MOLECULAR GENETICS OF AVIAN PROTEINS. IX. INTERSPECIFIC AND INTRASPECIFIC VARIATION OF EGG WHITE PROTEINS OF THE GENUS GALLUS

C. M. ANN BAKER

2, Bridge End, Berwick-upon-Tweed, England

Received July 11, 1967

THE egg white of the domestic fowl, *Gallus gallus* L., consists of at least ten different proteins: ovalbumin, conalbumin, ovomucoid, ovoinhibitor, mucin, flavoprotein, avidin, and globulins G_1 (= lysozyme), G_2 and G_3. In starch gel electrophoresis many of these are heterogeneous and four have genetically controlled polymorphisms, which are described below. (The electrophoretic patterns of many of the variants are in Figure 1.)

Ovalbumin (*ov* locus). Alleles *ov A* and *ov B* result in phenotypes A, B, and AB (LUSH 1961, 1964b). In the present paper, however, the variants are named with reference to their relative mobilities as the letter A is used in A_1, A_2 and A_3 terminology for the sub-fractions of ovalbumin (LONGSWORTH, CANNAN and MACINNES 1940; CANN 1949). Thus ovalbumin A = slow (S) and ovalbumin B = fast (F). A recent preliminary communication suggests that the primary structure of the fast and slow ovalbumins differs in a single peptide (WISEMAN and FOTHERGILL 1966).

Other ovalbumin variants have been described. BAKER and MANWELL (1962) found polymorphism which involves a more rapid migration of A_1 and A_2 and a lack of A_3 in one homozygote category (A_3^{--}); the heterozygote (A_3^{+-}) has less of the A_3 subfraction than the "normal" homozygote (A_3^{++}). CROIZIER (1966) has discovered yet another variant (ovalbumin Faverolles) which has subfractions extra to those of the fast and slow variants. He also found it possible to classify each of LUSH's (1964b) ovalbumin phenotypes into two subtypes on the basis of quantitative differences in A_3. As ovalbumin is a phosphoglycoprotein, it is possible that some or all of these additional ovalbumin variants represent differences in enzymes involved in attaching or removing carbohydrate or phosphate moieties, rather than mutation at the ovalbumin locus *per se*.

Conalbumin (*Tf* locus). There are at least four alleles at this locus: Tf^a and Tf^b (OGDEN, MORTON, GILMOUR and MCDERMID 1962); Tf^C (CROIZIER 1966) which is probably the same as Tf^{SJ}—conalbumin Sam Jackson—of BAKER (1967); and Tf^{BW}—conalbumin Bill Wilkinson—mentioned in BAKER (1967) and discussed in more detail in the present paper.

G_2 *globulin* (G_2 locus). This locus has at least two alleles $G_2{}^C$ and $G_2{}^D$ (BAKER and MANWELL 1962). There may be a third allele in an American Sebright and the Red Jungle Fowl, the protein product of which is distinguished from $G_2{}^D$

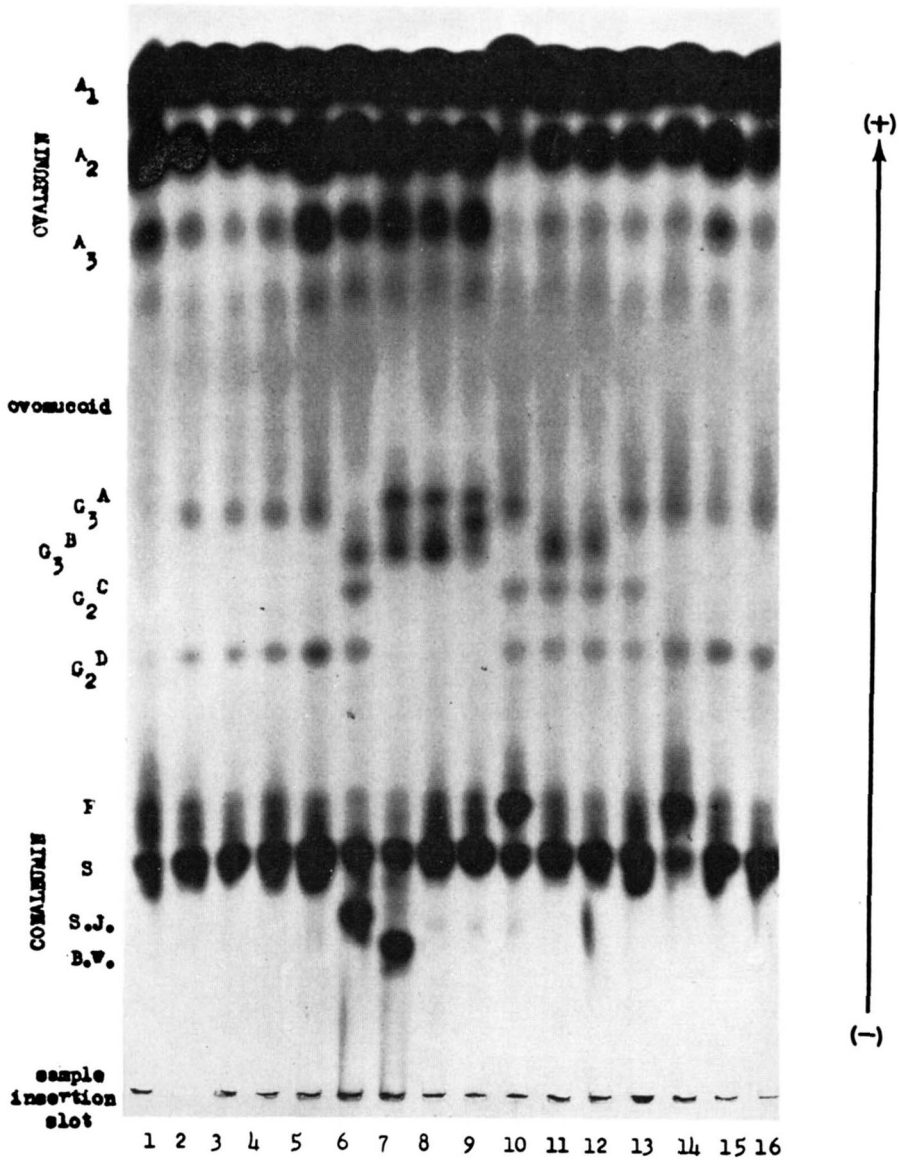

FIGURE 1.—Egg white polymorphism in *G. gallus* and *G. sonnerati* egg white subjected to electrophoresis in the discontinuous buffer system of FERGUSON and WALLACE (1961). The major egg white proteins visible in this gel photograph are described briefly here, starting with those at the top of the photograph (anodal end of the gel) and working down: 1. The heavily staining proteins at the top of the picture are ovalbumin. The fastest ovalbumin zone is A_1 and the second heavy zone is A_2. The slowest ovalbumin zone, A_3, varies greatly in amount in the different samples. Sample No. 10 (counting from left to right) is a *BA* heterozygote (LUSH, 1964b); the others are all *A* homozygous. 2. Ovomucoid forms a diffuse, weakly staining zone, migrating more slowly than ovalbumin. 3. The small, sharp protein bands, approximately half-way up the photograph are the G_3 and G_2 globulins. Samples 1–5, 10, and 13–16 are homozygous G_3^A;

under special conditions (BAKER 1964). G_2 (LONGSWORTH *et al.* 1940) corresponds to LUSH's (1961, 1964a) unidentified "locus III" and the "globulin A" of FEENEY, ABPLANALP, CLARY, EDWARDS and CLARK (1963) (BAKER and MANWELL 1962; BAKER 1964).

G_3 *globulin* (G_3 locus). $G_3{}^A$ and $G_3{}^B$ are common alleles (BAKER and MANWELL 1962). $G_3{}^J$ was first found in the Cornell flock of Red Jungle Fowl (BAKER 1964), and an electrophoretically similar protein has been found in the Marans breed (CROIZIER 1966). G_3 (LONGSWORTH *et al.* 1940) corresponds to LUSH's (1961, 1964a) "locus II" (BAKER and MANWELL 1962).

The existence of this genetic variation of egg white proteins in the chicken raises three questions: (1) Do other variants exist in domesticated and wild *Gallus gallus*? (2) What variants are there in other species of Gallus? (3) How do populations of *Gallus gallus* differ in the frequency of the variants and what bearing might this have on such problems as the evolution of the domestic fowl and the selection of poultry for special purposes?

MATERIALS AND METHODS

Terminology: The names and characteristics of breeds and varieties of chickens follow the American Standard of Perfection (1958) and British Poultry Standards (1954).

Sources of eggs: The breeders who supplied eggs are listed in the acknowledgments.

The main object of the study—to survey as many breeds as possible—was achieved by getting eggs from four fanciers, identified in this paper as I, II, III, and V, who keep a large number of different breeds. As fanciers often keep relatively few individuals of a given breed, the problem exists of obtaining a reasonable sample size for scarce varieties. This problem is reduced in some cases in that a number of breeds were obtained from several different sources, including two other breeders, IV and VI, who keep pure breeds for the production of commercial stock. Between these six enterprises it was possible to survey 37 different breeds; these are listed in Table 1. As some breeds are kept by more than one breeder, 62 distinct flocks are involved. In addition, the Maran breed was of such interest that eggs from three additional breeders, designated here VII, VIII, and IX, were examined.

The identities of the fast ovalbumin (LUSH 1964b) and conalbumin a (OGDEN *et al.* 1962) found in some flocks were checked by comparison with samples provided by MR. E. M. MCDERMID, then of Thornber Bros.

The *Gallus sonnerati* eggs were provided by MR. F. E. B. JOHNSON, Stagsden, Bedford, and by the Paignton Zoological Gardens, Devon.

Biochemical techniques: Vertical starch gel electrophoresis and other biochemical methods used in the study have been described in earlier papers, notably by BAKER and MANWELL (1962) and BAKER, MANWELL, LABISKY and HARPER (1966). The identification of genetic polymorphism of conalbumin presents special difficulties arising from chemically induced heterogeneity and phenocopy (BAKER 1967).

---

samples 6, 11 and 12 are homozygous $G_3{}^B$. Samples 1–5 and 14–16 are homozygous $G_2{}^D$; samples 6 and 10–13 are heterozygous $G_2{}^C G_2{}^D$. Samples 7, 8 and 9, which have neither $G_2{}^C$ nor $G_2{}^D$, are *Gallus sonnerati*. 4. The slowly migrating, heavily staining protein is conalbumin. All samples in this photograph have the "slow" conalbumin. Samples 10 and 14 are heterozygous for "slow" and "fast" conalbumin. Sample 6 is heterozygous for "slow" and "Sam Jackson" conalbumin. Sample 7 is heterozygous for "slow" and "Bill Wilkinson" conalbumin. 5. The protein at the very bottom edge of the photograph is lysozyme; its electrophoretic variation can not be detected in this buffer.

TABLE 1

Phenotypic frequencies of egg white proteins in 37 breeds of domestic fowl

Breed	No. of varieties	No. of flocks	No. of birds	Ovalbumin F	Ovalbumin S	Ovalbumin FS	G_3 globulin A	G_3 globulin B	G_3 globulin AB	G_2 globulin C	G_2 globulin D	G_2 globulin CD	Conalbumin F	Conalbumin S	Conalbumin FS	Breed origin*
Andalusian	1	2	5	..	5	..	2	1	2	..	3	2	..	5	..	**M** (Minorca)
Araucuna	1	1	1	..	1	..	1	..	..	..	1	..	..	1	..	**SA**
Australorp	1	2	37	..	37	..	7	14	16	..	35	2	..	37	..	*E* Minorca, Plymouth Rock, Langshan
Barbu d'Anvers	1	1	2	..	2	..	2	..	..	..	2	..	..	2	..	**B**
Barnevelder	1	1	22	..	22	..	16	..	6	5	6	11	..	22	..	**D**
Breda	1	1	1	..	1	..	1	..	..	..	1	..	..	1	..	**D (B, F)**
Campine	2	3	6	..	6	..	..	..	6	..	5	1	..	6	..	**B**
Cochin	2	2	6	..	6	..	..	4	2	..	4	2	..	6	..	**A**
Dorking	1	1	3	..	3	..	3	..	..	..	3	..	..	3	..	**E** (Spanish, Chittagong, Brahma, Indian Game, Langshan, Old English Game)
Faverolle	2	2	2	..	2	..	1	..	1	..	2	..	..	2	..	*F* Brahma, Cochin, Houdan, Dorking, Malines
Frizzle	1	1	2	..	2	..	2	..	..	..	2	..	..	2	..	**A**
Hamburgh	4	4	13	..	12	1	13	..	..	..	13	..	..	13	..	**E (D)**
Houdan	2	3	5	..	4	1	5	..	..	..	5	..	..	5	..	**F**
Indian (Cornish) Game	1	1	1	..	1	..	1	..	..	..	1	..	..	1	..	*E* Old English Game, Aseel, Sumatra Game
Japanese	1	1	4	..	4	..	3	..	1	..	4	..	..	4	..	**A**
Lakenvelder	1	2	3	..	3	..	3	..	..	..	3	..	..	3	..	**G (D)**
Leghorn	4	4	35	1	32	2	35	..	..	..	35	..	..	35	..	**M** (Minorca, Old English Game, Malay, Yokohama, "Asiatic blood")

Malay	1	1	2	..	1	1	2	..	..	..	2	..	..	2	..	**A** (Indian Game)
Maran	1	9	99	10	59	30	67	5	27	21	30	48	..	94	..	*F* Malines, Croad Langshan, Rennes, Faverolle, Barred Rock, Braekel, Gâtinaise
Minorca	1	2	7	..	7	..	6	..	1	..	7	..	..	7	..	**M** (La Fleche, Cochin, Old English Game, Langshan)
Naked Neck	1	1	1	..	1	..	1	..	..	..	..	1	..	1	..	**T**
North Holland Blue	1	1	25	..	18	7	18	1	6	2	8	15	..	25	..	*D* Malines
Old English Game	7	6	19	..	18	1	19	..	..	1	13	5	..	13	6	**E**
Orpington	1	1	1	..	1	..	1	..	..	..	1	..	..	1	..	*E* Langshan, Cochin
Pekin	3	3	7	..	6	1	6	..	1	..	7	..	..	7	..	**A**
Plymouth Rock	1	1	1	..	1	..	1	..	..	..	1	..	..	1	..	*US* Cochin, Dorking, Malay, Dominique, Spanish, Java, Langshan Orpington
Polish	4	4	9	..	9	..	9	..	..	..	9	..	..	9	..	? Poland? or Padua, Italy?
Redcap	1	1	2	..	2	..	2	..	..	..	2	..	..	2	..	**E**
Rumpless	1	1	7	..	7	..	6	..	1	..	7	..	..	7	..	**A** or **E** (from Old English Game bantams?)
Sebright	1	1	1	..	1	..	..	1	..	..	1	..	..	1	..	*E* Polish, Hen-feathered bantam
Sicilian Buttercup	1	1	1	..	1	..	1	..	..	..	1	..	..	1	..	**M**
Silkie	1	2	3	..	3	..	..	..	3	..	3	..	..	3	..	**A**
Sumatra	2	4	8	2	6	..	5	2	1	..	4	4	..	7	1	**A**
Sussex	3	4	77	..	77	..	24	22	31	12	38	27	8	54	15	**E** (Brahma, Cochin, Dorking, Old English Game)

TABLE 1—Continued

Breed	No. of varieties	No. of flocks	No. of birds	Ovalbumin F	Ovalbumin S	Ovalbumin FS	G_3 globulin A	G_3 globulin B	G_3 globulin AB	G_2 globulin C	G_2 globulin D	G_2 globulin CD	Conalbumin F	Conalbumin S	Conalbumin FS	Breed origin*
Welsummer	1	1	15	..	15	..	8	4	3	..	13	2	..	15	..	*D* Cochin, Wyandotte, Leghorn, Barnevelder, Rhode Island Red
Wyandotte	3	3	8	..	8	..	1	2	5	3	5	..	..	8	..	*US* Cochin, Hamburgh, Brahma, Leghorn, Polish, Indian Game, Plymouth Rock
Yokohama	1	2	4	..	4	..	3	..	..	..	4	..	..	4	..	**A**

Data on the Maran and the Sussex breed for different individual flocks appear in Table 3.

Frequency data for the rarest variants are not shown on the table. Five of the 99 Marans were heterozygous for conalbumin S and conalbumin S.J. Two of the Polish were heterozygous for lysozyme (G_1 globulin), $G_1^F G^{S}_1$; all other eggs screened in acetate buffers have the lysozyme phenotype G_1^F. One Yokohama had the G_3 globulin phenotype $G_3^J G_3^A$.

* Abbreviations for countries of origin: A—Asiatic, B—Belgian, D—Dutch, E—English, F—French, G—German, M—Mediterranean, SA—South America, T—Transylvania, US—United States. **Bold-face type** indicates a native breed; breeds in parentheses have contributed to some strains of the native breed. *Italics* indicate a synthesized breed. Ancestral breeds are listed when they are known. When a native breed of a particular type has been imported from more than one source, all sources are shown.

RESULTS

1. *New variants in* Gallus gallus: *Ovalbumin.* A number of flocks have quantitative variation of ovalbumin. This can be seen in Figure 1; samples 1, 5, 6, 7, 8, 9 and 15 have a very heavy A_3 zone; as the A_1 and A_2 zones are not diminished the high A_3 is unlikely to be the result of dephosphorylation (PERLMAN 1952; BAKER and MANWELL 1962). The effect is more one of doubling the A_3 of these samples; it has some resemblance to the variation reported by BAKER and MANWELL (1962) but does not resolve in the same way in barbiturate gels. This ovalbumin variation is not included in the tables: it is difficult to score some samples and the basis of the variation is obscure. Further biochemical characterization and breeding experiments should make it possible to use the quantitative A_3 variation in population studies.

Lysozyme (G_1 *globulin*). This protein resolves best in acetate gels, pH 4.7 and pH 5.7 (BAKER and MANWELL 1967). Two out of over 100 eggs screened in one or both of these buffers had a second lysozyme band migrating more slowly to the cathode than the usual lysozyme band. Birds with this pattern are presumed to be heterozygous at the lysozyme locus in that: (*a*) lysozyme is a single polypeptide chain and, thus, the heterozygote would be expected to have only the two zones (reviewed by JOHNSON 1966); (*b*) the two chicken lysozyme phenotypes parallel two of the three Coturnix lysozyme phenotypes, which are clearly a genetic polymorphism (BAKER and MANWELL 1967). The Gallus and Coturnix lysozyme polymorphisms differ slightly in that the Coturnix phenotype FS consists of two equal bands while in the corresponding Gallus phenotype, the F band is in higher concentration than the S band.

It is suggested that the chicken lysozyme locus be denoted G_1 (LONGSWORTH *et al.* 1940) and that the alleles be designated G_1^F, which is the more common type, and G_1^S, so far observed only in one flock of Polish bantams.

G_3 globulin. Some hens have a slightly different G_3^A protein, here called $G_3^{A'}$. This migrates very slightly faster to the anode in Ferguson-Wallace gels (FERGUSON and WALLACE 1961) and also slightly faster to the cathode in pH 4.7 acetate gels than the usual G_3^A. The possible heterozygote cannot be detected unequivocally. As all the globulin bands exhibit slight quantitative variation, it is almost impossible to tell a large spreading G_3^A zone from the overlapping G_3^A $G_3^{A'}$ phenotype. There is also a possibility that $G_3^{A'}$ may represent a chemical artefact, for chemically induced heterogeneity occurs in several egg white proteins (e.g., PERLMAN, 1952; BAKER 1967; BAKER and MANWELL 1967). So far, $G_3^{A'}$ has been found most frequently in Marans; but $G_3^{A'}$ is not included in the tables of variants in this paper for the reasons given above.

During the present work, one Yokohama was found to have a protein electrophoretically similar to G_3^J (BAKER 1964). The Yokohama G_3^J is not identical with the G_3^J of CROIZIER (1966) (BAKER, MANWELL, CROIZIER and STRATIL, unpublished data). It is not known if either of the G_3^J proteins is the same as the original G_3^J (BAKER 1964). Therefore it is suggested that, for the present, the G_3^J nomenclature include the names of the breeds in which it occurs, e.g., Red

Jungle Fowl, Maran or Yokohama. If either the Maran or Yokohama G_3^J is identical with the original G_3^J, no protein has been found in the Red Jungle Fowl that does not occur in some domestic breed of chicken. The same result is found in studies on hemoglobin, esterases, serum proteins and yolk proteins (BAKER 1964).

Two other rare variants have been found in a small flock of Old English Game bantams. One variant, $G_3^{J-B\ 1}$, migrates slightly faster than G_3^B; the other variant, $G_3^{J-B\ 2}$, is slightly slower than G_3^B. In Ferguson-Wallace gels, pH 8, and acetate gels, pH 4.7, the two new proteins are distinct from G_3^A and G_3^B and there is no overlap.

Two of the Old English Game bantams produce eggs with both the rare variants as the sole G_3 proteins. A third bantam produces the rare variants *and* G_3^A. This so far unique finding is not easy to explain. Chemical heterogeneity is difficult to exclude and, so far, no bird has been found which produces one of the new variants without the other. The birds are all in good health. It might be that the bird in question is trisomic for the part of the genome which determines the G_3 phenotype. The growing number of reports describing human subjects with trisomic cells indicates that such an hypothesis is not unreasonable. Also, partial and complete trisomy may be induced by viral infections (EVANS 1967). Until further information is available, the two new rare variants are omitted from the general consideration of G_3 phenotype frequencies.

2. *Egg white proteins of* Gallus sonnerati: Samples from the three *Gallus sonnerati* eggs are in positions 7, 8 and 9 in Figure 1. The patterns differ from those of *G. gallus* egg whites in the globulin region. All three *G. sonnerati* have a band which migrates slightly faster than G_3^A and a band with the mobility of G_3^B. Sample 9 also has a band which migrates in a position between those of G_3^A and G_3^B. *Gallus sonnerati* has no protein at all in the region which corresponds to the G_2 zones of *G. gallus*. These differences are maintained in several buffers including pH 4.7 acetate. As far as the author knows the *G. sonnerati* globulin region pattern has not been observed in any eggs from *G. gallus*.

One *G. sonnerati* (sample 7 in Figure 1) has an additional very slow conalbumin band, conalbumin Bill Wilkinson (BAKER 1967). The faster conalbumin zone of this *G. sonnerati*, and the only conalbumin zone in the other two *G. sonnerati*, is electrophoretically identical to the most common conalbumin of *G. gallus* (conalbumin b). The order of electrophoretic mobility in most alkaline gels is, from fastest to slowest: a, b, S.J., and B.W., in borate and Ferguson-Wallace buffers. The distance between a and b is the same as that between b and S.J.; however, this distance is greater than that between S.J. and B.W. When iron is added all the four Fe-conalbumins show the same relative increase in mobility. Thus, Fe-conalbumin B.W. migrates slightly faster than conalbumin S.J. without iron. These points are important in view of the variability in the iron contamination in the commercially available starch used in gel electrophoresis.

In "tris-EDTA-borate" gels the mobility difference between conalbumin S.J. and conalbumin B.W. disappears although the differences between conalbumins

a, b, and S.J. remain. In pH 4.7 acetate gels it is only possible to distinguish between the various conalbumin phenotypes under favourable conditions.

Studies in progress indicate that the conalbumin C of CROIZIER (1966), the conalbumin c (STRATIL, 1967 personal communication), and conalbumin S.J. (BAKER 1967) are electrophoretically identical but that conalbumin B.W. is clearly different (BAKER, MANWELL, CROIZIER and STRATIL, unpublished data). Thus it could be that conalbumin B.W. represents another difference between *G. sonnerati* and the domestic fowl.

3. *The occurrence of egg white polymorphisms in different populations of domestic fowl:* The results of the survey are summarized by breed in Table 1, which also contains an indication of the origins of the breeds. Native countries or regions are abbreviated to capital letters. Heavy type denotes what breeders call a "natural" breed, which has evolved from the indigenous barnyard fowl of a district. Italics are used for the homelands of synthesized ("artificial") breeds. When there is a record that a native breed has been altered by crosses, the crosses are listed in parentheses. Synthesized breeds have the ancestral breeds listed without parentheses. The information in the last column of Table 1, and in other parts of this paper concerning breed origins, is from ATKINSON (1924), *British Poultry Standards* (1954), BROWN (1929), DARWIN (1875), LEWER (not dated), and WEIR (1902).

The history of domestic breeds will not be discussed in detail in the present paper but three points must be emphasized: 1. Synthesized breeds may have been originated by several breeders, not all of whom used identical crosses. 2. Alterations to natural breeds were not made to all flocks; individual breeders "improved" their stock with various breeds thought to have desirable qualities. 3. Varieties of breeds were formed by crossing to introduce different colour patterns. The amount of crossbreeding between varieties ranges from regular interbreeding (e.g., between some of the thirty-odd color varieties of Old English Game), through occasional crossing, to no crossing at all.

Thus, within a breed, all varieties and all strains within varieties do not necessarily come from the same sources. However, the varieties available from each breed are not listed separately at this stage. Although differences between varieties exist, many involve small numbers of birds or are no bigger than differences between flocks within a variety. For the same reasons bantams which correspond to large breeds have been considered as varieties of these breeds (see *British Poultry Standards*, 1954, pp. 209–210 for the distinction between "true bantams," listed separately in Table 1, and "bantamized varieties.").

Twenty-six of the 37 breeds were found to be polymorphic at one or more egg-white loci. The polymorphic breeds are listed in Table 2 under the headings of the number of various loci: The findings of other authors are included for comparison. The latter emphasize the point, mentioned in the previous paragraph, that differences occur between populations within breeds.

Variation between flocks within three breeds is summarized in Table 3.

The possibility exists that where several breeds are kept on a single holding

TABLE 2

Summary of breeds polymorphic at one or more egg white loci

Genetic loci	Breeds	References
Single locus		
Ov	Hamburgh, Houdan, Leghorn, Malay	This paper
G_3	"Jungle Fowl"	Lush 1961
	Columbian	Baker and Manwell 1962
	Red Jungle Fowl	Baker 1964
	Bresse	Croizier 1966
	Faverolle, Japanese, Minorca, Rumpless, Silkie, Yokohama	This paper
G_2	Leghorn	Feeney *et al.* 1963
	Silkie	Baker 1964
	Leghorn	Croizier 1966
	Naked Neck	This paper
G_1	Polish	This paper
Two loci		
$Ov + G_3$	Leghorn	Buvanendran 1964
	Pekin	This paper
$G_2 + G_3$	Rhode Island Red	Lush 1961
	Leghorn, New Hampshire	Baker and Manwell 1962
	Langshan	Croizier 1966
	Leghorn, New Hampshire	Pöder and Pavel 1966
	Andalusian, Australorp, Barnevelder, Campine, Cochin, Welsummer, Wyandotte	This paper
Three loci		
$Ov + G_3 + G_2$	Leghorn	Lush 1961
	Faverolle, Gâtinaise	Croizier 1966
	North Holland Blue	This paper
$Ov + G_2 + Tf$	Old English Game	This paper
$G_2 + G_3 + Tf$	Light Sussex	Morton *et al.* 1965
	Light Sussex	Lush 1964a
	Wyandotte	Croizier 1966
	Light Sussex	This paper
Four loci		
$Ov + G_3 + G_2 + Tf$	Maran	Croizier 1966
	Maran, Sumatra Game	This paper

the genetic differences between them might be reduced by crossing or by selection. This does not appear to be the case in the present study, even when the numbers of any one breed are small. Table 4 gives the phenotype frequencies for five breeds kept on one of the two commercial farms sampled in this study. The Maran and North Holland Blue which are very similar morphologically and which have a common ancestry, are also similar in their protein types and frequencies. The Barnevelder and its descendent, the Welsummer are also biochemically similar to each other but differ from the Maran and North Holland Blue breeds. The Light Sussex is distinct from all four other breeds, in origin and in egg white protein frequencies. Thus, in this case the similarity between flocks is

TABLE 3

Frequencies of egg white proteins for two different flocks within each of three breeds

Breed and Flock	Number of birds	Ovalbumin			G_3 globulin			G_2 globulin			Conalbumin			S.S.J.	Gene frequencies			
		F	S	FS	A	B	AB	C	D	CD	F	S	FS		*Ov S*	G_3^A	G_2^D	*Tf S*
Australorp A	19	..	19	..	7	2	10	..	19	..	..	19	..	..	1.00	0.63	1.00	1.00
Australorp B	18	..	18	..	..	12	6	..	16	2	..	18	..	..	1.00	0.17	0.94	1.00
Maran A	24	2	17	5	17	1	6	3	10	11	..	24	..	..	0.81	0.83	0.65	1.00
Maran B	30	4	20	6	22	..	8	12	7	11	..	27	..	3	0.77	0.87	0.42	0.95
Light Sussex A*	51	..	51	..	16	17	18	10	21	20	8	29	14	..	1.00	0.49	0.61	0.71
Light Sussex B	24	..	24	..	6	5	13	2	16	6	..	23	1	..	1.00	0.52	0.79	0.98

* The Light Sussex population, in contrast to other chicken populations, deviates from the Hardy-Weinberg Equilibrium with a deficit of heterozygotes at all three egg protein loci: for the G_3 globulins [$\chi^2 = 6.56$ (1 df)]; for the G_2 globulins ($\chi^2 = 2.0$); for the conalbumins ($\chi^2 = 4.47$). The total $\chi^2 = 13.03$ (3 df); this indicates a highly significant deviation ($0.01 < P < 0.001$). A highly significant deficit of heterozygotes for the X-protein locus, but not other protein loci, occurs in two *Coturnix* populations with different breeding structures (BAKER and MANWELL 1967).

TABLE 4

Frequencies of egg white proteins in five breeds kept by one commercial breeder

Breed	Number of birds	Ovalbumin			G_3 globulin			G_2 globulin			Conalbumin			Gene frequencies			
		F	S	FS	A	B	AB	C	D	CD	F	S	FS	*Ov S*	G_3^A	G_2^D	*Tf S*
Light Sussex	24	..	24	..	6	5	13	2	16	6	..	23	1	1.00	0.52	0.79	0.98
Maran	24	2	17	5	17	1	6	3	10	11	..	24	..	0.81	0.83	0.65	1.00
North Holland Blue	25	..	18	7	18	1	6	2	8	15	..	25	..	0.86	0.24	0.62	1.00
Barnevelder	22	..	22	..	16	..	6	5	6	11	..	22	..	1.00	0.86	0.52	1.00
Welsummer	15	..	15	..	8	4	3	..	13	2	..	15	..	1.00	0.63	0.93	1.00

(Maran and North Holland Blue are placed side by side as they are related through a common ancestral breed, the Malines. Similarly the Barnevelder and the Welsummer are grouped in adjacent rows as the Welsummer was formed from crosses between the Barnevelder and other breeds.)

more a function of origin than of conscious selection by a single commercial operation. This conclusion is reinforced by comparing the genotype frequencies of the two Australorp flocks in Table 3. These belong to the same breeder but are kept as separate breeding units.

DISCUSSION

The ancestry of the domestic fowl: These studies extend previous investigations (BAKER 1964) indicating the great protein similarity between the Red Jungle Fowl and the domestic fowl. In fact, the one unique Red Jungle Fowl egg protein, G_3^J, may be the same as the G_3^J found in some Marans by CROIZIER (1966) or the G_3^J found in a Yokohama (present paper). In that event the only reported electrophoretic protein difference between wild and domestic *Gallus gallus* is eliminated.

There has been considerable disagreement as to whether *G. gallus* was the sole progenitor of the domestic fowl or whether one or more of the other species of Gallus were involved (e.g., DARWIN, 1875; LOTSY and KUIPER 1922–1924; GHIGI 1922, 1927; BEEBE 1927; DELACOUR 1964). It is known that *G. gallus* and *G. sonnerati* hybridize in captivity and in the wild and that their hybrids are fertile (see GRAY 1958). Furthermore, LOTSY and KUIPER (1924) noted that *G. sonnerati* characteristics were not marked in the F_2, F_3 and F_4 generations descended from crosses between *G. gallus* and *G. sonnerati*. Thus, it is probable that any *G. sonnerati* ancestors of the domestic fowl would be difficult to trace by morphological characteristics. That starch gel electrophoresis is sufficiently accurate to detect hybridization and genetic introgression has been established (MANWELL, BAKER and CHILDERS 1963).

In this context the G_3^B protein and the electrophoretically similar *G. sonnerati* protein are of interest. If they are identical it will be necessary to consider further the higher frequency of G_3^B in some breeds. Several of these also lay brown eggs, a characteristic occurring in *G. sonnerati* but not in *G. gallus* (DELACOUR 1964, pp. 109, 117). G_3^B is very rare in *G. gallus* (BAKER 1964).

At present, however, the data support *G. gallus* as the main ancestor of the domestic fowl. The G_2 globulin difference between *G. gallus* and *G. sonnerati* is at present complete; well over a thousand *G. gallus* eggs have been screened by various workers without revealing a single bird lacking both G_2^C and G_2^D, whereas none of the three *G. sonnerati* has either of these proteins. This difference is as great as or greater than the differences between the egg white proteins of such species pairs as *Phasianus versicolor* and *P. colchicus* (BAKER *et al.* 1966) and *Chrysolophus pictus* and *C. amherstiae* (BAKER 1965).

The breeds of the domestic fowl: In the present study related breeds were found to resemble each other in the occurrence of egg white protein variants (Tables 1, 3). In addition, certain other trends were observed.

G_3^B seems to be associated with Asiatic breeds and breeds known to have Asiatic stock in their make-up. This observation is supported by the results of other studies (LUSH 1961; BAKER and MANWELL 1962; BAKER 1964; CROIZIER 1966; PÖDER and PAVEL 1966). There are also exceptions: a few Asiatic breeds have

high frequencies of G_3^A. In the case of the Frizzle and the Japanese, the distinguishing features of each breed are qualitative. As the genes involved are known to have occurred more than once (e.g., HADORN 1961) it is possible that the flocks examined are not really Asiatic in origin. It is unlikely that this explanation applies to the Malay or the Pekin. In their case (and also in that of the Frizzle and the Japanese) it could be that the small flocks available are not typical of the whole breed. Loss of G_3^B by random fixation is, of course, more likely to take place in the unusual fancy breeds which are maintained in very small breeding populations.

Another exception is the Sebright. Only two eggs have been available but both the American bird (BAKER 1964) and the English bird are homozygous for G_3^B. The Sebright was bred from English stock around 1800, before the importation of Asiatic breeds is recorded (e.g., DARWIN 1875, p. 29 of Vol. II). Furthermore, most fanciers believe that individual strains of Sebright should be inbred as closely as possible, which suggests that "improvement" by crossing with other breeds is unlikely.

The comment of LUSH (1966) that G_3^B occurs always less than fifty percent in the gene frequency is not only contradicted by data in Table 1 of the present paper, but also by data on Rhode Island Reds in LUSH (1961) and on New Hampshires in BAKER and MANWELL (1962).

Many of the native English breeds and varieties are homozygous for G_3^A: Old English Game, Hamburgh, Redcap, Dorking and Buff Sussex. With the exception of Old English Game these birds are also homozygous for G_2^D. A similar pattern was found in most of the indigenous Northern European breeds examined and the Breda, Houdan, Lakenvelder and Polish are also homozygous G_3^A and G_2^D. So are some of the less "improved" Mediterranean breeds and varieties such as the Fayoumi (BAKER 1964) and the Black Leghorn.

Many breeds with the Fast ovalbumin variant have very mixed ancestry. An exception is the Old English Game, but the author has found no evidence to suggest that this breed is the main source of the Fast ovalbumin in other breeds. The distribution of the ovalbumin polymorphism could be the result of independent occurrence of the Fast variant in a number of populations. A parallel ovalbumin polymorphism has been found recently in some populations of *Phasianus colchicus* (BAKER and MANWELL, unpublished data) which suggests that phenotypically similar mutations of the ovalbumin locus have occurred more than once.

The *conalbumin a* gene has been found in Light Sussex, Old English Game, Sumatra Game and Wyandottes (BAKER 1967 and this paper; CROIZIER 1966). The Old English Game contributed to some strains of Brown Sussex but the author has not found any record of a connection between the Old English Game, Light Sussex and Wyandotte. The Old English Game and the Sumatra Game could be related by some Aseel crosses (Mr. W. WILKINSON, personal communication). Another possibility is that multiplication of small imported flocks of Sumatra Game could have included top-crossing onto English fowl with similar characteristics, i.e., Old English Game.

All the published data concerning conalbumin S.J. refer to the Maran breed

(CROIZIER 1966; BAKER 1967 and this paper). However, an electrophoretically identical conalbumin has been found in native Indian barnyard fowl (BAKER, MANWELL, JAYAPRAKASH and FRANCIS, unpublished data). The finding may be significant as DR. A. STRATIL (personal communication) found his conalbumin *c* in White Cornish. This breed originated from crosses involving the Aseel, an Indian breed (see Table 1 under Indian Game). It has not yet been possible to find pure Aseels; the breed is very difficult to propagate.

The possible significance of the egg white protein polymorphisms: The breeds surveyed were developed for different economic characteristics, namely, broodiness, egg production, fighting ability and meat production. There is no obvious connection between any of these traits and the egg white polymorphisms. However, many of the breeds with high frequencies of G_3^B are plump and placid; it would be useful to know if, within breeds generally considered to be nervous, the flocks with higher G_3^B frequencies were the more docile.

It has been suggested that variation of egg white proteins may influence hatchability, possibly because of maternal-offspring incompatability (MORTON, GILMOUR, MCDERMID and OGDEN 1965; BAKER and MANWELL 1967). Such incompatability could be more critical when other detrimental genetic factors are present.

Apart from these indications there is no evidence that either monomorphism or polymorphism of egg white proteins confers any obvious advantage. Yet it cannot be without some significance that three of the most successful pure breeds —Light Sussex, Maran and Old English Game—are polymorphic at a minimum of three egg white loci. Of the six small flocks of Old English Game (Table 1), one flock has been closed for over 70 years and another for 48 years—and both have polymorphism of egg white proteins. These findings agree with the data of LUSH (1961), who found egg white polymorphism in flocks with coefficients of inbreeding ranging from 0.31 to 0.9255. It is also significant that many of the synthesized breeds are still polymorphic at the egg white loci, even after many years of selection which would be expected to eliminate or reduce unfavourable phenotypes.

It is concluded that, assuming equilibrium, the egg white protein polymorphisms convey an advantage to some flocks of poultry. The advantage is not so strong as to show up in a departure from Hardy-Weinberg equilibrium; nor is there a clear correlation between any egg white protein variant and a particular commercial characteristic, although certain commercially successful breeds (e.g., Maran and Light Sussex) have polymorphism at several loci and with relatively high gene frequencies of the different variants.

The author is most grateful to the United States National Science Foundation for support (GB-3037, administered through the Florida State University) without which this research would not have been possible. The author thanks DR. CLYDE MANWELL for helpful advice and continual encouragement. The author also thanks the following people who supplied her with eggs and who lent her some of the books on poultry: MRS. E. BRAY, NORWOOD HALL, Yks.; COL. F. W. DEWHURST, Crapstone House, Devon.; MR. SAM JACKSON, Silsden, Yks.; MISS R. JACKSON-BENNETT, Saltash, Cornwall; LT. COL. A. A. JOHNSON, M. C. Ixworth, Suffolk; MR. and MRS. F. E. B.

JOHNSON, Stagsden, Bedford; MR. E. M. MCDERMID, then of THORNBER BROS., Mytholmroyd, Yks.; MR. M. MICHELMORE and MR. W. WILKINSON, Paignton Zoological Gardens, Devon.; M. O. and V. M. PELLOW, Callington, Cornwall; and, MR. and MRS. REX WOODS, Ashe House, Devon.

SUMMARY

Twenty-four out of 37 breeds of domestic fowl are polymorphic for egg proteins; five different proteins are involved. Many of the egg proteins of *Gallus sonnerati* are electrophoretically identical to those of *G. gallus*; however, whereas either G_3^C globulin or G_2^D are present in all chickens and Red Jungle Fowl examined, neither is present in *G. sonnerati*. The data support the opinion that the Red Jungle Fowl is the main progenitor of the domestic fowl—Discrete variation of chicken egg white lysozyme and an ususual G_3 globulin variant are described for the first time.—The G_3^B allele is associated to some extent with Asiatic ancestry. Many of the "purer" breeds native to England, Northern Europe and the Mediterranean are monomorphic for G_3^A and (except Old English Game) G_2^D.—Neither monomorphism nor polymorphism of egg white proteins was found to confer an obvious advantage, although the presence of extensive polymorphism with relatively high gene frequencies for the variants in some commercial breeds, and in some long isolated flocks with small breeding numbers, is suggestive that polymorphism of egg white proteins can convey a selective advantage to certain flocks.

LITERATURE CITED

The American Standard of Perfection, 1958 American Poultry Association, Great Falls, Mont.

ATKINSON, H., 1924 *The Old English Game Fowl*. The Feathered World, 9, Arundel St., London.

BAKER, C. M. A., 1964 Molecular genetics of avian proteins. III. The egg proteins of an isolated population of Jungle Fowl, *Gallus gallus* L. Comp. Biochem. Physiol. **12**: 389–403. —— 1965 Molecular genetics of avian proteins. IV. The egg white proteins of the Golden Pheasant, *Chrysolophus pictus* L., and Lady Amherst's Pheasant, *C. amherstiae* Leadbeater, and their possible evolutionary significance. Comp. Biochem. Physiol. **16**: 93–101. —— 1967 Molecular genetics of avian proteins. VII. Chemical and genetic polymorphism of conalbumin and transferrin in a number of species. Comp. Biochem. Physiol. **20**: 949–973.

BAKER, C. M. A., and C. MANWELL, 1962 Molecular genetics of avian proteins. I. The egg white proteins of the domestic fowl. Brit. Poultry Sci. **3**: 161–174. —— 1967 Molecular genetics of avian proteins. VIII. Egg white proteins of the Migratory Quail, *Coturnix coturnix*—new concepts of "hybrid vigour". Comp. Biochem. Physiol. **23**: 21–42.

BAKER, C. M. A., C. MANWELL, R. F. LABISKY, and J. A. HARPER, 1966 Molecular genetics of avian proteins. V. Egg, blood and tissue proteins of the Ring-necked Pheasant, *Phasianus colchicus* L. Comp. Biochem. Physiol. **17**: 467–499.

BEEBE, W., 1927 *Pheasants: Their Lives and Homes*. Robert Hale, London.

British Poultry Standards, 1954 *Poultry World*, Dorset House, Stamford St., London.

BROWN, E., 1929 *Poultry Breeding and Production*. Vols. I and II. Ernest Benn, London.

BUVANENDRAN, V., 1964 Evidence of linkage between two egg albumen loci in the domestic fowl. Genet. Res. **5**: 330–332.

CANN, J. R., 1949 Electrophoretic analysis of ovalbumin. J. Am. Chem. Soc. **71**: 907–909.

CROIZIER, G., 1966 Polymorphismes biochimiques de la poule domestique. I. Analyse génétique des protéines du blanc d'oeuf chez des poules de races Françaises et étrangères. Ann. Biol. Anim. Bioch. Biophys. **6**: 379–388.

DARWIN, C., 1875 *The Variation of Animals and Plants under Domestication.* 2nd Edition. John Murray, London.

DELACOUR, J., 1964 *Pheasants of the World.* Country Life, London.

EVANS, H. J., 1967 The nucleolus, virus infection and trisomy in man. Nature **214**: 361–363.

FEENEY, R. E., H. ABPLANALP, J. J. CLARY, D. L. EDWARDS, and J. R. CLARK, 1963 A genetically varying minor protein constituent of chicken egg white. J. Biol. Chem. **238**: 1732–1736.

FERGUSON, K. A., and A. L. C. WALLACE, 1961 Starch-gel electrophoresis of anterior pituitary hormones. Nature **190**: 629–630.

GHIGI, A., 1922 L'hybridisme dans la genèse des races domestiques d'oiseaux. Genetica **4**: 364–374. —— 1927 On the origin of the domestic breeds of poultry and their classification. Proc. 3rd World's Poultry Science Congress, pp. 52–54.

GRAY, A. P., 1958 *Bird Hybrids. A Check-list with Bibliography.* Commonwealth Agricultural Bureaux, Farnham Royal, Bucks.

HADORN, E., 1961 *Developmental Genetics and Lethal Factors.* (translated by U. Mittwoch) Methuen, London.

JOHNSON, L. N., 1966 The structure and function of lysozyme. Science Progress **54**: 367–385.

LEWER, S. H., not dated (about 1914) *Wright's Book of Poultry.* Waverley Book Co., London.

LONGSWORTH, L. G., R. K. CANNAN, and D. A. MACINNES, 1940 An electrophoretic study of the proteins of egg white. J. Am. Chem. Soc. **62**: 2580–2590.

LOTSY, J. P., and K. KUIPPER, 1924 A preliminary statement of the results of MR. HOUWINK's experiments concerning the origin of some domestic animals. Genetica **6**: 221–282.

LUSH, I., 1961 Genetic polymorphisms in the egg albumen proteins of the domestic fowl. Nature **189**: 981–984. —— 1964a Egg albumen polymorphisms in the fowl: loci II and III. Genet. Res. **5**: 39–49. —— 1964b Egg albumen polymorphisms in the fowl: the ovalbumin locus. Genet. Res. **5**: 257–268. —— 1966 *The Biochemical Genetics of Vertebrates except Man.* North-Holland Publishing Co., Amsterdam.

MANWELL, C., C. M. A. BAKER, and W. CHILDERS, 1963 The genetics of hemoglobin in hybrids. I. A molecular basis for hybrid vigor. Comp. Biochem. Physiol. **10**: 103–120.

MORTON, J. R., D. G. GILMOUR, E. M. MCDERMID, and A. L. OGDEN, 1965 Association of blood-group and protein polymorphisms with embryonic mortality in the chicken. Genetics **51**: 97–107.

OGDEN, A. L., J. R. MORTON, D. G. GILMOUR, and E. M. MCDERMID, 1962 Inherited variants in the transferrins and conalbumins of the chicken. Nature **195**: 1026–1028.

PERLMAN, G. E., 1952 Enzymatic dephosphorylation of ovalbumin and plakalbumin. J. Gen. Physiol. **35**: 711–726.

PÖDER, O. O., and U. G. PAVEL, 1956 Concerning the polymorphism in the globulins of egg albumen of domestic fowl. Genetika 1966 (**12**): 120–121. [Russian with English summary.]

STRATIL, A., 1967 The effect of iron addition to avian egg white on the behaviour of conalbumin fractions in starch gel electrophoresis. Comp. Biochem. Physiol. **22**: 227–234.

WEIR, H., 1902 *Our Poultry and All about Them.* Hutchinson, London.

WISEMAN, R. L., and J. E. FOTHERGILL, 1966 Structural aspects of the immunological cross-reaction of genetic variants of hen ovalbumin. Biochem. J. **99**: 58P.