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Evolution in Columbidae: Additional Relationships Among the Antigenic Specificities Produced by Gene Interaction

(cellular antigens/gene interaction/species relationships/birds/antisera)

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ABSTRACT **Two of the antigenic characters of erythrocytes, that in either identical or related forms differentiate four species of Streptopelia (*chinensis*, *humilis*, *orientalis*, and *senegalensis*) from a fifth (*S. risoria*) by interaction of the causative genes, are involved in the appearance in backcross hybrids of additional antigenic specificities to those of the parental species. The cells of backcross hybrids, resulting from matings of *S. chinensis* x *S. risoria* and backcrossing to *S. risoria*, in which the two independently inherited antigenic characters (*ch*-4 and *ch*-8 peculiar to *S. chinensis*) are combined as homozygotes, exhibit an antigenic specificity not present in backcross birds carrying separately these two characters. This result indicates an interaction within *S. chinensis* of the genes for these two antigenic characters.**

An antigen (C^g) by which *Columba guinea* differs from *C. livia* has behaved as a unit in over 400 backcross offspring. It is antigenically related to both *ch*-4 and *ch*-8 of *S. chinensis*. It is also related to, but not identical with, this interaction antigen of *S. chinensis*. Further, the C^g character is also related to an interaction product that appears in all backcross offspring carrying the group-4 antigen in a *S. risoria* genome.

Genes related to those that in modern species or in species hybrids interact to effect new antigenic specificities presumably possessed the same abilities in ancestral forms of Columbidae.

One of the unexpected findings in comparisons of the relationships of the antigenic characters of erythrocytes peculiar to species of Columbidae is that an antigenic character that behaves as a unit in one species (*Columba guinea*) is antigenically related to each of two genetically independent antigens of another species (*Streptopelia chinensis*) (1). Specifically, the C^g antigen of *C. guinea*, which distinguishes it from *C. livia*, has behaved as a single character in over 400 offspring in the population generated by backcrosses of the species hybrid and backcross hybrids to *C. livia*. This antigen is related to each of two independently inherited antigens (*ch*-4 and *ch*-8) of *Streptopelia chinensis* (1), which are among the 9 or 10 that differentiate *S. chinensis* from *S. risoria* (2). The genes responsible for each of these three antigens are involved in the production of antigenic specificities (hybrid substances or interaction products) in species hybrids and backcross hybrids, in addition to those present in the parental species. These hybrid substances may be effected either by the interaction (*a*) of alleles from the respective parental species (3), or (*b*) of independently inherited genes derived from the parental species (4).

Evidence for additional antigenic specificities *within S. chinensis* resulting from interaction between the nonallelic genes for *ch*-4 and *ch*-8, differentiating it from *S. risoria*, is given in this paper. Also, crossreactivity of the C^g antigen of *C. guinea* with this interaction product, and also with a fraction of the hybrid substance of species and backcross hybrids carrying the *ch*-4 antigen, is reported.

MATERIALS AND METHODS

The isolation as genetic units of the antigenic characters peculiar to *S. chinensis*, *S. humilis* (*tranquebarica*), *S. orientalis*, and *S. senegalensis* in comparison with *S. risoria* has been described (2, 5). Similarly, the cellular antigens of *C. guinea* have been obtained as units in backcrosses to *C. livia* (1, 6). Each of these four species of *Streptopelia* contains, among others, two antigens that are dealt with in this report. Those respectively composing group-4 and group-8 are *ch*-4 and *ch*-8 of *S. chinensis*, *hu*-4 and *hu*-8 of *S. humilis*, *or*-4 and *or*-8 of *S. orientalis*, and *se*-4 and *se*-8 of *S. senegalensis*. The members of each of these two groups in the four species are either identical or similar to each other. An antigen in *S. risoria* (*ri*-8) antithetical to those of group-8 has been demonstrated (3), but none antithetical to group-4 has been shown.

Antisera produced in rabbits were fractionated by suitable absorptions to provide reagents to detect various antigenic specificities of the different cells tested.

EXPLANATORY

One antiserum of over 200 produced against erythrocytes of *S. chinensis* was unique in that its antibodies reacted far more strongly with the group-4 antigens than did the others. Tests with the reagents made by suitable absorptions of this antiserum furnished evidence (7) for the existence of three antigenic specificities ($\overline{abc}$) of the group-4 antigen in *S. chinensis*, *S. humilis*, *S. orientalis*, and *S. senegalensis*, and in C^g of *C. guinea* (Table 1, row 1).

Antisera against the cells of *ch*-4 in backcross hybrids contained antibodies against antigenic specificities $\overline{def}$ (Table 1, row 5) in one antiserum, and towards $\overline{gh}$ in another (Table 1, row 6) (7). Factors $\overline{def}$ were normal constituents (although interaction products) of the cells of *S. humilis* and of those birds of *S. orientalis* and *S. senegalensis* that possessed the group-4 antigen, and thus differentiated these species from *S. chinensis* and *S. risoria* (7). If in the backcross hybrids to *S. risoria* the gene for the group-4 antigen from any of these four species was combined wth the homozygous gene for *ch*-8 (*ch-8/ch-8*) from *S. chinensis*, only antigenic factors $\overline{abc}$ were present, as in *S. chinensis* (8). Further, if a gene for any mem-

TABLE 1. *Fractionation of antisera to demonstrate various antigenic specificities*

Row	Antiserum	Absorbed by cells of:	Antibody specificities	Agglutination of test cells with antisera absorbed as indicated: *S. chinensis*	*S. humilis*	*S. risoria*	Backcross hybrids: *ch-4** / *ch-4*	*ch-8* / *ch-8*	*ch-4*/—; *ch-8*/*ch-8*	*ch-4*/—; *hu-8*/*hu-8*	C^g/C^g	F_1 *livia* (C^g) / *risoria*
1	*S. chinensis* (no. 1)	*S. risoria* + *ch-8*/*ch-8*	$abcz_1$	++	++	0	++	0	++	+	+	N.D.
2	*S. chinensis* (no. 1)	*S. risoria* + $\frac{ch\text{-}8}{ch\text{-}8} + \frac{C^g}{C^g}$	z_1	+	N.D.	0	0	0	+	0	0	N.D.
3	*S. chinensis* (no. 1)	*S. risoria* + $\frac{ch\text{-}4^*}{ch\text{-}4} + \frac{ch\text{-}8}{ch\text{-}8}$	z_1	+	N.D.	0	0	0	+	0	±	N.D.
4	*S. chinensis* (no. 1)	*S. risoria* + $\frac{ch\text{-}4}{ch\text{-}4} + \frac{ch\text{-}8}{ch\text{-}8} + \frac{C^g}{C^g}$	z_1	+	N.D.	0	0	0	±	0	0	N.D.
5	*ch-4*/— (no. 1)	*S. risoria* + *S. chinensis*	def	0	+	0	+	0	0	+	0	0
6	*ch-4*/*ch-4* (no. 2)	*S. risoria* + *S. humilis*	gh	0	0	0	++	0	0	0	±	+
7	*ch-4*/*ch-4* (no. 2)	*S. risoria* + *S. humilis* + $\frac{C^g}{C^g}$	gh	0	0	0	++	N.D.	N.D.	N.D.	0	+
8	*ch-4*/*ch-4* (no. 2)	*S. risoria* + *S. humilis* + $F_1 \frac{C.\ livia\ (C^g)}{S.\ risoria}$	—	N.D.	0	0	0	0	N.D.	N.D.	0	0

Symbols: ++, +, strong agglutination; ±, definite but weak agglutination; 0, no agglutination at the first dilution of the reagent; N.D., not done.

* Cells of the backcross hybrids carrying *ch-4*, *hu-4*, *or-4*, or *se-4* were indistinguishable in all tests.

ber of group-4 in a backcross hybrid was combined with the homozygous gene for *hu-8* from *S. humilis*, *or-8* from *S. orientalis*, or *se-8* from *S. senegalensis*, the specificities $\overline{abcdef}$ were present, duplicating the presence of these factors in the three species (4). Since the four kinds of backcross hybrids, carrying *ch-4*, *hu-4*, *or-4*, or *se-4* as units, but otherwise indistinguishable from *S. risoria*, contained specificities $\overline{gh}$ in addition to those normally present in the parental species, $\overline{abcdefgh}$, the rational explanation is that the gene for group-4 is homologous in the four species, effecting specificities $\overline{abc}$ in each, and may react with a gene for group-8 to effect specificities $\overline{def}$ (as in *S. humilis*, *S. orientalis*, and *S. senegalensis*) or $\overline{defgh}$ (as in backcrosses with *ri-8* of *S. risoria*) (4).

RESULTS AND DISCUSSION

An interaction product of genes within S. chinensis related to C^g of C. guinea. The ability to combine in a backcross bird the respective genes for *ch-4* and *ch-8* made it possible to determine whether there was an interaction within *S. chinensis* between the genes for *ch-4* and *ch-8*. Absorption of the antiserum to *S. chinensis* erythrocytes with the cells of *S. risoria*, plus those of backcross birds carrying *ch-4* or *ch-8* (*ch-8*/*ch-8*) (Table 1, rows 2, 3), did not remove reactions with the cells of *S. chinensis* or of backcross hybrids carrying *ch-4*/?; *ch-8*/*ch-8*. (Agglutination of the cells of *S. chinensis* is of limited significance, because the reagent contained antibodies against antigens of *S. chinensis* that were not available in backcross birds for use in absorptions.) This reagent was not reactive with cells of backcross hybrids carrying *ch-4*/?; *hu-8*/*hu-8*, thus indicating that this interaction specificity within *S. chinensis* is not the same as that reported by Miller and Weber (9) between the genes for *hu-4* and *hu-8* in *S. humilis*. The new specificity within *S. chinensis* is called $\bar{z}_1$ (10). Further, this reagent agglutinated the cells of backcross hybrids with the C^g character of *C. guinea* (Table 1, row 3), reacting somewhat more strongly with cells homozygous for C^g than with those heterozygous (C^g/C^1). However, C^g cells did not by absorption remove the antibodies for cells with *ch-4*/?; *ch-8*/*ch-8*, showing that this factor in C^g cells was antigenically similar to, but not identical with, the $\bar{z}_1$ specificity in *S. chinensis* (Table 1, row 4).

It is reasonable to conclude that the $\bar{z}_1$-like specificity in the C^g character of *C. guinea* is produced by interaction between presumably linked genes for the $\overline{abc}$ specificities and the *ch-8*-like antigen.

Relationship of interaction specificities of the group-4 antigen to C^g of C. guinea. Previous tests of the respective reagents for interaction specificities $\overline{def}$ and $\overline{gh}$ of group-4 with the cells of C^g birds produced no reactions (4). Numerous tests with the reagent for specificities $\overline{def}$ have consistently con-

firmed that these factors are not present on C^g cells (Table 1, row 5). However, recent tests with an unusually potent reagent for the $\overline{gh}$ factors revealed agglutination of cells of both C^g birds and of a hybrid between a *C. livia* bird carrying the C^g antigen and *S. risoria* (F_1—*C. livia* (C^g)/*S. risoria*) (Table 1, row 6). Absorption of this reagent with the cells of C^g birds failed to remove the antibodies for the generic hybrid (F_1—*C. livia* (C^g)/*S. risoria*) (Table 1, row 7). However, all the antibodies of the reagent were removed by the cells of the hybrid (Table 1, row 8). These reactions differ from those reported previously (4), in which a different antiserum for the group-4 antigen was used. From these results, it may be concluded that the cells of backcross birds with C^g contain an antigenic specificity related to the $\overline{gh}$ specificities of group-4 as these occur in backcross hybrids in the *Streptopelia*. Presumably, this $\overline{gh}$-like specificity of the C^g antigen is produced by the interaction of the genes for the $\overline{abc}$ specificities of group-4 and for the *ch*-8-like antigen. That there is a difference between the gene or subgene for the group-4 antigen in the C^g birds from that in the *Streptopelia* is evident in that only the $\overline{gh}$ specificities are produced in combination with the gene for *ri*-8 of *S. risoria* in the generic hybrid (F_1–*C. livia* (C^g)/*S. risoria*). Otherwise, the $\overline{def}$ specificities should also be present.

Is the C^g a unit character? Although the C^g character of *C. guinea* has behaved as a unit in the backcrosses to *C. livia*, involving over 400 birds to date, its crossreactivity with the independently inherited antigens of *S. chinensis*, *ch*-4 and *ch*-8, raises the question as to whether a single gene or linked genes are involved in its production. If only one gene effects C^g, the resulting product includes antigenic factors $\overline{abc}$ of *ch*-4 (8), at least two factors relating it to *ch*-8 (3, 10), and two separate interaction specificities ($\overline{z}_1$ and $\overline{gh}$-like), in addition to a complex of antigenic factors that relate it to those of many other species of Columbidae (1). These relationships suggest strongly that linked genes are involved in causation of the C^g trait.

On the basis of linked genes, the question arises as to whether linkage between the genes for antigens of group-4 and group-8 prevailed in an ancestral form of the *Columbidae*, or whether this presumed linkage resulted from an event involving genes on independent chromosomes. The independent inheritance of the genes for group-4 and group-8 in four species of *Streptopelia* is established by virtue of their behavior in the respective backcrosses (2, 5, 10). In contrast, if but one gene effects the C^g antigen, it would be rational to assign to it the status of an ancestral gene.

Equally as pertinent as the question of linkage or independence of the ancestral genes for group-4 and group-8 is evidence for the stability of the gene or subgene effecting antigenic specificities $\overline{abc}$. These appear in 7 of the 9 species of Streptopelia that have been tested (10), although in three (*S. capicola*, *S. orientalis*, and *S. senegalensis*) some individuals lack the factors of group-4, as do *S. risoria* and *S. semitorquata* (10). These factors are present also in three species of *Columba* —*C. guinea*, *C. picazuro*, and *C. palumbus* (10). [*C. palumbus* contains all the antigenic specificities of C^g of *C. guinea* (10) and must, therefore, have factors $\overline{abc}$.]

Other antigenic characters have been notably stable in evolution. For example, Landsteiner and Miller (11) demonstrated the identity of the $\overline{A}$ and $\overline{B}$ factors, wherever found in the higher apes, to those of human blood, implying identity of the causative genes. Also, cytochrome *c* in man and chimpanzees has identical sequences of amino acids, presumably specified by identical genes (12). Examples of heterophile antigens have been given by Buchbinder (13), of which one group would include antigens found both in vertebrate tissues and in certain bacteria. What appeared to be a single antigen was found widely distributed in many species of class Aves, suggesting strongly that a causative gene in distantly related species of class Aves had remarkable stability.

The evidence presented here and previously (10) adds to the rationality of the proposal of a gene complex (cistron) effecting the antigenic specificities of the group-4 antigen. One subgene a^1 produces specificities $\overline{abc}$, a^2 effects $\overline{ab}$, and a^3 produces only $\overline{a}$ (10). A second subgene (x), by interaction with the gene complex for the group-8 antigens, effects primarily, but not necessarily only the interaction specificities. Since the C^g cells contain factors $\overline{abc}$, subgene a^1 is present, but an allele of subgene x different from any thus far recognized is assumed. This allele interacts with the group-8-like gene in *C. guinea* to effect two different specificities. One of these is related to the $\overline{z}_1$ specificity in *S. chinensis*, the other to the $\overline{gh}$ factors of the backcross hybrids carrying any member of group-4. The allele of the subgene in *S. chinensis* may be termed x^6, that in *C. guinea* as x^7. Allele x^7 interacts with the gene for the group-8-like antigen of C^g, the product resembling both $\overline{z}_1$ and $\overline{gh}$, and with the gene for *ri*-8 of *S. risoria* to effect $\overline{gh}$ in the generic hybrid.

The appearance of various combinations of the interaction specificities ($\overline{defgh}$) associated with one or more factors ($\overline{abc}$) of the group-4 antigen has been listed for different species of Columbidae (10). It can be confidently assumed that these interaction specificities are the result of interaction between the genes for group-4-like and group-8-like antigens, irrespective of whether these genes are linked or independent. Because of the presence of these interaction products in species now only distantly related, one can state with confidence that some form of the interacting genes was present in species ancestral to those now constituting the Columbidae.

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