A GENETIC ANALYSIS OF SPECIES DIFFERENCES IN COLUMBIDAE[1]

M. R. IRWIN
University of Wisconsin, Madison, Wisconsin

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EVIDENCE was presented in a previous paper (IRWIN and his collaborators 1936) that certain of the antigenic characters of the red blood cells, by which *Columba guinea* is differentiated from *C. livia domestica*, had been obtained as units following, respectively, backcrosses of the species-hybrid and backcross hybrids to *livia*. Since a segregation of the cellular characters peculiar to Pearlneck (*Streptopelia chinensis*)[2] as contrasted with Ring dove (*Streptopelia risoria*) has been observed in individuals of the first and second backcrosses to Ring dove (IRWIN and COLE 1936, 1937), it would be anticipated from the results in the Columba species that isolation of the specific Pearlneck components may be accomplished. Investigations to this end are reported in this paper. References to earlier and related publications have been made previously.

EXPERIMENTAL PROCEDURE

The cellular characters under study are recognizable at present only by immunological techniques. The details of these methods have been adequately described elsewhere (IRWIN and COLE 1936, 1937), but a brief exposition of the principles involved may be helpful. The antiserum obtained from a rabbit which has been injected with the erythrocytes of a particular species, as Pearlneck, will not by direct agglutination tests differentiate the cells of the donor species from those of any other species of the same family. That is, the highest dilution of antiserum (that is, the titer) which will cause clumping of the homologous cells will usually produce clumping also of the cells of other species of the same genus or family.

However, if the antiserum for Pearlneck, at a relatively low dilution depending upon its titer, is mixed with an excess of the cells of Ring dove or some other species, it becomes by the absorption a differentiating "reagent," or "test-fluid," which will agglutinate the homologous but not the absorbing cells. Usually, following the absorption of anti-Pearlneck serum by Ring dove cells, the titer of the absorbed serum for Pearlneck cells is reduced slightly from that of the unabsorbed antiserum.

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[2] In former papers, Pearlneck was designated as *Spilopelia chinensis*. Recently PETERS (1937) has proposed that this species be included in the genus Streptopelia.

The explanation of the above results is as follows. The injection of the blood cells into rabbits produces specific antibodies (agglutinins) for the various biochemical constituents of Pearlneck cells. Further, the interaction of Pearlneck antiserum with Ring dove cells presumably is due to the presence within these corpuscles of antigenic substances at least similar to, and probably identical with, a part of the antigenic complex of Pearlneck cells. When, therefore, Ring dove cells are mixed with Pearlneck antiserum, the agglutinins specific for those cellular substances which are *common* to the two species are adsorbed on the surfaces of the cells and are thereby removed from the serum, leaving in it only those antibodies which are specific for Pearlneck (as contrasted with Ring dove).

As has been shown in reports cited above, that part of the antigenic complex of Pearlneck which is not common to Ring dove must itself be composed of several characters. The evidence for this statement is based upon the segregation of the specific Pearlneck characters in the backcross offspring.

The recognition of the different types of cells among the backcross birds is made possible by an extension of the absorption technique, in that Pearlneck antiserum, first absorbed by Ring dove cells, is further absorbed by the cells of individual backcross hybrids. These different "reagents" would then contain the agglutinins for specific Pearlneck substances which were not removed by the cells of the respective birds. For example, let us assume that Pearlneck antiserum following absorption by Ring dove

TABLE 1

Agglutination interactions of the species-specific Pearlneck components.

CELLS	TITERS FOLLOWING ABSORPTION OF		TITERS FOR THE DIFFERENT CELLS OF ANTI-PEARLNECK SERUM, FIRST ABSORBED BY RING DOVE CELLS, THEN BY CELLS OF BACKCROSS BIRDS CONTAINING, RESPECTIVELY, ONE OF THE FOLLOWING PEARLNECK CHARACTERS									
	ANTI-F_1 SERUM BY PEARLNECK AND RINGDOVE CELLS	ANTI-PEARLNECK SERUM BY RINGDOVE CELLS	d-1	d-2	d-3	d-4	d-5	d-6	d-7†	d-4 d-8	d-9	d-11
Ringdove	o	o	o	o	o	o	o	o	o	o	o	o
Pearlneck	o	7, 8										
F_1	2, 3, 4	7, 8, 9	8	8	8	8	7, 8	7, 8	7, 8	8	8	6, 8
d-1	o	3, 4	o	3, 4	3, 4	3, 4	2, 3	3, 4	4	2, 3, 4	4	4
d-2	o	2, 3, 4	2, 3, 4	o	3, 4	2, 3	2, 3	3	3, 4	2, 3, 4	3	4
d-3*	++	1, 2	2	1	o	1, 2	1, 2	2	1, 2	2	1	2
d-4	2, 3, 4	2, 3, 4	2,++	2	2	o	1, 2	2	1, 2	o	1, 2	2+
d-5	o	5, 6	5, 6	5, 6	6	5, 6	o	5, 6	6	5, 6	6	6
d-6	o	2, 3, 4	3	3, 4	2, 3	2, 3	2, 3	o	3	3, 4	3	2
d-7†	o	2, 3, 4	3,++	3	2, 3	3	2	3	o	1, 3	3	2, 3
d-4; d-8	2, 3	3, 4	2, 3:3		2, 2+	2	2, 3	2, 3	1, 2	o	2	2, 3
d-9	o	2, 3	2	2	2, 3	1, 2	1	2	1, 2	1	o	2
d-11	2, 3	7, 8	8	8	7, 8	8	8	7, 8	7, 8	8	8	o

The digits represent the highest dilution of serum at which agglutination was visible microscopically; see text for explanation. Symbol: ++ = strong agglutination at the first serum dilution.

* This substance usually required a special serum for identification; see text.

† Two substances.

cells contains agglutinins for substances A to J, respectively. If, then, the cells of a particular backcross individual removed, by further absorption, all the agglutinins except those for components A and B, such a reagent would in subsequent trials agglutinate only those cells which contained either A or B, or both.

Similarly, "second absorptions" by cells which contained only a single Pearlneck substance, as A, B or C, etc., would in each case remove only the corresponding agglutinin, and the clumping of the other cells would still be by virtue of the specific agglutinin for the respective cellular characters. (As will be explained below, although the respective Pearlneck antigenic characters, except as noted, behaved as if they were definite units and presumably each may have been due to the action of a single gene, more than one gene may be concerned in the production of any or of all. The terms "character" and "agglutinin" will be used throughout this paper, although the probable complexity and possible plurality of both should be recognized.)

IDENTIFICATION OF THE DIFFERENT CELLULAR COMPONENTS SPECIFIC TO PEARLNECK

Following successive backcrosses to Ring dove of birds selected within families for their content of different Pearlneck characters, it has been possible to identify by the procedures outlined above different constituents of the antigenic pattern of the specific Pearlneck complex (not in Ring dove). The results of immunological tests which differentiate the Pearlneck components, each from the others, are given in table 1. These Pearlneck substances are numerically designated, respectively, d-1, d-2, d-3 . . . d-11, the letter d indicating dove.

In order that the results obtained by the use of antisera derived from different rabbits in these experiments may be comparable, the dilution for each antiserum used in absorptions by Ring dove cells has been adjusted so that the last trace of agglutination of Pearlneck cells, following absorption, was usually at the eighth dilution. Thus if the first dilution of the reagent with the cells was one part serum in 45 parts of saline, in table 1 the digit $1 = 45$, $2 = 90 \cdots 8 = 5760$; the dilutions always increasing by halves. The majority of the combinations of the cells and test-fluids as given in table 1 have been made repeatedly. Only minor fluctuations have been observed in the quantitative expression of the reactions of the cells with the different reagents, and these are given whether they occurred with reagents derived from a particular antiserum, or from different antisera.

It is not proposed that slight differences in the dilution of a particular reagent, at which cells from two individuals will agglutinate, constitute

definite antigenic differences. The only sure criterion of an antigenic difference in the cells from two individuals, is that of agglutination of one kind of cell, as compared to no clumping of the other, with a particular reagent.

The second column of data in table 1 gives what may be termed the quantitative expression of the different Pearlneck substances; that is, the highest dilutions of Pearlneck antiserum, absorbed only by Ring dove cells, at which the last trace of agglutination of these respective characters was observed microscopically. The antiserum from only one rabbit, out of more than 30 immunized with Pearlneck cells, has contained an appreciable amount of agglutinin for substance d-3. It has therefore been necessary at times to use other means of detecting this substance, as will be described elsewhere.

Substances d-5 and d-11 gave the strongest reactions of these different substances. Indeed, by interaction with this reagent the cells of d-11 were indistinguishable from those of the F_1, since both were usually agglutinated at the same end-dilution. Because of the finding that substance d-11 produces agglutination quantitatively equal to that of the F_1 cells, it seems advisable to seek a different interpretation of the distribution of the antigens in the first and second backcross generations. It would be expected that d-11 would appear in approximately half the backcross offspring of any individual whose cells contained it, and on this explanation, the ratios of birds in the different backcross generations with this component present agree fairly well with those expected. Previously (IRWIN and COLE 1936), it was proposed that, in the first backcross generation, those cells giving the same quantitative expression as the cells of the F_1 did so by virtue of the tendency of several Pearlneck antigens to stay together, inferring a like tendency on the part of several chromosomes bearing the causative genes. Since the d-11 antigen alone would give the same effect, the previous interpretation need no longer be invoked.

The other substances generally showed the last trace of clumping (microscopically observed) between the second and the fourth dilutions of this test-fluid, and a distinction between them was impossible by this particular reagent, despite differences in their rate and type of agglutination, optimum temperature required, etc. The non-additive effect in agglutination of two or more of these substances has also been noted previously in another species cross (IRWIN, COLE and GORDON 1936).

However, when second absorptions of Pearlneck antiserum, first absorbed by Ring dove cells, were made by cells representing each of these substances, respectively, not only was there no change for each reagent in the dilution at which F_1 cells were agglutinated, but, likewise, very little if any change in the dilutions at which the cells bearing the individual

substances, other than those absorbing, were clumped. Thus, d-1 cells removed all of the agglutinins for that component, and for that component only, as attested by the plus reactions of the d-1 reagent with all other cellular characters. A similar statement can be made concerning each of the other reagents, except that the substance d-8 has never been obtained except in combination with d-4, and it would be expected that absorption by cells containing the combination of d-4·d-8 would remove the specific antibodies for both characters, as was observed.

Differences between components d-4 and d-9 were not always sharply defined, and not always present. These are, however, tentatively classed as two distinct substances.

The interactions of the different "reagents" and cells as given in table 1 may be briefly explained as follows. Cells representing each of the particular Pearlneck components, d-1, d-2, . . . d-11, react with Pearlneck antiserum, absorbed only with Ring dove cells, by virtue of antibodies specific for each particular component. The removal of any one of these different antibodies, by a further absorption by specific cells, still allows each of the remaining antibodies to interact with its specific substance. Whether or not each of these different substances may be single or complex can be determined only if there should be further separation of the respective characters in the offspring of backcrosses to Ring dove.

GENETICAL FINDINGS

The cells of the species hybrid contain nearly all of the specific components of both of the parental species (IRWIN and COLE 1936), although in the F_1 these different substances must, if genetically determined, be produced by genes which are simplex. The ratios expected for such characters in backcrosses to either parent would then presumably simulate those expected in the usual backcross of a monohybrid, dihybrid, etc., to a single recessive, double recessive, etc., respectively. (An exception would occur if there were a tendency for two or more chromosomes bearing genes for the specific components of one species not to separate independently at reduction division.) Thus, in backcrosses to Ring dove, if the specific part of Pearlneck cells were a single component, only two types of cells would be observed in the offspring; that is, those with and those without the Pearlneck character. If only two substances, as $\overline{A}$ and $\overline{B}$, comprised the specific part of Pearlneck, four kinds of cells would be found in the backcross offspring; that is, $\overline{A}\,\overline{B}$, $\overline{A}$, $\overline{B}$, and $\overline{O}$ (the $\overline{O}$ type of cell would naturally denote the absence of both $\overline{A}$ and $\overline{B}$, and would therefore be the same as the Ring dove cells). On this basis, the number of different types of cells possible from a backcross of the F_1 to Ring dove would be 2^n, in which n represents the number of Pearlneck characters.

TABLE 2

Distribution of the specific Pearlneck characters (of table 1) in progeny of mating to Ring dove of backcross birds carrying the different substances.

PEARLNECK SUBSTANCE	NUMBER OF OFFSPRING WITH	
	SUBSTANCE PRESENT	SUBSTANCE ABSENT
d-1	22	29
d-2	5	9
d-3	12	10
d-4	18	17
d-5	17	19
d-6	27	29
d-7*	14	2
d-4, d-8*	4	4
d-9*	3	2
d-11	25	29

* Only one backcross hybrid in each mating to Ring dove.

Obviously, if each of the different antigens represented in table 1 were but one substance, the progeny of matings to Ring dove of birds carrying any one of these components would be divided into (a) those which possessed the character and (b) those which did not, simulating the results expected within a species in the backcross of a monohybrid to the recessive. A summary of tests of the different progenies is given in table 2. It will be noted that there is an approximate equality in the proportions of individuals showing one or the other of the two kinds of cells produced from each of the different matings,[3] except for that of d-7. Some of the cellular characters were represented in the matings by several backcross hybrids, others by only a single bird. In this latter category were matings involving components d-7, d-4 · d-8, and d-9.

The distribution of the progeny of the bird possessing substance d-7 more nearly approximates that expected if two, rather than a single Pearlneck component were present. Such an explanation agrees with a previous proposal (IRWIN and COLE 1936, table 4) that the cells of the individual concerned ($458A_2$) carried at least two Pearlneck characters. The results (unpublished) of reciprocal absorptions and subsequent agglutinations of the cells of the progeny also point to the presence of at least two specific Pearlneck components in the cells of this backcross individual.

[3] Matings in which d-5 was present in combination with another character produced a statistically significant excess of progeny carrying d-5, usually in combination and sometimes alone. A few other combinations of two characters have not departed from the expected equality of of the four possible kinds of cells in their backcross progeny, but in these latter the numbers have been comparatively small.

Similarly, a segregation would probably have been observed in the cells of the offspring of the individual containing d-4·d-8, given adequate numbers. The eight offspring of this mating were produced over a period of two years, and d-8 was not obtained alone. Also, although a sufficient number of offspring have not been obtained as yet in the mating of d-9 to Ring dove to state definitely that this component is not divisible, a reasonable assurance of its unit-nature has been provided by the ratios of the kinds of cells produced in progeny of matings to Ring doves of birds possessing the combinations d-6·d-9 and d-9·d-10. In each of these, only four types of cells have been observed in the offspring, indicating that d-9 is a single substance.

Adequate tests were performed on nearly all of the birds of the different progenies listed in table 2, to determine whether or not the cells giving the positive agglutinations were identical with those of the respective parents. For example, the cells of all of the offspring of different birds containing substance d-1 were used in individual "second absorptions," each reagent thus produced then being tested for the complete absorption of the agglutinin for d-1 by mixing with "tester" cells for the d-1 substance. It would be expected on a genetic basis that the cells of a backcross hybrid parent would by absorption remove all antibodies for the specific Pearlneck character or characters in the cells of its backcross offspring; that is, the offspring could, barring mutation, possess no such genetic character not present in the backcross parent. However, if the cells of a particular backcross parent contained two or more antigenic substances, by virtue of one or more genes on each of two or more different chromosomes, or of two or more genes on the same chromosome each producing different effects, a separation of the antigens in either case would be possible. The latter alternative is the more probable, and a very few interactions have been observed which suggest that certain of the specific Pearlneck components listed above are not single substances. Further studies are required, however, before interpreting these exceptions as indicative of the action of more than one gene in the production of any of the several Pearlneck substances.

It is not proposed that these ten or eleven (counting d-7 as at least two substances) cellular components of Pearlneck constitute the total number which differentiate these cells from those of Ring dove. The characters described above represent major differences between the two species, in that they are all expressed at a relatively high dilution of the antiserum. What appears to be another major Pearlneck character has been isolated, and it is probable that there are many others which are as yet undiscovered.

These data show that there are at least ten biochemical characters in

the erythrocytes of Pearlneck which distinguish it from Ring dove. The segregation of these different components in the respective backcross progenies simulates that expected if each were the product of the action of a single gene. However, identical results would be obtained if two or more genes, on as many chromosomes of Pearlneck as there are specific characters, together produced each of the different substances, and if there were no crossing over in the backcross hybrids of such Pearlneck chromosomes with their (partial, at least) homologues in Ring dove.

The possibility that any two, or more, Pearlneck characters may have been separated in the backcross generations as the result of crossovers cannot definitely be excluded. However, the manner in which a separation has occurred in the progeny of backcrosses of individuals containing a combination of two such characters (unpublished data) makes it more reasonable to assume that each of these different characters is produced by one or more genes on independent chromosomes.

THE "HYBRID SUBSTANCE"

In view of the finding (IRWIN 1932) that the cells of the hybrid between Pearlneck and Ring dove contain one or more components not found in the cells of either parent, it is of interest to determine which, if any, of the specific unit-characters of Pearlneck are associated with the "hybrid substance." Agglutination of the cells containing any of the specific Pearlneck characters, in anti-hybrid serum absorbed by both Pearlneck and Ring dove cells, would indicate that the particular Pearlneck substance was correlated with the "hybrid substance." The results of such tests are given in the first column of data in table 1.

From the reactions it will be seen that only the cells containing d-3, d-4 and d-11 contain the "hybrid substance."[4] (Substance d-3 was weakly agglutinated, if at all, by the various anti-F_1 sera.) Up to the present this correlation has been positive and perfect; that is, cells not containing any one of these three Pearlneck components have not been found to have the "hybrid substance," and the presence of any one of the three, alone or in combination, has assured the presence of the "hybrid" component or components. Evidence that this "hybrid substance" is divisible into two, and probably more, parts will be presented in another paper.

SEPARATION OF SPECIFIC RING DOVE CHARACTERS IN THE PROGENY OF BACKCROSSES TO PEARLNECK

On the basis of previous work, it would be anticipated that, following

[4] Preliminary trials of a newly produced anti-hybrid serum, absorbed by the cells of both Pearlneck and Ring dove, indicate that cells bearing Pearlneck substances other than these three (d-3, d-4 and d-11) may carry also a part of the "hybrid substance." If confirmed in future work, these findings will be considered in publications to follow.

a backcross of the species-hybrid to Pearlneck, there would be a segregation in the progeny of the specific Ring dove components. Such offspring are obtained only with difficulty under our laboratory conditions. The eggs from hybrid females rarely, if ever, produce a living squab, leaving the mating of hybrid males to Pearlneck females as probably the only source of viable progeny. The Pearlneck females are extremely wild, and only an occasional individual has produced eggs. The few offspring available for testing have been hatched over a period of several years. It being very unlikely that many others will be obtained, these few observations will be presented, in order to establish definitely that the experimental findings agree with the expectations; that is, that there would be segregation of specific Ring dove components.

The individuals of family 795 in table 3 are from the mating of a species-hybrid male to a Pearlneck female, as is the single individual $778E_3$. A bird of family 795 (795V), also mated back to Pearlneck, produced family 993.

The differences between individuals in their content of specific Ring dove substances were analyzed by methods comparable to those described in this and in previous reports for determining the segregation of specific Pearlneck characters, specific *guinea* characters, etc. A summary of the tests performed is given in table 3.

The cells of each of the individuals of family 795 were agglutinated in anti-Ring dove serum absorbed only by Pearlneck cells (see second column of data, table 3). Presumably these agglutinations were produced by virtue of specific Ring dove substances, not in Pearlneck. Further absorptions of this absorbed serum, by the cells of the individual backcross hybrids listed, provided specific reagents, which, by interactions with the different cells, allow the probable number of specific Ring dove components to be estimated. Thus, the absorption by the cells of 795W removed the antibodies for itself only, whereas the cells of 795Z exhausted the agglutinins not only for its own cells but for those of 795W as well. Further, the cells of 795X removed by absorption the antibodies for itself, as well as for both 795Z and 795W.

The results as summarized in table 3 may be hypothetically explained as follows, on a minimum basis as to the number of specific Ring dove characters involved. By the above reactions, one substance ($\overline{A}$) is required in the cells of 795W, two ($\overline{A}\,\overline{B}$) in those of 795Z, and three ($\overline{A}\,\overline{B}\,\overline{C}$) in the corpuscles of 795X. Since the "reagent" for 795V agglutinates the cells of all other birds within this family, another component ($\overline{D}$) must be added to the list. Further, the antigen of 795U removes antibodies for itself only, but this substance is found also in the cells of 795H, as are the components of 795G. These latter cells, however, share the component ($\overline{A}$) of 795W;

TABLE 3

Interactions of the cells of individuals of the first and second backcrosses to Pearlnecks with various absorbed sera.

TITERS AND REACTIONS FOLLOWING ABSORPTION OF			AGGLUTINATION OF THE VARIOUS CELLS LISTED, WITH ANTI-RING DOVE SERUM FIRST ABSORBED BY PEARLNECK CELLS, THEN BY CELLS OF EACH OF THE FOLLOWING BIRDS:																	
CELLS	ANTI-F_1 SERUM BY* PEARLNECK AND RING DOVE CELLS	ANTI-RING DOVE SERUM BY PEARL-NECK CELLS	778E_3	795D	795E	795G	795H	795U	795V	795W	795X	795Z	993S	993X	993Y	993K_2	993N_2	993P_2	993R_2	993C_3
Ring dove	o	7, 8	6	4	3	6	5, 6	5, 6	4, 5, 6	6	4	4	5	5	5+	5	5	5	5	5
Pearlneck	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
F_1	C	6, 7		±		4	4	4	+	4										
778E_3	+	3	o	o			o	o	o	+	o									
795D	++	5, 6	++	o	+	++	++	++	++	++	+	+								
795E	C	6, 7	++	+	o	++	++	++	++	C	±	±	++		++	++	++	++	+	
795G	O	4, 5	++	+	+	o	o	+	++	++	+	+	++		++	++	++	++	+	
795H	+	5, 6	++	++	+	+	o	+	++	++	+	+								
795U	+	5	++	+	+	+	o	o	++	++	+	±								
795V	C	6, 7	++	o	o	++	++	++	o	++	±, +	±	+, ±	o	++	o	o	±	±	±
795W	o	4	+	o	o	o	o	±	++	o	o	o								
795X		7		+	o	++	++	++	++	++	o	+								
795Z		5, 6		o	o	++	++	++	+	++	o	o								
993P	o	o													o					
993S	C	6			o	++			o				o	o	++	o	o	o	o	o
993X		6			o	++			o				±	o	++	o	o	o, o+	o	o
993Y	o	1, 2			o	o			o				o	o	o	o	o	o	o	±
993B_2	o	o													o					
993F_2	o	o													o					
993K_2	C	6			o	++			o				o	o	++	o	o	o	o	o
993N_2		6			o	++			o				o	o	++	o	o	o	o	o
993P_2	C	6			o	++			o				o	o	++	o	o	o	o	o
993R_2		6			o	++			o				o	o	++	o	o	o	o	o
993V_2	o	o													o					
993C_3	C	6							o					o	++	o	o	o	o	o

* First dilution of the serum-cell mixture = 1:45 or 1:90.

The digits indicate the serum-dilution at which the last trace of agglutination was observed. The first serum-cell-dilution was 1:90, 1 = 90, 2 = 180, 3 = 360, · · · 8 = 11, 520; o = no trace of clumping at the first dilution.

Symbols: C = complete agglutination; ++ = marked agglutination; + = agglutination; ± = trace; o = no agglutination—all at first dilution of the serum-cell mixture.

their respective phenotypes would be, for 795U, $\overline{E}$, for 795G, $\overline{A}$ $\overline{F}$, and for 795H, $\overline{A}$ $\overline{E}$ $\overline{F}$. Also, by the reciprocal interactions of their cells and particular "reagents," birds 795D and E are surely not identical in their cellular patterns.

The interactions of the respective reagents with the other cells show that the antigens of 795V, W and Z are present also in 795D, while those of 795V, W, X and Z are found in 795E. The presence of a different antigen must, therefore, be assumed in the cells of each of these two birds (795D and E), giving the phenotypes $\overline{A}$ $\overline{B}$ $\overline{D}$ $\overline{G}$ for the cells of 795D, and $\overline{A}$ $\overline{B}$ $\overline{C}$ $\overline{D}$ $\overline{H}$ for those of 795E. Finally, if the one or more components of $778E_3$ be considered, another antigen ($\overline{I}$) would be required. This would be present also in the cells of 795D, E, H, U, V and X, requiring then a minimum of nine different specific Ring dove characters in the cells of the species hybrid to explain the results of the table.

Illustrating the principle set forth earlier in this report, the Ring dove antiserum, after absorption by Pearlneck cells, contains specific antibodies for each of the different antigens peculiar to Ring dove. As a specific example, the cells of 795D by absorption remove agglutinins a b d g i, and this reagent then gives agglutination with the cells of 795E ($\overline{A}$ $\overline{B}$ $\overline{C}$ $\overline{D}$ $\overline{H}$ $\overline{I}$) by virtue of substances $\overline{C}$ and $\overline{H}$ in these latter cells; with those of 795G, H, U and X by virtue of the substances $\overline{F}$, $\overline{E}$ $\overline{F}$, $\overline{E}$ and $\overline{C}$, respectively. A similar analysis of the interactions of the reagent for each individual with the other cells would explain agglutination, or lack of it, for each combination of reagents and cells. Presumably, these cellular characters particular to Ring dove are produced by genes contributed by the Ring dove parent. These genes then are simplex in the species and backcross hybrids, and the phenotypes and genotypes are the same in respect to the specific Ring dove characters of the cells.

Additional evidence regarding the specificity of the "hybrid substance" is furnished by the presence or absence of agglutination of the cells of certain of the backcross birds when tested by anti-F_1 serum from which the antibodies for the cells of both parents had been removed (see first column of data, table 3). Of the cells of the birds of the 795 family available for testing with this reagent, five (795D, E, H, U and V) agglutinated, while those of 795G and 795W did not. Within the offspring of 795V, family 993, and exclusive of 993Y, only those individuals showing the presence of specific Ring dove components also possessed the "hybrid substance."

The findings are in accord with the specificity proposed in the production of this hybrid substance or substances. For example, the cells of $778E_3$ contained at least a part of this character, and thus all other cells in which the one or more Ring dove antigens of $778E_3$ were found should also possess the hybrid substance, as was indeed noted. Furthermore, no other cells

containing only $\overline{A}$ or $\overline{F}$, alone or together, can carry the "hybrid component." A partial test of this condition is found in that the cells of 795W, containing $\overline{A}$ alone, have no trace of this substance. It presumably is produced by the interaction of particular genes which, in each of the two species, are linked to others producing the respective specific characters, or themselves produce such effects. Further consideration of this interaction will be presented elsewhere.

DISCUSSION

In this paper are summarized the results of experiments to obtain, as separate entities, the different cellular characters which distinguish the Pearlneck from the Ring dove species. Also given are findings which indicate that the specific Ring dove characters, not in Pearlneck, are divisible and hereditary. There seems little doubt, in view of the segregation of both Pearlneck and Ring dove specific characters as noted, that the different antigenic constituents peculiar to the cells of each species are gene-determined.

That the cellular components *common* to both Pearlneck and Ring dove are divisible and multiple is made probable, since evidence has already been presented (IRWIN 1938) that two of the unit-characters of *C. guinea*, not in *C. livia domestica*, are shared by both Pearlneck and Ring dove. A comparison of the interrelationships of the cellular components of Pearlneck, Ring dove, *C. guinea* and *C. livia*, as previously given (IRWIN 1938), would further substantiate the above statement.

If, within the Pearlneck species, each of these genes for specific Pearlneck characters has an allele with a different effect and assuming ten such genes, the number of combinations of these species-specific characters may be readily calculated. Assuming no dominance, and with each gene giving an independent expression if heterozygous, the number of possible combinations would be 3^{10}, or 59,049. This number would be changed if there were dominance in one or more pairs of characters, or if there were multiple alleles active at one or more loci. The genes affecting the cellular pattern of Pearlneck shared with Ring dove may be equally numerous, and thus the number of possible combinations of characters (that is, the phenotypes) for the species would indeed be very great.

The suggestion has already been made (IRWIN and COLE 1936) that the antigens of the erythrocytes may be considered as more or less direct products of the gene. The finding by LANDSTEINER and LEVINE (1926) that human sperm contain the same antigens, $\overline{A}$ and $\overline{B}$, as the blood cells of the individuals tested, may be considered as very pertinent evidence to this point. That the genic effect on the cellular antigen may not always be direct is indicated by the finding of the "hybrid substance" in certain spe-

cies hybrids, not in all, and that it or substances closely related to it are found normally in the cells of certain other species (Irwin 1935).

More recently HALDANE (1938) has discussed the possibilities of the relationship of genes to cellular antigens, proposing that "The gene is a catalyst making a particular antigen, or the antigen is simply the gene or part of it let loose from its connexion with the chromosome." Further, HALDANE (1938) and the writer appear to be in agreement in advocating that advances in our knowledge of the agglutinogens should also increase our information of the nature of the gene itself.

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SUMMARY

These data show that the species-specific components of Pearlneck may be separated into so-called unit-substances as a result of backcrosses to Ring dove. At least ten specific Pearlneck characters have been isolated by this procedure, each of which is immunologically distinct from the others. Data relative to the separation of specific Ring dove characters are also included.

LITERATURE CITED

IRWIN, M. R., 1932 Dissimilarities between antigenic properties of red blood cells of dove hybrids and parental genera. Proc. Soc. Exp. Biol. and Med. **29**: 850–851.

1935 Complementary action in a generic hybrid of genes having a biochemical effect. Abstract. Amer. Nat. **69**: 67.

1938 Immunogenetic studies of species relationships in Columbidae. J. Gen. **35**: 351–373.

IRWIN, M. R., and COLE, L. J., 1936 Immunogenetic studies of species and of species hybrids in doves, and the separation of species-specific substances in the backcross. J. Exp. Zool. **73**: 85–108.

IRWIN, M. R., COLE, L. J., and GORDON, C. D., 1936 Immunogenetic studies of species and of species hybrids in pigeons, and the separation of species-specific characters in backcross generations. J. Exp. Zool. **73**: 285–308.

IRWIN, M. R., and COLE, L. J., 1937 Immunogenetic studies of species and of species-hybrids in doves; the separation of species-specific substances in the second backcross. J. Immunol. **33**: 355–373.

HALDANE, J. B. S., 1938 Essay in "Perspectives in Biochemistry," edited by Needham and Green. Cambridge: University Press.

LANDSTEINER, K., and LEVINE, PHILIP, 1926 On group specific substances in human spermatozoa. J. Immunol. **12**: 415–418.

PETERS, J. L., 1937 Check-list of Birds of the World. 311 pp. Cambridge: Harvard University Press.