the pigeon genus *Columba* remain uncertain despite the considerable attention accorded this group of species. Ridgway (1916) placed 14 of the New World species in 10 genera to emphasize their morphological differences. Peters (1937) disagreed with these assignments, combined Ridgway's genera, and placed 52 species in the genus *Columba*. Cumley and Irwin (1944), Irwin (1953), and Irwin and Miller (1961) studied the blood cell antigens of 11 species of *Columba* and concluded that each of the six American species of the genus tested was more closely related serologically to one of the other five than to any one of the five Old World species examined. Miculicz-Radecki (1949) published a partial phylogeny of the genus based on studies of plumage pattern and coloration. She proposed that certain New World species are most closely related to the ancestral stock of the genus. The phylogeny of the genus prepared by Boetticher (1954) is more extensive than that of Miculicz-Radecki, places certain New World species closest to the ancestral line, derives the Old World species from two major lines of descent branching from New World types, and gives subgeneric status to each of Ridgway's New World genera. Verheyen (1957) placed 49 species into 11 genera on the basis of his osteological data. This action resulted in a complete separation of the Old and New World species. The large genus *Columba* was retained by Goodwin (1959) who examined the color patterns of the 52 species in the genus as designated by Peters. However, Goodwin proposed the division of the genus into four subgenera, one of which he further subdivided into five species-groups (this term is defined by Cain 1954). The first four species-groups and the subgenera *Turturoena* and *Nesoenas* contain the Old World species while those of the New World are placed in the fifth species-group and the subgenus *Oenoenas*. Goodwin's illustration of the relationships within the genus shows four major lines of descent from the ancestral type. Sibley (1960) and Corbin (1965) examined the egg-white proteins of five and 10 *Columba* species, respectively, using the techniques of paper and starch-gel electrophoresis. These studies revealed species differences in the electrophoretic mobilities of the ovalbumins that could be correlated with the Old and New World distribution of the species. An examination of the angle on the skull at the frontal hinge and a study of the structure along the trailing edge of the tenth primary indicated to Johnston (1962) that the genus should be split into three genera. The result was a nearly complete separation of the Old and New World species.

The results of several other investigations are of value for a clarification of the pigeon relationships. These include the studies of hybrid formation (Cavazza 1931; Ghigi 1932; Taibel 1934, 1935, 1947; Hertwig 1936; Frank 1940; Tomek 1958; Creutz 1961), additional serological studies (Irwin and Cole 1936; Irwin *et al.* 1936; Cumley *et al.* 1942, 1943; Irwin and Cumley 1942, 1943; Miller 1953; Cavalli-Sforza *et al.* 1954, 1955; Bryan and Irwin 1961), and observations on distribution, ecology, behavior, or plumage (Cumley and Cole 1942; Cumley 1943; Miculicz-Radecki 1950; Boetticher 1960; Goodwin 1960, 1964, 1966; Harrison 1960; Vaurie 1961; Murton and Isaacson 1962; Peeters 1962; Pitwell and Goodwin 1964).

It is evident that there is still a lack of agreement concerning those species that should be included in the genus *Columba*. In this paper *Columba* is synonymous with the comprehensive genus proposed by Peters. Evidence in support of this position will be presented.

The ovalbumin tryptic peptide maps of 18 pigeon species including 10 species of *Columba* are shown. For comparative purposes the peptide maps of chicken and Mallard ovalbumins also are included. The genetic relationships of the 10 *Columba* species examined have been inferred using the similarities and differences among the peptide maps as a basis for comparison. The relationships so indicated are compared with previous classifications of the genus.

MATERIALS AND METHODS

Baker and Manwell (1962) observed that the catalatic activity of chicken egg white was coincident with the ovalbumin components after electrophoresis in starch-gels. But Corbin and Brush (1966) showed that catalase is not present in egg white and that the

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catalaselike activity is an artifact. This finding and electrophoretic tests of purity indicated that purified ovalbumins suitable for peptide analyses could be obtained by the techniques of ammonium sulfate equilibration and ion-exchange chromatography. The basic techniques have been described by Kekwick and Cannan (1936), Cohn *et al.* (1940, 1946), Sober *et al.* (1954), Peterson and Sober (1956), and Feeney *et al.* (1960). The purification scheme used throughout this study is outlined below.

Ovalbumin was partially isolated from the other egg-white proteins by salting-out with ammonium sulfate and then purifying by ion-exchange column chromatography. The ovalbumin crystals obtained in the salting-out step were dissolved in distilled water, the salts removed by dialysis against distilled water and Amberlite MB-3, and the ovalbumin solution dialyzed against the starting ion-exchange buffer. That buffer was composed of 0.02 M glycine, 0.017 M K_2HPO_4, 0.029 M KH_2PO_4 and adjusted to *p*H 6.0 with 0.1 N HCl. After the final period of dialysis, the sample was applied to a diethylaminoethyl (DEAE)-cellulose column. Ovalbumin and the protein contaminants, ovotransferrin and ovomucoid, were eluted from the column by a gradual *p*H gradient that dropped from 6.0 to 3.5 over a volume of 450 ml. The gradient was produced and maintained by a Buchler Varigrad mixer. A Technicon autoanalyzer proportioning pump was used to maintain a constant flow rate during the elution, and the eluate was monitored and collected by a Gilson Medical Electronics fraction collector. The UV absorption curve (at 280 mμ) was recorded by a Texas Instruments Recti/riter.

The fractions included in the ovalbumin portion of the optical density curve were pooled, dialyzed against distilled water and Amberlite MB-3, lyophilized and stored in rubber-capped serum bottles at –60° C if not used immediately. An aliquot of the chromatographic fractions was examined by starch-gel electrophoresis using a discontinuous buffer system (Ashton and Braden 1961; Ferguson and Wallace 1961) to monitor the progress of ovalbumin purification. The ovalbumin was considered to be free from contamination if the electrophoretic pattern did not contain other protein fractions. If contaminants were present, the sample was rechromatographed on a DEAE-cellulose column. It is possible that immunological techniques might have demonstrated the presence of other protein components that were present in amounts too small to be detected by the Amido Black 10B protein stain. However, the tryptic peptides of such trace proteins would not be detectable on a peptide map because of their low concentrations.

The final procedures involved the digestion of the purified ovalbumins with trypsin and the comparison of these tryptic digests by two-dimensional thin-layer chromatography and electrophoresis. Prior to each digestion, the ovalbumins were reduced with sodium borohydride and alkylated with iodoacetamide following the procedure of Brown (1960). Two-times crystallized trypsin (Worthington Biochemical Corporation, Freehold, New Jersey) was used to digest each sample of reduced and alkylated ovalbumin. The ratio of trypsin to ovalbumin was 1:100, and the digestion, performed under a nitrogen atmosphere in a semi-automatic *p*H stat at *p*H 8.0 and 38° C, was complete at the end of 2.5 hours. The sample was acidified to *p*H 2.0 and then lyophilized.

Two-dimensional thin-layer chromatography and electrophoresis on Silica Gel G was used to produce the peptide maps. A description of a thin-layer technique similar in many respects to that used in this study has been reported by Ritschard (1964), Stegemann and Lerch (1964), and Wieland and Georgopoulos (1964). These papers were published after some of the work reported here had been completed. Some of the differences in the techniques are noted below.

To insure that separation distances were equivalent from one thin-layer plate to another, 5 μl of Gelman RBY dye were spotted onto the corner of each thin-layer plate prior to electrophoresis. A pyridine, acetic acid, and water buffer (1 : 10 : 289) were used for the electrophoretic separation, which was effected by a constant voltage of 40 volts/cm for 30 minutes. After electrophoresis, the plates were dried in a forced draft oven preheated to 105° C. The peptides were chromatographed in the second dimension using a solvent composed of *n*-butanol, acetic acid, and water (13: 2 : 5). After the primary solvent front had migrated 12 cm from the point of sample application, the thin-layer plate was placed in a hood and partially dried before spraying with a ninhydrin reagent (0.4 per cent ninhydrin in ethanol, water, and collidine, 20: 5 : 1). Maximum color development was obtained by placing the sprayed plate in the oven for 10 minutes. When the ninhydrin spots were fully developed, the plate was cooled and placed on a light box where the spots could be outlined by dotting their margins with a pin-tipped stylus. The plate was next sprayed with a modified Ehrlich's reagent (1 per cent *p*-dimethylaminobenzaldehyde in ethanol, acetone, and HCl, 30 : 15 : 2). This combination reduced the flaking of the Silica Gel G without decreasing the intenstiy of the purple color produced by the reaction of the reagent with tryptophan. Those peptides that contained tryptophan were marked and the peptide pattern recorded by using the plate itself as a negative to produce a contact print of the peptide map. Each ovalbumin sample was digested two or more times and at least three peptide maps were prepared from each digest. The peptide map of each species examined is a tracing of one of the original patterns and not a composite of the six or more replicates.

TRYPTIC PEPTIDE MAPS

Chicken ovalbumin has a molecular weight of approximately 47,000 and may have zero, one, or two phosphoric acid groups complexed with the protein moiety (Linderstrøm-Lang and Ottesen 1949; Perlmann 1950, 1952). It contains 15 residues of lysine, 20 residues of arginine, and three residues of tryptophan, and the total number of amino acid residues is approximately 400 (Fevold 1951). Comparable information is not yet available for the ovalbumins of the columbid species.

Since trypsin splits protein molecules at the carbonyl peptide bonds of lysine and arginine, a knowledge of the number of residues of these amino acids in the protein being digested makes it possible to predict the number of peptides that should be present after digestion. But ninhydrin does not react equally well with all peptides. Generally, the larger the peptide, the poorer the reaction. Consequently, not all peptides in a digest mixture

TABLE 1. Peptide similarities and differences obtained from figures 1–4. The lower left portion of the table lists the number of peptides that are identical for the respective pairs of species. The upper right portion lists the number of peptides by which the ovalbumins of species pairs differ. The numbers in parentheses on the diagonal equal the total number of peptides in the maps of the respective species.

Species		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)
Columba																			
leucocephala	(1)	(25)	3	4	8	10	9	7	9	13	11	8	12	14	12	12	14	14	16
cayennensis	(2)	22	(22)	3	7	9	8	6	6	10	12	11	13	13	11	13	13	13	13
maculosa	(3)	22	21	(23)	6	12	9	9	7	11	11	10	14	16	12	16	16	16	16
fasciata	(4)	22	20	21	(26)	8	11	9	8	13	9	9	11	18	10	14	15	15	14
flavirostris	(5)	19	18	17	20	(24)	15	11	13	13	11	12	12	14	12	14	13	15	16
picazuro	(6)	20	19	19	19	16	(25)	8	10	8	10	9	13	12	12	11	11	11	15
palumbus	(7)	21	20	19	20	18	21	(25)	8	8	8	11	13	11	10	9	11	7	15
guinea	(8)	18	18	18	19	15	17	18	(21)	6	11	9	10	14	11	12	13	12	12
oenas	(9)	16	16	16	16	15	19	19	18	(22)	8	11	14	12	12	7	8	10	13
livia	(10)	19	16	17	20	17	19	20	16	18	(24)	6	10	13	10	9	11	10	13
Leptotila																			
rufaxilla	(11)	21	18	19	21	18	20	19	18	17	21	(25)	6	14	8	11	12	10	11
Columbigallina																			
talpacoti	(12)	18	16	16	19	17	17	17	17	15	18	21	(23)	12	8	15	15	14	10
Zenaida																			
macroura	(13)	17	16	15	15	17	20	19	14	16	17	17	17	(26)	11	13	12	9	16
Streptopelia																			
decaocto	(14)	19	18	18	20	18	19	20	17	17	19	21	20	19	(25)	15	15	11	10
Phaps																			
chalcoptera	(15)	18	16	15	18	16	19	20	16	19	20	19	16	17	17	(25)	9	10	14
Chalcophaps																			
indica	(16)	17	16	15	16	18	19	19	16	20	18	18	16	19	17	20	(26)	13	10
Gallicolumba																			
luzonica	(17)	18	17	16	18	17	19	21	16	18	20	20	17	21	20	20	19	(26)	15
Ducula																			
goliath	(18)	18	18	17	20	17	18	18	19	18	19	21	21	17	21	19	21	19	(29)

necessarily will be detected. However, because of the specificity of trypsin, homologous peptides will or will not be detected as the ovalbumins of related species are examined.

Those peptides that are detected in the map of one species will be homologous to those detected in the map of another species if the species are closely related. The more distantly related the species being compared, the greater is the probability that amino acid substitutions will have altered the number of lysine residues, arginine residues, or both. Each time an additional lysine or arginine is effectively substituted for one of the other 18 amino acids, an additional peptide will be present in a tryptic digest mixture of that protein. One of the peptides of the prototype will now be split into two peptides. Thus, in comparing tryptic peptide maps, a single peptide in one map occasionally may be homologous to two peptides in the map of another species.

Ideally, the complete amino acid composition of each peptide would provide the basis for determining peptide homologies (sequence of amino acids would be necessary in some cases). Another method takes advantage of specific amino acid reagents in conjunction with the resolving power of the two-dimensional thin-layer system. Since the two-dimensional technique potentially can detect single amino acid substitutions, two homologous peptides that differ by one or more amino acids should migrate to different positions in their respective maps. If in the comparison of two peptide maps only one peptide of each map is in a unique position, it is logical to conclude that these two peptides are homologous. If two or more peptides of a map are in unique positions, it is virtually impossible to designate their homologues in other maps when amino acid compositional data are not available. Therefore, peptides in the maps shown below are labelled or lettered alike only if they are in identical positions in their respective patterns. This procedure will not be reliable if the techniques of analysis are a source of nongenetic variation. This possibility has been studied and detailed elsewhere (Corbin 1967). It was found that peptide 17′ may not be detected because of the similarity of its electrophoretic and chromatographic properties to those of peptide 17. Otherwise the variation caused by the techniques of analysis (reduction, alkylation, digestion, electrophoresis, and chromatography) is not considered appreciable.

Representative ovalbumin tryptic peptide maps of the 20 species examined are shown in figures 1–4. Because nonidentical peptide homologues that migrate to different positions in their respective maps cannot be identified by the techniques used in this study, such peptides will be assigned different numbers

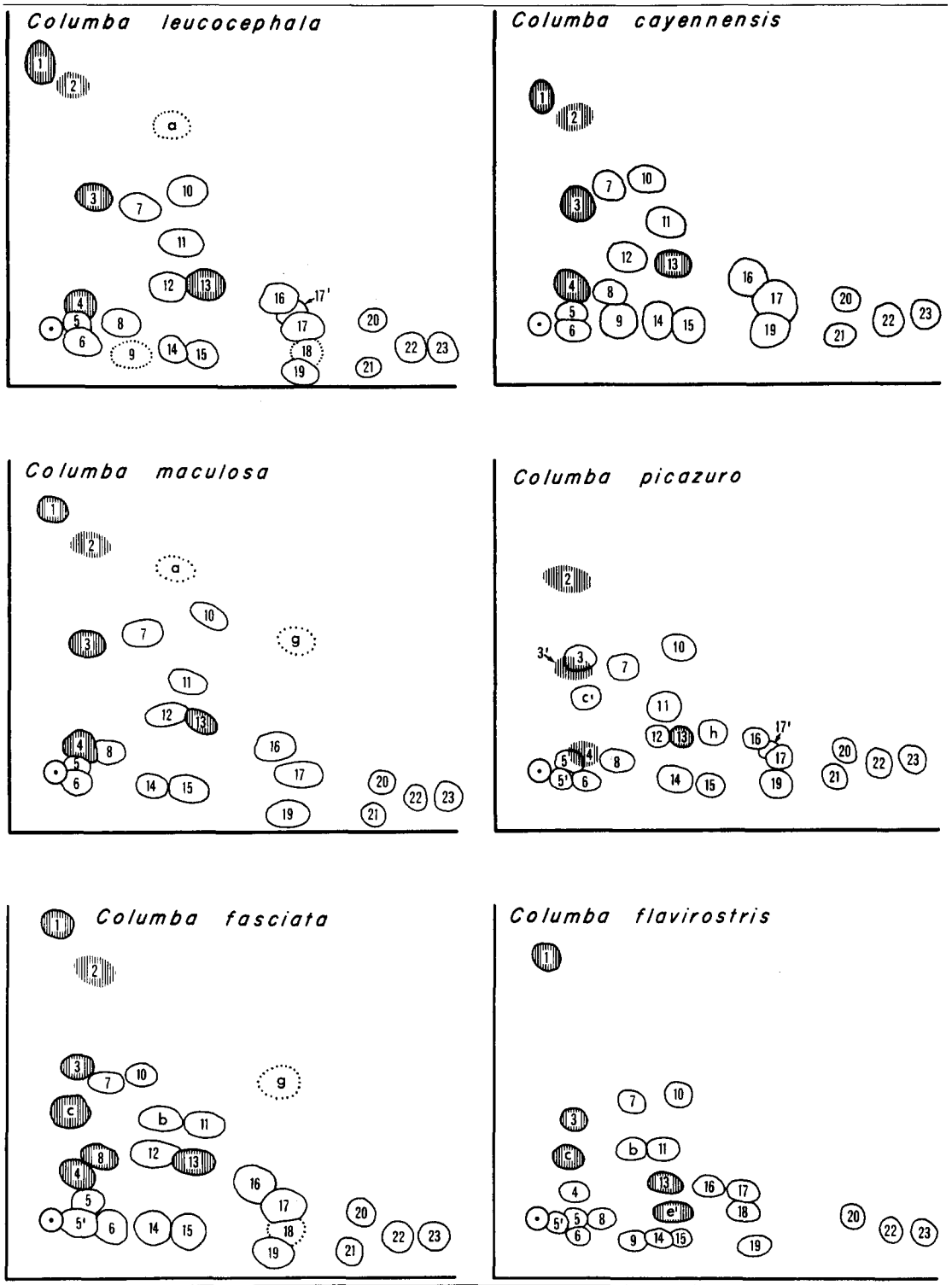

FIGURE 1. Tryptic peptide maps of ovalbumins of the respective species. Peptides were separated electrophoretically in the horizontal dimension and chromatographically in the vertical dimension. Peptides circled by a solid line reacted with ninhydrin. Peptides circled by a dotted line showed very little color after reaction with ninhydrin. Shaded peptides reacted with a reagent specific for the amino acid tryptophan.

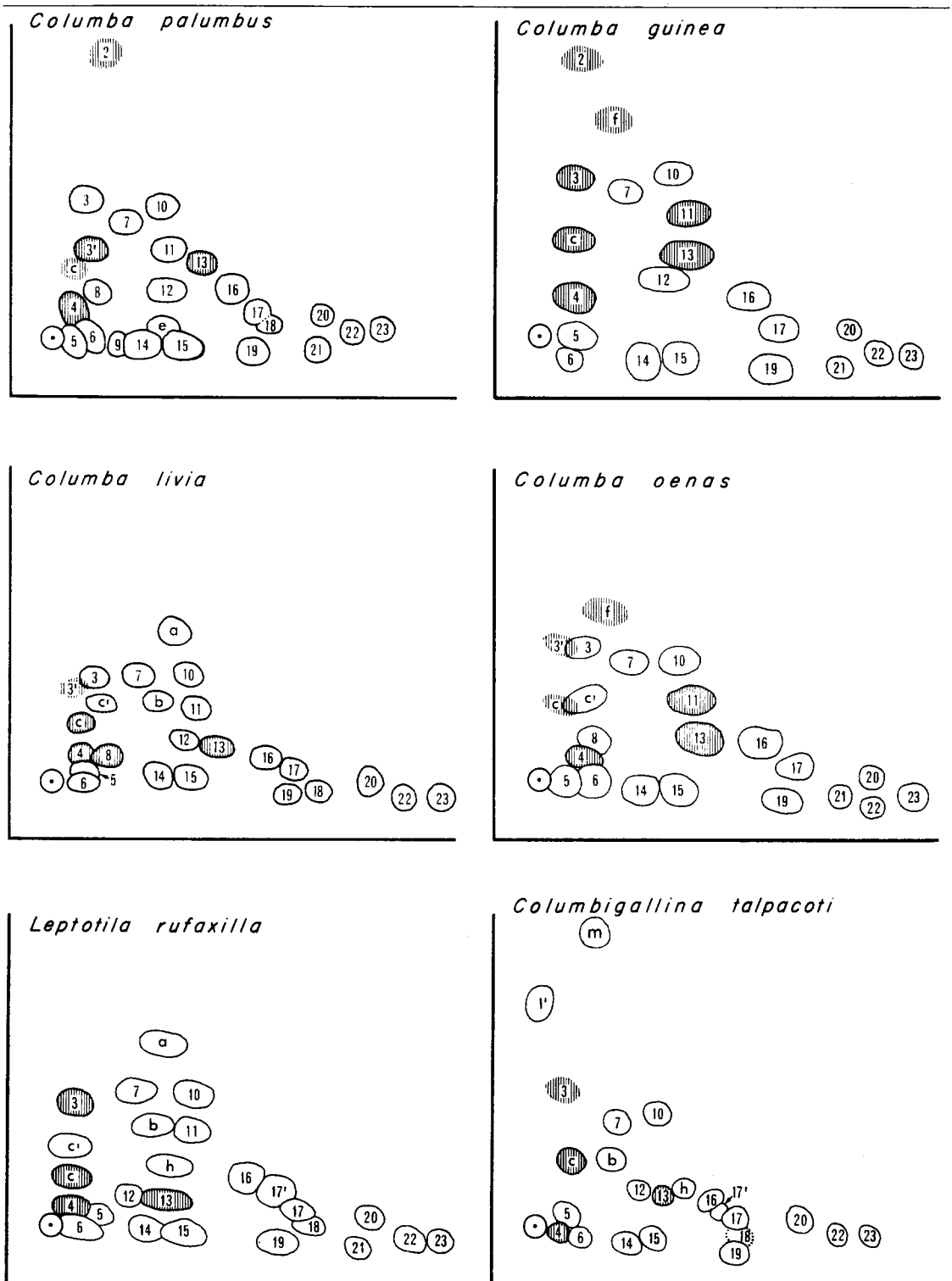

FIGURE 2. Tryptic peptide maps of ovalbumins of the respective species. Identifications as in figure 1.

or letters. This technique has the disadvantage that it sometimes underestimates the differences between peptide maps. If two homologous peptides differ by more than one amino acid residue, the additional alterations cannot be detected. That is, homologous peptides that differ by one or more amino acids will appear in different positions in their respective maps, but their quantitative differences cannot be determined. This should

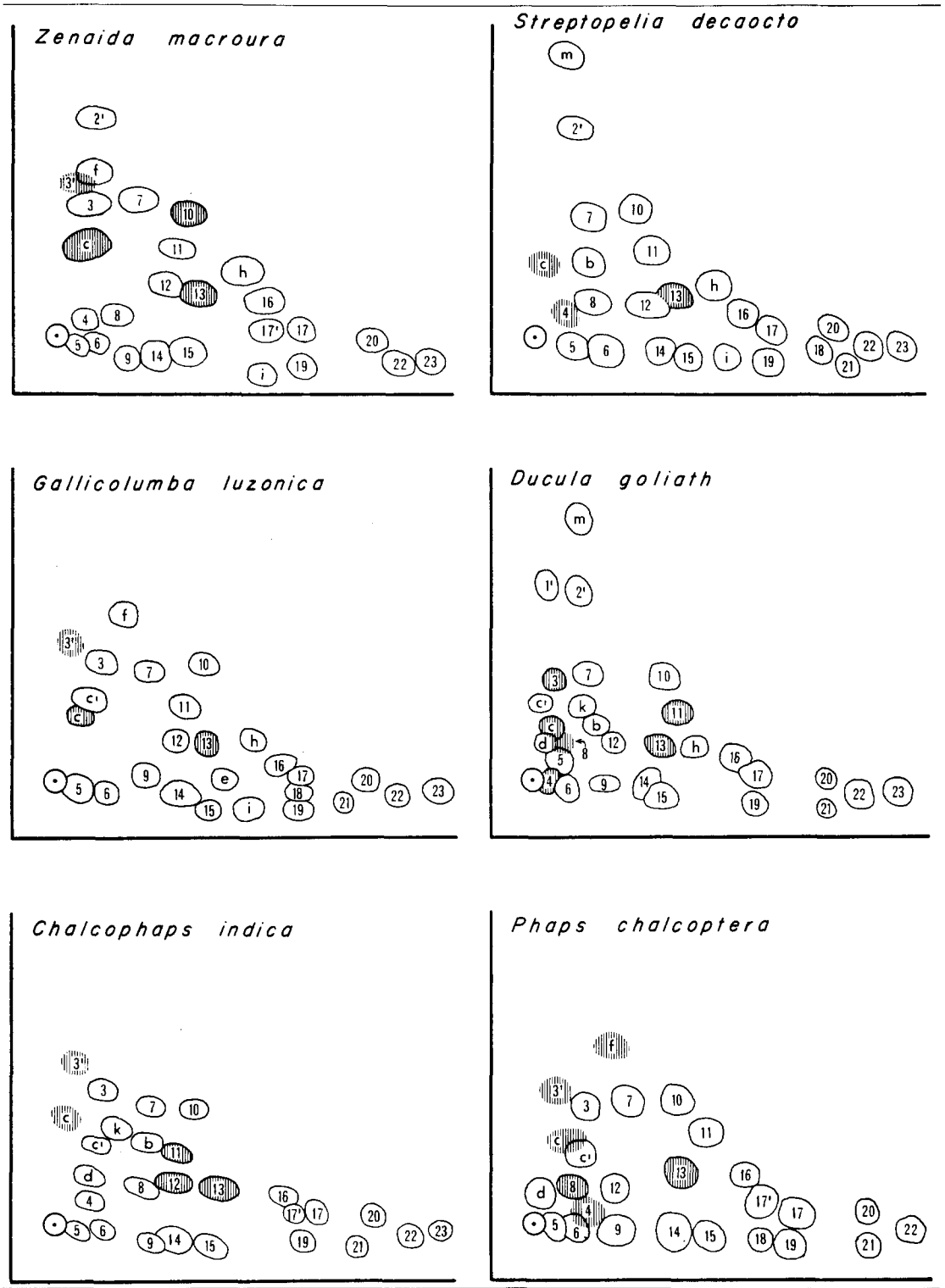

FIGURE 3. Tryptic peptide maps of ovalbumins of the respective species. Identifications as in figure 1.

not be a serious problem unless the species being compared are distantly or not at all related. For species as closely related as the 10 species of *Columba* it is reasonable to assume that where differences occur among homologous peptides, they will be of the order of one amino acid per peptide.

A summary of the peptide map data is given in table 1. (The peptide map data of the chicken and Mallard ovalbumins could

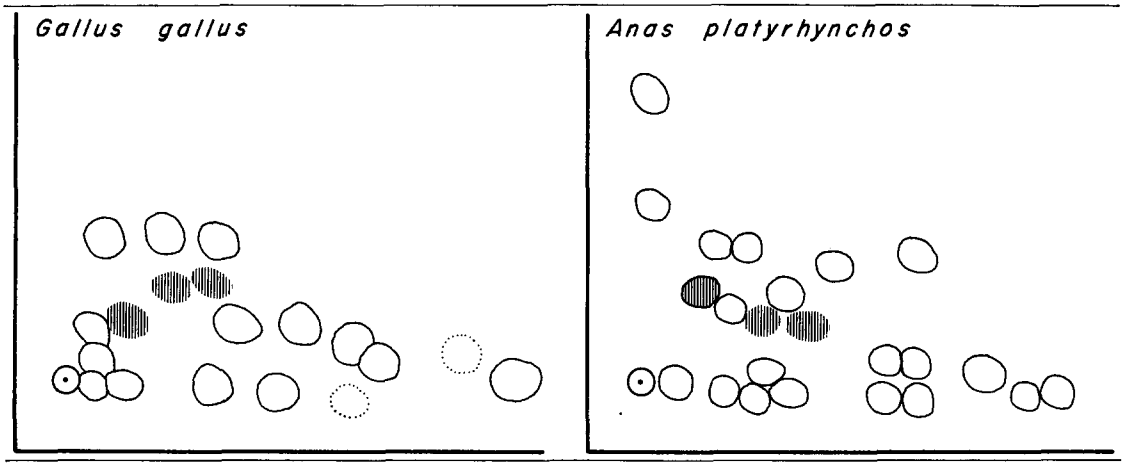

FIGURE 4. Tryptic peptide maps of ovalbumins of the respective species. Identifications as in figure 1.

not be summarized because none of the peptide homologies of these maps could be determined.) A peptide in one map is considered to be both homologous and identical to a peptide in another map only if both peptides are in the same positions in their respective maps and they must either both contain or both lack a tryptophan residue. Elsewhere I have shown how these data can be used to reconstruct the evolutionary history of the ovalbumins of 10 species of *Columba* (Corbin 1967). Since it is assumed that the evolutionary history of a selected group of ovalbumin molecules parallels the evolution of the species that produce them, these data also provide a basis for a discussion of the taxonomic relationships of these species.

TAXONOMIC IMPLICATIONS

In the discussion that follows, the relationships indicated by the peptide map data will be compared with the phylogenies of Miculicz-Radecki (1949:58), Boetticher (1954:63), and Goodwin (1959:17). Johnston (1962) would probably arrange these species in a scheme similar to that of Goodwin except that they would be placed in three genera and the *palumbus* species-group would contain *fasciata, araucana,* and *caribaea.* It is difficult to determine how Verheyen (1957) might have arranged his 11 genera.

Each of these arrangements of the species of *Columba* is unique in part. One point of disagreement concerns the origin of the species-groups of the Eastern and Western Hemispheres. The phylogeny prepared by Boetticher places the New World species, *cayennensis* and *flavirostris* in particular, closest to the origin of the genus and evolves the African and Eurasian species from those of South America. In the scheme of Miculicz-Radecki all the species of *Columba* evolve either directly or indirectly from *cayennensis* (= *rufina*). Goodwin's arrangement shows four major lines of evolution, one of which gives rise to the American species. None of the African or Eurasian species evolve from those of the New World.

The serological work of Cumley and Irwin (1944) and Irwin and Miller (1961) indicates that the species-specific antigens of the blood cells are more similar within the Old and New World species-groups of *Columba* than between these groups. This separation of Old and New World lines of *Columba* evolution also agrees with the evidence obtained from the electrophoresis of egg-white proteins (Sibley 1960; Corbin 1965). Of the columbid species examined, only the Old World species of *Columba* showed an electrophoretic pattern in which the ovalbumin fraction is juxtaposed to the ovomucoid fraction. Although this may be due to changes in only a few amino acid residues, this difference between the species-groups is consistent. If speciation has occurred in the manner indicated by the peptide data, a change of this type could have occurred after a divergence of the Old from the New World lines of *Columba* evolution was complete. This supposition is supported by the fact that the electrophoretic patterns of the American species of *Columba* are more similar to other columbid species, with respect to the ovomucoid region, than to the Afro-Eurasian species of *Columba.*

The peptide data suggest a common ancestry for the American and Afro-Eurasian species followed by speciation in the separate hemispheres. Of the species examined, *cayennensis* is presumably most similar to the ancestral type. This placement of *cayennensis* is consistent with the arrangements of Boetticher

and Miculicz-Radecki, but differs in that the Old World species are not evolved directly from those of the Western Hemisphere. In this respect the peptide data agree with the arrangement proposed by Goodwin.

Johnston and Verheyen advocate splitting the genus *Columba* as delineated by Peters (1937) into three and 11 genera, respectively. However, each agrees that there is little doubt the genus as it now exists is a monophyletic assemblage of species, and that the species of the genus are more closely related to one another than any one of them is to some other columbid species. This being the case, how does generic splitting better indicate the evolutionary relationships of these species? It seems the opposite is true and that the subgeneric stratification proposed by Goodwin, or a modification thereof, is to be recommended. If the peptide data are correct, there is evidence that some of the species of each hemisphere may not be most closely related to species in their own hemisphere, *e.g.*, *picazuro* is closest to *palumbus, palumbus* is closest to *cayennensis,* and *guinea* is equidistant from *cayennensis* and *oenas.* If these are the proper genetic relationships, then the genus should not be split.

The origin of the genus *Columba* is uncertain, and no recent author has dealt explicitly with this problem. An ancestral type that had a New World distribution presumably would be the choice of Miculicz-Radecki and Boetticher. Whitman (1919) thought the genus might have evolved from a *Streptopelia* lineage. Although a considerable number of hybrids between various columbid species have been described, only 10 of the many potential intergeneric crosses that involve *Columba* species have been recorded. (For a review of this literature see Gray 1958.) The majority of these intergeneric crosses involve *Streptopelia* species, but these data are not complete enough to answer the question of generic affinity.

Irwin (1953) and Irwin and Miller (1961) have tested a number of columbid species for the presence of species-specific cellular antigens that separate *Streptopelia chinensis* from *S. risoria* and *Columba guinea* from *C. livia.* The first study indicated that antisera to *cayennensis* contained antibodies to eight of the 10 antigenic substances that separate *chinensis* from *risoria.* Of the other species examined, only *S. senegalensis* possessed more than this number of antibodies to the species-specific antigens of *chinensis.* In the second study 24 intergeneric tests were made, and those that involved the species of *Streptopelia* were most similar in their antigenic complement to the species of *Columba.* Although this finding suggests a close relationship between *Columba* and *Streptopelia,* the possibility remains that species not yet examined by Irwin's group may be closer to the *Columba* species than are the *Streptopelia* species. Furthermore, the antigenic substances that differentiate the species pairs of *S. chinensis-S. risoria* and *C. guinea-C. livia* may not be the most useful characters for determining the origins of the genus *Columba.*

Of the 80 intergeneric comparisons of peptide maps that involve *Columba* species, the map of *Leptotila rufaxilla* is most similar to eight of the 10 *Columba* species. The peptide map of *Streptopelia decaocto* is next most similar. The peptide data also suggest that *Leptotila* is closely related to *Streptopelia.* There are two possible relationships that account for these data. Either *Streptopelia* gave rise to both *Leptotila* and *Columba,* or the former two genera evolved from *Columba.* Neither of these possibilities is eliminated by the peptide data.

Within the genus *Columba* it is obvious that the evidence presented here disagrees in several respects with earlier classifications. This is not surprising since most of these relationships have not been clarified unequivocally by previous studies. Uncertainty as to the correct phyletic position of *fasciata* is expressed by the number of different affinities proposed for this species. Johnston (1962) removed all except three of the American species from the genus *Columba* and assigned them to either *Patagioenas* or *Oenoenas.* The three exceptions are *fasciata, araucana,* and *caribaea,* which Johnston believes are most closely related to the *palumbus* species-group. This separation apparently is based on the presence or absence of a trailing fringe on the tenth primary. Since the angle on the skull at the frontal hinge is given as 150° or more for the *fasciata* super-species, this character does not separate these species from the other American *Columba* species.

Peeters (1962) did not believe that *fasciata* is more closely related to the Old World species, basing this conclusion on his behavioral observations. Verheyen (1957) placed *fasciata* closest to the other American species, but in a separate genus that includes *araucana* and *albilinea* but not *caribaea.* Whitman (1919) states only that the behavior of *fasciata* is separate from that of the others. Boetticher (1954) places *fasciata* in the subgenus *Chlo-*

roenas, which is closest to the subgenus *Picazuros* in his phylogeny. He also includes *araucana* and *albilinea* in this subgenus and excludes *caribaea.* Miculicz-Radecki (1949) places *fasciata* in a separate evolutionary line that is intermediate between those of *palumbus* and *trocaz* on the one hand and *guinea, arquatrix, plumbea, squamosa, picazuro,* and *speciosa* on the other. Irwin and Miller (1961) have shown that the blood cells of *fasciata* may possibly contain an antigen similar to antigen B′ of *livia.* If this is correct, *fasciata* and *cayennensis* are the only New World species of *Columba* that possess a B′-like antigen. In other respects *fasciata* is most similar serologically to *cayennensis* as measured by these tests. The peptide map of *fasciata* is most similar to that of *maculosa* and only a little less similar to those of *cayennensis* and *leucocephala.* Thus, I believe most of the available data ally *fasciata* with the American species and not with the Afro-Eurasian species as suggested by Johnston.

Columba picazuro is placed closest to *maculosa* by Goodwin and Johnston and to *gymnophthalmus* by Boetticher, Verheyen, and Miculicz-Radecki. Male hybrids between *picazuro* and *livia* may sometimes be fertile, but the females are sterile (Ghigi 1932; Taibel 1934, 1935, 1947). The same is true of *picazuro* × *guinea* hybrids reported by Cavazza (1931) and Hertwig (1936). Certain behavior patterns of *picazuro* are similar to those of *maculosa* (Goodwin 1964, 1966). The serological data are ambiguous in demonstrating the affinities of *picazuro.* If antisera to *picazuro* are tested for antibodies to the cellular antigens that differentiate *guinea* from *livia, picazuro* is equally similar to *cayennensis, fasciata,* most of the *Streptopelia* species, and the three species of *Zenaida* examined (Irwin and Miller 1961). However, if the blood cells of *picazuro* are tested for antigenic substances either identical or similar to those that differentiate *guinea* from *livia,* this species possesses only two antigenic substances, A and E, that are similar to the A and E of *guinea,* and none that are identical to those that separate *guinea* from *livia* or vice versa. None of the other 30 species tested had an antigenic complement of this type. The peptide data suggest that *picazuro* diverged from an ancestral type during the early stages of *Columba* evolution. Such an arrangement is partially supported by the evidence given above, since it could explain why *picazuro* is more similar to New World species in certain characters and more similar to Old World species in others.

The relationships of *cayennensis, leucocephala,* and *maculosa* to one another are not well defined. Verheyen places these species in three genera, Boetticher places them in three subgenera, and Goodwin places all three in the *picazuro* species-group but each is assigned to a different subgroup. Irwin and Miller (1961) have shown that antisera to *cayennensis* contain antibodies to cellular antigens A, C, and E of *guinea,* while antisera to *leucocephala* contain antibodies to A and C, and antisera to *maculosa* contain antibodies to A. Antisera to these same species contain antibodies to the following antigenic substances of *Streptopelia chinensis*: *cayennensis*—d-1, d-2, d-3, d-4, d-5, d-7, d-11, d-12; *leucocephala*—d-7, d-11; *maculosa*—d-5, d-7 (Irwin 1953). This information, together with the peptide data, suggests that the ancestral type of *cayennensis* successively gave rise to *maculosa* and to *leucocephala* genetic lines.

The arrangement of these three species as indicated by the peptide data is not in agreement with that of Boetticher. Goodwin has not explicitly indicated the relationships of *cayennensis,* so it is difficult to determine whether this arrangement agrees with his. If *maculosa* must remain in the *picazuro* subgroup as defined by Goodwin, there is disagreement since the peptide data indicate that *maculosa* and *leucocephala* are more similar to one another than either is to *picazuro.*

The affinities of *flavirostris* are uncertain. Boetticher places this species closest to the origin of the genus within the subgenus *Oenoenas,* while Goodwin places *flavirostris* at a point distant from the origin. The peptide data support the latter placement. In the production of antibodies to *guinea* cellular antigens, *flavirostris* is more similar to *cayennensis* than to the other American species, and next most similar to *fasciata* (Irwin and Miller 1961). The peptide evidence would place *flavirostris* closer to *fasciata* than to *cayennensis.* However, the possibility of a progressive loss of antigen-antibody specificity from *cayennensis* to *fasciata* to *flavirostris* (Irwin 1953) is not excluded by the peptide data.

Opinions concerning the correct relationships of *palumbus, guinea, oenas,* and *livia* are quite divergent. Boetticher places *palumbus* at the terminus of one evolutionary line that originates from the New World subgenus *Oenoenas.* The other three species are placed on the terminal branches of a separate line of evolution originating from the New World

subgenus *Notioenas*. He places *livia* and *oenas* in one subgenus, while *palumbus* and *guinea* are each placed in separate subgenera. Goodwin places these four species in the *palumbus* species-group, and within this species-group *livia* and *guinea* are combined in one subgroup while *oenas* and *palumbus* are placed in separate subgroups. Verheyen includes *livia* and *oenas* in the same genus and places *guinea* and *palumbus* in separate genera. Johnston places *livia* and *oenas* in the same species-group and *palumbus* in a separate species-group. He did not examine *guinea*. Miculicz-Radecki places *livia* and *oenas* close to one another, while *guinea* and *palumbus* are well separated both from each other and from *livia* and *oenas*.

When the antisera to these species were tested for antibodies to the species-specific antigens A, B, C, and E, which differentiate *guinea* from *livia*, antisera to *palumbus* contained antibodies to each of these four antigens. The antisera to *oenas* did not contain antibodies to antigen A (Irwin and Miller 1961). When the blood cells of these species were tested for the presence of antigens identical or similar to those that differentiate *guinea* and *livia*, *palumbus* cells contained antigens A and C that were indistinguishable from those of *guinea* and antigens B and E that were similar to those of *guinea*. The cells of *oenas* contained one antigen (A) that was identical and three (B, C, and E) that were similar to those of *guinea*. *Guinea*, *palumbus*, and *oenas* blood cells did not contain any of the antigens (A′, B′, C′, E′) specific to *livia*, whereas the blood cells of *cayennensis* and *fasciata* may have B′-like antigens. When antisera to *livia*, *oenas*, *guinea*, and *palumbus* were tested for antibodies to the antigens that differentiate *Streptopelia chinensis* from *S. risoria*, a progressive loss of antibody production was observed similar to that demonstrated for the New World species. Antisera to *livia* contained antibodies to five of these antigens, antisera to *oenas* contained four, and antisera to *palumbus* and *guinea* contained three.

Within *Columba* the maps of *palumbus* and *oenas* are most similar to that of *livia*. The maps of *oenas* and *guinea* are more similar to one another than either is to the map of any other species. The map of *palumbus* is equally similar to the maps of *livia*, *oenas*, and *guinea* but most similar to that of *cayennensis*. Thus the affinities of these four Old World species of *Columba* vary depending upon the characters examined. The peptide data indicate that *guinea* is closest to *oenas*, the serological data suggest *guinea* is closest to *palumbus*, and plumage color patterns and courtship displays suggest a close relationship between *guinea* and *livia*. A reconciliation among these mutually exclusive relationships does not seem possible at this time.

Although the variation observed in protein structure is the primary phenotypic expression of the genes controlling their synthesis, different types of proteins intraspecifically may have different numbers of mutants due to unequal rates of evolution. Therefore, the variation within a given protein must be carefully assessed before utilizing such information for taxonomic purposes. If a protein changes rapidly relative to speciation, its utility is essentially limited to the characterization of subspecific groups. On the other extreme are proteins that exhibit little variation among unrelated taxa. Clearly one cannot a priori know the kinds of variation that will be found for different types of proteins. But once established, it should not be difficult to apply this information to different taxonomic problems. The variation among ovalbumins immediately suggested the usefulness of ovalbumins in examining problems at the taxonomic level of the species. To fortify the conclusions of this study not only should additional ovalbumins be examined but also other types of proteins. If the relationships indicated by this study are correct, they should correlate well with other arrangements based on the examination of different kinds of proteins that have evolved at rates comparable to the rate at which ovalbumin has changed.

SUMMARY

Two-dimensional electrophoretic and chromatographic patterns of ovalbumin tryptic peptides of 20 avian species are presented. The peptide maps are compared on the basis of total numbers of differences and similarities. The positions of peptides within patterns and the presence or absence of tryptophan residues in specific peptides were the principal criteria used in designating peptide homologies. The genetic relationships of 10 pigeon species of the genus *Columba* have been inferred from these data, and the relationships so indicated are compared with previous classifications of these species.

The genetic relationships indicated are as follows: The Old and New World species-groups of *Columba* appear to have evolved from a common ancestor followed by speciation in the Eastern and Western Hemispheres. Since the ovalbumins of some *Columba* spe-

cies in each hemisphere are most similar to ovalbumins of *Columba* species in the other hemisphere, these data do not support the division of *Columba* into two or more genera, the species of which would be limited in distribution to either the Eastern or Western Hemisphere. Of the genera examined, the ovalbumins of *Leptotila* and *Streptopelia* are most similar to those of the species of *Columba*. Within *Columba* the ovalbumin of *fasciata* is most similar to those of other American species. *Picazuro* presumably diverged from other New World lines of evolution during the early stages of *Columba* speciation. *Flavirostris* ovalbumin is most similar to that of *fasciata*. The ovalbumin of *cayennensis* appears to have changed least from the ancestral type and is most similar to those of *leucocephala* and *maculosa*. Of the Old World species examined, *guinea* and *oenas* ovalbumins are most similar to one another. The ovalbumin of *palumbus* is equally similar to those of *livia*, *oenas*, and *guinea*, but is most similar to that of *cayennensis*.

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