

INTERRELATIONSHIPS OF THE CELLULAR CHARACTERS OF SEVERAL SPECIES OF COLUMBA¹

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Received July 6, 1942

IMMUNOLOGICAL methods have so often been employed as a means of differentiating species, particularly in animals but also in plants, that it is almost a truism to state that antigenic differences are one of the more important criteria by which qualitative differences between species may be assayed. In animal species, the serum antigens have been somewhat more widely used in such comparisons than have the antigens of the tissues or blood cells. LANDSTEINER and MILLER (1925a) suggested that this was due to the presumption that the species specificities of the precipitins and of the hemagglutinins are of the same order and that species specificity means protein specificity, whereas there is definite evidence that the antigens which engender antibodies against red blood cells do not consist simply of proteins.

Various reports from this laboratory (IRWIN and COLE 1936a, 1940; IRWIN, COLE, and GORDON 1936; IRWIN 1939) have shown that, following a comparison of the relationships of the cellular characters of several pairs of species of pigeons and doves, certain general statements may be made. First, each species possessed antigenic components in common with the other and, secondly, in addition to the common substances, each species possessed cellular constituents peculiar to itself (that is, species-specific characters). The hybrids between any two species studied invariably contained all the common components of the two parental species and all or nearly all the species-specific properties of both parents. Furthermore, segregation of the species-specific characters has been observed, by virtue of successive backcrosses of the species hybrid and selected backcross hybrids to one or both parental species. Thus, in three different species crosses—Pearlneck (*Streptopelia chinensis*) × Ring dove (*St. risoria*), Pearlneck × Senegal (*St. senegalensis*), and *Columba guinea* × *C. livia domestica*, respectively—at least certain of the cellular characters peculiar to Pearlneck in the first two crosses and to *guinea* in the third have been obtained in unit form—that is, no further separation of these characters has been observed following the appropriate backcrosses.

It has also been shown, first by immunological procedures and then verified by genetical findings, that some of the cellular characters which distinguish one species (Pearlneck) from another (Ring dove) may be shared with still a third species (Senegal) (IRWIN and COLE 1940). Also, at least one, and probably two, of the antigenic substances which differentiate *C. guinea* from *C. livia*

¹ Paper No. 302 from the Department of Genetics, Agricultural Experiment Station, UNIVERSITY OF WISCONSIN. This investigation was supported in part by grants from THE ROCKEFELLER FOUNDATION, and from the WISCONSIN ALUMNI RESEARCH FOUNDATION.

² Much technical assistance has been given in these studies by former and present Research Assistants in Genetics: DR. ALFRED GOLDEN, WARREN G. BLACK, J. R. DICK, and W. H. MCGIBBON.

are shared by *C. guinea* with both Pearlneck and Ring dove (IRWIN 1938), showing that several species may share one or more such characters to the exclusion of another species.

These and other results make highly probable the conclusion that all the antigens of the blood cells are gene-determined. Hence it can be stated with reasonable assurance that immunological studies of similarities and differences of the cells of related species will yield a fairly accurate approximation of the gross genetic relationships of such species, although hybridization may not be possible between them.

All information available at the present time leads to the conclusion that all or at least the majority of the cellular antigens are independently expressed, irrespective of the genetic complex in which each is found. If this be true, such characters should be eminently fitted for a study of the interrelationships of species.

MATERIALS AND METHODS

In the present investigation a comparison has been made of the interrelationships of the cellular components of each of 11 species of the genus *Columba*, with several or all of the other ten species. These species are *Columba fasciata*, *flavirostris*, *guinea*, *janthina*, *leucocephala*, *livia domestica*, *maculosa*, *oenas*, *palumbus*, *picauro* and *rufina*. (The normal habitat of these species will be given in a later report.) The comparisons of the cellular components have been made on the basis of the agglutination of the red blood corpuscles of the various pigeon species by specially prepared "test-fluids" or "reagents," as will be described below.

Antisera were produced by injecting rabbits with washed erythrocytes from representatives of each of the pigeon species. The details of these methods have been described elsewhere (IRWIN and COLE 1936a, 1936b; IRWIN 1938).

It was found in the earlier part of this work that an antiserum against the cells of any species usually agglutinated the cells of the other species of the genus at practically the same end-dilutions as were observed for the homologous cells (that is, the cells used in immunization). Therefore, such tests on the cells of the various species did not allow a definite differentiation of the cells of different species. Consequently, an antiserum against the cells of a particular species was not always tested with the cells of each of the other species. Although the data of such agglutinations are not listed in the table, they should be kept in mind as a starting point for the various comparisons. Furthermore, the agglutination of the corpuscles of these different species at approximately the same end-dilutions (usually 1:23,040 or 1:46,080) of an antiserum for any species indicates with certainty that an appreciable proportion of identical or related biochemical components was present in their respective bloods. In terms of the usual comparisons of species, these reactions would denote "homologies" in the different species.

However, a highly specific method of making a very clear cut differentiation of the cells of any two related species is possible following the absorptions of antibodies—in these experiments, the agglutinins. For example, *guinea* anti-

serum will agglutinate the cells of both *guinea* and *livia* at the same, or nearly the same, end-dilutions, but following absorption by an excess of *livia* cells, it becomes a "test-fluid," or "reagent," which will no longer agglutinate *livia* cells, even at the dilution used in the absorption (usually 1:60). However, this reagent will react with *guinea* corpuscles, ordinarily at a slightly lower titer than before the absorption (IRWIN et al. 1936). This kind of reagent, therefore, provides a highly specific and delicate test for distinguishing the cells of a pair of related species. Given such test-fluids for a pair of species, one drop of blood, even only a few blood cells from either species may be identified with ease. As explained in previous papers (*loc. cit.*), the antibodies of an immune serum to one (the homologous) species which are absorbed by the cells of another species (as by those of *livia* in the above example) were engendered by antigens presumably common to the two species. The antibodies which are not absorbed will react with the cells of the homologous species by virtue of components "species-specific" to that species. It follows, then, that the cells of any other species which possesses cellular antigens identical with, or chemically similar to those peculiar to the homologous species (in relation to the cells of the species used in the absorption) will interact with such a reagent. Such reactions will occur by virtue of antigens which two or more species share to the exclusion of a third. This kind of reaction allows an analysis of genetic relationships of cellular characters, and therefore of their causative genes, among species hardly possible by any other technic.

Complications would of course arise in the differentiation of these or other pairs of contrasted species if, for example, there were differences between individuals within *livia* in the antigens common to these two species, and if these were detectable at the dilution of *guinea* antiserum used in making the absorptions. However, no such differences have definitely been observed in the more than 200 representatives of *livia domestica* used in these tests. Undoubtedly antigenic differences between individuals of *livia* do exist and are numerous, but our experience to date in testing for such differences in birds leads to the belief that these would be found only infrequently, with antisera at the dilutions used in these studies of the interrelationships of species. Furthermore, the cells of several representatives of *livia*, and also of the different species among which these comparisons have been made, have usually been pooled at the various times of absorbing and testing, thereby probably eliminating any antibodies for possible antigenic differences between individuals whose cells were used in absorptions. It seems reasonable, therefore, to conclude that individual differences within the respective species probably have not appreciably influenced the interrelationships of these species, as elicited from these various tests.

The interactions of the blood cells and various reagents prepared from antisera against the cells of these eleven species of *Columba* are given in table 1. Antisera against each of the species were absorbed independently by cells of each of the other species, as far as was possible, and the respective reagents thus produced were tested against the cells of the available species. (In these tests, the deaths of representatives of certain species—particularly of *janthina*,

leucocephala and *palumbus*—and inability to obtain replacements have prevented the making of tests between every possible reagent and the cells of each of the species.)

The symbols used in describing the reactions given in table I indicate

TABLE I
Agglutination interactions of the cells of different species of *Columba*, with reagents prepared from the various antisera to each of the species.

ANTISERUM	ABSORBING CELLS	TEST CELLS										LINE	
		<i>fasciata</i>	<i>flavivirostris</i>	<i>guinea</i>	<i>janthina</i>	<i>leucocephala</i>	<i>livia</i>	<i>maculosa</i>	<i>oenas</i>	<i>palumbus</i>	<i>pica-zuro</i>		<i>rufina</i>
<i>C. fasciata</i>	<i>C. flavivirostris</i>	C	o	+			++	++	C	++	++	++	2
	<i>C. guinea</i>	C	+	o			+	++	++	±	C	++	3
	<i>C. livia</i>	C	++	+	++	++	o	++	++	+	++	++	4
	<i>C. maculosa</i>	C	++	o, ++			++	o	C	C	C	++	5
	<i>C. oenas</i>	++	++	?			?	++	o		++	++	6
	<i>C. palumbus</i>	C	++	?	o, ±	++	+	++	o ¹	o	++	C	7
	<i>C. picazuro</i>	C	o, ±	o			o, +	++	++		o	++	8
	<i>C. rufina</i>	++	o	o			o ²	++	o, ±	②	o	o	9
<i>C. flavivirostris</i>	<i>C. fasciata</i>	o	C	?			+	++	+	o	++	C	10
	<i>C. guinea</i>	C	C	o			+	C	+	o	++	C	11
	<i>C. livia</i>	++	++	++	++	++	o	++	±	++	++	++	12
	<i>C. maculosa</i>	+	C	+			++	o	+	+	++	++	13
	<i>C. oenas</i>	++	++	?			±	++	o		++	++	14
	<i>C. palumbus</i>	C	C	++ ³			C	C		o		C	15
	<i>C. picazuro</i>	+	C	o, ±			++ ⁴	++ ⁵	?		o	++ ⁶	16
	<i>C. rufina</i>	o, +	C	o, ±			o, ±	o	o, ±	②	o	o	17
<i>C. guinea</i>	<i>C. fasciata</i>	o	o, ±	C	C	?	++	o, ±	++	++	o, ±	o, ±	18
	<i>C. flavivirostris</i>	+	o	C	++	+	++	+	++	++	±	+	19
	<i>C. guinea</i>	o	o	o	o	o	o	o	o	o	o	o	20
	<i>C. janthina</i>	++	++	C	o	++	C	++	++	++	++	++	21
	<i>C. leucocephala</i>	o	o ⁸	C	++	o	++	o	++	++	o	o	22
	<i>C. livia</i>	++	++	C	C	++	o	++	++	++	+	++	23
	<i>C. maculosa</i>	±	±	C	++	±	++	o	++	C	?	±	24
	<i>C. oenas</i>	+	o	C	++	+	+	+	o	++	?	+	25
	<i>C. palumbus</i>	o	o ³	C	?	?	?	o	o ¹⁰	o	o	o	26
	<i>C. picazuro</i>	o	± ⁸	C	++	o ^{7,9}	+	?	++	C	o	?	27
<i>C. rufina</i>	o	o	C	++	?	++	?	++	++	o	o	28	
<i>C. janthina</i>	<i>C. fasciata</i>	o	o	++			++	o, ±	C	C	o, ±	±	29
	<i>C. flavivirostris</i>	++	o	++			++	++	C	C	++	++	30
	<i>C. guinea</i>	+	+	o			++	+	++	++	++	++	31
	<i>C. livia</i>	++	++	++	C	++	o	++	++	C	++	++	32
	<i>C. maculosa</i>	C	C	C			++	o	++	C	C	++	33
	<i>C. oenas</i>	++	++	++			++	o	o		++	++	34
	<i>C. palumbus</i>	+	±	o			++	o		?	②	±	35
	<i>C. picazuro</i>	±	o	+			++	?	++	C	o, +	?	36
	<i>C. rufina</i>	±	o	++			++	o	++	++	o	o	37
<i>C. leucocephala</i>	<i>C. fasciata</i>	o	±	++			++	++	++	++	++	++	38
	<i>C. flavivirostris</i>	C	o	++			+	++	C	++	++	++	39
	<i>C. guinea</i>	++	++	o			++ ^h	++ ⁱ	±	o	++	++	40
	<i>C. livia</i>	++	++	++	++	++	o	++	±	++	++	++	41
	<i>C. maculosa</i>	++	++	++			+	o	++	++	++	++	42
	<i>C. oenas</i>	C	++	+			o, ±	+	o		++	C	43
	<i>C. palumbus</i>	++	++	②			② ^h	② ⁱ		o	C	C	44
	<i>C. picazuro</i>	+	?	++ ⁷			+	+	++		o	+	45
	<i>C. rufina</i>	o	o	±			+	±	++	+	o	o	46

TABLE I—(Continued)

ANTISERUM	ABSORBING CELLS	TEST CELLS										LINE		
		<i>fasciata</i>	<i>flavistrotris</i>	<i>guinea</i>	<i>janlhina</i>	<i>leucocephala</i>	<i>livia</i>	<i>maculosa</i>	<i>oenas</i>	<i>palumbus</i>	<i>pica-zuro</i>		<i>rufina</i>	
<i>C. livia</i>	<i>C. fasciata</i>	o	?	+	±	±	C	±	++	++	±	±	47	
	<i>C. flavistrotris</i>	++	o	++	±	+	C	+	++	++	±	+	48	
	<i>C. guinea</i>	o, +	±	o	?	?	C	±	++	?	±	+	49	
	<i>C. janlhina</i>	++	++	++	o	++	C	++	++	++	++	++	50	
	<i>C. leucocephala</i>	o	o	±	±	o	C	o	+	±	?	?	51	
	<i>C. maculosa</i>	++	++	++	+	++	C	o	++	++	+	++	52	
	<i>C. oenas</i>	+	+	++	o	+	C	+	o	+	+	+	53	
	<i>C. palumbus</i>	o, ±	+ ^a	?	?	?	C	o	++	o	o	+	54	
	<i>C. picazuro</i>	±	o ^{a, b}	++	±	?	C	±	++	±	o	o, ±	55	
<i>C. rufina</i>	± ²	?	+	+	±	C	±	++	±	o	o	56		
<i>C. maculosa</i>	<i>C. fasciata</i>	o	C	±			+	C	++	o	++	++	57	
	<i>C. flavistrotris</i>	++	o	+			++	C	++	+	+	++	58	
	<i>C. guinea</i>	C	C	o			+	C	+	±	++	++	59	
	<i>C. livia</i>	C	C	±	+	++	o	C	+	++	++	C	60	
	<i>C. oenas</i>	++	++	+			+	++	o		C	C	61	
	<i>C. picazuro</i>	+	o ^b	o, ±			+	C	±	±	±	± ⁸	62	
	<i>C. rufina</i>	+	o	o, ±			+	++	+		o	o	63	
<i>C. oenas</i>	<i>C. fasciata</i>	o	o, ±	++			++	+	C	C	±	+	64	
	<i>C. flavistrotris</i>	++	o	C			C	+	C	C	++	++	65	
	<i>C. guinea</i>	++	o, +	o			++	+	++	++	±	++	66	
	<i>C. livia</i>	++	±	++	++	C	o	±	C	++	++	++	67	
	<i>C. maculosa</i>	++	++	++			C	o		o ⁹	++	++	68	
	<i>C. palumbus</i>	C ¹	+	o, + ¹⁰			++	②	C	o	②	++	69	
	<i>C. picazuro</i>	++	o	C			++	C	±	C	C	o	++	70
<i>C. rufina</i>	+	o	++			++	±	C	C	o	o	71		
<i>C. palumbus</i>	<i>C. fasciata</i>	o	o	++			±	o ^d	++ ^b	C	o	o, ± ¹	72	
	<i>C. flavistrotris</i>	+	o	++			±	o, ±	++	C	o, ±	±	73	
	<i>C. guinea</i>	o, ± ¹	o	o			o	o, ± ^k	±	C	o	o, ± ¹	74	
	<i>C. livia</i>	++	±	++	C	++	o	o, ±	C	C	+	++	75	
	<i>C. maculosa</i>	+ ^c	o, ?	++			+	o	o ^{e, b, f, g}	C	±	±	76	
	<i>C. oenas</i>	o ^c	o	++			⑧	± ^d	o		±	±	?	77
	<i>C. palumbus</i>	o	o	o		o	o	o	o	o	o	o	78	
	<i>C. picazuro</i>	o ^j	o	+			±	o ^k	++ ^e	C	o	o ¹	79	
<i>C. rufina</i>	o	o	+			±	o	+ ^f	C	o	o	80		
<i>C. picazuro</i>	<i>C. fasciata</i>	o	++	o, ±			±	++	?	o	C	C	81	
	<i>C. flavistrotris</i>	C	o	±			+	+	+	++	C	++	82	
	<i>C. guinea</i>	C	++	o			o, ±	C	o, ±	?	++	++	83	
	<i>C. livia</i>	++	++	+	++	++	o	++	+	++	C	++	84	
	<i>C. maculosa</i>	C	++	+			++	o	++	++	C	++	85	
	<i>C. oenas</i>	++	++	±			++	++	o		++	++	86	
<i>C. rufina</i>	o	o	o			o	o	?	②	+	o	87		
<i>C. rufina</i>	<i>C. fasciata</i>	o	+	o, ±			+	++	?	o	C	C	88	
	<i>C. flavistrotris</i>	++	o	+			±	+	C	±	C	C	89	
	<i>C. guinea</i>	++	++	o			+	++	++	±	C	C	90	
	<i>C. livia</i>	C	++	+	C	C	o	C	++	C	C	C	91	
	<i>C. maculosa</i>	++	++	+			++	o	++	+	C	C	92	
	<i>C. oenas</i>	++	C	++			++	C	o		C	C	93	
<i>C. picazuro</i>	++	o ⁶	±			±	o ⁸	++		o	C	94		

whether or not agglutination of the cells (one drop of a 2.5 percent suspension) occurred when mixed with (two drops of) a particular reagent. (Because of the small volume of reagents obtainable, the tests were usually performed at double the dilution of antiserum that was used in the absorption. If no agglutination

was noted, the mixture was repeated, whenever possible, with the reagent at the absorbing dilution.) Thus "o" indicates that no clumping of the cells could be noted by microscopical examination. A 2 indicates no clumping of cells in the second dilution of the reagent but does not preclude the possibility that there might have been agglutination in the first dilution had it been used. In some combinations the cells were not distributed entirely at random nor were there well-defined clumps. Such indefinite reactions have been labelled "?." The symbols " \pm ," "+," "++" and "C" indicate definite agglutination, in various degrees, from numerous small clumps, usually plainly visible to the naked eye (" \pm "), to one large clump of cells, or complete agglutination ("C").

Each reading recorded in the table, except for those involving a few reagents prepared by the absorptions of different antisera by the cells of *oenas*, represents the results of at least two separate tests, performed at different times. Such separate tests always were made with reagents obtained by absorptions of different portions of the antisera, rather than with the same reagents. Occasionally, at different times of testing, the same combination of reagent (from a particular antiserum) and cells produced discordant results—that is, no agglutination occurred at one test as compared with definite clumping at another. In such instances both kinds of reactions have been recorded in the table, as o, \pm , etc., and may have been caused by errors in technic, by the disappearance of antibodies from an antiserum because of ageing, or by other causes.

RESULTS

The reactions of the various combinations of reagents and cells, presented in table 1, provide information as to whether the antigens specific to one of a pair of contrasted species are shared, at least in part, by other species. For example, anti-*guinea* serum absorbed by *livia* cells agglutinates not only the cells of *guinea* but those of each of the other species as well (line 23, table 1) That is, at least a part of one or more of the antigens of *guinea*, not shared with *livia*, are found in these other species. However, it does not necessarily follow that each of these other species shares the same antigenic pattern of *guinea* which sets it and therefore, to that extent, each of them apart from *livia*.

Since most, if not all, of the specific antigens of *guinea*, not shared with *livia*, have been recognized as single characters (antigens A, B, CD, E and F; IRWIN et al. 1936), following backcrosses of the species hybrids and selected backcross hybrids to *livia*, appropriate tests would tell which of these specific characters of *guinea* was shared, in whole or in part, with the other species. Such an analysis has been reported (IRWIN 1938), showing that certain of these specific characters of *guinea* are shared with Pearlneck and Ring dove. Similar assays of these characters of *guinea* have also been done with various species of the genus *Columba*, and these will be reported in detail elsewhere.

EXPLANATION OF DISCREPANCIES

As stated above, practically all the combinations of the different reagents and cells of the various species listed in table 1 have been made at least twice, many have been repeated several times. Only a few discrepant reactions have

been noted for any one antiserum to the cells of a particular species, although the reactions of reagents prepared from two or more antisera to the same species have not always agreed. That this may have been caused largely by the differential response of rabbits to immunization appears to be reasonable from the general experience in this and other laboratories (IRWIN and GOLDEN 1942).

The cross relationships of species can be tested reciprocally, and therefore very precisely, according to the principle exemplified in the following example. As stated above, *guinea* shares with each of the other species certain antigens which are not common between *guinea* and *livia*, as evidenced by the observation that the antiserum to *guinea*, when absorbed by *livia* cells, agglutinates those of the other species (line 23). Therefore, the respective antisera to each of these species, after absorption with *livia* cells, should agglutinate the corpuscles of *guinea*. The table shows that the results expected on this basis really were obtained (lines 4, 12, 32, 41, 60, 67, 75, 84, and 91 in the column under *guinea* cells).

By far the greater majority of the reciprocal reactions of the table are in agreement for presence or absence of agglutination. A few, however, have given discrepancies on this basis of comparison. For example, although the absorption of anti-*guinea* serum by the cells of *palumbus* removed the agglutinins not only for themselves but for those of *flavivirostris* as well (as shown by a lack of agglutination of *flavivirostris* cells with this reagent, line 26), the antiserum to *flavivirostris* after exhaustion by the corpuscles of *palumbus* reacted definitely in duplicate tests with *guinea* cells (line 15). Although the fractionation of various *guinea* antisera did not show that this species shared any antigen with *flavivirostris* which was not also shared with *palumbus*, proof of this relationship was obtained by the reciprocal tests with *flavivirostris* antiserum. Thus the conclusion seems reasonably valid that the antibodies for that particular part of *guinea* were not contained in the different *guinea* antisera used. Notations in the table, calling attention to such discrepancies in the reciprocal relationships, are marked at the pertinent combinations of the different reagents and cells, using a common superscript, in this particular comparison the superscript 3 (lines 15 and 26). The results of all the interactions of reagents and cells have been similarly examined for reciprocal agreement, and only ten such discrepancies have been observed in the many combinations of the table.

Another type of discrepancy is possible and has been found among the interactions of the cells from the different species with reagents prepared from a particular antiserum. An example of this kind may be noted in the interaction of *flavivirostris* cells with reagents prepared from *livia* antiserum by absorption with the cells of *palumbus* and *picazuro*, respectively (lines 54 and 55). The corpuscles of *flavivirostris* are agglutinated by the first reagent (line 54), but not by the second (line 55). These results are at variance, because the reagent prepared by the use of *palumbus* cells removes the antibodies for those of *picazuro*, whereas *palumbus* cells are agglutinated by the second reagent. Thus, of the antigens of *livia*, *palumbus* shares the same (as X, Y, and Z), and more

than does *picazuro* (as Y and Z). It would be expected, therefore, that the cells of *palumbus* would be by absorption remove more antibodies from *livia* antiserum than would those of *picazuro*. Hence the cells of another species might not be agglutinated by the reagent prepared by exhausting this antiserum of antibodies for *palumbus* cells but could be clumped by the reagent produced by absorption with *picazuro* cells. The lack of agglutination of *flavivirostris* cells, as shown in line 55 of the table, is therefore not in accord with expectation, since these cells are agglutinated by the reagent of anti-*livia* serum absorbed by *palumbus* corpuscles. These paired comparisons are both marked with the superscript "a." Comparable discrepancies of differences in expected results of reactions of the species cells with reagents prepared from a single antiserum are similarly marked with letters as superscripts.

The expected agreement in reactivity between cells and reagents for a particular antiserum may be stated as follows. After absorptions of any antiserum by the cells of any two or more species, if the homologous corpuscles are found to share the same components with these two or more species, the reactive capacities of their respective cells should agree when in combination with all other reagents produced from this antiserum. Furthermore, the reactions of the cells of other species toward the reagents produced by the respective absorptions of this antiserum with the corpuscles of these species should also be parallel.

On the other hand, the results may show that a particular species shares a part of its complex with one species, and this same complex plus additional antigens with another, as *guinea* shares with *oenas* all the components, and additional ones, which it has in common with *flavivirostris* (line 25). Then within *guinea* antiserum there may not legitimately be a zero (o) reaction for any cells with the *flavivirostris* reagent and a plus reaction for the same cells with the serum absorbed by the cells of *oenas*. Further, converse agglutinabilities should obtain for the cells of these two species—that is, cells of *flavivirostris* should not react with any reagent from *guinea* antiserum which fails to agglutinate the corpuscles of *oenas*, whereas the cells of *oenas*, by virtue of the additional antigens, may readily be agglutinated by reagents which do not react with those of *flavivirostris*.

ANALYSIS OF GUINEA

In explanation of the meaning of the various tests given in table 1, it may be helpful to consider in detail the relationships of *guinea* to the other species as shown by the assay of its immune serum. One should keep in mind that the antibodies of the antiserum to a species represent what may tentatively be termed specific counterparts of the antigens of the cells of that species, so that fractionating an antiserum by the various absorptions is a means of separating the antibodies for the respective cellular antigens of a species.

Furthermore, two kinds of relationships are possible between particular antigens that are shared by any two or more species. They may be indistinguishable and therefore presumably identical, as was demonstrated by LANDSTEINER and MILLER (1925b) for the **A** and **B** characters of human blood

cells when present singly or together in the blood of anthropoid apes. Or these cellular characters in different species may be similar in structure but not identical. Examples of the latter kind have been demonstrated in lower monkeys with antigens similar to, but distinguishable from, the **B** character of human cells (LANDSTEINER and MILLER 1925c). Similarly, substances related to but not identical with either the **M** or **N** characters of humans have been found in some of the apes and lower monkeys (LANDSTEINER and WIENER 1937; WIENER 1938). Examples of antigenic similarities within the latter category have been observed in different species of birds (IRWIN, 1938; IRWIN and COLE 1940), although in these the respective antigens assayed may not have been produced by single genes, as presumably are those of humans given above. Some of the genetic implications of these relationships have already been discussed (IRWIN and CUMLEY 1940).

It is a fundamental tenet of immunology that antibodies will be engendered in an organism only to the antigens used in immunization. (A few possible exceptions to this rule appear to be satisfactorily explained on other grounds.) Therefore, the absorption of *guinea* antiserum by the cells of *guinea* and subsequent tests on the cells of the different species (line 20) are of significance. Since this absorbed serum showed no agglutination whatever with the cells of any species, we may conclude that *guinea* antiserum contained antibodies for no other antigens than those found in *guinea* cells. Results duplicating these were obtained following the absorption of anti-*palumbus* serum by the corpuscles of *palumbus* (line 78).

As stated above, the reagent produced from *guinea* antiserum by the absorption with *livia* cells reacts with the cells of each of the other species as well as with those of *guinea* (line 23). On the basis of these reactions, however, it cannot definitely be told whether the antigenic complexes which *guinea* shares with any two other species, as *fasciata* and *oenas*, to the exclusion of *livia*, are the same or different. This kind of assay of the different relationships requires an extension of the absorption technic and will be given in a later report. Although different degrees of agglutinative reactions to this and other reagents have been noted for the cells of certain species, it is doubtful if such differences can be considered as more than tentative indices of significance. In our opinion, the only criterion of a trustworthy differentiation is the lack of reaction of a particular reagent toward the cells of one species, as compared with definite agglutination with the cells of another.

When anti-*guinea* serum was absorbed by the cells of *flavivirostris*, the reagent so produced agglutinated the corpuscles of each of the other nine species (line 19). Each of these species, then, shares with *guinea* one or more antigens not common to *guinea* and *flavivirostris*. As stated above, the antigens of *guinea*, not found in *livia*, are shared in part with *flavivirostris*. *Guinea* therefore shares some antigens with *livia* that are not found in *flavivirostris* and some with *flavivirostris* that are not held in common with *livia*, and, presumably, *guinea* shares many with both *flavivirostris* and *livia*. (For somewhat comparable relationships, see the diagrammatic representations of the antigens of *guinea*, *livia*, Pearlneck and Ring dove, IRWIN 1938.)

Exhaustion of anti-*guinea* serum with the cells of *janthina* and *maculosa*, respectively, provided reagents which also agglutinated in various degrees the cells of the other species (lines 21 and 24), except those used in the individual absorptions. These results therefore parallel those obtained with the reagents produced in this antiserum following independent absorptions by the cells of *livia* and *flavivostriis*, except that a different species is involved in each comparison with *guinea*. Unfortunately the relationships of *guinea* to these four species, as well as to the others, cannot be accurately diagrammed from the data presented. That is, from the results obtained with the reagent produced by exhausting *guinea* antiserum with cells of *flavivostriis*, for example, no statement can be made as to whether the agglutinations of the cells of any two species, as *fasciata* and *leucocephala*, are by virtue of the same or different characters. But, on the basis of the reactions observed following fractionation of the antiserum to *guinea* cells by these four absorptions, it can be reasonably concluded that the antigens which engendered these antibodies must have been numerous.

The reagent prepared by absorbing *guinea* antiserum with the corpuscles of *oenas* agglutinated the cells of each of the other species except *flavivostriis*, although it produced only a faint trace of agglutination, if any, with the corpuscles of *picazuro* (line 25). Since there is agglutination of the cells of *oenas* following absorption of this antiserum by those of *flavivostriis* (line 19), as stated above, it appears that *guinea* shares with *oenas* the same and more substances than are shared with *flavivostriis*. *Guinea* cells are therefore undoubtedly more closely related in antigenic structure to those of *oenas* than to those of *flavivostriis*. Thus, the cells of *oenas* will be expected always to be agglutinated by the different reagents prepared from *guinea* antiserum, which clump those of *flavivostriis*; *oenas* cells may also be agglutinated in combinations with reagents which do not clump *flavivostriis* corpuscles. According to the principles stated above, this latter reaction is possible by virtue of the antigens of *guinea* shared with *oenas* but not with *flavivostriis* (for examples, see lines 22 and 28).

A slightly different picture of species relationships may be noted from the results of tests with reagents obtained by the respective absorptions of *guinea* antiserum with the cells of the remaining species. Following absorption with *fasciata* cells (line 18), the test fluid agglutinated strongly the cells of *guinea*, *janthina*, *livia*, *oenas*, and *polumbus*. Discrepancies in results have been observed for this reagent with the cells of *flavivostriis*, *maculosa*, *picazuro*, and *rufina*, in that no agglutinations, in contrast to only a relatively few clumps of cells, have been noted for the cells of each of these species at different times of test. It is probable, however, that *guinea* shares with each of these latter species a very minute fraction of its cellular antigens not common to *fasciata*. The suspicious reaction of this reagent (*guinea* antiserum absorbed by the cells of *fasciata*) with the cells of *leucocephala* has been repeatable and therefore presumably represents a definite but minute antigenic similarity of *guinea* and *leucocephala* to the exclusion of *fasciata*. This relationship is verified

by the reciprocal test—that is, agglutination of *guinea* cells by *leucocephala* antiserum absorbed by *fasciata* cells (line 38).

Following exhaustion of *guinea* antiserum by the cells of *picazuro*, no clumping was noted with the corpuscles of *fasciata* or *leucocephala*, and only suspicious reactions with those of *maculosa* and *rufina* (line 27). The antibodies were removed in this absorption for the cells of *fasciata* and *leucocephala* as well as for the absorbing cells. However, the reciprocal test of *leucocephala* antiserum absorbed with *picazuro* cells (line 45) showed definite agglutination of *guinea* cells (discrepancy 7). It is therefore probable that the *guinea* antisera, from which these reagents have been derived by exhaustion with cells of *picazuro*, were deficient in the antibody specific for that part of the pattern shared with the cells of *leucocephala* to the exclusion of *picazuro*. (The possibility of a non-specific absorption of the antibody cannot be entirely excluded in explanation of the discrepancy.)

Since absorption by *rufina* cells removed from *guinea* antiserum the agglutinins for *picazuro* cells and for those of *flavivirostris* as well (line 28), it may be concluded that all the cellular antigens which *guinea* shares with *flavivirostris* and *picazuro* are also shared with *rufina*. It is probable, also that *guinea* and *rufina* have a small fraction of antigenic components in common to the exclusion of *picazuro* (compare reactions of cells and reagents in lines 27 and 28). Furthermore, since *rufina* cells by absorption likewise remove the antibodies from *guinea* antiserum for the corpuscles of *flavivirostris* but not for those of *oenas* there is pertinent evidence for the statement that *guinea* shares a complex of antigens with the three species, *flavivirostris*, *oenas*, and *rufina*. In addition to the complex held in common with these three species, *guinea* shares other characters with *oenas* and still others with *rufina*. *Guinea* is therefore more closely related to both *oenas* and *rufina* than to *flavivirostris*, but these tests do not allow a statement concerning any possible difference in the degree of relationship of *guinea* to either *oenas* or *rufina*.

Following absorption of *guinea* antiserum with the cells of *leucocephala* (line 22), no antibodies remained for either *picazuro* or *rufina* corpuscles, nor for those of *maculosa*. Naturally, antibodies were also removed for the cells of any species which had less of *guinea* substances than any one of these—that is, *fasciata* and *flavivirostris*. On this basis, *guinea* is more closely related to *leucocephala* than to *rufina*, *picazuro*, *fasciata*, *flavivirostris*, or *maculosa*. The data, however, do not permit a statement as to what extent *guinea* shares the same antigens with *oenas* and *leucocephala*, respectively.

Finally, when the antiserum to *guinea* was absorbed by the cells of *palumbus*, only suspicious reactions were observed with the cells of *janthina*, *leucocephala*, and *livia*, and none with those of the others except with the homologous cells (line 26) and possibly with *flavivirostris* (discrepancy 3). These particular results show that *guinea* shares with the other nine species very few, if any, of its cellular antigens not common to *palumbus*. Reciprocally, *guinea* cells exhaust *palumbus* antiserum (line 74) of antibodies for the cells of *flavivirostris*, *livia*, and *picazuro*, and leave antibodies capable of reacting at the first dilution only

weakly with the corpuscles of the other species. *Palumbus* cells, however, were agglutinated strongly by this latter reagent. Thus it seems reasonably definite that *guinea* and *palumbus* are more closely related to each other in these cellular characters than to any other species of those tested.

The logical conclusion to be drawn from the fractionation of *guinea* antiserum, as described above, is that the cellular characters of *guinea* form a complex pattern within which are woven interlocking relationships with each of the other ten species. Just how these antigens of *guinea* are interwoven among the other species cannot be determined precisely from the data here presented, but are susceptible to experimental assay, as will be described elsewhere.

GENERAL RELATIONSHIPS

The antiserum for each species might be analyzed in detail in the same manner as has been done above for *guinea* antiserum. From such analysis would emerge a picture of the antigens of each species interlocking in intricate but somewhat dissimilar patterns with those of the others. In general, the interrelationships of the cellular characters of one species to those of the others may be divided into several reasonably well defined groups, as follows:

(1) The antiserum to one species, when absorbed by the cells of another, may still react in various degrees with the corpuscles of all the other species. For example, anti-*guinea* serum when absorbed with *livia* cells reacted with the cells of each of the other species. Likewise, the antisera to each of the other species (excluding *livia* antiserum), when exhausted by *livia* cells (with but one possible exception—line 75), agglutinated the cells of all other species. Thus the antigens of one species, not shared with another, may be shared at least in part with all the other species.

(2) Absorption of an antiserum by the cells of another species may remove antibodies not only for the absorbing cells, but for those of one, two, three, or more other species. That is, a given species may share (a) a complex of antigens with one species, it may share (b) either the same complex or this same complex plus additional antigens with another species, or (c) all the antigens of the second species plus others with a third, etc. Different degrees of relationships between species may be seen in the data of the table.

Thus anti-*fasciata* serum, when absorbed by *guinea* corpuscles, agglutinated in varying degrees the cells of the ten other species (line 3). *Fasciata* then shares cellular antigens, but not necessarily the same antigens, with each of the other species which it does not share with *guinea*. Furthermore, when *fasciata* antiserum was exhausted by the cells of *picazuro* (line 8), no reaction was obtained between the reagent so produced and *guinea* corpuscles. Therefore, *fasciata* possesses in common with *picazuro* all and more cellular antigens than are shared with *guinea*. Similarly, since the reagent produced by absorption of *fasciata* antiserum by *rufina* corpuscles (line 9) failed to agglutinate either *guinea* or *picazuro* cells (as well as those of other species), whereas *rufina* cells are agglutinated by each of the other reagents produced from this antiserum, it seems certain that *fasciata* cells in common with those of *rufina*

have all the antigens which are shared with *picazuro* and *guinea*, plus others in addition.

(3) It is conceivable that two species could be so closely related that neither would share with any other species the cellular characters which distinguish the one from the other. No examples of this kind are to be found in the data of table 1, although, as cited above, *guinea* and *palumbus* are very closely related. A slightly different picture of antigenic relationships may be seen for *picazuro* and *rufina*. *Picazuro* shares practically no cellular characters with any other species that are not shared with *rufina* and seemingly has only a small proportion of antigens particular to itself (line 87). On the other hand, at least some of the antigens of *rufina*, not in *picazuro*, are shared by *fasciata* and *oenas*, and a minute fraction by *guinea* and *livia* (line 94). Therefore, it appears that *picazuro* and *rufina* are very closely related, but that *picazuro* has less of antigens specific to itself than has *rufina*.

The question naturally arises as to whether the antigenic components, and therefore the causative genes, of one species may simply be the sum of those found in several related species. That is, does any one species have cellular characters which are particular to that species alone and are not found in any other? It is conceivable that some species may possess genes with antigenic effects quite unlike those of any combination of other species, while most, if not all, of the antigens of others may well be nothing more than the sum of the components of certain combinations of related species. This question will be considered further in future reports.

OTHER ANALYSES

Another step in the analysis of the interrelationships of the antigenic characters of these species that can be made would be to determine if there are any parallel reactions of the various species' antisera, when each is separately absorbed with the cells of a single species and then tested with the cells of each of the other species. That is, do the reactions of the respective antisera thus absorbed, toward the corpuscles of all the various species, suggest a common complex of antigens between any two or more species which are shared to the exclusion of a single species? Such comparisons may very easily be made by a rearrangement of the data of table 1, listing together the reactions of the various antisera, following absorption by the cells of a single species, toward the cells of each of the different species.

An example of this kind may be noted in the reactions of antisera to *flavirostris*, *maculosa*, *picazuro*, and *rufina*, respectively, following the absorptions of each by the cells of *fasciata* (compare lines 10, 57, 81, and 88). Except for differences in the degree of reactivity of these four reagents toward their homologous cells, their reactions toward the cells of other species are very similar. There were relatively weak or uncertain agglutinations of the respective reagents with the cells of *guinea* and none with those of *palumbus*. Thus, these four species share no antigens with *palumbus*, and very few with *guinea*, that they do not possess in common with *fasciata*.

Substantiating evidence of the statement made earlier that *guinea* and

palumbus are closely related in their content of cellular antigens may be derived from the interactions with the cells of either *guinea* or *palumbus* to reagents produced by absorption of antisera to the remaining nine species by the cells of the other. When the absorptions were done with *guinea* corpuscles, the reactions for *palumbus* cells were completely wiped out in the antisera from *flavivirostris* and *leucocephala*, with faint if any reactions with those from *livia*, *maculosa*, *picazuro*, and *rufina*. Exhaustions of the antisera to *picazuro* and *rufina* were not done with *palumbus* cells; the other antisera, except that to *flavivirostris*, when exhausted by *palumbus* cells gave only questionable, if any, reactions with *guinea* corpuscles.

A similar substantiation of the close relationship proposed previously between *picazuro* and *rufina* will be obtained if the data of table 1 are examined in like manner. The cells of *picazuro* exhausted the respective antisera appreciably, but not entirely, of the antibodies for *rufina* corpuscles, while exhaustion of the different antisera by the antigens of *rufina* in every instance removed also the antibodies for *picazuro* cells. Thus the conclusion seems valid that whereas *picazuro* and *rufina* both share antigenic components with the other species, in every comparison of these two with a third species, *rufina* shares all and usually more cellular components than does *picazuro*. These results agree entirely with the statements made above, that *rufina* has more cellular components specific to itself than does *picazuro*.

Furthermore, the reagents produced by absorptions of these different antisera by *picazuro* cells (except in anti-*flavivirostris* serum) reacted only faintly, if at all, with *flavivirostris* cells. Results paralleling these were obtained following the absorptions of the same antisera with the corpuscles of *rufina*—that is, no reaction of *flavivirostris* cells except with this type of reagent from the homologous antiserum. The reactions for the corpuscles of *fasciata* were generally faint, if any existed at all, with the reagents obtained by absorptions of the respective antisera by the corpuscles of either *picazuro* or *rufina*.

There are many other ramifications of the relationships of the species that could be discussed in detail. Perhaps the most important single conclusion that can be drawn from an analysis of these antigenic relationships is that each of the species studied, in its interrelationships with the others, appears definitely to be an entity. How far variation between individuals within any of these species may be found to reduce the distinction between the species is an open question. It must be admitted that the number of representatives from these different species which were available for testing was extremely small, and the hazards of extrapolation from these few to the species as a whole should not be minimized. However, as stated above, we question, in the light of our experience, whether differences in the antigenic composition of individuals would materially change the results of the relationships reported here, particularly at the level (dilution of antisera) at which the tests were made.

For example, two recently imported birds of the *picazuro* species were obtained early in 1937 from a dealer in California. Later, two more birds of this species were provided by courtesy of Drs. HOLMBERG and ROSELLI of the ZOOLOGICAL GARDENS OF BUENOS AIRES. Still later, 11 of these birds, coming

from two recent importations from South America, were purchased from a dealer in Maryland. Although not all the tests involving absorption by, or agglutination of, *picazuro* cells were repeated for these different samples of the species, in no case in which duplicate tests were made was there a suggestion of different reactivities.

Another general conclusion is that there appears to be a reasonably well-pronounced tendency for certain of these species to resemble each other more than others. Thus *fasciata*, *flavirostris*, *maculosa*, *picazuro*, *rufina* and, insofar as the data show, *leucocephala* appear to be more closely interrelated each to the others than they are to *guinea*, *livia*, *oenas*, *palumbus*, and probably *janthina*, and vice versa. Since these groupings coincide with the native habitat of these different species in the Old and New Worlds, respectively, there may well be for these species a significant correlation of geographical habitat with evolutionary progression. A paper to follow will discuss these relationships.

Although the evidence points strongly to the conclusion that the gene complexes affecting the cellular characters of these species may be roughly divided into two primary groups, it seems reasonable to conclude from the above tests that the characters common to any two of the species are probably more numerous than are those which are particular to either of the species. As stated above, the cells of these different species very seldom, if ever, are clearly differentiated by their reactivities with untreated antiserum against any species—that is, each antiserum agglutinates the homologous cells and those of the other species to approximately the same end-dilutions. The logical inference to be drawn from such interactions is that a species shares a considerable proportion of its cellular antigens with all these other species. It is not unreasonable to assume that a goodly proportion of the characters common to two species are also common to all the species.

Granted that an accurate estimate cannot now be reached of the number of characters shared by any two of these species, and even less of those probably shared by all, nevertheless an approximation may be made of the relative proportion of the chromosomes of *guinea* which bear genes making for common and species-specific cellular characters, respectively, in contrast with *livia*. From such information, it is possible to postulate a somewhat similar relationship between other pairs of species. It was previously shown (IRWIN et al. 1936) that there were six cellular characters (**A**, **B**, **C**, **D**, **E**, and **F**) which differentiate the blood cells of *guinea* from those of *livia*. At the present writing only five of these are available in unit form; antigen **C** has not been perpetuated singly. These characters have segregated in the offspring of the backcrosses to *livia* of the species hybrids between *guinea* and *livia*, and selected backcross hybrids. Each of these *guinea* components is assumed to be produced by the action of one or more genes on a chromosome in *guinea*; unless there is a linkage between some of these genes, there are five or six chromosomes of *guinea* which produce "major" cellular antigens, specific to *guinea*. (It is entirely possible, of course, that any one or all of these five or six chromosomes may also carry genes which produce effects common to the two species, as well as particular to *guinea*.) These are called major characters because they

are expressed at relatively high dilutions of the absorbed antiserum; **A** and **F** usually are agglutinated at end-dilutions of 1:180 or 1:360, and **B**, **D**, and **E** ordinarily react at end-dilutions of 1:1440, often as high as 1:5760. It is, of course, conceivable that there may also be "minor" characters peculiar to *guinea*, but these supposedly would be expressed only at lower dilutions (less than 1:60) of *guinea* antiserum than have been used in these tests. Therefore, except that there may be minor characters specific to *guinea*, any other genes in *guinea* producing antigenic effects in the red blood cells should produce components shared with *livia*. (It is possible, in fact probable, that the so-called "major characters" of *guinea* may individually be the result of joint effects of several genes on the respective chromosomes. Since it has been shown (Irwin 1938) that qualitatively different component parts of the **CD** and **E** characters of *guinea* may be shared by Pearlneck and Ring dove, respectively, to the exclusion of *livia*, it appears rather improbable that these antigens are each produced by a simple gene, or by the action of the individual chromosomes as a whole in *guinea*, as proposed in essence by GOLDSCHMIDT (1940).

Evidence applying rather directly to this point may be adduced from the results of experiments on the number of cellular antigens within a species. The greatest number of these known at the present writing for any species has been found in this laboratory in the cells of cattle (FERGUSON 1941; FERGUSON et al., 1942). The 30 cellular antigens now recognized in cattle furnish substantiating evidence for the proposal that one or more genes on each of the chromosomes of a species may have effects on the antigens of the blood cells. (The work of LANDSTEINER and LEVINE (1932), TODD (1930), and unpublished results from this laboratory indicate very strongly that a similar situation holds for the cells of the chicken.) If this be true, and if, therefore, one or more genes on each of the presumed 30 pairs³ of chromosomes of *guinea* have such effects, the genes on only five or six of these serve to distinguish *guinea* from *livia*. Presumably all the other antigens of *guinea* would be shared by the two species, although, as stated above, there might be "minor" antigens, not expressed at the level of the recorded tests, which would be species-specific to *guinea* but not readily detectable. That is, it is entirely possible that one or more genes on each of the probable 24 other chromosomes of *guinea*, than these five or six which produce specific *guinea* characters, initiate antigens common to both *guinea* and *livia*.

Each of the cellular characters which differentiate *guinea* from *livia* has so far behaved as a unit in inheritance and might therefore be construed as being determined by a single gene. On such an interpretation, there would be the somewhat anomalous situation in which the erythrocytes of *guinea* differed from those of *livia* (a different species) in the effects of only six genes, whereas the cells of individual cattle (the same species) theoretically may differ by the

³ Actual counts of the chromosomes of *guinea* have not been made. On the supposition that the chromosome numbers of the species of *Columba* and *Streptopelia* would be very similar, we are assuming that these species of *Columba* have the same number as *livia* and Ring dove—that is, approximately 30 pairs (unpublished data by T. S. PAINTER, personal communication).

effects of as many as 30, since no two of the causative genes seem to have a simple allelic relationship. (Actually, however, the lowest number of known antigens observed in the cells of any individual in cattle has been four or five, while the greatest number has been rarely, if ever, above twenty. Furthermore, nearly all the cellular antigens recognized in cattle might be classed as "minor" characters. That is, they are reactive at dilutions of their respective reagents much lower than those which have detected the five or six "major" characters specific to *guinea*.) It therefore seems more probable that each of the five or six cellular antigens specific to *guinea* is produced by two or more genes on the individual chromosomes.

Furthermore, any species which possessed all the cellular characters which are common to *guinea* and *livia*, as well as a part of the components specific to *guinea* in contrast with *livia*, would certainly differ from *guinea* only to the extent that it did not share the *guinea* specific substances *in toto*. Only *palumbus* appears to have in common with *guinea* practically all the components shared between *guinea* and *livia* (line 26) as well as a part of those specific to *guinea*, in contrast to *livia*. Unpublished evidence suggests that *palumbus* contains all the specific *guinea* characters **A** and **F**, but only a part of **CD** and **E**, and no more than a minute fraction, if any, of **B**. Hence *guinea* differs from *palumbus* in the biochemical composition of its erythrocytes in the effects of genes on three or four chromosomes—namely, those with genes affecting, respectively, character **B** and parts of characters **CD** and **E**,

Although there is definite evidence that the blood cells of *guinea* differ from those of *livia* in five or six major antigens, and by inference differ from those of *palumbus* in three characters, each determined by one or more genes on as many chromosomes, there is no reason to assume that the chemical differences between these species are limited to these cellular characters or to the effects of the genes causing them. Recent results have shown a segregation of antigens specific to the serum of one of the parental species, in backcross individuals from each of three different species crosses (CUMLEY, IRWIN and COLE, 1941; CUMLEY and IRWIN, 1942, unpublished data; IRWIN and CUMLEY 1942). The species-specific constituents of the serum have separated independently of those of the cells in each of the four kinds of backcross progeny, implying independent and specific action of the genes in each species producing the species-specific effects in the cells and serum, respectively. Hence in *guinea* it is reasonable to assume that chromosomes other than those which carry genes producing the five or six *guinea* specific characters of the cells may have genes influencing the chemical composition of the serum (proteins). Whether the chromosomes of *guinea*, carrying genes for any kind of biochemical differentiation of that species from any other, will also carry genes affecting other characters distinguishing that species is still an open question. Could this be answered, it would undoubtedly provide information as to the relative importance of the various kinds of characters by which differentiations of species are attempted.

All these comparisons of species relationships have been made herein on the basis of characters which definitely distinguish one species from another (that

is, species-specific characters). Also, an extension of the comparisons has been made to determine whether the specific characters of one species are shared, at least in part, with still other species of the genus. The emphasis is therefore placed first of all on the differences between a pair of related species, with the underlying assumption, based on a reasonable amount of experimental evidence, that the differences as well as the resemblances are genetically determined. Moreover, in the light of our present knowledge, the genes responsible for the cellular characters appear to produce their effects irrespective of the total genetic complex. Thus, effects of both external and internal environment on these characters are presumably at a minimum.

In some respects this kind of biochemical assay of relationships between species differs slightly from the general picture of relationships obtained by the methods of either classic taxonomy or cytology. ANDERSON (1937) states that "cytology, or more properly karyology, concerns itself with the architecture of the germplasm; taxonomy with the adult forms which result from germplasms." In general, these two disciplines deal primarily with homologies between species, whether the homologies are in the nature of a morphological character or in the banding of a salivary chromosome. The examination of the salivary gland chromosomes of various species and strains of *Drosophila* has resulted in a picture of differences in gene rearrangements, or inversions, in these forms. Such studies are somewhat comparable, then, to these biochemical studies of differences between species. For example PATTERSON (1942) has compared, among others, five species of the group of *D. virilis* and has proposed that one species, *americana*, evolved from hybrids between *novamexicana* and *texana*. Although STURTEVANT (1942) has questioned the specific status of certain members of the *virilis* group, they serve to emphasize the well-recognized fact that it is possible that gene rearrangements have played a significant role in the evolution of species. Similar studies have been made by several workers on populations of races A and B of *D. pseudoobscura*, as a result of which a phylogenetic chart of relationships has been constructed (DOBZHANSKY 1941). Should it be found that gene rearrangements are accompanied by biochemical changes, their probable role in evolutionary processes could hardly be questioned. It has already been shown (CUMLEY 1940) that grouping of *Drosophila* species by serological methods corresponds relatively closely to the grouping by the use of morphological characters. In our opinion, these methods may be employed to determine whether the various kinds of gene rearrangements of themselves produce biochemical changes.

In conclusion, the experimental evidence from all species crosses, from which an assay of the segregation of antigens has been made, indicates strongly that the biochemical characters of the blood cells of such pigeon and dove species by which one species is differentiated from another appear to be produced by the action of one or more, probably usually of several, genes on each of a relatively small proportion of the chromosomes of the respective species, rather than by genes scattered over most of the chromosomes. In our opinion, parallel relationships obtain between the various species reported in this paper, although species hybrids and backcross hybrids have not yet been

obtained between them. These findings suggest that at least the major changes of this kind, which have taken place in the germinal material, have been confined to a few chromosomes. Eventually it should be possible to assay the chemistry of these or comparable characters, presumably thereby gaining some knowledge of the structural changes (DOBZHANSKY 1941, p. 85) that must have occurred in genes during the evolution of species. Such a statement is based on the supposition, as previously proposed (IRWIN and COLE 1936a; HALDANE 1938) that the cellular antigens are more or less primary products of their causative genes.

ACKNOWLEDGMENTS

We are particularly indebted to DR. CARLOS A. MARELLI, Director, and to DR. ADOLFO D. HOLMBERG, Manager, of the ZOOLOGICAL GARDENS OF BUENOS AIRES, Argentina, for their courtesy in furnishing us with a pair of *Columba picazuro*, and to DR. WILLIAM H. MANN, Director of the NATIONAL ZOOLOGICAL PARK OF WASHINGTON, D. C., who kindly brought these birds to the United States and forwarded them to us.

PROFESSOR L. J. COLE, with characteristic generosity, has allowed all birds under his supervision to be bled for these tests.

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