

**STUDIES ON THE RELATIONSHIP BETWEEN PROTEIN
POLYMORPHISMS AND PRODUCTION CHARACTERS IN
THE FOWL**

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Part 1INTRODUCTION

Artificial selection methods are practised by plant and animal breeders to change the mean of the population for some character in the direction of greater economic benefit. The selection techniques employed for animals till 1940 were designed to use mainly the additive genetic variance in the population and the methods used (mass selection or family selection) were dependent on the proportion of additive genetic variance of the trait under selection. However, after some generations of selection on the additive genetic variance, there was gradual attenuation of improvement and the selected stocks tended to plateau in performance (Lerner, 1958). Genetic variability was still demonstrable and this remaining variance was probably non-additive.

The non-additive genetic variance in a population exists because of dominance, overdominance, epistatic deviation and interactions between loci. If this is to be utilised, selection methods have to be modified. A number of different techniques are available (Lerner, 1958) but they are all modifications of one basic plan. In general, it involves selection of two populations for different alleles in such a way that crosses between them made anew in each generation are characterised by the desired genotype. (This is the system of cross-breeding, which had been used as an empirical procedure previously.) Therefore strains or inbred lines are developed and crossed in all likely combinations to find the successful groups. A considerable capital investment and labour is necessary for this type of selection, since the method of detection of the/

the lines which will produce superior crosses is a matter of trial and error, and a large number of crosses have to be tested. For example, twenty strains can be made to give 380 two-way crosses and 116,280 four-way crosses. It is obvious therefore that any breeder can test only relatively few strains.

It would therefore be very desirable to have ancillary tools which could be used for preliminary selection of basic lines to be tested in crosses. Such ancillary guides may be physiological variation which influence economic characters or variations of major genes, i.e. genetic polymorphisms. If easily identifiable polymorphic genes could be shown to have major effects on economically important traits, this could be utilised in commercial animal breeding. Blood groups in poultry were shown to have such effects (see reviews by Gilmour, 1960 and Rendel, 1961). The discovery of the biochemical genetic polymorphisms in the egg white proteins by Lush (1961) posed the question whether these genes also could exert such effects and it was therefore decided to examine this question in three closed poultry populations. Further, if any effect on the traits examined was found, it will also help to elucidate some of the mechanisms which maintain genetic polymorphisms in populations.

The concept of polymorphism.

Before examining how one may exploit genetic polymorphisms in animal improvement, it would be useful to define polymorphisms and the situations where polymorphisms would be expected. Genetic polymorphism has been defined by Ford (1940) "as the occurrence together in the same habitat of two or more discontinuous forms of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation".

If/

If genetic polymorphisms are confined to the limitations of the definition given above, the following causes of stable polymorphisms may be listed (Haldane & Jayaker, 1963).

1. The conflict between selection and mutation. An equilibrium is reached when in each generation in a population of a given number, as many 'mutant' genes are destroyed by selection as appear by mutation. The equilibrium frequency of the 'mutant' gene would then depend on the selective force and the mutation rate. As an example of this, one may quote the pre-zygotic selection at the T locus of mice where males that are heterozygous for almost any t lethal transmit it to much more than half their offspring (Dunn, 1957).
2. The conflict between selection and segregation. The best known example of this type is when a heterozygote is fitter than either of the two corresponding homozygotes. This is called overdominance. Sickle cell anaemia in man is a classical example of this type of stable polymorphism. Here, it was shown, that homozygous normal humans were susceptible to malaria, homozygous Hb^S individuals to sickle cell anaemia and that the heterozygotes enjoyed a relative resistance to both diseases (Allison, 1954).
3. The conflict between fitness and abundance. A genotype may become less fit as it becomes more abundant and vice versa. Batesian mimicry is an example of this, where mimics are better protected when greatly outnumbered by distasteful models than when they are common (Sheppard, 1965).
4. The conflict between selection and migration. If a population is split up into a number of small non-interbreeding groups, different alleles would be fixed in the different subpopulations depending on the environment and selective forces acting on them. If migration now occurs between two genotypes which are fitter in different areas, there will be

a cline in which polymorphism for the two forms would occur.

5. The conflict between selection in different directions in the diploid and haploid generations as in *Primula vulgaris* (Crosby, 1949) or in the two sexes.

6. The conflict between selection in two directions i.e. selection which sometimes favours one phenotype and sometimes another. For example, Hiraizumi (1964) after examining the selective forces on blood groups in man concluded that 'the elements favoured by pre-zygotic selection were somehow disadvantageous for post-zygotic selection'. Since an element which is disadvantageous for both pre- and post-zygotic selection will be eliminated from a population and conversely an element favoured by both selections will be fixed, only those having selection coefficients of opposite sign in the pre- and the post-zygotic stage will successfully maintain the polymorphic state.

7. Finally a polymorphism may be transient. A phenotype which was formerly common may be disappearing as the result of changed conditions.

A general question that is often raised is whether a population derives an advantage from being polymorphic. Genetic polymorphism is a mechanism of maintaining genetic diversity. As a broad generalisation genetic diversity may be said to be an adaptive mechanism for coping with the diversity of environments (Dobzhansky, 1965) and in this sense polymorphisms are beneficial. But the presence of the polymorphism implies that some individuals less fitter than the optimum phenotype are produced in each generation and this puts a 'genetic load' on the population (Crow, 1963). This implies that the number of polymorphisms that can be maintained cannot be indefinitely large but this optimum number would depend on the selective forces and size of the population.

Polymorphisms/

Polymorphisms in animals (particularly poultry).

a) Visible polymorphisms.

Polymorphisms of visible characters or 'Fordian polymorphisms' are common in domestic animals. These often involve the coat colours in large animals such as the black and red coats of Holstein Friesian COWS.

In poultry, polymorphism of the comb was first examined by Bateson in 1898, when he made his cross between the rose comb and single comb fowls. Besides polymorphism of the comb, genes controlling plumage colour are common. Some of the loci controlling plumage colour have the following alleles

- a) Gold vs silver
- b) Barred vs non-barred
- c) Inhibitor of black vs non-inhibitor of black.

Drawing a parallel with the findings of the polymorphisms seen in the wild species such as the Scarlet tiger moth or Cepaea, it may be visualised that these different forms in the present day domestic animals are relics of some past adaptation to natural environments before they were domesticated.

Some of these visible polymorphisms may in fact be related to fitness of the individuals. Rose comb, determined by a completely dominant autosomal gene is characteristic of the Wyandotte breed of fowls. The breed standard requires a rose comb and therefore there is conscious artificial selection for this type but a considerable proportion of single combed birds appear in most flocks. It was suggested (Lerner, 1954) that this may be due to ~~some~~ form of selective advantage of the heterozygote and/

and the evidence now available shows that this is in fact a case of operational overdominance. Natural selection operates against the rose comb males (as these have low fertility) and artificial selection by the breeder against the single combed birds. Therefore operationally there is overdominance and both forms exist in the population (Fox et al, 1964). But this does not explain why the rose comb existed before the breeder applied selection for it. The most likely explanation is some selective advantage for this gene in some environment which perhaps does not exist now.

Cock (1956) took a different approach to study the roles of the plumage colour and pattern genes in natural selection. He observed the amount of segregation for five of these loci in a large number of inbred lines and compared this frequency with that which would be expected on the basis of their inbreeding coefficients. Excessive segregation was found in some highly inbred lines suggesting that natural selection favoured heterozygotes. It must however be remembered that an inbred population is less homeostatic than an outbred one and the individuals are therefore less able to adapt themselves to small changes in their environment. If individuals carrying different alleles are selected by these small environmental changes, then there will be residual heterozygosity in the population which is not due to any selection operating in favour of heterozygotes.

b) Cryptic polymorphism.

Polymorphisms may also involve characters that do not affect the external morphology of the individual. Amongst the well known of these are the chromosomal polymorphisms in *Drosophila* and blood groups in man and other animals.

The question that is often raised is - what are the causes of this polymorphism/

polymorphism? In trying to answer this question, one should distinguish between wild populations existing in their natural environment and populations living in artificial environments created by man.

In wild populations of *Drosophila*, there is clear evidence that ecological selective factors are responsible for the existence of chromosomal polymorphisms, particularly selection operating in favour of heterozygotes (Dobzhansky, 1951). In man however, the evidence is not quite clear. Blood groups are thought by some to be neutral in survival value, but evidence has been accumulating recently of some relationship between these genes and diseases. (Roberts & Fraser, 1959 and Ford, 1964). There is also some indication of heterozygote superiority for the MN locus by the presence of a significant excess of heterozygotes, though no relationship with survival value could be found (Morton & Chung, 1959).

Among domestic animals the most extensive study on polymorphisms has been done on cattle and poultry. With domestic animals, it should be borne in mind, that if the polymorphisms had any selective value in the past when the animals were in the wild, they may not be detected now for two reasons. (1) The environments in which domestic animals are now reared and maintained are very different from those to which they were exposed in the wild. Further in many instances (for example, poultry) they are exposed to a "constant environment" throughout life so that they are not exposed to environmental changes which are some of the main causes for polymorphism in wild populations. (2) The genetic environment of these polymorphic genes are again quite different. Most domestic species have been subject to rather intense selection in the direction of characters that are useful to man. The extreme situation is to be found in some populations of poultry which are very highly inbred and this genetic situation/

situation is a very artificial one, different from that of natural population.

Confining our attention now to blood groups only, the studies done in cattle have been inconclusive, in that no superiority between animals with different blood group genotypes has been detected for the characters studied. (Niemann Sørensen & Robertson, 1961). The inability to detect differences does not rule out the possibility that real differences may exist though not detectable due to the limitations of the techniques used.

In poultry, the investigations done so far on the blood groups, point to a general superiority in fitness of heterozygotes. There is both direct and indirect evidence of this heterotic effect. The indirect evidence comes from an examination of highly inbred lines for segregation of the blood group loci. It was shown conclusively that despite high levels of inbreeding the blood group genes failed to become homozygous (Briles et al, 1957 and Gilmour, 1960). This implies that heterozygous birds were being selected in each generation possibly because of their higher reproductive performance and vigour.

The direct evidence comes mainly from comparisons of homozygotes and heterozygotes in inbred lines. Schultz & Briles (1953) studying several inbred lines stated that they could not find/

find the heterozygotes at the A locus to be superior for any one character, but since a higher proportion of heterozygotes were selected in each generation under artificial selection they concluded that heterozygotes though not superior in any one character, were above average for the characters being considered - these being egg number and full sister family size. Since this evidence is based on artificial selection, it is strictly not correct to conclude that heterozygotes are superior in the Darwinian sense. But, as the characters considered are components of fitness, a real superiority may be present. Briles & Allen (1961) from a study of seven inbred lines also concluded that there was overdominance at the B locus when the total viability was considered but dominance effects when juvenile and adult viability was each considered separately.

Though these results suggest that heterozygosity at these loci confers increased fitness in the populations studied, it is important to remember that these are findings strictly confined to inbred lines. It is known that interaction between genes and the rest of the genotype may play an important part in the effect of selection on genes (Dobzhansky, 1951 and Mayr, 1963). For example, it is known that identical phenotypes of Cepaea nemoralis from different colonies do not always show the same physiological reactions, indicating an interaction between the gene for polymorphism and the residual genotype (Lamotte, 1959). An inbred line has a relatively homozygous background and individual genes or gene combinations with certain/

certain beneficial effects on that genetic background may be deleterious on a more heterozygous background. In the specific case of blood groups, the magnitude of a heterozygous blood group advantage in a panmictic population may be much smaller than in an inbred one as to make it useless in practical breeding schemes. This may perhaps be the reason "for the inability of some workers to find any practical value in them except for checking of identity." (Robertson, 1963).

Very recently, new biochemical techniques have enabled the discovery of another class of polymorphisms - the biochemical polymorphisms. The techniques essentially depend on the migration of protein molecules under an electrical gradient in specially treated paper, on blocks of starch gel or other media (acrylamide, agar etc). The separation depends on the existence of different electrical charges on the different molecules and also on the size of the molecules.

The biochemical polymorphic systems are well suited for physiological studies, since the polymorphic forms of the protein molecules could be separated and studied to see if they have any superior physiological properties. Among domestic animals, some extensive investigation of this sort has been done on the haemoglobin polymorphism in sheep. In sheep, two alleles A & B control the haemoglobin differences between adult animals giving three phenotypes A, AB & B (Harris & Warren, 1955). The distribution of these genes (Evans, Harris & Warren, 1958) is predominantly Hb^B in lowland breeds and Hb^A in hill breeds suggesting an adaptation of each type to its particular environment. Huisman et al (1958/

(1958, a & b) therefore studied the oxygen dissociation curves of the two sheep haemoglobins and found that the A type, the predominant haemoglobin in mountain breeds, had a greater affinity for oxygen. This suggests that natural selection could favour Hb A at high altitudes, but this selective advantage would be lost at low altitudes where the oxygen content of the atmosphere is sufficient so that there is no superiority of Hb A over Hb B as regards oxygen saturation. But the Hb B will be superior at low altitudes since it will be able to release its oxygen to a greater extent because of its lower oxygen affinity. Investigations by King et al (1958) on the Blackface mountain sheep and more recently by Watson & Khattab (1964) on the Welsh mountain sheep have not revealed superiority of any haemoglobin type for reproductive performance. It may be that the haemoglobin polymorphism exists because of crossbreeding between the hill and lowland breeds in the past.

The other interesting biochemical polymorphism is in cattle, where two alleles control the type of β -lactoglobulin produced in the milk. These genes not only control the type of protein produced but they also control the amount of β -lactoglobulin in the milk, the homozygote AA producing almost twice as much as the other homozygotes while the heterozygote shows an intermediate output (Aschaffenburg & Drewry, 1957). This would make it appear that the enhanced production of lactoglobulin by the Lg^A allele would be nutritionally advantageous to the calf and hence the Lg^A gene should be superior to the Lg^B . But in all the breeds examined, the frequency of Lg^B gene predominates ranging from 0.8 to 0.6. This may be due to the early weaning of calves so that any benefit derived from the extra nutrition from the milk/

milk of LGA dams is diminished.

Practical applications of genetic polymorphism in animal breeding

Practical applications of the polymorphism in animal breeding depends on the pleiotropic effects of these genes. By pleiotropy is meant the capacity of a gene to affect different aspects of the phenotype. Every gene that has been studied intensively has been found to be pleiotropic to a greater or lesser extent (Mayr, 1963). It is therefore quite probable that these polymorphic genes may have effects on other characters of animals and some of these may be of economic importance.

The methods employed to detect such pleiotropic effects are mainly statistical and in essence consist of comparing the different phenotypes with respect to the characters in large populations. In such statistical analyses, there is one important complication that may give spurious association between an allele and production. In cattle, large groups of progeny can be born to one sire and if a sire has a rare allele and is also above average for some production trait, then a spurious association between this allele and production may be obtained if this group of progeny form a significant proportion of a population of young cows which are being analysed for this trait. Therefore in populations where there is concentration of blood from a few sires, it is necessary to work within sire groups to remove sire effects and also within herds where the animals are in different herds. The sire effects are unlikely to be a major complication in poultry where large numbers of sires are used for breeding.

Before discussing the specific applications of polymorphism

to/

to breeding of different farm animals, it may be useful to classify the type of characters in farm animals, the type of polymorphic systems and the relation between these two. The characters of economic importance can be broadly classified into two groups

- a) Characters associated with or forming a major component of fitness. Examples of this type would be fertility, viability or egg number in poultry.
- b) Characters not closely related to fitness but are important in determining economic profitability. Examples are milk composition in dairy cows, egg weight in poultry or carcass conformation in the beef animal.

The polymorphic genes may also be crudely classified as being of two types.

1. Those maintained actively by selection of heterozygotes.
2. Those whose maintenance is not due to any selective force.

They may have originated as a result of past pressures of natural selection but their present existence may be due to selective neutrality of the genes concerned.

Let us first consider polymorphic loci showing overdominance and see how they may be used in poultry breeding techniques practiced now. As mentioned earlier, overdominance would be expected with respect to characters related to fitness. These are traits which generally have low heritability but display considerable hybrid vigour and breeding techniques employed are intended to utilise this property. The methods involve crossing different inbred lines, strains or breeds. But this method of cross breeding is entirely an empirical practice since there is no means of predicting which two/

two lines of a large number being tested would give the best hybrid. Hence if any ancillary aids could be used in preliminary selection of the lines, a considerable amount of labour involved in testing would be saved. It is in this context that genetic polymorphisms may be useful. Parent lines can be selected which carry particular genes at these loci and used in crosses so that heterozygous combinations occur in progeny. They may also be used to purify lines that have already been selected on cross performance i.e. by eliminating from the selected lines animals which will not give desired combinations in the cross progeny.

Though this looks attractive, many criticisms can be levelled at this method. Most commercial chickens that are marketed today are four-way crosses. Therefore if each of the inbred lines was homozygous for a gene such that the two-way cross was heterozygous, only 50% of the progeny in the four-way cross would be expected to be heterozygous at that locus because of genetic segregation. This is therefore not satisfactory and theoretically the possible solution is to make each of the two-way crosses homozygous for an allele such that all the four-way crosses were heterozygous.

As to how far overdominance could be useful in breed and strain crosses is important. If the advantage of the heterozygote is due to modifying genes (Fisher, 1930; Ford, 1964) i.e. genes which make the beneficial effects of alleles dominant and the disadvantageous ones recessive then these modifiers would differ in different strains and breeds of poultry since the modifying genes selected would depend on the environment in which they are selected. In a strain or breed cross, therefore, the modifying genes that accumulate will be of different/

different sorts and it can hardly be expected that they would influence the polymorphic locus in the same way. But evidence from *Drosophila* casts doubt on this conclusion. Bracic (1954), Wallace (1955) and Vetukchiv (1957) have found cases in *Drosophila* where inter-strain hybrids have proved fitter than either parent.

With the second category of characters, namely those trivial from the point of the animal's fitness, we would expect the genetic variance to be mainly additive and the second class of polymorphic genes may by chance affect this type of character. In this case we would expect the homozygous type to be the economically better animal and knowledge of such associations may be used in selection methods employing the additive genetic variability such as mass selection and family selection. For example selection among the cockerels of a dam family could be based on the criterion of the beneficial polymorphic type. Polymorphism expressed only in the hen may prove to be of use in sire family selection (without trap-nesting) where entire half sib families are selected. In these cases a certain amount of selection among hens within the family may be done on the basis of the polymorphic genes.

LINKAGE.

Pleiotropy has been mentioned as one of the causes for expecting correlations between polymorphic 'marker' genes and metric characters. However correlations may also be found due to another cause, namely linkage. It may be that genes controlling production are linked to these marker genes. This is very probable if we accept the findings of Newcomer (1957) that the chicken has only five pairs of autosomes corresponding with the five known autosomal/

autosomal linkage groups (Hutt, 1963) though recent evidence (Ford & Woollam) ¹⁹⁶⁵ refutes Newcomer's findings. But, linkage between marker genes and genes for production is not likely to be of use as an aid in selection programmes. Linkage can exist in both coupling and repulsion phases and will reach equilibrium between the two in the absence of forces to stop this. Suppose we have in a flock of poultry, two linked loci, one the A/B system determining a polymorphism and the other containing two alleles or polygenic blocks α/β such that α/α animals are superior to β/β . If the flock had reached linkage equilibrium, then A will not be associated with α any more than B is i.e. $\frac{A\alpha}{A\beta} = \frac{B\alpha}{B\beta}$ where $A\alpha$ is the frequency of the A chromosome and so on. In such a case, selection for A will not change the frequencies of α and β . Therefore, to utilise linkage, the linkage must be very close so that we could use a certain sire $A\alpha/B\beta$ and then choose offspring that derived the A gene from their father in the knowledge that in a high proportion of offspring the α gene would come along too. But as linkages become very tight, the situation becomes operationally more and more similar to pleiotropy. However, it should be mentioned that in the chicken, where the number of 'markers' is large, there are at present 12 autosomal blood group antigenic loci and 5 biochemical polymorphic loci, the possibility of linkages does exist. But it is very difficult to establish such linkages merely by looking at the effect on a metric trait, say egg production unless (a) a reasonably large population is studied and (b) an appreciable portion of the genetic variance of the trait under investigation is controlled by genes actually linked with the 'marker'.

Review/

Review of studies on biochemical polymorphism in farm animals.

The present position with regard to the well established polymorphisms in farm animals is given in Table 1. The literature on blood group polymorphism and production traits in poultry and other animals has been covered in recent reviews (Gilmour (1960), Briles (1964) & Rendel (1961)). It might therefore be relevant to limit the discussion here to work with biochemical polymorphism and performance in animals.

Practically all studies with polymorphisms of domestic animals suffer from two rather severe limitations.

- a) All observations are made in one environment and the environments are not atypical.
- b) Observations are limited to only two or three years.

Since the selective forces acting on these polymorphisms may depend on both spatial and temporal changes any negative findings will have to be viewed in the light of these two limitations.

In sheep both haemoglobin and red cell potassium polymorphisms have been studied by King et al (1958) and Watson & Khattab (1963) to see whether animals with different alleles differ in performance. Both groups of workers studied various body measurements and fleece characters but the former group also studied reproductive performance. Essentially no differences between the different phenotypes were observed.

In cattle, detailed reports have appeared only on the influence of the transferrin (Tf) locus. Ashton (1960) grouped progeny tested (for milk yield) bulls on the basis of their genes at the Tf locus and/

TABLE 1. Present position of biochemical polymorphisms in domestic animals.

Species	Polymorphism	No of alleles	Reference
Horses	Transferrin	5	Braend & Stormont (1964)
	Plasma albumin	2	Stormont & Suzuki (1963)
Cattle	Haemoglobin	3	Bangham & Blumberg (1958)
	Transferrin	5	Ashton (1958 and 1959)
	Post albumins	2	Bo Gahne (1965)
	Serum amylase	3	Ashton (1958)
	Serum albumin	2	Ashton (1964)
Milk			
Pig	α - Lactalbumin	2	Bhattacharya et al (1963)
	β - Lactoglobulin	3	Aschaffenburg & Drewry (1958)
	β - Casein	3	Aschaffenburg (1961 & 1963)
	α_s - Casein	3	Kiddy et al (1964)
	Haptoglobin	4	Hesselholt (1963)
	Transferrin	2	Kristjansson (1960)
	Pre albumins	2	Kristjansson (1963)
	Thread Proteins	2	Ashton (1960b)
	Ceruloplasmin	2	Imlah (1964)
	Arylesterase	5	Augustinson (1961)
	Transferrins	12	Ashton (1958 & 1963)
	Haemoglobin	2	Harris & Warren (1955)
Sheep	Na-K levels	2	Evans & King (1955)
	Haemoglobin		Harris & Warren (1955)
Goat	Haemoglobin		Harris & Warren (1955)
	Transferrins	2	Millson & Pattison (1961)

and observed that the proportion of bulls above and below the mean progeny test values were different in the different transferrin groups. Bulls of the DD genotype were significantly in excess in the 'high' group whereas a larger proportion of AA animals was in the low group. The heterozygotes had equal numbers above and below the mean. This indicates an additive effect of this locus on milk yield. Ashton has been able to confirm this finding from an independent sample of Jersey and Illawara shorthorn cows in Australia (Ashton et al, 1964). This latter data is subject to the criticism that both first and repeat lactations were treated in the same way without applying any correction. Further, he does not state whether the cows were naturally or artificially mated, as in the latter case, it would be necessary to do a within sire comparison to remove sire effects. In both these papers, he concludes that the transferrin locus controls about 10 to 15% of the genetic variation in milk yield. This is certainly an overestimate as he assumes that all the variation between the groups of cows with different Tf genotypes as being due to genetic variation at this locus. A better estimate would be about 4% (Robertson, 1961). But, what is puzzling is that, if the Tf^D allele is beneficial for milk yield then one would expect the frequency of this allele to be greater than Tf^A because of the artificial selection that has been applied for this trait. But the data on gene frequencies shows a general trend of high frequency for the Tf^A allele. For example, in the RDM and Jersey populations in Denmark (Brummerstedt-Hansen, Moustgaard and Moller, 1963) the Tf^D allele is at a low frequency of 0.286 and 0.395 respectively and also in Jerseys in Queensland the frequency/

frequency of Tf^D is only 0.33 (Ashton et al, 1964). But the frequency of this allele in Friesian cows in England is close to 0.5 and in Illawara shorthorns it is 0.68 (Ashton et al, 1964). This implies that there is some other larger selective force maintaining the Tf^A allele in populations where it is at a high frequency. Even if one assumes selection of heterozygotes to explain the frequency of the two alleles, one would have to postulate greater selection intensity against the Tf^D allele (in populations where it is at a low frequency), since the frequencies of the two alleles at equilibrium would be $\frac{Tf^A}{Tf^D} = \frac{S^D}{S^A}$ where Tf^A and Tf^D are the gene frequencies and S^A and S^D the selection intensities against Tf^A and Tf^D respectively.

The other type of study with these polymorphisms is that involving fertility and embryonic mortality. In essence, the studies consist of comparing the observed segregation ratios of matings between different genotypes with that which would be expected on the basis of random fertilisation and embryonic survival. In cattle, it has been shown (Ashton, 1961 and Ashton & Fallon, 1962) that genes at the Tf locus influence fertility and embryonic survival. Their evidence indicates that matings between animals homozygous at the Tf locus are significantly more fertile than those involving heterozygotes, but heterozygous embryos are more viable than homozygotes in utero. They conclude therefore that the Tf locus in cattle affects reproduction in two ways, at fertilisation and in utero. It should be noted, however, that the number of bulls used was very small particularly for the first part of the experiment showing fertility differences. For example in one comparison involving 1166 inseminations (in 2 years), in one year the comparisons were between 2 homozygous and 2 heterozygous bulls/

bulls and in the other year between 1 homozygous and 3 heterozygous bulls. Some of these differences between genotypes may well be differences between bulls irrespective of their transferrin types.

In sheep too, there is evidence of transferrin influence on embryonic survival (Khattab, Watson & Axford, 1964) but it seems to be of a specific kind involving only a particular allele. It appears that in matings involving homozygous CC dams with $\pm/c$ heterozygous sires, excess of offspring of the same genotype as the mother are produced. They conclude that this is due to differential embryonic mortality and not selective fertilisation as matings involving heterozygotes at the T_f locus with T_f^C as one of the alleles did not give any deviation from expectation. It must however be noted that the total number of matings of this specific type was only 14 and more evidence is necessary to confirm this.

A rather similar effect has been reported in the pig (Kristjansson, 1964). It was shown that sows of $T_f^{BB} \times T_f^{AB}$ matings show a greater likelihood of returning to service (this being an indication of ^{no} viable embryos in the uterus) than other matings. He concludes that this is due to greater embryonic mortality as only about 5% of the ovas are lost due to failure of fertilisation in the pig. If this effect is to be explained as due to embryonic mortality, a sow returning to heat should lose all her embryos. In a multiparous animal like the pig where 50% of the embryos in a BB x AB mating would be BB and the other half AB one would have to postulate that both types of embryos have relatively high death rates in these matings. Since matings giving only BB or AB embryos have normal survival values/

values, the mechanism may possibly be some sort of incompatibility effect between AB and BB embryos when both are simultaneously present in utero.

If transferrins do exercise a role in embryonic survival and the above data suggest that they may, then an explanation of their effects on their known function as iron binding proteins may be attempted. From Turnbull and Giblett's (1960) work it does not appear that human transferrins differ in their iron binding capacity but there is a possibility that they may differ in the facility with which they transfer it to tissues, thus endowing some genotypes with an advantage that others lack.

Biochemical polymorphism in the fowl.

Lush (1961) using starch gel electrophoresis reported the occurrence of polymorphism in the egg albumen protein of the domestic fowl. The albumen proteins were separated into nineteen fractions and in each of the three regions of the electrophoretogram (I, II and III) a protein was found which was genetically variable in its electrophoretic mobility. The polymorphism in region I is due to genetic variation in the ovalbumin molecule. The identity of proteins in regions II and III is not known but have been given preliminary identification as globulin (Feeney, et al, 1963). Ogden, Morton, Gilmour & McDermid (1962) reported a fourth genetic variant from a flock of Light Sussex fowls. The protein involved in this variant has been identified as conalbumin. This iron binding protein has been shown by Williams (1962) to occur in a slightly different form in the plasma; hence the polymorphism can be followed in samples from the egg albumen or plasma and has the added advantage in that the males can be typed phenotypically.

The/

The fifth biochemical polymorphism in the fowl was that of the plasma albumins reported by McIndoe (1962).

In Table 2, the protein composition of the egg white (Forsythe & Foster, 1950) and the genetic loci controlling these proteins is given. It will be seen that the ovalbumin locus controls the major portion of the protein in egg white.

Table 2. Protein composition of egg white and known genetic loci.

Constituent	Percent	Genetic locus	Alleles
Ovalbumin	64.9	Ovalbumin	Ov ^A Ov ^B
Conalbumin	13.8	Transferrin	Tf ^a Tf ^b
Ovomucoid	9.2	*	-
Lysozyme	3.4	*	-
Globulin	8.7	Loci II & III	II ^A II ^B and III ^A III ^B
Ovomucin	1.9 ⁺	*	-
Avidin	-	-	-

* No genetic variants of this protein are known.

⁺ Calculated by chemical methods (Warner, 1953).

If one accept the present views on polymorphism (Ford, 1964 and Mayr, 1963) then the existence of these biochemical polymorphisms/

polymorphisms should be due to survival advantage in fowls possessing some of the types. There is no published evidence pointing to selective advantage. similar to the work with blood groups in poultry. However, some of the populations examined by Lush (1961) were highly inbred, but some lines remained heterozygous at some of these loci. It is possible to examine whether the heterozygosity in these lines is greater than expected by dividing the proportion of heterozygotes by the term $(1-F)$ where F is the coefficient of inbreeding. This should remain constant as inbreeding increases and hence if the ratio exceeds 0.5, selection for heterozygosity may be inferred. (This assumes that the original population from which this inbred line was derived had 50% of heterozygotes.) In Table 3 this is analysed for the lines which were still heterozygous in Lush's data.

Table/

Table 3. Analysis of Lush's data (1961)

Line	Coefficient of inbreeding	Proportion of Heterozygotes (1-F)	Locus
Breeding	31	0.285	Ov *
Intensity	46	0.626	Ov *
White Plumage	62	0.675	Ov *
Breeding	31	0.425	II
R.I.R. Q.Line	92.55	6.85	II
Red Plumage	74	1.515	III
Non moult	57	0.086	III
Small egg	79	0.790	III
Dwarf body	58	1.280	III
R.I.R. Q.Line	92.55	2.960	III

*The frequency of heterozygotes were calculated assuming Hardy Weinberg equilibrium in populations.

Though most of the lines that are still segregating show that the proportion of heterozygosity is greater than expected, many other lines have in fact been fixed for one or other of the alleles. This makes it improbable that there is any strong selective advantage of the heterozygotes at this locus.

THE/

THE PROBLEM.

Since there was no published evidence on the relationships between these polymorphisms and production characters in the fowl (one has appeared since this work was begun, (Morton et al, 1965) it was decided to tackle this problem using three commercial strains of poultry.

In a study of this nature the findings may be one of the following:

- a) A real significant detectable difference may exist between genotypes.
- b) Differences may exist but may not be detectable because
 - (i) the differences may be so small that random environmental changes may mask them.
 - (ii) the population size may be small so that the difference may not be statistically significant.
- c) No differences may exist.

This particular study also has certain limitations owing to the nature of the polymorphism.

1. Compared with a locus which is expressed in both sexes from the time of hatching, a sex limited inheritance with the added complication of the phenotype not being expressed till after sexual maturity, imposes limitations on the quantitative studies that could be undertaken. For example, differences in mortality between individuals with different albumen phenotypes cannot be studied directly because the phenotype of the individuals that do not survive till they lay an egg cannot be ascertained. Similarly, since the phenotype/

phenotype of the sires cannot be ascertained directly, studies on fertility and effect of embryo's own phenotype on mortality cannot be carried out unless the sire's genotype can be determined independently from family data.

2. Population studies on segregating egg albumen loci by other workers (Law, 1963; Cochrane & Annan, 1962; Baker & Manwell, 1962) have indicated that closed flocks of poultry often show segregation at only one or two of the egg albumen loci. A flock of birds segregating at all 4 egg albumen loci has still not been reported. This, with the added limitation that the gene frequency of one of the segregating alleles at a locus may approach zero, imposes restrictions on the number of comparisons that could be made. In Table 4, the available data from published papers on gene frequencies at these loci is summarised.

TABLE 4. Gene frequencies of egg white loci in different populations.

Author	Breed	Ovalbumin locus		Locus II		Locus III		Transferrin	
		Ov ^A	Ov ^B	II ^A	II ^B	III ^A	III ^B	T ^a	T ^b
Lush (1961)	Brown Leghorns (8 inbred lines)	1.0 to 0.13	0.87 to 0	1.0 to 0.85	0.15 to 0	0.9 to 0	1 to 0.1	0	1
"	Bush Crossbred	1.0	0.0	0.60	0.40	0.05	.95	-	-
Cochrane & Annan	White Rock x Leghorn	-	-	0.80	0.20	0.41	0.59	-	-
Baker & Manwell	White Leghorn	-	-	0.9	0.1	0.25	0.75	-	-
	New Hampshire	-	-	0.47	0.53	0.49	0.51	-	-
	Columbian	-	-	0.65	0.35	0.0	1.0	-	-
	DeKalb Line I	0.90	0.10	0.66	0.34	0.0	1.0	-	-
	" Line II	0.62	0.38	1.0	0.0	0.5	0.5	-	-
	" Line III	-	-	0.87	0.13	0.51	0.49	-	-
Law (1963)	" Line IV	-	-	1.0	0.0	1.0	0.0	-	-
	Line 79(1964)	0.96	0.04	-	-	0.64	0.36	-	-
	" (1963)	0.92	0.08	-	-	0.46	0.54	-	-
	Line 60	-	-	0.49	-	-	-	-	-
	Line 45 (1962)	-	-	-	-	0.34	-	-	-
	" (1963)	-	-	-	-	0.57	-	-	-
	Line 79 (1962)	-	-	-	-	0.20	-	-	-
	" (1963)	-	-	-	-	0.18	-	-	-

PART II MATERIALS & METHODS

Experimental flocks:

Most of the birds used in this work were from two closed strains of White Leghorns of the Edinburgh School of Agriculture which were used in a selection experiment. The strains to be described will be called strain 2 and strain 5. Both strains were built up from rather small numbers: strain 2 originated from ten males and ten females in 1955 and a similar number in 1956. It was then closed, and the flock expanded to a thousand in 1957. From 1957, the strain was kept as a closed random breeding population until 1962. In Spring 1962, two selection lines, called the maximum egg weight and minimum body weight lines - were begun. A random mated "control" line was also kept. The following year, two further selection lines, the ratio and regression lines - were started from the random control. The selection procedures used will be given below.

Strain 5 has a similar history except that it was developed in 1956 from an even smaller number of parents, the exact number being unknown. Four selection lines, similar to those of strain 5 were started, the first two in Autumn 1962 and the other two in the Autumn of the following year. The history of the two lines to 1964 is summarised below.

Strain 2/

	Strain 2	Strain 5
1955	Originated	-
1956	Built to)	Originated
1957	1000)	Built to)
1958	Random -)	1000)
1959-1961	Bred)	Random bred
1962	B.W. + E.W. + R	B.W. + E.W. + R [*]
1963	B.W. + E.W. + R + Ratio + Reg	B.W. + E.W. + R + Ratio + Reg ⁺
1964	Ditto	Ditto

* B.W. = Minimum body weight line

* E.W. = Maximum egg weight line

+ Ratio = Ratio line

+ Reg = Regression line

+ R = Random control line

Individual selection was used for all four selection lines, while the males were selected on the basis of full sib averages.

Selection criteria in the four lines were as follows:

- a) Minimum body weight line: Based on body weight measurements taken at 12 weeks of age.
- b) Maximum egg weight line: Based on mean egg weight of eggs laid at 28 weeks of age.
- c) Ratio line: Ratio of egg weight at 28 weeks of age to body weight at 28 weeks was calculated and birds having the highest ratios were selected.
- d) Regression deviation line: Birds having the maximum positive deviations/

deviations from the regression of egg weight on body weight were selected.

Each selection line had about 240 progeny which were the result of matings of 15 sires to 4 hens each. There were thus 15 half sib groups of approximately 16 progeny and 60 full sib groups of 4 in each selection line. Selection intensity was approximately the same in all lines and was 0.25 in females while the males were from the best 10 full sib families i.e. a selection intensity of 0.17.

The random control line had 150 to 180 progeny and was from 15 sires and 60 dams in each year. The 15 sires were rotated each week during the breeding season of 4 weeks.

Prior to 1964, breeding was carried out by natural mating in single sire pens. From the Autumn of 1964, with a change in management from deep litter to battery cages, artificial insemination was used in breeding. From each strain, chicks were hatched in four fortnightly hatches. Hatches 1 and 2 consisted of the eggs from maximum egg weight and minimum body weight selection lines while hatches 3 and 4 consisted of the ratio and regression lines. The control line eggs were included in all four hatches. The chicks were wing banded at hatching, sexed and the sexes separated. Up to 6 weeks of age, they were reared under brooders and then transferred to deep litter rearing houses where they remained until 20 weeks of age. They were then leg-banded and transferred to laying quarters. No culling was practiced, but only a proportion of the pullets was transferred to laying quarters to equalise the number of progeny in each full sib and half sib family. Selection of pullets in a sib family was at random. The laying/

laying pens were lit by artificial roof lighting. A commercial mash was fed ad lib.

The other flock that was examined was a Light Sussex flock of approximately 400 laying hens at the Royal Dick Veterinary field station, Edinburgh. This flock was built up in 1952 from 5 widely differing sources in U.K. It has been closed since then except for the introduction of two outside cockerels in 1962. The birds were housed in individual cages. No pedigree records were kept and in each year the semen of males used as breeders was pooled for insemination. Selection was applied to the hens only for egg number based on part individual records.

Another crossbred flock of about 1500 females used in a breeding study at the University of Durham was also studied. It was formed between 1957 and 1961 by crossing together four breeds - Rhode Island Red, Light Sussex, Brown Leghorn and White Leghorn. The population examined was the 1963 generation. Only a sample of 43 hens from this population was typed to see if some of the loci which were not segregating in strains 2 and 5 were segregating in this population. There was one such locus - locus III, but since the gene frequencies were such that the frequency of one class of homozygotes were very low, no further studies were undertaken.

Collection of samples and electrophoresis.

Eggs were collected either from trapnests or battery cages, the identifying number written on them and stored at 3° to 5°C as soon as possible after collection. For samples that could be used within two months, whole eggs were stored at the above temperature. Storage for this period had no effect on the electrophoretic analysis.

Longer/

Longer storage at this temperature caused the electrophoretic pattern to be diffuse causing difficulty in identification of certain phenotypes particularly in region II. Samples that had to be kept longer than two months, were stored at -20°C but only the albumen was stored in test tubes. For routine typing, the egg shell was carefully broken (after cleaning the exterior with alcohol in cases where the shell was dirty) and the thin albumen transferred directly to 3" x $\frac{1}{4}$ " test tubes.

Electrophoresis: Partially hydrolysed starch, specially standardised for gel electrophoresis, manufactured by Connaught Laboratories, Toronto, was used throughout. The buffer system employed was the discontinuous system of Paulik (1957), slightly modified (Lush, 1961).

The electrolyte concentrations were as follows:

Gel	(	(0.076 M tris (hydroxy methyl) amino methane p^H 8.6
	(	(0.005 M citric acid
Electrode	(	0.3 M boric acid
end trays	(	0.1 M sodium hydroxide p^H 8.8

The electrode vessels were commercial polystyrene sandwich boxes. The electrodes were 6" to 9" lengths of platinum wire (90% platinum and 10% iridium) 0.025" diameter. The wicks between the two end trays on each side were five thicknesses of surgical lint, while the wicks from the tray on to the gel were five thicknesses of Whatman No 3 MM chromatography paper. The gel trays were made of $\frac{1}{8}$ " thick glass and enclosed a space for the gel of 22.75 cm x 19.75 cm x 0.3 cms. In each apparatus a total of 2000 ml of buffer was evenly distributed among the end trays. This buffer was used for 4 to 5 times and then renewed. It was thought that changing the polarity of the apparatus/

apparatus between alternate experiments would balance the electrolyte concentrations but no improvement resulted when this procedure was used and hence it was not routinely adopted. The source of stabilised d.c power was a Siemen-Ediswan R1280 Power unit.

The procedure for a typical experiment was as follows:

About 20 grs of starch powder (the exact amount varied from batch to batch as recommended by the manufacturers) was weighed in a beaker and tipped into a 500 ml conical flask. About two-thirds of the required amount of buffer was added and the starch swirled into a suspension as quickly as possible. The starch remaining in the beaker was washed with the remaining third of the buffer and added to the flask. The suspension was heated with a bunsen flame for about 4 minutes with constant agitation. The flask was taken away from heat as the suspension came to boil, degassed for about 12 to 15 seconds with a water vacuum pump at 600 mm of mercury and poured quickly into a glass tray. This was covered with a thin sheet of polythene, care being taken to prevent air bubbles being trapped between the sheet and the starch. A sheet glass cover was placed on the polythene and excess starch removed by pressure on the glass. A weight was placed on the glass and the polythene sheet pulled taut over the starch. Care was taken to ensure that the glass cover did not slip sideways during the early stages of cooling while the gel was still liquid. The gel could be used for electrophoresis after three hours, or could be kept at room temperature and used the following day. A series of 14 to 20 slits (depending on the size of the filter paper inserts) were cut in a straight line about 3.75 cm from the cathode end of the gel by sticking pieces of Ever-Ready razor blades cut to size/

size. The sizes of the slits used in the experiment were one of three sizes - 1.25 cm, 1.0 cm or 0.8 cm. The egg albumen was absorbed on to rectangles of Whatman 3 MM filter paper (3 mm broad and the length depending on the size of the slit), the excess removed by absorption on filter paper and inserted one by one into the slits in the gel. The number of samples that could be accommodated in the gel were 14, 18 or 20 with the three different sizes of filter paper inserts. The paper wicks were laid on each end of the tray, overlapping the gel by about 1 cm. The gel and wicks were carefully covered with the polythene sheet and the glass cover replaced to maintain good electrical contact between gel and wicks. A stabilised d.c. voltage gradient was now established. Two methods were employed.

- a) a voltage gradient of 10 to 12 V/cm for 4 to 5 hours at a temperature of 4°C, and
- b) a voltage gradient of 3 to 4 V/cm for 16 hours either at 4°C or at room temperature (18°C).

Both systems gave satisfactory results. System (a) was superior for distinguishing the ovalbumin region phenotypes whereas the latter gave clear patterns for regions II and III. During the run, the brown buffer boundary described by Poulik (1957) moved along the gel from cathode to anode. This was used as an indication of the progress of the electrophoresis and the experiment was ended when it was about to disappear beneath the anode wick.

When electrophoresis was completed, the gel was removed from the circuit and cut into two halves of approximately 11.5 cms wide. These/

These pieces were each transferred on to a slicing glass piece. Perspex guides $\frac{1}{8}$ " thick were kept close to the two sides of the gel and the gel sliced into two pieces $\frac{1}{8}$ " thick by running a thin taut steel wire through its centre.

Staining for protein: The lower slice of each gel was stained for 10 minutes in maphthalene black (1% in a 5 : 5 : 1 mixture of methanol, water and acetic acid) while the upper half was stained with 0.05% nigrosine in the same solvent for 15 minutes. The excess background stain was washed with several changes of the stain solvent.

The gels were stored in stain solvent in plastic boxes. They showed little deterioration if covered with the solvent all the time, though they were difficult to handle after a period of a few months. Gels were photographed by transmitted light, using rapid process panchromatic plates (Ilford). The exposure was 2 seconds at f.32 and the developer I.D.2.

A typical electrophoretogram of egg white is shown in Figure 1. In one batch of starch, it was observed that the conalbumin band (band 17, Lush 1961) split into two components one migrating faster than the other (Fig.2). At first this was thought to be due to polymorphism at the Tf locus (Ogden et al 1962), but comparison of the same egg white sample in other batches of starch gel confirmed that this was an artefact. Since conalbumin binds iron (Lush, 1963) it was suspected that this artefact may arise if there is insufficient iron in the gel to combine with all the iron binding sites available in the conalbumin molecules. If only a fraction of the conalbumin molecules bind iron and others do not, then the two fractions would be expected to move at different rates owing to the difference in charge. This was tested (a) by adding ferric chloride to the buffer before making up the gel and (b) by adding ferric chloride to egg white samples

Fig 1. A typical electrophoretogram of
egg white protein.

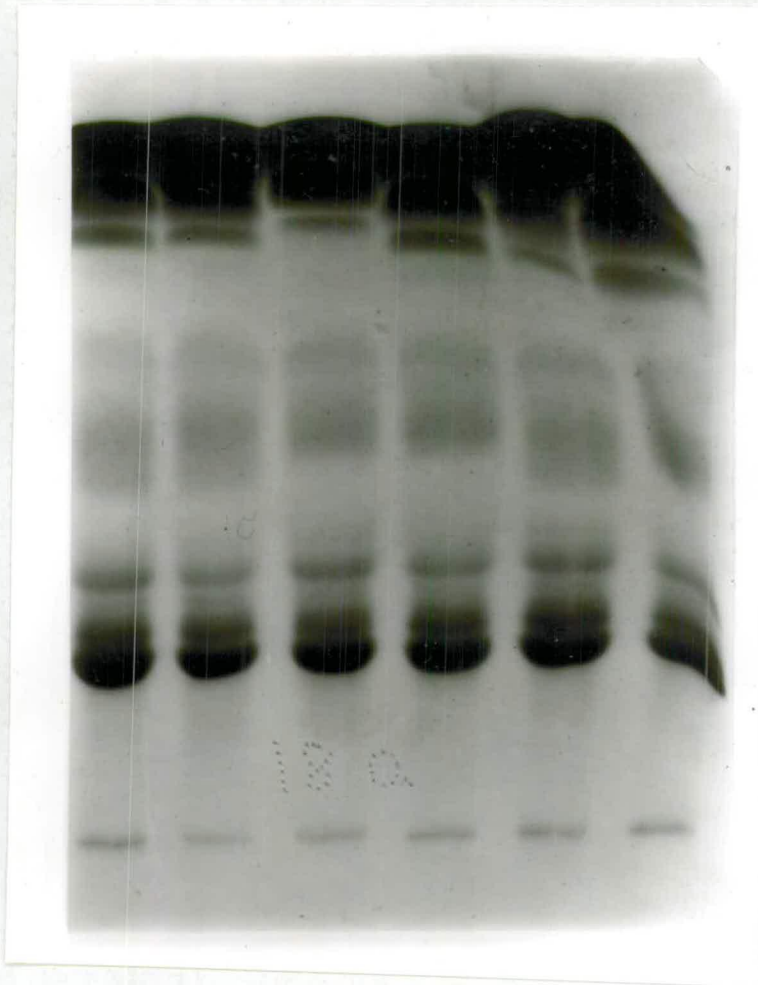
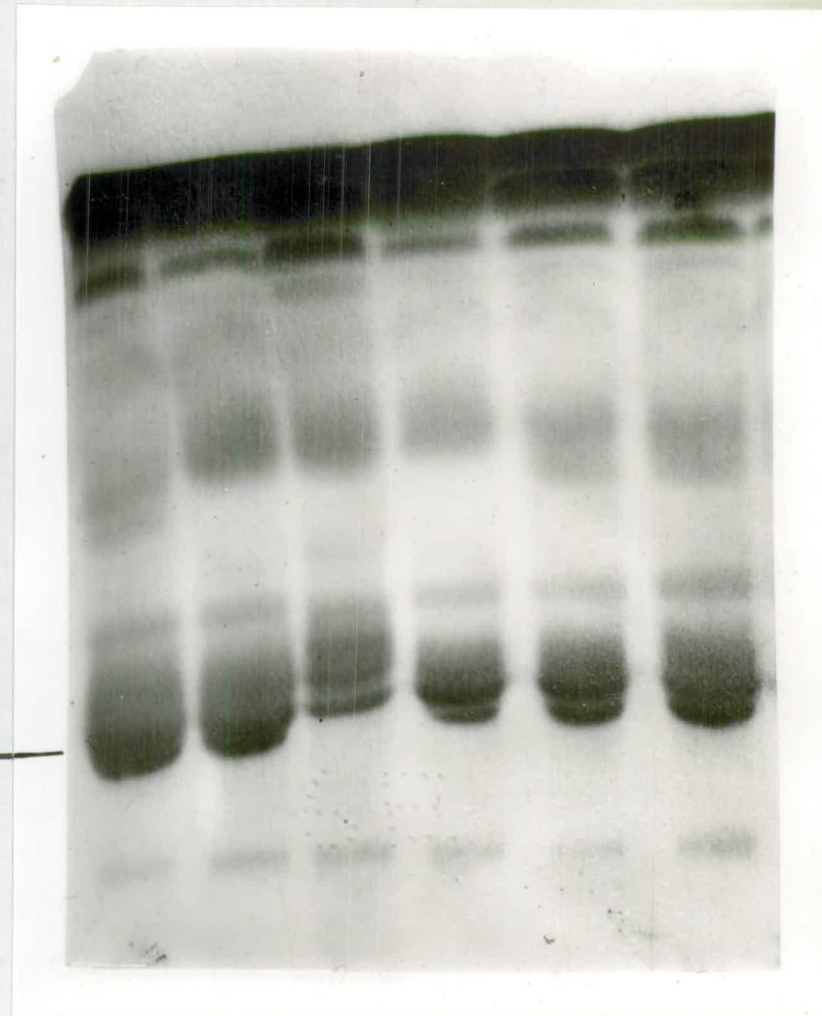


Fig. 2. An electrophoretogram showing splitting
up of the conalbumin component.

Con-
albumin ←



and running them adjacent to controls. In the former case, all samples gave a uniform one banded pattern, while in the latter case, only the samples to which the iron was added gave the normal pattern. This confirmed the view that lack of iron was the limiting factor and about 10 drops of a 0.025% solution of ferric chloride was routinely added to the 175 cc of buffer necessary for making up a gel.

Characters studied.

Since this study was intended to detect associations between the polymorphisms and production characters, the characters chosen for study should be such that overdominance or simple homozygote superiority would be detected. Since overdominance has generally been associated with characters related to fitness, some of the characters included should be those contributing to the fitness of the population. Fitness is measured by the proportionate contribution of offspring by an individual to the next generation. In poultry, the major components of fitness will be a) Number of eggs laid, b) fertility, c) hatchability and d) viability. The characters chosen for detailed study were hatchability i.e. embryonic mortality and egg number.

The second category of characters should be those that are trivial for the fitness of the individual, since these would have a large proportion of additive genetic variance and any additive effect of the polymorphic genes may by chance be on these characters. Various egg weight and body weight measurements were taken to represent this group of characters.

Measurement of characters:

1. Body weight. Body weight measurements to the nearest 10 grams was taken at the following ages:- 12, 20, 28, 36 and 44 weeks.
2. Egg weight. Eggs were weighed to the nearest gram and the mean of all/

all eggs laid during the week was calculated. Records were obtained at 28, 36 and 44 weeks of age.

3. Egg number. The birds were trapped 3 days per week. The total number of eggs laid up to two ages - 44 weeks and 72 weeks were calculated. The 44 week age was chosen because most artificial selection programmes for egg number are based on part egg production records to 44 weeks only. Therefore under artificial selection this trait would be a major component of fitness.
4. Embryonic mortality. All incubated eggs were candled on the 6th and 13th day of incubation. The infertile eggs and those in which the embryo had died were removed. The eggs removed were broken and examined macroscopically to determine the age at which the embryo died. A study of the literature giving morphological changes in the chick embryo with age (for example, Hamburger, 1951) revealed that classification of age of embryo by each day was difficult to achieve when large numbers are involved as some of the changes, particularly during the latter part of the incubation, were mainly quantitative increases of body measurements. The embryos were therefore classified into 3 day age groups using the following criteria. These are based on the classification given by Hamburger (1951)
 - 1 day. Enlargement and organisation of blastodisc but no blood formation.
 - 2 to 4 days: Evidence of blood formation but eye is not pigmented.
 - 5 to 6 days. Limb bud formation has begun and eye pigmentation is distinct but there is no evidence of beak.
 - 7 to 9 days. Feather germs and beak appear but eyelids are not quite clear.

10 to 12 days. The opening between eyelids narrows and feather formation is advanced. No evidence of overlapping scales on leg.

13 to 17 days. Feather formation well developed with overlapping scales on legs.

18 to 20 days. Absorption of yolk sac begins and is completed by the 20th day.

21 days. Shell pipped.

PART III. LINKAGE STUDIES

Introduction

Any attempt to find if the albumin polymorphisms contribute to the levels of the characters listed earlier, depends on finding populations in which the loci are segregating in adequate numbers. A survey of the four populations already described, showed genetic segregation, as follows:-

Strain 2 - Ovalbumin locus and locus II. (Fig.3)

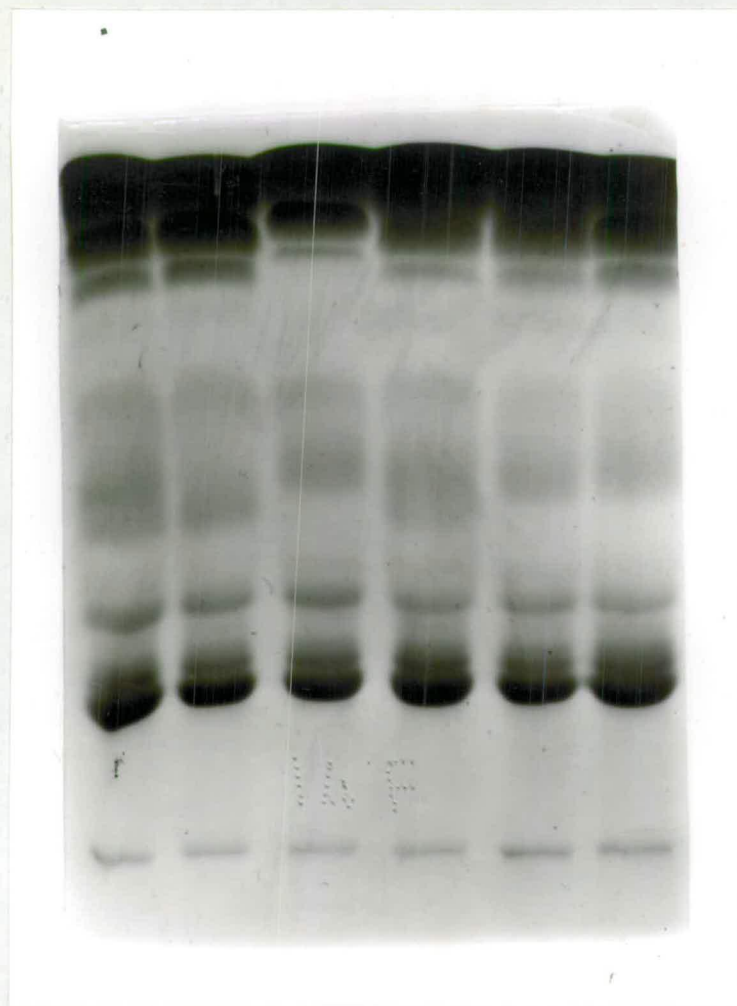
Strain 5 - Locus II.

Light Sussex - Loci II,III and Tf (Fig. 4)

Newcastle Crossbred - Ovalbumin locus, loci II and III.

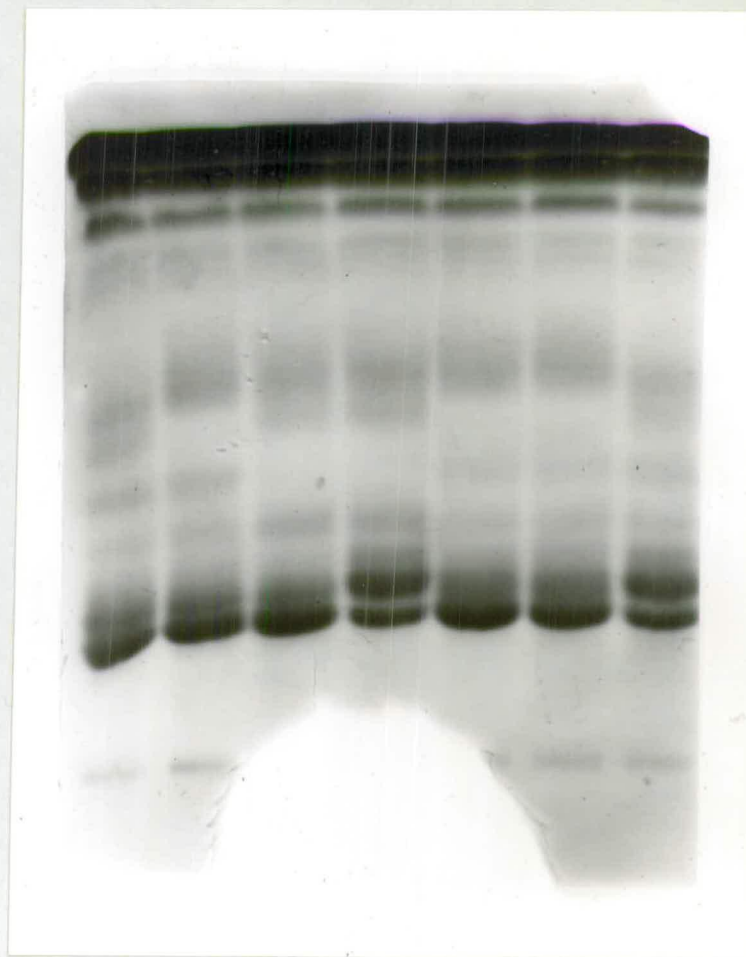
Not all of the classes were present in sufficient numbers for comparisons with respect to production and other characters. But since the polymorphisms are all (assumed) due to pairs of alleles each at an autosomal locus, the genotypic frequencies could be compared with expectations. This was done for each locus separately and the expected and observed numbers are shown in Tables 5a, b, c, and d. There is good agreement between expected and observed numbers for strains 2, 5 and the Newcastle flock. The Light Sussex flock is in equilibrium at loci II and III, but at the Tf locus there is a slight deficiency of heterozygotes ($P < 0.05$). Since one of the assumptions of Hardy-Weinberg equilibrium is absence of differential selection in favour of or against any genotype, we should not expect any strong selective forces on genes at Ovalbumin locus (Ov), locus II and locus III. The deficiency of Tf heterozygotes in the Light Sussex flock may have arisen by chance due to the small number of breeding males used or it may actually be due to selective forces/

Fig 3. An electrophoretogram of representative variants of Strain 2.



Ov.	A	A	B	AB	AB	A
II.	AB	B	A	AB	A	A
III.	B	B	B	B	B	B
Tf.	B	B	B	B	B	B

Fig 4. An electrophoretogram of representative variants of the Light Sussex population



Ov.	A	A	A	A	A	A
II.	AB	A	AB	AB	A	AB
III.	A	AB	B	B	AB	AB
Tf.	B	B	B	AB	B	AB

forces acting against this genotype. This will be discussed later.

TABLE 5. Classification of hens with reference to the Ovalbumin locus, loci II, III and T_f, each considered separately. Expected numbers are worked on the basis of Hardy-Weinberg equilibrium.

a. Strain 2.

Locus	AA		AB		BB	
	Obs	Exp	Obs	Exp	Obs	Exp
Ov. locus	290	286.5	463	469.7	196	192.9
Locus II	689	687.8	238	240.1	22	20.9
				χ^2	D.F.	
Segregation for Ov. locus				0.202	1	
Segregation for locus II				0.072	1	

b. Strain 5.

Locus	AA		AB		BB	
	Obs	Exp	Obs	Exp	Obs	Exp
Locus II	295	293.7	423	425.3	155	154.0
				χ^2	D.F.	
Segregation for locus II				0.023	1	

c. Light Sussex.

Locus	AA		AB		BB	
	Obs	Exp	Obs	Exp	Obs	Exp
Locus II	140	145.8	196	184.1	52	58.1
Locus III	6	10.9	118	108.4	264	268.6
T _f locus	13	7.1	79	90.5	296	290.4
				χ^2	D.F.	
Segregation for locus II				2.32	1	
Segregation for locus III				1.64	1	

d. Newcastle flock.

Loci	AA		AB		BB	
	Obs	Exp	Obs	Exp	Obs	Exp
Locus II	33	32.7	9	9.6	1	0.7
Locus III	6	5.6	19	19.8	18	17.6
T _f locus	1	1.3	13	12.4	29	29.3

Independence or non-independence of segregation:

It is also necessary to test if the pairs of loci segregate independently or if they are on the same chromosome.

Ovalbumin locus and locus II: These two loci segregate in strain 2 and the Newcastle flock. We shall consider only the first strain here. The expectations for each subclass calculated on the basis of independent segregation are shown along with the observed values in Table 6. They differ significantly from the observed values ($\chi^2=154.9$, $P < 0.001$). The joint distribution shows a complete absence of three combinations of types.

TABLE 6. Classification of hens with reference to the ovalbumin locus and locus II. The figures in parentheses give the numbers of each phenotype expected on the basis of independent segregation.

Ovalbumin locus				
		AA	AB	BB
Locus II	AA	160 (211.3)	333(335.0)	196 (242.7)
	AB	108 (72.2)	130(116.8)	0 (49.0)
	BB	22 (6.5)	0(11.2)	0 (4.3)
			χ^2	D.F.
Segregation for Ov.locus			0.202	1
Segregation for Locus II			0.072	1

Lush (1961) showed that both the Ov. and locus II. variants were inherited in a straightforward, Mendelian manner. The present finding that the two kinds of variants do not occur independently is strong presumptive evidence of linkage. The results are then interpreted as indicating that the genes determining Ov. and locus II. variants lie on the same chromosome. Table 6 shows that all Ov.B homozygotes are also IIA homozygotes. This would be explained by an absence of chromosomal type Ov^BII^B in the population. If this chromosomal type is absent, the frequencies of the other three possible chromosomal types can be calculated by the maximum likelihood method (Mather, 1956) and are shown in Table 7. From a knowledge of these frequencies, the expected number of individuals in each class are calculated; and this is found to fit the observed data as shown in Table 8 ($\chi^2=0.73$, $P>0.95$).

TABLE 7 Maximum likelihood estimates of gametic frequencies (assuming gametic type Ov^BII^B to be absent).

Ov ^A II ^A	0.397
Ov ^A II ^B	0.150
Ov ^B II ^A	0.453

TABLE /

TABLE 8. Comparison of actual and expected numbers in each class assuming gametic frequencies of Table 3.

		Ovalbumin locus					
		AA		AB		BB	
		Act	Exp	Act	Exp	Act	Exp
Locus II	AA	160	149.4	333	341.3	196	196
	AB	108	113.0	130	129.1	0	0
	BB	22	21.4	0	0	0	0
				χ^2	D.F.		
Segregation for Ov. locus				0.157	1		
Segregation for locus II				0.106	1		
Correlation				0.727	4		

The absence of the zero classes (Table 8) may be due to the lethality of these gene combinations. But this is not possible since two of these combinations, the Ov.BB,II.AB and Ov BB II BB have been reported by Lush (1961) in the BR (Breeding) line of Poultry Research Centre Brown Leghorns.

As my experimental population (see materials and methods) was built up from a very small initial sample of birds (about 20 females and 20 males), it is possible that, though the combination Ov^BII^B was present in the population from which the sample came, it was by chance absent from the sample. (since the II^B gene is at a low frequency.). If this were so and the two loci were linked, then some gametes of this type should have been formed by crossing over in heterozygotes of the type Ov^A II^B/Ov^B II^A and the frequency of this gamete in the flock would have gradually increased during the time it was randomly/

randomly bred. Since there were 130 such heterozygotes in the present generation and as the population had been random bred at a population size of a thousand, it may be estimated that about 600 birds, which were the progeny of such heterozygotes, must occur in the ancestry of the present generation. If each bird contributes a gamete to the next generation, 150 gametes of the type $\underline{\text{Ov}^{\text{B}} \text{II}^{\text{B}}}$ should be expected on the assumption of no linkage. Since no such gametes were detected in the present sample, the distance between the two loci must be less than one cross over unit.

In the absence of evidence of crossing over, the results could be explained as a single locus with multiple alleles, three of which are present in this population. On this hypothesis, each and such postulated allele should control two regions in the electrophoretic pattern; region I and region II of Lush (1961). If we designate the alleles as A^1 , B^1 and C^1 , the electrophoretic pattern of the three homozygous types would appear as shown in Figure 5. On this postulate a gene should produce several protein fractions differing in electrophoretic mobilities. Homozygous A^1A^1 would have 4 fractions in region I, 2 in region II and so on. If the current view concerning the genetic determination of protein structure i.e. that "one gene determines the specificity of one polypeptide chain" is to be accepted, then the electrophoretic heterogeneity should be due to the binding of variable amounts of charged 'prosthetic groups' to small groups of protein molecules. In fact, the electrophoretic heterogeneity of ovalbumin has been shown by Perlmann (1952 and 1953) and Lush (1964) to be largely due to the different phosphate contents of the electrophoretic fractions. If the gene now mutates causing a demonstrable/

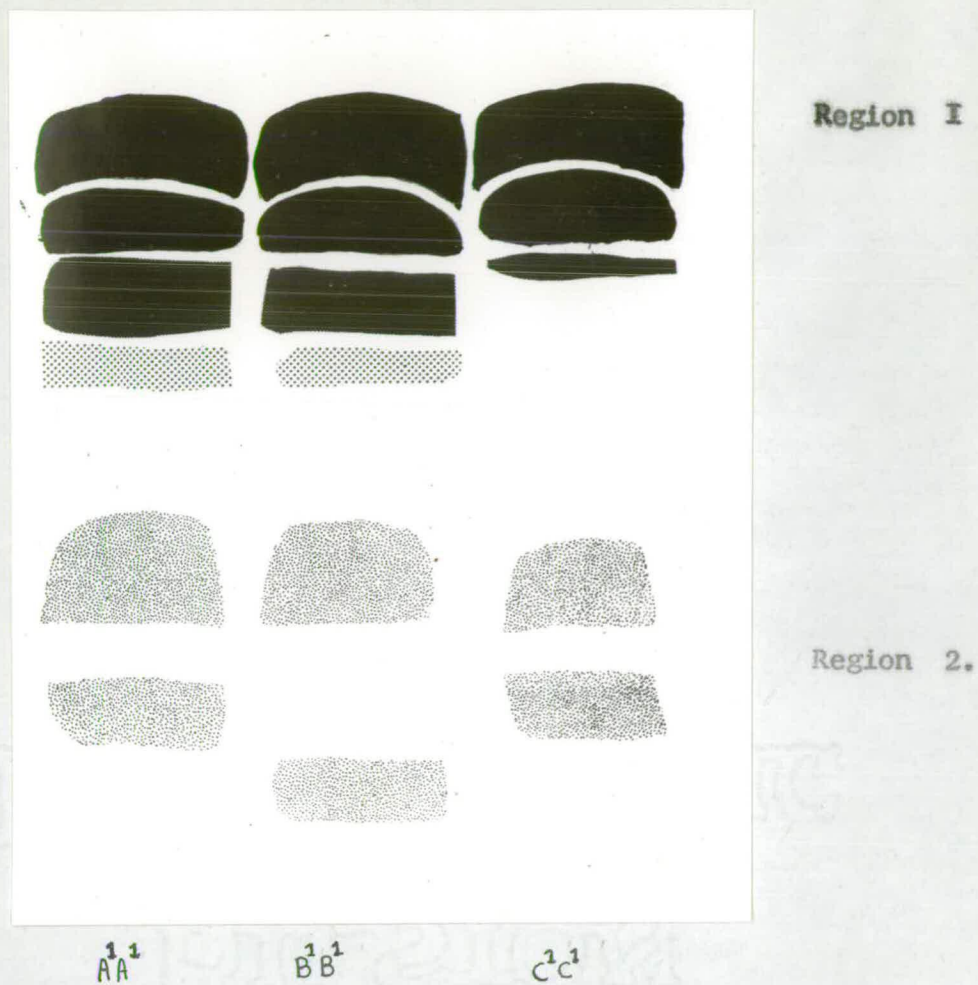


Fig 5. Diagram of homozygous types of region I & II variants on the hypothesis that the two regions are controlled by a tri-allelic locus.

change from the electrophoretic pattern of the original protein, this variation should affect all the fractions of the original protein. But on the hypothesis of multiple alleles as shown in Figure 5, one would have to postulate that mutation from A^1 to B^1 changes the electrophoretic fractions of region II but not of region I and that mutation of B^1 to C^1 alters only region I. This is incompatible with the 'one gene - one protein' hypothesis and hence the explanation of the segregation on a multiple allelic theory is unacceptable. On the basis of two separate loci however, variation in each region is ascribable to each locus and this satisfies the one gene (cistron) one polypeptide chain hypothesis.

There are published reports of two other populations segregating at these two loci - the Brown Leghorn population examined by Lush (1961) and a DeKalb line reported by Baker & Manwell (1962). The latter sample is too small for any comparison. Lush's population and the Newcastle crossbred flock reported here were also analysed to see if there was supporting evidence of association between the two loci. In both cases, there was agreement between observed and expected values ($\chi^2=5.01$, $P>0.05$ for Lush's data and $\chi^2=2.85$, $P>0.10$ for the Newcastle flock) indicating no evidence of linkage. The agreement for Lush's data, however, was not close. Probably these two populations have attained linkage equilibrium with both coupling and repulsion heterozygotes at equal frequencies.

Loci II, III and T_F : The detection of linkage between Ov and locus II raised the possibility that similar linkages may exist between the other loci and a preliminary investigation of this was made in the Light Sussex flock. A comparison of observed and expected numbers for each pair/

pair of loci is shown in Tables 9a, 9b and 9c. Since there are very few individuals of genotype IIIIAA and T_f -aa, these have been lumped together with their respective AB subclasses leaving six cells in each paired loci comparison. The value of χ^2 is large enough only for loci II & T_f to warrant a rejection of the hypothesis of no association. As this discrepancy may be due to other causes such as non-random mating or selection against some genotypes, it has to be checked by an independent experiment.

TABLE 9. Classification of hens in Light Sussex flock with reference to loci II, III and IV for pairs of loci taken in turn. The figures in parentheses refer to the numbers of each type expected on the basis of independent segregation.

a. Loci II and III

		Locus II		
		AA	AB	BB
Locus III	AA	3.0 (3.6)	3.0 (5.2)	0 (1.6)
	AB	33 (40.7)	68 (51.4)	17 (16.2)
	BB	101 (100.9)	128 (127.4)	35 (40.2)
		χ^2		D.F.
Segregation for locus II		2.32		1
Segregation for locus III		1.64		1
Linkage (IIIA & IIIB pooled)		1.69		2

b/

b. Loci II and T_f

		Locus II		
		AA	AB	BB
T_f locus	AA	0 (2.6)	10 (3.3)	3 (1.06)
	AB	19 (34.0)	54 (43.0)	6 (13.6)
	BB	121 (109.1)	132 (137.7)	43 (43.5)
		χ^2		D.F.
Segregation for locus II		2.32		1
Segregation for T_f locus		6.4		1
Linkage (T_f -a & T_f -ab pooled)		16.05**		2

** $P < 0.001$

c. Loci III and Loci IV

		AA	AB	BB
T_f locus	AA	0 (0.2)	2 (1.97)	11 (4.9)
	AB	2 (2.6)	22 (25.3)	55 (62.7)
	BB	4 (8.2)	94 (81.1)	198 (200.9)
		χ^2		D.F.
Segregation for locus III		1.64		1
Segregation for T_f locus		6.4		1
Linkage		5.3		2

Linkage experiment:

Because the evidence given above for linkage between the Ov locus and locus II was indirect, and as an association was found between loci/

loci II and T_f it was decided to investigate if the loci were linked by two experiments - one to test linkage between Ov and locus II and the other between II, III and T_f . The design is the same for both experiments and will be illustrated using Ov. and locus II. Backcross matings, (males of Ov.AA,II.AA x Ov.AA³¹,II.AB hens) were made and segregation of genes in their progeny examined by electrophoretic 'typing'. The heterozygous dams used were of unknown phase, as there was no information about the egg white types of their parents. It is therefore not possible to ascertain directly the parental and crossover types in the offspring unless large progeny groups are obtained per mating. This raises a practical problem, since raising up of large progeny groups requires several hatches with the attendant increase in labour of brooding and rearing the separate small hatches. An optimum progeny size which would give an accurate estimate should be determined and this is dealt with in Appendix Ia. It is seen that for low recombination values of 1.0 to 5.0 percent the gain in accuracy with increase in size of progeny groups is negligible after $n=4$ (n =progeny size). However, for higher recombination values, say 30 percent, the gain is almost linear with increase in progeny size. Since earlier indirect evidence suggests that Ov. and locus II are closely linked, progeny size is not important for the experiment involving these two loci whereas for the other experiment, larger progeny size is desirable.

Choice of parents:

In both experiments, the males used were from the L line (large egg) of Brown Leghorns kept at the Poultry Research Centre. Two generations of hens of this line were previously examined and found/

found to be homozygous for all the known egg albumen loci (Lush, personal communication). The entire 1963-64 generation consisting of 30 hens were again typed and all were homozygous at the four loci being OVA, IIA, IIIB, T_F -b. It was therefore assumed that the males of that generation which were sibs of the hens examined would also be homozygous at these loci.

Dams:

a) Ov and locus II. White Leghorn strain 200 females were used.

Twenty Ov,AB,II,AB females were chosen each from a different sire family. In addition, two hens each of genotype Ov.A,II.B and Ov.B,II.A were used as controls and mated to the same sires (to confirm that the sires used were of the assumed genotype).

The hens were mated in a pen with ~~two~~ of the males and eggs collected for incubation a week following introduction of males to the pen.

b) Loci II, III and T_F : Ten females heterozygous at these three loci and one homozygous hen IIB, IIIB, T_F -b were chosen from the Light Sussex flock for the experiment. The latter hen was chosen to confirm the genotype of the males used. Mating was by artificial insemination, the semen used being pooled from four L line males. Insemination was carried out twice weekly and eggs collected were incubated weekly for seven weeks. Chicks were wingbanded and pedigreed to their dams. Eggs were collected from these progeny and at least one egg white sample from each pullet was examined by starch gel electrophoresis.

The results for Ov. locus and locus II are shown in Table 10 and for the other three loci taken in pairs in Tables 11a, 11b, 11c.

TABLE 10. Details of segregation in each dam family in matings between Ov.A,II.A♂ x Ov.AB,II.AB♀. Only the gamete inherited from the dam is indicated.

Dam	Ov ^A II ^B or Ov ^B II ^A	Ov ^A II ^A or Ov ^B II ^B
1	10	1
2	5	0
3	7	0
4	1	0
5	11	0
6	4	0
7	4	0
8	3	0
9	8	0
10	4	0
11	4	0
12	3	0
13	8	0
14	4	0
15	3	0
16	9	0
17	13	0
18	9	0
19	6	0
20	<u>4</u>	<u>0</u>
Total	120	1



TABLE 11. Numbers segregating in each family in matings between IIA,IIIA, $T_f a\delta$ x II.AB,III.AB, $T_f ab\phi$ for each pair of loci taken in turn. Only the gamete from the dam is indicated.

a. Loci II and III

Dam	II ^A III ^B or	II ^B III ^B or
	II ^B III ^A	II ^A III ^A
H 38	9	6
J 31	0	9
K 39	1	0
E 10	9	8
D 7	4	9
J 20	6	8
E 29	4	6
L 29	5	4

b. Loci II and T_f

Dam	II ^A T_f^b or	II ^B T_f^b or
	II ^B T_f^a	II ^A T_f^a
H 38	10	5
J 31	4	5
K 39	0	1
E 10	9	8
D 7	6	7
J 20	8	6
E 29	5	5
L 29	2	7

c. Loci III and T_f

Dam	III^B	T_f^b	or	III^A	T_f^b	or
	III^A	T_f^a		III^B	T_f^a	
H 38		8			7	
J 31		5			4	
K 39		0			1	
E 10		13			4	
D 7		9			4	
J 20		6			8	
E 29		3			7	
L 29		4			6	

The analytical procedures used for calculation of recombination values are shown in Appendix Ib.

Ov. locus and locus II. From the earlier population data, it was argued that the results could only be explained by assuming that the chromosomal type, $Ov^B II^B$ was absent. If this is true, then none of the double heterozygotes used in the experiment should be of the coupling type, $Ov^{A A/B} II^{B B}$. This hypothesis could easily be verified from the segregation in the offspring, since all progeny inheriting the non cross-over chromosome from the repulsion dam will be either $Ov^{A B} II^{A A}$ or $Ov^{B A} II^{A A}$. This expectation is entirely confirmed from the results (Table 10). Further, since the sample of pullets used was such that twenty sire families were represented (to exclude the possibility of the inheritance of the same chromosomal type by more than one experimental dam) the earlier hypothesis for the whole population must be valid.

The/

The recombination value between these two loci calculated by the method in Appendix Ib is 0.0083 ± 0.0082 . Skewness in distribution makes the standard error practically useless, but the true value must be very close to zero.

Loci II, III and T_f : From the distribution of the segregation data given in Tables 11a, 11b, 11c, it is clear that these loci are not closely linked. Linkage distances were estimated by the method given in Appendix Ib. Details of calculation are also shown in the Appendix. The values obtained were as follows.

	<u>Recombination value</u>	<u>S.e</u>
Loci II and III	0.367	0.065
Loci III and T_f	0.426	0.091
Loci II and T_f	0.500	-

Maximum likelihood estimates of the linkage values were also obtained with the aid of a computer. I am grateful to Professor D.J. Finney for the programme used in this analysis. The following results were obtained

Loci II and III	0.414 ± 0.075
Loci III and T_f	0.410 ± 0.072
Loci II and T_f	0.500 ± 0.091

The results show that for loci II and T_f , there is not the slightest sign of linkage and the results agree almost perfectly with random segregation. The other two pairs of loci also do not show any significant evidence of linkage, but they would also agree with a belief of a rather loose linkage in either or both pairs.

Discussion.

The linkage studies show that Ov. and locus II are closely/

closely linked and that the other two loci (loci III and T_f) are on separate chromosomes. The suspicion of linkage between II and T_f loci from the non-random segregation in the population data is not confirmed in the linkage studies. These four loci could therefore serve as markers for three autosomal chromosomes. Recent cytological studies (Owen, 1965) suggest that the fowl has 38 pairs of chromosomes, but it is doubtful whether the small 'micro-chromosomes' are genetically significant (Ford and Woollam, 1964). Even if only the five large autosomes and the sex chromosome are genetically important, it is still surprising to find close linkage between two of the loci.

The linkage between Ov and locus II is interesting as it adds to the growing number of cases in which linkage has been found between related characters or chemically related proteins. Sheppard (1963)⁵¹, in a review, states that "most of the polymorphic species which have been investigated show linkage between many of the genes, ranging in degree from so little crossing over that they behave as multiple alleles, up to crossing over values of the order of 20 percent". For example, in Cepaea nemoralis, the genes for pink shell and unbanded are linked (Fisher and Diver, 1934). Among the cryptic polymorphisms, the A and E blood group loci in the fowl are linked with a recombination frequency of one percent (Briles, 1958). The Rh blood group loci in man may also be due to three closely linked genes (Race and Sanger, 1958). Among the chemically related proteins, the best known example concerns the loci controlling haemoglobin chains in man: there is strong evidence suggesting that the loci controlling the β , γ and δ haemoglobin chains are closely linked (Neel/

(Neel, 1961). Recently, more reports have appeared on linkages between genes controlling related proteins. These are: the mouse γ -globulin loci Ig-1 and Ig-2 (Herzenberg, 1964), the mouse amylase loci Amy-1 and Amy-2 (Sick and Nielsen, 1964) and the cattle casein loci α_s -Cn and β -Cn (King, Aschaffenburg, Kiddy and Thompson, 1965).

How can one explain the close proximity of loci controlling Ovalbumin and locus II proteins. Explanations may be attempted on two hypothesis - (1) linkage due to common origin (duplication of loci) and (2) linkage caused by selection.

The first hypothesis is really an extension to egg white proteins of Ingram's postulate on the evolution of haemoglobin genes - in the human. According to Ingram (1961) evolution occurred by duplication of loci, so that in addition to the original locus, the population has a spare locus which can be changed by mutation. He therefore suggested that the α gene originated from some ancestor and that the γ , β and δ all originated from each other in that order by a process of duplication of loci, followed by mutation. This would explain the similarity in structure of the haemoglobin molecules and also their proximity on a chromosome. Speculating on the same possibility for ovalbumin and locus II proteins, one may consider the genetic loci controlling these proteins to have originated from a single gene and to have diverged by a process of duplication of loci and accumulation of mutational changes.

Selection could also act to suppress crossing over between loci or to bring them closer. If there is strong epistatic interaction between loci, selection would be expected to bring the two loci together in such a way as to reduce the production of 'undesirable' or 'less fit' genotypes. Examples of such linkage due to natural selection/

selection are to be seen in wild populations of *Paratettix Texanus* (Fisher, 1939). The presence of two dominant colour genes in an individual in this species is disadvantageous and Fisher postulated that natural selection reduced crossing over between them by selecting genes suppressing crossing over. Similarly, if there is interaction between Ov.[&]locus II genes on characters related to fitness, one would expect natural selection to bring them together.

Summary: The linkage relationship between the four loci controlling egg white proteins, namely Ov., locus II, locus III and T_f were examined. Ov and locus II were found to be very closely linked (less than 1 percent recombination value). III and T_f were not linked to each other or to locus II. It is concluded that the four loci reside on three autosomal chromosomes.

PART IV

Studies on association of polymorphisms with production traits.

The production characters of poultry are greatly influenced by non-genetic and environmental factors such as feeding, management and age. This is reflected in the heritabilities of the characters. Investigations in different poultry populations have revealed heritabilities of 0.3 to 0.5 for body weight and egg weight, but much lower values for egg production; 0.05 to 0.2 (Dickerson, 1955). Further, production traits are polygenically controlled and therefore high correlations between the egg white polymorphic genes and production characters are hardly to be expected. Since the effects (if any) are likely to be small, it is important (in order to aid their detection) to eliminate all non-genetic causes of variation in the experimental animals. However, since the flocks studied were not kept specifically for this purpose, this could not be arranged.

A Ovalbumin locus and locus II polymorphisms and production traits.

Studies on strain 2 population.

The Ovalbumin locus and locus II are polymorphic in one of the populations studied (strain 2). Because these two loci are closely linked and one chromosomal type is absent (see Part III) it would be incorrect to analyse the effect due to each locus separately, since the number of individuals in a class at a particular locus would not be independent of the others. Hence it is necessary to classify the birds by both loci simultaneously and consider their effects as if a three-allelic locus is being analysed.

As stated in the section on materials and methods, this strain has four selection lines and a control. Table 12 shows the number of birds in each class, classified by egg white type and selection line.

Analytical methods:

Essentially, the statistical method employed was an analysis of variance to test whether the means of the different genotypes for a particular character were different. Since the number of birds in the Ov.A,II.B class was very small, ranging between 2 and 10 in the different lines, these were excluded from the analysis, leaving five egg white classes.

Each selection line was composed of birds from two hatches and the random control from four hatches. Since line means and hatch means within a line were different, individual records of birds within each line were converted to deviations from their hatch means. This is a valid and useful procedure for eliminating an extraneous source of environmental variation provided that hatch means are accurately estimated and there is no egg white genotype $\times$ hatch interaction.

Two-way analysis of variance was carried out, the egg white genotype being one way of classification and selection line the other. The model used was an additive one

$$y_{ijk} = \mu + g_i + l_j + (lg)_{ij} + e_{ijk}$$

where $i = 1, 2 \dots .5$ number of genotypes

$j = 1, 2 \dots .5$ number of lines

$k = 1, 2 \dots$ number of birds

The/

TABLE 12 Number of birds in egg white classes in different lines

Line	Ov.A,II.A	Ov.AB,II.A	Ov.B,II.A	Ov.A,II.AB	Ov.AB,II.AB	Ov.A,II.B	Total
Max.egg weight	44	70	37	27	34	2	214
Minu body weight	22	76	32	34	36	10	210
Ratio	33	68	56	31	21	3	212
Regression	41	58	35	9	17	3	163
Control	14	55	32	9	23	3	136
Total	154	327	192	110	131	21	935

The genotype effects and line effects were considered fixed so that for significance tests the between genotype mean square could be compared with the error term. The interaction mean square would give an indication of whether the ranking of the genotypes was the same in all lines.

From the distribution of the birds in line-genotype subclasses (Table 12) there appears to be disproportionality in numbers compared to the marginal totals (χ^2 for 16. D.f = 46.5, $P < 0.05$) and it may be considered necessary to do a least squares analysis by fitting constants. This exact solution (Snedecor, 1962) was done for one trait (28 week body weight) and compared with the results obtained on the assumption that cell numbers were exactly proportional to marginal totals. The results obtained by the latter method agreed closely with the exact results (Table 13). All analyses were therefore performed by the latter method.

The analysis may be open to the criticism that differences between sires would act as a confounding element in the data and give spurious significant differences. But since 15 sires were used in each line and the number of progeny per sire was only 16, it could be assumed that the sires were proportionately distributed among the different egg white classes. However, to obviate this criticism, whenever significant differences between genotypes were detected, an analysis was done within sires, comparing the best genotype with the rest, within each sire progeny group using the method of Neimann Sørensen and Robertson (1961). If d is the difference in production trait means of two groups of individuals in a comparison within a sire, the best estimate of this difference is obtained by weighting d with a factor/

TABLE 13. Analysis of variance of 28 week body weight

a) assuming proportional cell numbers

	D.F.	Mean square	Variance Ratio
Between Genotypes	4	1318.4	5.510***
Between lines	4	16.72	
G x L Interaction	16	406.8	1.700
Error	889	239.3	

b) by fitting constants

	D.F.	Mean square	Variance Ratio
Between Genotypes	4	1315	5.516***
Between line	4	13	
G x L Interaction	16	407.6	1.703
Error	889	239.3	

*** Significant at $P < 0.001$

w , where $w = n_1 \cdot n_2 / (n_1 + n_2)$, n_1 and n_2 being the numbers of individuals in the two groups being compared. The estimate of this difference summed over all progeny groups, $\bar{D}$, is given by

$$\bar{D} = \frac{\sum wd}{\sum w}$$

and this has standard error

$$S.E. \bar{D} = \frac{\sigma^2 \sum w}{\sum w}$$

$$\sqrt{\frac{\sum w^2}{\sum w}}$$

If D is the estimate of the difference in a line between the two groups, we may calculate the variation between the differences D in the separate lines from the expression

$$SS_D = \sum WD^2 - \frac{(\sum WD)^2}{\sum W}$$

(W = total weight/line and $\sum W$ = Weight summed over all lines)

which is a sum of squares with L-1 degree of freedom (where L is the number of lines) and the corresponding variance V_D may be compared with the estimates of σ^2_W by the usual variance ratio test, the F test. This tests the homogeneity of the differences in the separate lines.

Estimation of variance components.

In the model for the analysis we have considered the genotypic effects to be fixed. The expectations for mean squares would be as follows.

$$\begin{aligned} \text{Between genotypes} &= \sigma^2_e + k_1 \cdot \sigma^2_g \\ \text{G x L Interaction} &= \sigma^2_e + k_4 \cdot \sigma^2_{gl} \\ \text{Error} &= \sigma^2_e \end{aligned}$$

An approximate estimate of the components of variance in the unbalanced case would be obtained by performing a weighted squares of means analysis for which Harvey, (1960) gives a method for obtaining the weighting coefficients. Using this method, the results of expectations of the means squares are as follows.

$$\begin{aligned} \text{Between Genotypes} &= \sigma^2_e + 150.1 \sigma^2_g \\ \text{G x L Interaction} &= \sigma^2_e + k_4 \sigma^2_{gl} \\ \text{Error} &= \sigma^2_e \end{aligned}$$

Using the coefficients given above the variance component between genotypes could be obtained for each trait.

In order to put an upper limit to the percentage of the total variance due to genotypes, it is necessary to calculate the sampling variance of this estimate. No reliable methods are available, but an approximate value is obtained by modifying the formula of Niemann/

Niemann Sørensen & Robertson (1961) to the analytical procedure used here. The formula is

$$V_{kf} = \frac{2f + 4NWkf}{N^2W}$$

where k_f is the excess χ^2/NW , i.e. χ^2_f for f degrees of freedom minus the degrees of freedom, f divided by NW the degrees of freedom within sires. In the present case, an estimate of the $\chi^2_{(4)}$ due to genotypes is obtained by dividing the sum of squares between genotypes by the mean square for error. Nw would be the degrees of freedom for error. Therefore the formula used would be

$$V(V_p) = 2 \times 4 + 4 \times \left(\frac{\text{S.S. between} - 4}{\text{M.S. error}} \right) / 889^2$$

where $V(V_p)$ would be the sampling variance of the proportion of the total variance due to the loci being considered.

Results

Body weight:

Body weight measurements were recorded at 12, 20, 28, 36, 44 weeks and at sexual maturity. The lines differ considerably in the mean body weights (Table 14). The adult weight at 44 weeks for example ranges from 1970g to 2130g in the two extreme lines

TABLE 14. Mean body weight (in decagrams) at various ages in different lines.

Line	12 wks	20 wks	1st egg	28 wks	36 wks	44 wks
Max egg wt	101.2	161.3	181.9	195.1	204.8	210.0
Min B. wt	99.6	150.3	173.5	184.6	194.6	197.0
Ratio	93.0	155.7	168.0	183.8	195.0	197.1
Regression	97.4	162.7	183.2	198.7	211.7	213.7
Control	98.4	159.2	174.1	187.3	197.4	199.7

The deviations of each egg white-line subclass from the line trait mean are shown in Table 15. This presentation in the form of deviations enables easy comparisons between classes, as positive signs would indicate that the birds are heavier than the mean and vice versa. The genotype mean for the trait given in the last row is a weighted line mean.

The only regular pattern of differences between the genotypes in the different lines seems to be between the Ov.A II.A and Ov.B₂ II.A classes. Ov.A birds are heavier than Ov.B at all ages (Fig.6), the mean difference being about 20g at 12 weeks and 85g at 44 weeks. The Ov.AB, II.A class is often intermediate between the two homozygotes though it is not consistent. In the overall mean (averaged over all lines) the mean of Ov.AB, II.A however is close to the population mean. Ov.A, II.AB and Ov.AB, II.AB classes seem to be both above and below the mean at random in the different lines, but in comparison with Ov.A, II.A, these two classes are generally inferior in weight.

An analysis of variance of body weights of the different classes confirms that there are marked differences between them (Table 16). Interaction with lines is not generally large and is significant for only one measurement -28 week body weight. But since genotype effects are considered fixed, the interaction term will not enter into the mean square between genotypes and the latter can be tested against error. Wherever significant effects were observed, Duncan's multiple range test (Snedecor, 1962) was done to identify the means that were different at 5% significance level. In all cases Ov.A, II.A was significantly heavier than Ov.B, II.A whereas/

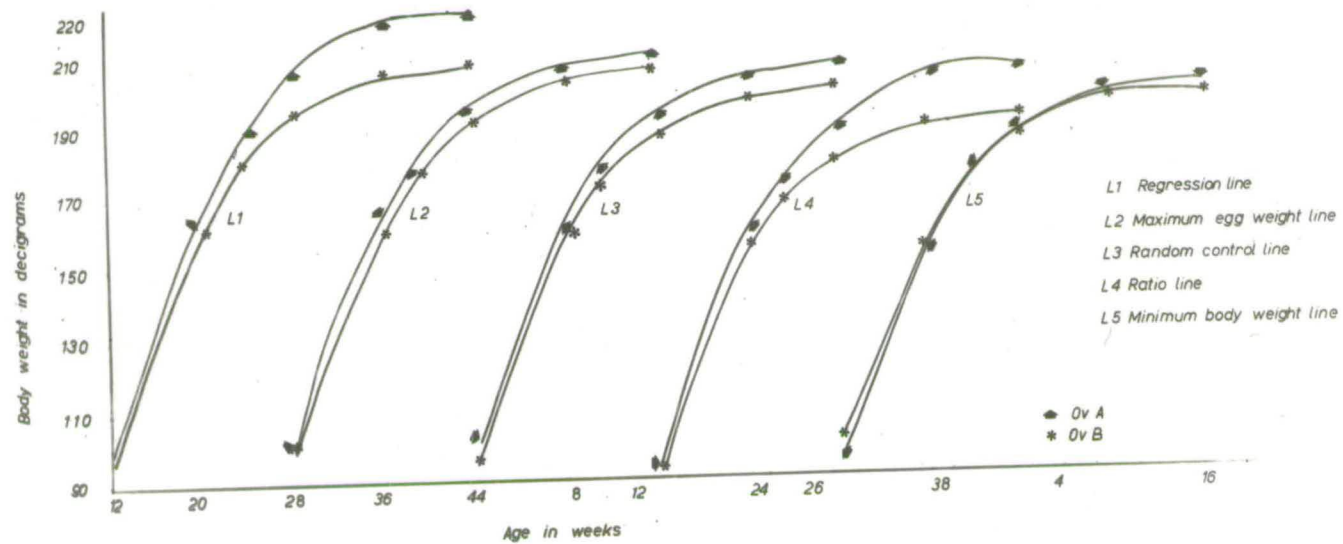


Fig 6. The mean growth of Ov.A and Ov.B birds in the five lines.

Note that the growth of each successive line has been displaced by sixteen weeks.

TABLE 15 Mean deviations of egg white classes from line means

a) 12 weeks body weight

Line	Ov.A,II.A	Ov.AB,II.A	Ov.B,II.A	Ov.A,II.AB	Ov.AB,II.AB
Max egg wt	0.978	-2.065	-0.692	0.250	3.488
Min B. wt	0.222	0.394	+2.198	-1.207	-1.325
Ratio	0.985	0.123	-0.847	0.538	-0.472
Regression	2.625	0.279	-0.953	-3.540	-1.888
Control	2.375	0.693	-1.408	2.314	-1.302
Mean	1.437	-0.159	-0.422	-0.204	-0.008

b) 20 week body weight

Max egg wt	2.769	-2.398	-2.533	0.246	2.263
Min B wt	0.352	1.268	+3.912	-1.284	-4.802
Ratio	3.686	-0.760	-1.134	+1.278	-2.222
Regression	2.498	-0.069	-0.934	-2.773	-0.601
Control	4.656	-0.780	-0.483	4.781	-1.232
Mean	2.720	-0.520	-0.418	0.163	-1.383

c) Body weight at 1st egg

Max egg wt	-1.237	-1.546	-2.335	-2.198	7.305
Min B. wt	2.750	0.642	0.439	-1.149	-2.485
Ratio	3.700	2.145	-4.368	-0.432	-0.030
Regression	7.867	-0.731	-3.817	-8.477	-0.633
Control	3.087	-0.877	-2.211	3.546	0.344
Mean	+3.207	-0.012	-2.715	-1.419	1.186

d) 28 week body wt

Max egg wt	1.09	-1.65	-4.66	0.15	4.94
Min B. wt	1.46	2.15	0.91	-1.73	-3.19
Ratio	5.30	1.50	-5.58	2.21	-0.31
Regression	7.75	0.02	-4.78	-10.04	+0.33
Control	7.06	-2.38	-1.18	4.78	1.16
Mean	4.364	-0.062	-3.442	-0.375	0.603

whereas for 28 week and 36 week weights the mean weight of the former was superior to all the other classes.

TABLE 16. Analysis of variance showing mean squares of body weight measurements.

Source of variation	D.F.	12 wks	20 wks	1st egg	28 wks	36 wks	44 wks
Between genotypes	4	90.4	378.7	851.2**	1318.9***	1916.0***	1643.7**
Between lines	4	1.15	6.61	12.1	16.7	13.5	43.9
G x L Interaction	16	94.6	174.9	338.6	406.9*	410.5	588.9
Error	889	74.0	180.8	217.2	239.3	368.7	433.8

* Significant at $P < 0.05$

** " " $P < 0.01$

*** " " $P < 0.001$

Because of the consistent trend of the difference in weight between Ov.A,II.A and Ov.B,II:A classes, the 12 and 20 week weights were also analysed taking only these two genotypic classes into consideration. On this analysis, the differences were significant (Table 17). It may therefore be concluded that the ovalbumin locus has some influence on body weight from 12 weeks of age (or even earlier).

TABLE 17. Analysis of Variance of Ov.A and Ov.B phenotypes for 12 and 20 week body weight. (Values shown are mean squares.)

	D.F.	12 wks	20 wks
Between Genotypes	1	352***	1317.5***
Error	346	68.70	185.7

*** Significant at $P < 0.001$

The influence of this locus on weight with age may perhaps be better appreciated if the difference in weight between the two ovalbumin homozygotes is expressed as a fraction of the mean body weight at that age. (Table 18). It is seen that the proportion of the difference in weight almost doubles itself from 2 percent to 4 percent from 20 to 28 weeks of age suggesting some association with maturity (see Fig. 6).

TABLE 18. Difference in body weight between Ov.A and Ov.B classes at different ages.

Age	Mean Body wt	Difference	Difference expressed as a percentage
12	979.4	18.6 grs	1.89%
20	1575.2	31.4 grs	1.99%
28	1896.8	78.1 grs	4.11%
36	2004.1	91.2 grs	4.55%
44	2033.1	85.3 grs	4.19%

The results of Table 18 indicate that the weight gain from 20 weeks to later ages is different in the different ovalbumin classes. An analysis of variance was therefore performed on the weight gains between different ages to see if this confirmed the observation. The analysis was carried out in the following manner. If x and y are body weights at two ages, then the variance of body weight gain ($y - x$)

$$V_{y-x} = V_x + V_y - 2Cov_{xy}$$

The mean square, V_{y-x} is easily obtained since variances of x and y are known and the covariance can be obtained by the usual methods.

Results of the analysis of body weight gains are shown in Tables/

Tables 19 a, b and c.

TABLE 19. Analysis of variance of body weight gains.

a) 12 weeks to adult weights

	M.S. between Genotypes	Error M.S.	V.R.	P
12 to 20 wks	138.7	96.8	1.432	< 0.05
12 wks to 1st egg	460.8	190.1	3.792	< 0.01
12 to 28 wks	783.8	206.6	4.059	< 0.01
12 to 36 wks	1194.7	294.3	2.810	< 0.05
12 to 44 wks	1011.7	360.0	2.423	< 0.05

b) 20 weeks to adult weights

	M.S. between Genotypes	Error M.S.	V.R.	P
20 wks to 1st egg	568.5	247.5	2.30	< 0.10
20 to 28 wks	708.2	216.1	3.28	< 0.05
20 to 36 wks	799.2	296.6	2.69	< 0.05
20 to 44 wks	903.1	353.0	2.56	< 0.05

c) First egg to adult weights

	M.S. between Genotypes	Error M.S.	V.R.	P
1st egg to 28 wks	118.3	163.5	1	
1st egg to 36 weeks	348.9	221.5	1.57	> 0.10
1st egg to 44 weeks	176.4	254.0	1	

It is clear that body weight gains from 12 and 20 weeks to later ages were different for the different egg white types. The genotype/

genotype mean square for body weight gains from 12 and 20 weeks to later ages were significant in all cases (Tables 19 a and b). However, when one considers the weight gain after sexual maturity, the differences between genotypes were small and none of the effects were significant.

Before drawing any conclusions from this analysis, one has also to consider the fact that growth rate in multicellular organisms is dependent on initial weight and the weight gain will be proportional to the weight at a particular age. A rational approach would therefore be one when the observed weights are adjusted to make them the best estimates of what they would be if all individuals had the same initial weight and then compare these adjusted weights between egg white classes. This is done by an analysis of covariance in which the mean square for error and between genotypes is reduced by a quantity depending on the regression of body weight at one age on the other. The phenotypic correlation coefficients (r_p) between body weights at different ages (Table 20) indicate the closeness of association between the difference traits.

TABLE 20. Phenotypic correlation coefficients between body weights at different ages.

	20 wks	1st egg	28 wks	36 wks	44 wks
12 wks	0.684	0.391	0.411	0.450	0.412
20 wks		0.380	0.489	0.487	0.465
1st egg			0.643	0.696	0.647
28 wks				0.796	0.715
36 wks					0.847

The analysis was performed only for 28 week body weight (Tables 21, a, b and c). The results show that even after removal of/

TABLE 21 Analysis of covariance of 28 week
body weight and

A) 12 week weight

	D.F.	Mean square	V.R.	P
Between Genotypes	4	929.2	4.54	< 0.01
G x L Interaction	16	267.5	1.30	
Error	888	204.5		

b) 20 week weight

	D.F.	Mean square	V.R.	P
Between Genotypes	4	989.5	5.24	< 0.001
G x L Interaction	16	228.0	1.20	
Error	888	188.5		

c) Weight at 1st egg

	D.F.	Mean square	V.R.	P
Between Genotypes	4	340.2	2.2	> 0.05
G x L Interaction	16	152.2	< 1.0	
Error	888	153.7		

Of the covariance for 12 and 20 week weights separately on 28 week weight, the differences in mean body weights of the egg white classes are significant. But removal of the covariance

for 28 week weight on weight at first egg is sufficient to decrease the mean square between genotypes below the 5% level of significance.

It therefore appears that the ovalbumin locus influences body weight difference at two stages (1) early weight, i.e. weight at 12 weeks or earlier and (2) weight gain from 12 weeks to sexual maturity (age at first egg). Early weight could be a function of chick weight at hatching and growth rate, and weight gain from 12 weeks to sexual maturity would probably depend again on the growth rate as well as the age at sexual maturity. It would be possible to determine differences in growth rate by the use of equations which describe the growth of multicellular organisms. One such equation which has been useful in describing the growth of the fowl is the logistic equation (Sang, 1962). The formula is

$$W = \frac{A}{1 + ce^{-kt}}$$

where W is the weight at time t (in logarithm); A, the upper limit of growth; e the exponential constant; c the constant determined by size at zero time and k the growth rate constant. This formula therefore depends on the intrinsic growth rate, initial weight and adult weight.

Rewriting this in another form

$$\frac{A-W}{W} = ce^{-kt}$$

$$\text{or } \log \frac{A-W}{W} = \log C - k \cdot \log e \cdot t$$

in

This is the form of a general regression equation where log c determines the intercept, k log e the slope and $\log \frac{A-W}{W}$ and L are the variables. The data for Ov.A and Ov.B genotypes were transformed into the above variables and a regression analysis done for the mean values of these two genotypes in each line. From the results (Table 22), no regular pattern/

pattern of differences in growth rate ($k \log e$) are detectable.

TABLE 22. Growth Constants for Ov.B genotypes in different lines

	$k \log e$		$\log c$	
	Ov. A	Ov.B	Ov.A	Ov.B
Max egg wt	3.545	3.316	3.956	3.649
Min B wt	3.231	3.572	3.346	3.961
Ratio	3.017	3.206	3.506	3.523
Regression	3.819	3.772	3.351	3.257
Control	3.487	3.691	4.127	3.872

In some lines Ov.A types seem to have the faster growth rate while in others Ov.B are superior. But the constant $\log c$ which determines the hatching weight-adult weight relationship shows a consistent higher value for Ov.A than Ov.B (with the exception of the minimum body weight ^{ratio} lines). Therefore this analysis seems to support partially the earlier conclusion that differences in body weight may be due to differences in chick weights. The constant k which determines growth rate neglects the onset of sexual maturity and this equation therefore will not detect any differences arising out of differences in age at maturity. The average difference in age at maturity between the two classes is about 2 days with the Ov.A maturing later. It is doubtful whether this small difference would explain the difference in mature body weights. However, differences in egg production may cause differences in weight by the poorer layers depositing more fat. The evidence (Table 23) on egg production to 28 weeks indicates that Ov.B birds do in fact lay about 3.2 eggs more/

more (3-day trapnest record converted to 7 days) than Ov.A up to this age. Hence causes for the differences in weight gains between 20 and 28 weeks may well be in the age at maturity and rate of egg production.

TABLE 23 Mean deviations of egg white genotypes for egg number to 28 weeks (from line mean).

	Ov.A,II.A	Ov.AB,IIA	Ov.B,II.A	Ov.A,IIAB	Ov.AB,II.AB
Max egg wt	0.642	-0.430	0.175	0.922	-0.832
Min body wt	-0.931	-0.327	0.967	0.443	0.091
Ratio	-0.679	-0.612	1.919	-1.218	-0.406
Regression	-2.017	1.291	0.235	1.528	-0.825
Control	1.544	0.111	0.531	-2.937	-0.036
Mean	-0.491	-0.047	0.785	-0.095	-0.133

Analysis of body weight data within sires:

It was stated earlier (under analytical procedures) that sire differences might give spurious associations of these polymorphic marker genes with certain traits if the different genotypes are not proportionately distributed within progeny groups. A within-sire analysis would remove such spurious associations if they exist. Ideally, a comparison of Ov.A with Ov.B birds within sires would be expected to show highly significant differences, but when this was attempted it was found that matings of AB x AB class (which would produce the two homozygotes) were too few to be meaningful. The total weight for weighting the differences ($\sum W$) (see analytical methods)

when these two classes were compared was only 25.4. This would raise the sampling variance of the mean difference and therefore mask significant effects. It was therefore decided to compare Ov.A,II.A on one hand with all other types. Even on this basis, the number of birds available for analysis was considerably less than that used earlier, since both classes were not represented in some progeny groups. Further, in the control line, progeny were not pedigreed to sires and therefore dam progeny groups were used in the analysis.

TABLE 24. Within sire comparison of Ov.A,IIA with all other classes for 36 week body weight.

Line	No of progeny groups	No of animals	Weight w	Average difference D
Max egg wt	10	146	26.58	+ 75.3
Min body wt	9	139	17.05	+ 18.4
Ratio	8	119	19.25	+ 57.8
Regression	10	108	16.57	+ 75.9
Control	8	37	7.37	+ 67.6
Total	45	512	86.82	+ 59.7

Mean difference = 59.71 ± 20.6

Test of homogeneity of average differences in lines

	D.F.	M.S.	V.R.
Between lines	4	102.2	1
Error	466	295.7	

Only one trait, 36 week weight was analysed in this way owing to the considerable amount of labour involved in calculating deviations of each bird's weight from its hatch mean. The results would however be/

be applicable to body weights at other ages owing to the close similarity in the data between weights at different ages. The results of this analysis (Table 24) confirms the earlier finding that Ov.A birds are heavier than the other four types and on a within sire analysis they are about 60grs heavier than the other four classes taken together which is fairly close to the figure of 70.0grs observed for the whole population. Also, the analysis testing homogeneity of the observed differences in the lines indicates that the effects are not significantly different in different lines.

Egg weight:

The egg weight means at various ages in the different lines are shown in Table 25 and the deviations of the egg white genotypes from their hatch means in Table 26a, b and c. Among the egg white genotypes Ov.A,II.A birds laid heavier eggs than the other groups but the worst ranking group was Ov.A,II.AB with Ov.B,IIA the second lowest class. It therefore appears that locus II alleles when present in the heterozygous condition with Ov.A homozygotes have a depressing effect on egg weight. When the three genotypes of Ov. locus alone are compared within the II.A class, the effect on egg weight appears to be additive or a partial dominance of Ov^A allele. But, when the effects of both Ovalbumin and locus II are considered simultaneously the picture is not quite clear and there seems to be considerable interaction between the two loci.

TABLE 25. Mean egg weight at various ages in the different lines.

Line	1st egg	28 wks	36 wks	44 wks
Max egg wt	44.50	50.42	56.62	59.60
Min body wt	42.39	47.61	53.22	56.05
Ratio	42.07	48.94	54.14	56.90
Regression	45.74	52.03	57.32	60.10
Control	41.67	47.77	53.35	56.24

TABLE 26. Mean deviation of egg white classes from line means.

a) 28 weeks.

	Ov.A,IIA	Ov.AB,IIA	Ov.B,II.A	Ov.A,IIAB	Ov.AB,II.AB
Mean egg wt	-0.040	0.041	0.746	-1.430	-0.307
Min body wt	0.086	0.176	-0.832	0.047	0.501
Ratio	0.471	0.034	-0.683	0.393	0.622
Regression	0.579	0.449	-0.421	-1.831	-0.869
Control	1.069	0.121	-10.937	0.504	-1.813
Mean	+0.353	0.158	-0.114	-0.338	-0.273

b) 36 weeks.

	Ov.A,II.A	Ov.AB,II.A	Ov.B,IIA	Ov.A,II.AB	Ov.AB,II.AB
Max egg wt	0.473	-0.206	0.484	-0.804	-0.512
Min body wt	0.405	0.416	-0.864	-0.208	0.167
Ratio	0.408	0.514	-0.496	-0.497	1.047
Regression	1.111	0.193	-0.550	-1.901	-1.328
Control	1.071	-0.196	0.499	-0.587	-0.984
Mean	0.674	0.086	-0.212	-0.605	-0.264

c) 44 weeks.

	Ov.A,II.A	Ov.AB,II.A	Ov.B,II.A	OvA,II.AB.	Ov.AB,IIAB
Max egg wt	0.560	-0.462	0.866	-0.969	-0.386
Min body wt	0.039	0.303	-0.924	0.155	0.251
Ratio	1.098	0.186	-0.776	-0.450	0.578
Regression	1.418	0.036	-0.819	-3.083	-0.312
Control	0.760	0.303	0.502	-0.338	-1.045
Mean	0.848	0.068	-0.279	-0.597	-0.163

Analyses of variance (Table 27) of these data show that only the differences in means of 36 and 44 week egg weight are significant, but it should be noted that even at earlier ages the trend is in the same direction. In fact, removal of the covariance of 36 and 44 week egg weights with 28 week weight is sufficient to reduce the mean square between genotypes for 36 and 44 week weights below the 5% level of significance (Table 28). This is to be expected if the genes affecting early egg weight are the same as those affecting egg weight at a later stage (Clayton, unpublished data)

TABLE 27. Analysis of variance (showing mean squares) of egg weight measurements.

Source of variation	D.F.	1st egg	28 wks	36 wks	44 wks
Between Genotypes	4	15.5	13.06	32.65*	42.47*
Between lines	4	0.3	0.98	0.54	0.84
B x L Interaction	16	18.15	18.91	11.69	17.5
Error	889	14.84	9.46	9.83	11.06

* $P < 0.05$

TABLE 28 Analysis of covariance - 36 and 44 week egg weights with 28 week egg weight

a) 36 week egg weight.

	D.F.	M.S.	V.R.
Between Genotypes	4	6.805	1
Error	888	15.960	

b) 44 week egg weight.

	D.F.	M.S.	V.R.
Between Genotypes	4	13.605	1
Error	888	17.140	

It was noted earlier that there were significant differences in body weight means of the egg white classes at 28, 36 and 44 weeks. Body weight is positively though not strongly correlated with egg weight (Table 29). It would therefore be expected that egg weight differences between polymorphic types would partly reflect differences in body weight. Therefore in order to investigate any independent effect on egg weight, one should remove the covariance of egg weight at a particular age on body weight at that age. Analysis of variance, after removal of this covariance decreases the difference in egg weight at 36 weeks below the level of significance but differences in 44 week egg weights are still significant though at a lower level of significance (Table 30). Therefore, it may be concluded that egg white genes influence adult egg weight and that this influence is at least in part due to their effect on body weight.

TABLE 29. Phenotypic correlation (r_P) between body weight at 28 weeks and egg weight at different ages.

	<u>Phenotypic correlation</u>
Weight 1st egg	0.1309
Egg weight 28 wks	0.1766
Egg weight 36 wks	0.1714
Egg weight 44 wks	0.1593

TABLE 30. Analysis of covariance - 36 and 44 week egg weights with body weight at those ages.

a) 36 week egg weight.

	<u>D.F.</u>	<u>M.S.</u>	<u>V.R.</u>	<u>P</u>
Between Genotypes	4	18.59	2.01	>0.05
Error	888	9.24		

b) 44 week egg weight

	<u>D.F.</u>	<u>M.S.</u>	<u>V.R.</u>	<u>P</u>
Between Genotypes	4	28.72	2.72	<0.05
Error	888	10.53		

The data on 36 week egg weights were also analysed on a within sire basis and the results (Table 31) confirm that real differences exist between classes. The difference in egg weight at this age between Ov.A,II.A birds and the other four classes is 0.86 ± 0.34 grs which is close to the difference of 0.82 grs in overall means. As before there is no evidence of significant heterogeneity between lines.

TABLE 31. Within sire comparison of Ov,A,II,A with all other classes for 36 week egg weight.

Line	No of progeny groups	No of animals	Weight w.	Average difference $\bar{D}$
Max egg wt	10	144	26.597	+ .3615
Min body wt	8	124	15.892	+ .9148
Ratio	8	119	19.863	- .1115
Regression	10	103	15.514	+2.0861
Control	8	37	8.0	+2.4582
Total	44	527	85.868	+0.8614

Mean difference = $0.8614 \pm .3383$

Test of homogeneity of differences in lines

	D.F.	M.S.	V.R.	P
Between lines	4	14.73	2.1	>0.05
Error	482	7.05		

Additional support for the findings that Ov.A,II.A birds are heavier and lay larger eggs comes from the observed distribution of the egg white genotypes in different lines. The selection programme being carried out with this flock can be considered to be a two-way selection experiment, with the maximum egg weight and regression lines being selected upwards and the minimum body weight and ratio lines downwards for egg weight and body weight. This is apparent from a comparison of body weight and egg weight line means (Tables 14 and 25). If the egg white loci have an influence on body weight and egg weight as stated above, it would be expected that artificial selection would select a higher proportion of Ov.A,II.A birds to be parents for maximum egg weight and regression lines and similarly Ov.B,II.A as parents for the other two lines. If this happened, one would expect the/

the progeny distribution to be disturbed in the same direction. The observed distribution of the egg white genotypes compared with the expectations (based on marginal totals) is shown in Table 32. In the maximum egg weight line, the results are in accordance with expectation, there being more of Ov.A,II.A and less of Ov.B,II.A. In the minimum body weight line, there is a deficiency of Ov.A, II.A according to expectation, but the number of Ov.B birds is also lower. The distributions in ratio and regression lines are disturbed in the expected direction, the Ov.B and Ov.A numbers being greater in the ratio and regression lines respectively.

The association between these two loci and body weight was also studied in another way. From each sire progeny group, 3 birds having the highest and lowest body weight were selected. From the standard deviation within progeny groups, we can calculate the expected difference between the high and low groups. For example, when 3 progeny are selected out of 16 (16 being the average progeny size per sire), the deviation of the selected group mean from the population mean (obtained from tables of deviations of ranked data, Fisher & Yates, 1943) is 1.343 standard deviations. Therefore, the expected difference between the high and low groups would be 2.686 times the within progeny group standard deviation. For body weight at 28 weeks, this value is 415 grs. It is clear then, that any direct effect of these loci, if it is appreciable at all, should appear as differences in egg white type frequencies between high and low groups. The results displayed in Table 33 confirm the previous findings. There are more progeny of Ov.A in the high body weight group than in the low group and vice versa for the Ov.B types. The differences however are not significant ($\chi^2_{(2)} = 4.8, P < 0.10$).

TABLE 32 Comparison of observed numbers in different lines with expectations on the basis of proportional marginal totals.

Line	Ov.A,II.A		Ov.AB,II.A		Ov.B,II.A		Ov.A,II.AB		Ov.AB,II.AB		Total
	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	
Max egg wt	44	35.7	70	75.8	37	44.5	27	25.5	34	30.4	212
Min body wt	22	33.7	76	71.6	32	42.0	34	24.1	36	28.7	200
Ratio	33	35.2	68	74.8	56	43.9	31	25.1	21	25.1	209
Regression	41	26.9	58	57.2	35	33.6	9	19.2	17	19.2	160
Control	14	22.4	55	47.6	32	27.9	9	16.0	23	16.0	133
Total	154		327		192		110		131		914

TABLE 33 The number of birds of egg white genotypes in sire progeny groups selected on the basis of the three highest or lowest body weight at 28 weeks.

Line	Ov.A,II.A		Ov.AB,II.A		Ov.B,II.A		Ov.A,II.AB		Ov.AB,II.AB	
	High wt	Low wt	High wt	Low wt	High wt	Low wt	High wt	Low wt	High wt	Low wt
Max egg wt	12	6	11	15	5	5	2	6	10	8
Min body wt	7	4	19	17	5	9	4	4	8	5
Ratio	7	9	16	7	7	13	5	6	3	3
Regression	11	6	10	10	3	5	1	2	3	5
Total	37	25	56	49	20	32	12	18	24	21

The variance controlled by the two egg white loci.

One important aspect of this work has been the possibility that these loci might be useful in selection procedures. Here we are concerned with the direct effect which these genes have on production. This effect is measured by the fraction of the variance in the trait due to the polymorphism.

In order to understand whether information on the polymorphism has any value in individual selection we have to view the situation from the theory of a correlated response. The correlated response in a Character Y when selection is applied for another character x is given by $CR_y = ih_x g \sigma_{GY}$ (Falconer, 1960) where i is the intensity of selection, h_x the square root of heritability of character x, and σ_{GY} the genetic variance in Y. Direct selection on Y would give a response

$$R_y = ih_y \sigma_{GY}$$

It is apparent therefore that the correlated response would be greater than direct response only if $h_x g$ is greater than h_y .

Now consider a polymorphism like the ones studied here. Since the genotype of an animal for the polymorphic loci could be determined accurately, the heritability of the character is 1. Suppose we make a correct prediction of the animal's breeding value from a knowledge of these loci as equal to X. Then, the correlation between X and its true breeding value G is

$r_{XG} = \sqrt{\frac{V_x}{V_G}}$ where V_x is the variance in the character due to the polymorphism. Equating this r_{XG} in the earlier formula, it follows that selection on the basis of the polymorphism would lead to a greater improvement than direct selection only if r_{XG} is greater than h_y

i.e./

$$\text{i.e. if } \sqrt{\frac{V_x}{V_G}} \text{ is greater than } \sqrt{\frac{V_G}{V_y}}$$

In other words, selection on the basis of the polymorphisms will not be superior to selection based on the animal's own performance unless the proportion of the genetic variance in the trait controlled by these loci is greater than the heritability of the trait under consideration.

From the results in Table 34, it is seen that the highest proportion of the phenotypic variance which these loci control is about 2.3 percent for body weight and 1.4 percent for egg weight with an upper limit of 5.5 percent and 4.1 percent. Since these characters have heritabilities of the order of 0.4 to 0.5, to obtain the proportion (approximate) of genetic variance, these estimates have to be multiplied by a factor of 2. Even so, the upper limits do not go beyond 10 percent which is far below the heritabilities of the characters considered. It could therefore be concluded that the variances due to these two loci on the characters considered are not large enough to have any practical importance. It should however be mentioned that the variance due to a locus is a property of the gene frequencies of its alleles and in a two allele locus is at a maximum when the frequencies of the two alleles are intermediate (particularly for additive effects). In the present case, though the frequencies of the alleles at the ovalbumin locus are of this order, the frequencies at locus II are of the order of 0.85 and 0.15 and are therefore not at the optimum ranges. However, since the latter locus does not appear to be contributing much to the variance on body weight and egg weight, it is not likely that the estimates given here would be different if the locus II alleles were at intermediate frequencies.

TABLE 34. The variance controlled by
Ov. locus and locus II.

	Phenotypic variance(V_p)	Variance due to egg white loci	Proportion due to the loci	
			Estimate %	Upper limit %
Body wt 20 wks	180.8	2.32	1.2	3.0
Body wt at 1st egg	217.2	2.87	1.3	4.1
Body wt 28 wks	239.2	5.13	2.14	5.5
Body wt 36 wks	368.7	8.61	2.3	5.5
Body wt 44 wks	433.8	5.97	1.4	4.0
Egg wt 1st egg	14.8	0.0044	0.3	1.3
Egg wt 28 wks	9.5	0.024	0.2	1.6
Egg wt 36 wks	9.8	0.129	1.3	3.8
Egg wt 44 wks	11.1	0.152	1.4	4.1
Age 1st egg	3.0	0.0069	.2	1.6

Sexual maturity: Age at sexual maturity was recorded as the age in weeks when the first egg was laid. From the deviations of the genotype - line subclasses from their hatch means (Table 35) it can be seen that the range in differences between the genotypes in any one line is not greater than one week.

TABLE 35 Mean deviations of egg white classes from
line means for age at sexual maturity.

Line	Ov.A,II.A	Ov.AB,IIA	Ov.B,II.A	Ov.A,IIAB	Ov.AB,II.AB
Max egg wt	-0.587	0.351	0.025	-0.324	0.338
Min body wt	0.439	0.081	-0.319	0.010	-0.081
Ratio	0.013	0.232	-0.418	0.358	-0.044
Regression	0.606	-0.199	-0.151	-1.095	0.232
Control	-0.200	0.020	-0.270	0.410	-0.052
Mean	+0.013	+0.110	-0.243	-0.031	+0.079

Analysis of variance (Table 36) of differences between genotypes does not reveal any significant differences. The only consistent trend is in Ov.B birds which in all lines mature at a slightly earlier age than Ov.A,II.A birds. This difference may in part explain the difference between the two classes in adult weight.

TABLE 36 Analysis of variance of age at maturity.

Source of variation	D.F.	M.S.	V.R.	P
Between Genotypes	4	4.076	1.340	>0.05
Between lines	4	0.28		
G x L interaction	16	4.762	1.566	>0.05
Error	824	3.040		

Egg number. Production has been considered mainly on survivors' records since unequal mortality affecting any particular phenotypic class would materially distort the results (mortality analysis to be shown later, in fact, confirms that the deaths are not random among classes). Accordingly, all birds dying during the laying cycle, have been eliminated from the detailed analysis. This reduces the degrees of freedom for error from 889 to 812.

The analysis was done on two overlapping records of production, egg number to 44 weeks of age and 72 weeks respectively. The former character was chosen because selection of birds for egg number is often done on the basis of this part record.

Differences in egg number to 44 weeks between egg white types are not statistically significant, but the general pattern appears to be a superiority of Ov.B,II.A over Ov.A,II.A with the heterozygotes Ov.AB,II.A being intermediate (Table 37). This trend is/

is opposite to what was observed for body weight and egg weight and is what would be expected if these traits are negatively correlated with egg number. The analysis of egg number to 72 weeks of age shows statistically significant differences between egg white classes (Tables 38 and 39).

TABLE 37. Mean egg numbers to 44 weeks and 72 weeks in the different lines.

Line	To 44 weeks	To 72 weeks
Max egg wt	49.95	101.18
Min body wt	49.05	93.93
Ratio	52.78	110.37
Regression	50.44	104.39
Control	51.37	103.31

TABLE 38. Mean deviation of egg white classes from line means.

a) 44 week egg number

Line	Ov.A,II.A	Ov.AB,II.A	Ov.B,II.A	Ov.A,II.AB	Ov.AB,II.AB
Max egg wt	1.006	0.461	-0.698	-0.987	-0.870
Min body wt	-1.887	-0.789	1.827	3.252	-0.218
Ratio	-2.611	-0.047	3.485	-1.120	-2.774
Regression	-3.325	1.758	0.114	3.333	-0.591
Control	1.424	1.048	0.294	-9.032	-0.050
Mean	-1.289	0.361	1.275	0.229	-0.811

b) /

b) 72 week egg number

Line	Ov.A,II.A	Ov.AB,II.A	Ov.B,II.A	Ov.A,II.AB	Ov.AB,II.AB
Max egg wt	4.869	4.408	-1.886	-8.308	-5.983
Min body wt	0.474	1.984	-0.481	3.659	-7.868
Ratio	-2.265	2.176	5.511	-3.310	-11.258
Regression	-4.960	0.016	1.054	11.182	3.683
Control	0.507	3.188	2.065	-15.821	- 3.936
Mean	-0.220	2.302	1.706	- 1.921	- 5.455

TABLE 39. Analysis of variance (showing mean squares) for egg number to 44 and 72 weeks of age.

Source of variation	D.F.	M.S. 44 wks egg no.	V.R.	M.S.72 wks egg no.	V.R
Between Genotypes	4	163.9	2.24	1643.7	3.64 ^{***}
Between lines	4	12.1		50.2	
G x L interaction	16	143.4	1.96*	584.7	1.29
Within	812	73.0		451.7	

* Significant at $P < 0.05$ ** Significant at $P < 0.01$

Comparison of the ovalbumin locus phenotypes within the II.A class shows overdominance, but when all classes are considered, the double heterozygote Ov.AB,II.AB turns out to be the poorest class and in a multiple range test its mean is significantly different from all other classes.

A within sire analysis was done to see if the findings on the basis of the whole population would be confirmed (Table 40). The comparison was made between Ov.AB,II.A and Ov.AB,II.AB classes. This comparison has severe limitations on the number of animals available for study because of the absence of both classes in a half sib group.

Only/

TABLE 40 Within sire comparison of Ov,AB,II.A with
Ov,AB,II.AB for egg production to 72 weeks.

Line	No of progeny groups	No of animals	Weight w	Average difference $\bar{D}$
Max egg wt	6	47	8.0	+ 8.91
Min body wt	10	66	10.28	+18.13
Ratio	5	37	7.75	+14.48
Regression	7	41	8.42	+ 0.10
Total	28	191	34.45	± 10.76

Mean difference = 10.76 ± 3.57

Only 28 progeny groups comprising 191 birds (total weight $\Sigma w = 34.5$) were used. This limited material confirms the original finding, the difference between the two groups being 10.76 ± 3.6 which is significant at the 5% level. The large standard error is due to the small number of animals used.

Mortality. The total adult mortality in this flock after sexual maturity was 10.8 percent. Since some birds died before egg samples were obtained from them, the information on egg white type was available for only about 65% of the dead birds. This forms 7.9% of the total number with egg white information. Table 41 shows the observed distribution of mortality with expectations based on a probability of 0.079 for all classes. The observed distribution is in reasonable agreement with expectation except for Ov,AB,II.AA class where the observed figure is about 25% higher than expected. This implies that heterozygotes at the ovalbumin locus are less fitter than homozygotes, but with the small numbers involved, it is doubtful whether any reliance can be placed on the results.

TABLE 41 Comparison of observed mortality in different egg white classes with expectations (based on the same proportion in all classes).

		Ovalbumin locus					
		A		AB		B	
		Obs	Exp	Obs	Exp	Obs	Exp
Locus II	A	10	12.8	35	26.9	17	16.1
	AB	5	8.8	10	10.6	0	0
	B	0	1.5	0	0	0	0

Summary: Productions traits in a flock of poultry showing genetic variation at the ovalbumin locus and locus II were analysed to see if differences could be detected between individuals showing variation at these loci. Significant differences were noted for body weight, egg weight and egg number. For the former two traits, the association was entirely with the ovalbumin locus. An additive effect was observed, the Ov.A homozygotes being heavier and laying larger eggs. The effect on egg number was due to an interaction between the two loci. At each locus the heterozygotes were superior to the homozygotes when the genotype at the other locus was homozygous (these differences not being significant) but the double heterozygotes (Ov.AB,II.AB) were markedly inferior in production to all other available genotypic combinations, the differences being highly significant. The proportion of the variance in each trait due to these loci however was too small to be of practical importance in selection.

B. Locus II and production traits. Strain 5 results.

The account given below is based on analysis of the strain 5 birds. This strain is polymorphic at locus II but fixed at Ov.locus, locus III and T_f , the alleles at the latter three being Ov^A , III^B and T_f^b respectively. The frequency of the alleles at locus II are II^A , 0.58 and II^B , 0.42. The three phenotypes are therefore represented at moderately high frequencies enabling a comparison to be made between all three classes unlike in the previous strain (strain 2) where no comparisons were made with the II^B class because of its low frequency. Strain 5, like strain 2, is also made up of four selection lines and a control line. The number of birds in the three, locus II classes in the different lines is shown in Table 42.

TABLE 42. Number of birds in locus II genotypic classes in different lines.

Line	A	AB	B	Total
Max egg wt	36	60	27	123
Min body wt	59	99	22	180
Ratio	55	66	40	161
Regression	89	110	42	231
Control	56	88	24	168
Total	295	423	155	873

Analytical procedure.

The analytical procedure was the same as before. An analysis of variance was done using the deviations from hatch-line means for all birds that were typed for egg white protein polymorphism. It was stated earlier that large discrepancies in the/

the distribution of the different egg white types in sire-progeny groups may cause spurious significant differences between genotypes. Similarly the same cause may obscure real differences if they exist. An inspection of the distribution of locus II genotypic classes in sire progeny groups revealed that in 15 half sib groups only one genotypic class was represented. Differences in sire family means may therefore increase or decrease real differences between genotypic classes. Therefore, wherever the genotype class means showed the same trend in all lines, with or without statistical significance, a within-sire analysis was carried out to determine if the findings on the preliminary analysis were confirmed. In this analysis, two-way comparisons were made between weighted means of genotypic classes within sire progeny groups. Comparisons were therefore of the following form - II.A with II.AB; II.B with II.AB and II.A with II.B. The total number of birds available for this analysis was 680, but the numbers used in each of the three comparisons differed greatly. Since the II^A allele was at a higher frequency (0.58) the number available for the comparison of II.A with II.AB was large, about 550, while only about 360 birds were used in comparing II.AB with II.B. There were very few progeny groups with both homozygotes and therefore only 150 birds were available for the II.A vs II.B

comparison.

Results

Body weight. The mean deviations of locus II genotypes for body weight at different ages, weighted for the different numbers in the lines, are shown in Table 43. The II.AB birds consistently show positive deviations indicating larger body size than the two homozygotes at all ages. The differences however are not significant/

significant with the exception of the 44-week body weight which is significant at the 5% level (Table 44). Since a superiority of II.AB was observed, it was decided to do a comparison of the classes within half sib groups for 20 week weight. This trait was chosen as weight at this age is not complicated by differences in age at maturity. The results (Table 45a, b and c) show that the II.AB class is superior and the difference in weight between II.AB and II.B classes is significant, being 4.08 ± 1.71 ($P < 0.02$). The difference between II.AB and II.A is small and not significant but the effect is opposite to what was observed in strain 2 where the Ov.A,II.A birds were heavier than the Ov.A,II.AB type.

TABLE 43 Mean deviation of genotypic classes from line means for body weight.

Trait	A	AB	B	Overall mean at in decà-grams.
Body weight 12 weeks	+0.056	-0.055	+0.019	98.411
Body weight 20 weeks	-1.133	+0.892	-0.382	148.652
Body weight at sexual maturity	-0.148	+0.895	-1.979	174.379
Body weight at 28 weeks	+0.223	+0.550	-1.887	177.600
Body weight 36 weeks	-0.285	+1.202	-2.65	178.422
Body weight 44 weeks	-1.56	+1.841	-1.77	182.345

TABLE/

TABLE 44 Analysis of variance (showing mean squares) of body weight measurements.

Source of variation	D.F.	12wks	20wks	1st egg	28wks	36wks	44wks
Between genotypes	2	1.14	369.1	444.3	347.6	360.5	1317.7 ^a
Between lines	4	0.03	2.5	10.2	12.2	9.6	12.4
G x L Interaction	8	175.7	611.2	400.1	382.2	533.0	497.6
Error	859	73.45	205.7	311.0	342.6	363.0	514.5

^a Significant at $P < 0.05$

TABLE 45 Within sire comparison of locus II genotypes for 20 week body weight.

a) II.AB vs II.A

Line	No of progeny groups	No of animals	Weight w	Average differences $\bar{D}$
Max egg wt	10	77	15.42	+ 4.09
Min body wt	9	102	27.34	+ 1.55
Ratio	6	72	15.18	+ 1.86
Regression	14	188	38.44	+ 3.21
Control	13	86	17.96	- 5.46
Total	52	525	114.34	+ 1.392

Mean difference = $+1.39 \pm 1.4$

b) II.AB vs II.B

Max egg wt	10	71	13.57	- 2.5
Min body wt	7	71	13.48	+ 2.04
Ratio	9	80	16.87	+ 4.86
Regression	7	89	17.3	+10.94
Control	8	48	9.89	+ 2.55
Total	41	359	71.11	+ 4.08

Mean difference = $+4.03 \pm 1.7$

c) II.A vs II.B

Max egg wt	7	38	7.37	- 3.8
Min body wt	5	21	6.56	+3.84
Ratio	3	57	8.02	+8.42
Regression	6	21	7.87	+7.00
Control	3	13	4.17	-1.86
Total	24	150	33.99	+3.29

Egg weight: Egg weight means of locus II genotypic classes show no consistent trends at different ages or in the different lines (Table 46a and b). The differences too are small and not significant.

TABLE 46.

a) Mean deviation of phenotypic classes from line means for egg weight at different ages

Trait	II.A	II.AB	II.B
Egg weight 28 wks	-0.038	+0.141	-0.312
Egg weight 36 wks	0.221	-0.105	-0.129
Egg weight 44 wks	0.287	0.023	-0.117

B) Analysis of variance (showing mean squares) of egg weight measurements.

Source of variation	D.F.	28wks	36wks	44 wks
Between genotypes	2	11.94	12.5	0.65
Between lines	4	0.02	0.9	1.60
G x L Interaction	8	14.82	15.1	14.6
Error	859	9.54	10.5	12.5

Sexual maturity: The mean deviations of the three genotypic classes from their line means are shown in Table 47. It can be seen that there is a consistency in the sign of the deviations for the classes in all lines even though the lines differ considerably in mean age at maturity. The II.A homozygotes mature later than the II.AB and II.B classes and this difference is significant at $P < 0.025$. (Table 48). The within sire analysis supports the finding to the extent that the differences are/

are in the expected direction, but they are small and not significant (Table 49). It is therefore doubtful whether any real effect exists on this character.

TABLE 47. Mean deviations of locus II genotypes for age at sexual maturity from line means.

Line	II.A	II.AB	II.B	Line Mean
Max egg wt	+0.357	-0.171	-0.096	26.72
Min body wt	+0.378	-0.179	-0.208	27.51
Ratio	+0.356	-0.239	-0.096	23.63
Regression	-0.004	-0.015	+0.047	23.66
Control	+0.424	-0.055	-0.785	25.36
Mean	+0.265	-0.142	-0.115	

TABLE 48. Analysis of variance of age at maturity.

Source of variation	D.F.	Mean Square	V.R.	P
Between genotypes	2	15.66	4.27	<0.025
Between lines	4	0.52		
G x L Interaction	8	2.93		
Error	859	3.71		

TABLE/

TABLE 49. Within sire comparisons of locus II genotypes for age at sexual maturity.

a) II.A vs II.AB

Line	No of progeny groups	No of animals	Weight w	Average difference $\bar{d}$
Max egg wt	10	77	15.42	+ 0.263
Min body wt	9	102	27.34	+ 0.354
Ratio	6	72	15.18	- 0.062
Regression	14	188	38.44	+ 0.240
Control	13	86	17.96	+ 0.081
Total	52	525	114.34	+ 0.215

Mean difference = $+0.215 \pm 0.180$

b) II.AB vs II.B

Max egg wt	10	71	13.57	- 0.175
Min body wt	7	71	13.48	- 0.026
Ratio	9	80	16.87	- 0.35
Regression	7	89	17.30	- 0.05
Control	8	48	9.89	+ 0.73
Total	41	359	71.11	-0.032

Mean difference = -0.032 ± 0.23

c) II.A vs II.B

Max egg wt	7	38	7.37	+ 0.827
Min body wt	5	21	6.56	+ 0.098
Ratio	3	57	8.02	- 0.544
Regression	6	21	7.87	+ 0.327
Control	3	13	4.17	+ 1.09
Total	24	150	33.99	+ 0.159

Mean difference = $+0.159 \pm 0.33$

Egg production: Egg production records were based on 3 day per week trapnest records. Production was terminated at 60 weeks of age because of high mortality in this strain. Thirty one birds included in the earlier analysis died before they completed their 60 week production and these were eliminated from the present analysis. The mean deviation of the three classes for part (44 weeks) and full records (60 weeks) are shown in Table 50.

TABLE 50. Mean deviation of genotypic classes from line means for (a) 44 week and (b) 60 week egg production.

Trait	A	AB	B
44 week egg number	-0.197	+ 0.423	- 0.394
60 week egg number	-0.609	+ 0.717	- 1.407

The heterozygotes are superior and II.B homozygotes the worst for part and full egg production. These differences are found not to be significant in the analysis of variance (Table 51). The within sire comparisons support the mean values in Table 50. The heterozygotes are superior to both homozygotes, the difference in each case just approaching significance. The difference between II.AB and II.A classes is 2 ± 1.3 eggs and between II.AB and II.B 3.1 ± 1.7 eggs (Table 52). A comparison of heterozygotes against the two homozygotes pooled together gives a significant difference of 2.8 ± 1.11 eggs ($t = 2.52$). It was noted earlier that heterozygotes mature slightly earlier than the homozygotes. Since age at maturity is negatively correlated

phenotypically with egg number ($r = -0.30$), the significant differences between genotypes for egg number may be due to differences in age at maturity. The mean differences in the within sire analysis were therefore corrected for differences in age at maturity by subtracting an amount attributable to the regression of egg number on age at maturity (Snedecor 1956, p.401) and tested for significance against the adjusted standard error. From the results shown in Table 53, it is seen that the difference in production between heterozygotes and pooled homozygotes (II.AA and II.BB) is still significant though in the comparison of the heterozygote with each homozygote the difference falls below the level of significance. The within sire analysis in this case shows that the real effects of this locus were diminished in the overall mean (Table 50) by inequality in distribution of the genotype classes among sires since differences are greater when estimated within sire groups. Though there is a tendency for the heterozygotes to be superior to either homozygote, it is difficult to be certain that this is real because of the low level of significance.

TABLE 51. Analysis of variance (showing mean squares) for egg production.

Source of variation	D.F	44 wk egg no.	60 wk egg no.
Between genotypes	2	61.12	309.5
Between lines	4	7.2	5.4
G x L Interaction	8	66.3	181.7
Error	828	90.15	189.2

TABLE 52. Within sire comparison of locus II genotypes for 60 week egg number.*

a) II.AB vs II.A

Line	No of progeny groups	No of animals	Weight w	Average difference $\bar{D}$
Max egg wt	9	65	13.3	+ 5.62
Min body wt	11	125	26.9	+ 2.74
Ratio	6	72	14.8	+ 2.58
Regression	14	176	36.5	+ 1.72
Control	12	85	18.6	+ 1.93
Total	52	523	110.1	+ 2.59

Mean difference = 2.59 ± 1.3

b) II.AB vs II.B

Max egg wt	8	55	10.16	+ 1.81
Min body wt	8	76	13.98	+ 3.25
Ratio	9	70	15.34	+ 2.18
Regression	8	96	17.56	+ 4.31
Control	7	45	8.71	+ 3.59
Total	40	342	65.7	+ 3.11

Mean difference = $+3.11 \pm 1.7$

c) II.A vs II.B

Max egg wt	6	31	7.16	+ 1.75
Min body wt	6	35	6.56	+ 3.08
Ratio	5	43	6.35	+ 2.4
Regression	7	55	7.87	+ 0.61
Control	3	13	2.7	- 4.53
Total	27	177	30.64	+ 1.32

Mean difference = $+1.32 \pm 2.5$

d) II.AB vs (II.A + II.B)

Max egg wt	11	93	19.83	+ 4.52
Min body wt	13	163	33.05	+ 2.17
Ratio	10	122	26.11	+ 2.93
Regression	15	219	48.23	+ 2.29
Control	14	119	25.28	+ 3.15
Total	63	716	152.50	+ 2.805

Mean difference = $+2.805 \pm 1.11$

* 3 day trapnest record

TABLE 53. Within sire comparisons of locus II genotypes for 60 week egg number after adjusting for regression of egg number on age at maturity.

Comparison	Average difference	Standard error
II.AB - II.A	2.13	1.25
II.AB - II.B	3.04	1.63
II.AB - (II.A + II.B)	2.54	1.08

As was done with the previous strain, it was decided to see if the association of this locus with body weight and egg production could be confirmed by differences in numbers in phenotypic classes between hypothetical high and low selected groups. For this purpose, only the progeny groups used in the within sire analysis were considered since only these groups have sibs differing at this locus. The average half sib family size in these groups was about 10 and if on average 2.5 individuals with the highest and lowest values for these traits were selected, the deviation of each selected group mean from the population mean would be 1.35 standard deviations. The 'high' and 'low' groups would therefore differ by 387 grs in body weight at 20 weeks and 37.2 eggs in egg number to 60 weeks and this difference should appear as a difference in genotype frequencies between the two groups. The results classified by each line are shown in Table 54 and these confirm the previous findings. II.AB birds are found in higher proportions for both characters in the 'high' group while II.B is at a high frequency in the 'low' groups. The results however are not uniform in all lines and for body weight at 20 weeks the observed distribution in the control line is opposite to what would be expected.

Any large effect of this locus on body weight would also be expected to show up in the distribution of the locus II classes in the different lines. Since II.AB is the superior class for body weight in the high body weight lines (maximum egg weight and regression lines) one would expect a larger proportion of these to be selected as parents. Even if this happened, because of segregation/

segregation of the two alleles a difference in frequency in favour of II.AB class would not be observed in the progeny. In the low weight lines (minimum body weight and regression lines) a larger proportion of II.B birds would be expected to be selected and hence this should be evident as a higher frequency of II.B in the progeny groups. From the observed and expected distribution (Table 55) it is seen that the distribution in the ratio line is in the expected direction, while in the minimum body weight line it is in the opposite direction.

TABLE 54. Number of birds/locus II genotypes in sire progeny groups selected on the basis of the best and worst birds for

a) Body weight at 20 weeks.

Line	II.A		II.AB		II.B	
	High	Low	High	Low	High	Low
Max egg wt	5	9	9	8	5	2
Min body wt	9	9	16	14	1	3
Ratio	4	4	15	9	5	11
Regression	13	19	24	10	5	13
Control	9	5	7	11	2	2
Total	40	46	71	52	18	31

b) Egg production to 60 weeks.

Max egg wt	2	5	10	5	3	5
Min body wt	6	12	19	12	1	2
Ratio	7	5	9	8	5	8
Regression	13	14	19	14	4	8
Control	4	4	10	11	4	3
Total	32	40	67	50	17	26

TABLE 55. Comparison of observed numbers in different lines with expectations on the basis of proportional totals.

Line	II.A		II.AB		II.B		Total
	Obs	Exp	Obs	Exp	Obs	Exp	
Max egg wt	36	41.5	60	59.6	27	21.8	123
Min body wt	59	60.8	99	87.2	22	31.9	190
Ratio	55	54.4	66	78.0	40	28.6	161
Regression	89	81.4	110	116.7	42	42.7	241
Control	56	56.7	88	81.4	24	29.8	168
Total	195	295	423	423	155	155	873

The variance due to this locus: The effect of this locus on the production traits could be measured by the proportion of the total variance in the character that is due to this locus. The total variance between genotypic classes was therefore partitioned into its components. The expectations were as follows:

$$\text{Between genotypes} = \sigma_e^2 + 252.3 \sigma_G^2$$

$$\text{Error} = \sigma_e^2$$

σ_e^2 = Error variance and σ_G^2 = variance component due to locus II genotypes.

From the results (Table 56) it is clear that the proportion of the total variance which this locus controls for any character is very small. The highest value among body weights was 0.73% for weight at 44 weeks of age which is approximately 1.5% of the additive genetic variance. For age at sexual maturity and egg number to 60 weeks the proportions are 1.4% and 0.28% respectively. Since the heritabilities of these two traits are approximately of the order of 25 to 30% (Dickerson, 1955) the above estimates have to be multiplied by a factor of 3.5 to 4 to obtain the proportion of the additive/

additive genetic variance. It was shown earlier that the polymorphic loci could be of practical use only if the proportion of the additive variance they control approaches the heritability of the trait. This is not attained for any of the characters.

TABLE 56. The variance controlled by locus II.

Trait	Phenotypic variance	Variance due to locus II	Percent of phenotypic variance
Body wt 20 wks	205.7	0.72	0.34%
Body wt 1st egg	311.0	0.54	0.19%
Body wt 28 wks	342.5	0.026	0.009%
Body wt 36 wks	363.0	2.19	0.603%
Body wt 44 wks	415.6	3.97	0.73%
Egg wt 28 wks	9.54	0.009	0.095%
Egg wt 36 wks	10.52	0.009	0.086%
Egg wt 44 wks	12.55	-0.05	-0.42%
Age 1st egg	3.71	0.53	1.41%
Egg No 44 wks	90.15	-0.13	-0.14%
Egg No 60 wks	189.2	0.53	0.28%

Summary: A population of about 900 White Leghorn birds was surveyed to detect if associations existed between egg white polymorphisms and production traits. Genetic variation was observed only at locus III, the other three loci being fixed. All three genotypes at this two allele locus were represented at moderate frequencies. Significant differences between genotypes were observed for body weight and egg number. The II.AB heterozygotes were heavier than either homozygote but only/

only the difference between II.AB and II.B was significant.

The heterozygotes were also superior for egg number to either homozygote, the difference in each case just approaching significance. However, when both homozygotes were pooled and tested against the heterozygotes, the difference was significant at the 5% level. Consistent differences were also observed for age at maturity but these were not statistically significant.

C. Loci II, III and T_f and production traits. Studies on Light Sussex flock

In the two strains examined earlier the transferrin locus and locus III were fixed so that no studies on the association of these two loci with production traits were possible. However, in a flock of Light Sussex birds at the Royal (Dick) Veterinary Field Station these two were segregating in addition to locus II. This flock was originally typed to obtain triple heterozygotes at loci II, III and T_f for linkage studies. For this purpose, the entire flock consisting of 400 hens was typed, but two families consisting of 81 birds which had been purposely inbred were excluded from the present analysis.

This population was not pedigreed, and mating was entirely random. Artificial insemination was used, the semen of selected males being pooled before insemination. A certain amount of selection had been applied to the females based on individual egg production to 44 weeks of age. Only two production characters were recorded, these being age at sexual maturity and egg production to 65 weeks of age.

Analysis: The birds used in the present analysis were from six weekly hatches, but since the same parent stock were used in all, it was assumed that the distribution of the progeny of different genotypes would be the same in all hatches and that there would be no genotype x hatch interaction. Further, since this population was not pedigreed, no within sire analysis was possible, but this also was not necessary since mating was random and all sires had an equal chance of contributing a gamete for any particular progeny. The number of birds of genotypes III.A and T_f -a were very small and these were therefore/

therefore excluded from the analysis, since inclusion would cause a number of vacant cells when considering genotypes at all three loci simultaneously. The final distribution of birds used for analysis is shown in Table 57. From the subclass numbers, it is evident that the data are very non-orthogonal with one vacant cell and it is therefore necessary to use a least squares analysis by fitting constants. A detailed account of the two factor case is given by Harvey (1960) from which an extension to the three factor case considered here was done. The statistical model, considering genotype effects as fixed is as follows:

$$Y_{ijkl} = \mu + u_i + V_j + W_k + (UV)_{ij} + (UW)_{ik} + (VW)_{jk} + (UVW)_{ijk} + e_{ijkl}$$

where $i = 1, 2, 3$

$j = 1, 2$

$k = 1, 2$

$l = 1, 2 - - - - - n_{ijk}$

μ = Overall mean with equal subclass numbers

u_i = effect of i^{th} genotype of locus II

V_j = effect of j^{th} genotype of locus III

W_k = effect of k^{th} genotype of T_f locus

$(UV)_{ij}$ = interaction effects between loci II and III

$(UW)_{ik}$ = interaction effects between loci II and T_f

$(VW)_{jk}$ = interaction effects between loci III and T_f

$(UVW)_{ijk}$ = interaction effects between loci II, III and T_f

e_{ijkl} = random errors.

TABLE 57 Distribution of birds used in analysis among loci II, III and T_f genotypes.

	Loci			Number
	II	III	T_f	
Genotypes	aa	.	.	118
	ab	.	.	157
	bb	.	.	44
	.	ab	.	103
	.	bb	.	216
	.	.	ab	60
	.	.	bb	259
	aa	ab	ab	3
	aa	ab	bb	24
	aa	bb	ab	13
	aa	bb	bb	78
	ab	ab	ab	16
	ab	ab	bb	46
	ab	bb	bb	70
	bb	ab	ab	0
	bb	ab	bb	14
	bb	bb	ab	3
	bb	bb	bb	27

In addition to furnishing unbiased means for each genotype, the fitted constants also allow us to distinguish between additive and non-additive effects. Thus in locus II where all three/

three genotypes are represented, the estimated parameter for the heterozygote will lie between the two homozygotes if there is no dominance. In the other two loci, however, comparisons are only possible between a homozygote and the heterozygote and non-additive effects cannot therefore be detected.

Results.

Egg number: The results of the analysis of variance for this trait are shown in Table 58. Three significant effects are noted (a) effect of locus II, significant at $P < 0.05$, (b) effect of transferrin locus significant at $P < 0.01$ and (c) first order interaction involving T_f locus and locus II significant at $P < 0.005$. The nature of these effects can be better understood by examining the fitted parameters for the different genotypes at each of the two loci singly and in combination. The parameter for the three significant effects are shown in Table 59. It is clear that at locus II, the heterozygote is superior to either homozygote and that the II.B type is significantly inferior in egg production. At the transferrin locus, the heterozygote is inferior to the homozygote Tf_{-b} , the difference between the two being about 24.4 eggs. No comparison with the other homozygote is possible. The interaction between the two loci is complicated as could be seen from the parameters shown in Table 59. The T_f locus heterozygotes seem to be superior to the Tf_{-b} homozygotes in combinations with II.A or II.AB but the reverse is seen with II.B homozygotes, i.e. the Tf_{-ab} , II.B birds are inferior to Tf_{-b} , II.B. It should however be mentioned that there are only three birds in the former (Tf_{-ab} , II.B) class and hence the latter result cannot be relied on. At locus III, the heterozygotes III.AB are superior in egg production to III.B, but the difference is/

TABLE 58. Analysis of variance showing effects of the three egg white loci and egg production.

Source of variation	D.F.	M.S.	V.R.	P
Locus II	2	4253.3	3.23	< 0.05
Locus III	1	1144.1	1	
T _f locus	1	8461.9	6.43	< 0.01
II x III	2	3566.1	2.7	
II x T _f	2	8428.6	6.4	< 0.005
III x T _f	1	520.9	1	
II x III x T _f	1	2490.4	1.89	
Error	308	1316.5		

TABLE 59. Estimated parameters with approximate standard errors for locus II and T_f genotypes singly and in combination for egg production.

Loci		Parameter
II	T _f	
A	.	+7.2 ± 3.3
AB	.	+12.3 ± 2.8
B	.	-19.5 ± 5.5
.	AB	-12.2 ± 3.6
.	B	+12.2 ± 2.4
A	AB	+18.1 ± 9.0
AB	AB	+ 7.1 ± 5.6
B	AB	-25.2 ± 20.9
A	B	-18.1 ± 3.6
AB	B	- 7.1 ± 3.3
B	B	+25.2 ± 5.6

is not significant.

Sexual maturity: The detailed analysis of variance of this trait is shown in Table 60.

TABLE 60. Analysis of variance showing effects of the three egg white loci on age at sexual maturity.

Source of variation	DF	M.S.	V.R.	P
Locus II	2	390.1	3.2	<0.05
Locus III	1	21.4	1	
T _f locus	1	557.8	4.5	<0.05
II x III	2	53.3	1	
II x T _f	2	138.1	1.1	
III x T _f	1	98.4	1	
II x III x T _f	1	5.6	1	
Error	308	123.0		

Significant effects are again seen for locus II and T_f locus genotypes. From the estimated parameters for the genotypes (Table 61), it is seen that at locus II, the II.A homozygotes mature later than the other two genotypes, the latter two not differing in this character. At the T_f locus, the heterozygotes mature about 60 days earlier than the II.B homozygotes. The interactions between loci for this character are small and not significant.

TABLE 61. Estimated parameters for loci II and T_f singly for age at sexual maturity.

	Phenotype	Parameter
II	A	+ 3.6
	AB	- 2.0
	B	- 1.6
T _f	ab	-3.1
	b	+ 3.1

Since birds that mature early are likely to lay more eggs for the same period than those maturing later, it is necessary to know if the significant differences between genotypes for egg production are not simply due to differences in age at maturity. This is tested by an analysis of covariance (Table 62). It is seen that differences between genotypes for egg number are significant even after removing the covariance for regression of egg number on age at maturity. The differences between T_f genotypes were not tested in this manner because the T_f -ab birds that matured earlier laid fewer eggs and therefore the effect of age at maturity will only increase the differences in egg number (since phenotypic correlation between the two characters is negative $r = -0.26$).

TABLE 62. Analysis of covariance of egg number with age at maturity for locus II genotypes.

	D.F	Adjusted M.S	V.R	P
Between locus II genotypes	2	3771.4	3.48	<0.05
Error	308	1081.6		

Summary: The association between genotypes of egg white loci II, III and T_f and egg production (to 65 weeks) and age at maturity were analysed in a flock of Light Sussex birds. At locus II, all three genotypes were represented, while at loci III and T_f , the III.A and T_f -a homozygotes were not included in the analysis because of low frequency. Significant effects were observed for both traits with loci II and T_f . At locus II: the heterozygotes were superior to either/

either homozygote for egg production and also matured earlier.

The II.B homozygotes were markedly inferior for egg production while II.A birds were inferior for age at maturity (if delayed maturity could be considered an undesirable trait). The transferrin locus heterozygotes were inferior in production to the T_f-b homozygotes but were earlier maturing than the latter. Significant epistatic interactions were also observed between loci II and T_f for egg production.

DISCUSSION

D.

A major object of this study was to determine if there were associations between genes or genotypes at the four egg white loci and production characters in poultry. The value of finding such associations and potential modes of application were outlined earlier. They are also of interest as a possible explanation for the existence of extensive cryptic polymorphisms in many species.

Results from other species do not provide clear cut answers to these questions. The data from poultry blood groups, though by no means completely consistent, indicate important superiority in certain traits for particular individuals heterozygous at the B blood group locus (Gilmour 1960; Briles 1960). Data from dairy cattle on blood groups (Neimann - Sørensen and Robertson 1961; Rendel 1961) have provided only one instance of a consistently significant association, that between the allele B^{BO, Y, D} and butter fat percentage in milk. The situation for sheep blood groups is similar, no consistent effects being found (Stansfield, Bradford, Stormont & Blackwell, 1964). We have not a great deal of evidence as yet on the effects of loci controlling the biochemical polymorphisms in animals. Bangham & Blumberg (1958) found no suggestion of association of haemoglobin genes with resistance to protozoan parasitic infections in cattle. Ashton (1960, 1964) reported an additive effect of the Tf locus on milk yield from two independent populations but Datta et al (1965) in a recent study have not been able to confirm this though their results suggest a superiority of Tf D homozygotes similar to Ashton's findings. The studies on sheep biochemical

polymorphisms are concerned with only the haemoglobin and potassium polymorphisms (King et al, 1958 and Watson & Khattab 1964) and these have not shown any significant association with production characteristics.

The present investigation with poultry though showing evidence of some association of egg white polymorphisms with production characteristics is not very illuminating on why certain polymorphisms exist.

Before discussing the findings of this investigation, it may be useful to summarize the materials used and analytical methods employed.

The investigation was carried out in three closed flocks, the first one polymorphic at ovalbumin locus and locus II, the second at locus II only and the third at loci II, III and Tf. Two methods of analysis were used in the investigation. In the first case, the entire population was divided into classes on the basis of their egg white loci genotypes and the analysis of variance technique was employed to see if the mean values of the different classes differed significantly for the characters studied. This did not consider distribution of genotypes among parents and concentration of a proportion of individuals of a particular genotype among two or three good parental families would distort the picture in their favour. In the second analysis two way comparisons were done between progeny of different genotypes within sire families. This analysis should be reasonably free of the chance associations mentioned earlier and therefore significant effects detected by the latter method could be given more weight.

The first method of analysis is also subject to the criticism that when a large number of F tests are done, 5 percent of the F values based on 95% confidence limits would be significant by chance alone, in the absence of any real effects. In the first flock (strain 2) out of 12 tests 6 were significant and except for one the others were significant at more than 95% confidence limits. From the table of binomial probabilities (Parzen, 1960) 6 out of 12 tests would be significant at 5% level on a null hypothesis at $P = .0001$ i.e., the probability of 6 F tests out of 12 being significant at 5% level when in fact they are not is only 0.01%. It may therefore be correctly concluded that the number of significant F tests obtained in this case could not reasonably be due to chance. In the second flock (strain 5) only one F test was significant out of 12 and from the tables this will have a P value of 0.35 or 35%. This significant effect may therefore have arisen by chance.

The ovalbumin locus was found to have an additive effect on body weight and egg weight with the Ov^A allele having a positive and Ov^B a negative effect on these traits. The difference between the two homozygotes was half the standard deviation for body weight and a third of the standard deviation for egg weight. It was shown that differences in egg weight could explain part but not the whole of the differences in body weight between the two homozygotes. The additive effects shown here are consistent with the expectation put forward in the introduction that characters not closely related to reproductive fitness would be expected to show an additive relationship with genes influencing them.

Even though the effect is additive with respect to body weight and egg weight, there may be overdominance with respect to fitness if one accepts the data of Lerner (1951) and Lerner & Gunns (1952) that birds with intermediate egg weights have superior fitness.

Egg weight is a function of yolk, albumen and shell weights but albumen forms the major component - about 60 percent (Erasmus, 1954). Ovalbumin forms about 65 percent of the total albumen weight and should therefore contribute 40 percent to the total egg weight. Further, since it has been shown that large eggs have proportionately more albumen ie; the yolk albumen ratio is smaller than small eggs (Erasmus, 1954) it is not unreasonable to conclude that large eggs also have proportionately more ovalbumin than small eggs. It is therefore conceivable that the differences in egg weights between the two homozygotes of the ovalbumin locus may be due to quantitative differences in the amount of ovalbumin produced by the two genotypes. Other examples of such quantitative differences in support of this hypothesis are known. For example, in the β - lactoglobulin polymorphism in cattle, it has been shown that the AA homozygote produces almost twice as much β - lactoglobulin as the other homozygote (Aschaffenburg & Drewry, 1957); while the heterozygote shows an intermediate output.

Locus II also seems to exert an influence on body weight but this is only evident in a within sive analysis of strain 5. The hererozygotes in this case are superior in weight to the II BB homozygotes. The difference in body weights between II. A B and II A. is positive in this strain while it is the reverse in strain 2. In both cases however,

the differences are small and not significant. One can therefore only conclude that no real difference in body weights are demonstrable between II.A & II.AB types. Unfortunately, the significant effect found between II.AB & II.B types in strain 5 could not be confirmed by statistical analysis in the other strain because of the low frequency of the latter homozygotes. However the mean of the 21 II.B birds was calculated as a deviation from the population mean for body weights from 20 weeks to 44 weeks and compared with the means of II.AB heterozygotes as shown below.

Age.	II.AB	II.B
20 weeks	+ 1.6 grs.	+ 11.3
28 "	- 49.3 "	- 45.2
36 "	- 14.7 "	- 49.0
44 "	- 9.3 "	- 29.1

No definite conclusions can be drawn from this comparison because at earlier ages II.B types seem to be superior whereas it is the reverse at later ages. The effect of locus II on body weight therefore needs confirmation from studies on other strains.

There seems to be a rather consistent effect of locus II on sexual maturity in all 3 strains examined. In strain 5 and the Light Sussex flock the heterozygotes were earlier maturing than the homozygotes and the differences were significant. In a within sire analysis of strain 5 however, the effects detected earlier were much diminished but still in favour of heterozygotes. It is doubtful if it is a real effect even though the ranking of the genotypes is the same in all three populations because of the failure to reach

significance in two of them.

Three loci seem to have an influence on egg number. The effect of ovalbumin locus seems to be a superiority of Ov. B homozygotes and the heterozygotes over the Ov.A homozygotes but the differences are not significant. Locus II had significant effects in both strain 5 and the Light sussex flocks and in both cases there was overdominance with the II.B homozygotes the lowest in production. In strain 2, a similar comparison is possible only between II.A and II.AB classes ie; Ov.A, II.A Vs Ov.A,II.AB. When the entire population data means were analysed, the II.A had slightly better mean than II.AB which was the reverse of the two population data, but a within sire analysis of these two classes in strain 2 showed the following results confirming the results of the other two populations. It could therefore be reasonably concluded

Line	Weight Ew.	Difference II.AB-IIA.
Max. egg weight	8.63	+ 4.8
Min. Body weight	6.82	+ 7.7
Ratio	8.45	+ 1.4
Regressions	2.0	+ 5.4
Control	0.67	- 2.6
Total	26.57	+ 4.30+4.12

that there appears to be overdominance for egg number at locus II at least in two of the populations examined and in the third one the difference though not significant is in the direction favouring heterozygotes. The Tf locus and locus III effects were only determined

in the Light Sussex flock. The Tf locus effect was significant in a comparison of Tf - b homozygotes with the heterozygotes, the former having the better egg production. No significant differences were observed between locus III genotypes.

Significant interactions are also observed between some loci for egg production. At ovalbumin and locus II, each heterozygote is superior to both or one of its respective homozygotes ie; Ov.A,II.AB is superior to Ov.A,II.A and Ov.A,II.B and similarly for the other locus. But the simultaneous presence of heterozygotes at both loci seems to confer a marked disadvantage on individuals having these alleles so that OvAB,IIAB individuals are inferior to all other combinations of these two loci (except Ov.B,II.B on which no information is available). This is very similar to the situation observed by Fisher (1939) in wild populations of *Paratettix texanus* where there was a deficiency of individuals heterozygous for two or more dominant genes so that an individual that was heterozygous at both loci was at a 40% disadvantage. He postulated that under these conditions there would be very strong selection in favour of genes suppressing crossing over between the two loci, for in an individual with the chromosome constitution $\begin{smallmatrix} A & b \\ \hline a & B \end{smallmatrix}$ (capital letters being dominant) a cross over would produce the chromosomes $\begin{smallmatrix} a & b \\ \hline a & b \end{smallmatrix}$ and $\begin{smallmatrix} A & B \\ \hline A & B \end{smallmatrix}$ and an individual carrying the latter would be at a disadvantage to other genotypes.

In fact the genes controlling the dominant varieties of this species are very closely linked, crossing over between them being rare (Sheppard 1955). Similarly, since egg number is closely related to fitness, we would expect natural selection to act strongly on genes or genotypes controlling this character. If for example, the presence of 'B' alleles at both loci (Ov.B,II.B or Ov.AB,II.AB) makes the individuals disadvantageous for this character, we would expect natural selection to bring these two loci closely together and this would explain the tight linkage between them shown earlier.

Before concluding the remarks on egg number another important point should be discussed. The comparisons between genotypes were based on survivors' production and this therefore ignores mortality. The relationships between mortality and egg number are not clear, (Lerner, 1958) but if it is assumed that birds that die before they complete their full record are also poor layers, then analysis of survivors' records may be biased in favour of certain genotypes if mortality distribution among genotypes is not random. It was noted earlier that the mortality distribution in strain 2 was not random and that there was a greater proportion of deaths in Ov.AB,II.A birds. The observed superiority of Ov.AB,II.A in egg production in this strain may therefore have been biased in their favour. In strain 5, the mortality among the three genotypes were as shown below. Since the

Locus II

	AA	AB	BB
Mortality %	3.5%	2.6%	9.1%.

heterozygotes had the lowest mortality, the observed results on egg number would if at all have been biased against them and the production of II.B birds may have been even lower than the observed values. This therefore re-inforces the conclusions on egg number for locus II reached earlier.

It is not possible to determine from the data whether the associations detected are due to linkage. Linkage is unlikely to be the cause because two of the populations have been random bred for at least 5 generations and the other flock for a much longer period. Further, they have been kept as closed flocks and therefore linkage disequilibrium is unlikely to exist except for very closely linked genes. Though it is possible to distinguish between pleiotropy and linkage by statistical analysis (Lowry & Schultz, 1959), it has been shown by Niemann Sørensen & Robertson (1961) that the chance of detecting close linkage is 50% or greater only if the locus contributes more than 10% of the observed variance in the character. Since in no case was this limit reached for the characters considered, no conclusion could be reached from such an analysis.

PART VStudies on associations between maternal egg white genotypes and embryonic viability.

Hatchability is a major component of fitness: the fittest individuals are not the ones with intermediate values, but those which hatch the largest number of progeny. Therefore an association of polymorphic genes with this trait, if present, would throw some light on the forces maintaining these egg white polymorphisms.

Hatchability of fertile eggs is a compound trait made up of two components. The first is the ability of the embryo to survive and the second is the contribution of the dam to the extra-embryonic portion of the egg which provides the milieu for embryonic development. Therefore an analysis of the effect of egg white loci on hatchability should consider (a) the genotype of the zygote and (b) the genotype of the dam. But the published evidence indicates that the genotype of the dam has a much larger influence on hatchability than the former. For example, Crittenden and Bohren (1961) estimated that the hereditary and non-hereditary maternal effect on the hatchability of individual embryos was nearly ten times as great as the direct hereditary influence on the embryo. Other reports (Abplanalp and Kosin, 1953) also support this. Further, with the egg white loci, since the dam's genotype for these genes is expressed in her egg, there is a direct connection between the genes and embryonic environment. This therefore justifies an analysis of hatchability differences by dam's genotype though it also is possible that the egg white genotype of the embryos/

embryos may influence their ability to survive. It should however be mentioned that an analysis by embryo genotype was not possible in this case since the genotypes of the sires for these loci were not known.

Materials and methods.

The bulk of the data used in this analysis was from breeding birds of strain 2 and were collected during the spring hatches of two successive years 1964 and 1965. Approximately 250 pullets and 60 cockerels were used for breeding in each season. Eggs were collected for a fortnight before incubation. Four hatches were obtained, the first two being from the maximum egg weight, minimum body weight and control lines and the last two from the ratio, regression and control lines. Therefore except for the birds from the control line which appeared in all four hatches the parents of eggs used in the first two hatches were different from those used in hatches 3 and 4. The methods used in classifying the dead embryos into age groups were given under the section on materials and methods.

Statistical analysis: The analytical procedure used is somewhat similar to that of Morton et al (1965). Mortalities were calculated for seven periods as a percent of embryos alive at the beginning of each period as follows:

Early Embryonic Deaths (EED): deaths on days 1 to 6.

Dead Germ one (DG1): deaths on days 7 to 12

(EED + DG1) : deaths on days 1 to 12

Dead Germ two (DG2): deaths on days 13 to 20

Pips (PIP): embryos that pipped their shells

but failed to hatch out

(DG2 + PIP) : deaths from 13 days to end of hatch.

Total Embryonic Mortality (TEM): deaths from beginning to end of hatch.

Since percentage data characteristically show heterogeneity of errors caused by a relationship between the means and the variances all percentages were transformed to the arc-sine $\sqrt{p}$ scale as recommended by Bartlett (1947) to better meet the assumptions underlying the analysis of variance. Further a correction was applied for values of 0 and 100 percent as given by Bartlett (1937). Values of 0 were replaced by $\frac{1}{2}n$ and values of 100 by $1-\frac{1}{2}n$, n being the number of eggs set per dam. For percentage data based on denominators of varying numbers, Cochran (1943) suggests elimination from the analysis of all percentages based on a denominator of less than eight to minimise the error from the use of unweighted values. In order to satisfy this requirement, the results of the first two hatches in each year were pooled as these were from the same set of birds (and there was no marked heterogeneity between hatches) and similarly results of hatches 3 and 4 were pooled. Those birds that still failed to satisfy Cochran's restriction were eliminated and the population then available for analysis is shown in Table 63.

The number of hens in each hatch genotype subclass are not very non-orthogonal so that a preliminary analysis of variance was performed between genotypes, between hatches and between hens within genotypes for each year. Where the results were significant or approached significance, the data were analysed by the method of fitting constants (Snedecor 1956). By this method the main effects are correctly estimated only if the interaction term is not significant./

TABLE 63. Number of hens and eggs used in the analysis.

Year	Hatches	Ov.AA,II.AA	Ov.AB,II.AA	Ov.BB,II.AA	Ov.AA,II.AB	Ov.AB,IIAB
1964	1 and 2 No of hens	20	40	28	28	23
	No of eggs	327	677	453	316	387
	3 and 4 No of hens	30	43	42	9	16
	No of eggs	502	693	730	158	272
1965	1 and 2 No of hens	24	37	24	20	23
	No of eggs	364	579	345	315	340
	3 and 4 No of hens	17	52	39	13	18
	No of eggs	276	1010	740	257	351

Total No of hens = 536

Total No of fertile eggs = 9092

significant. In no case was the genotype x hatch interaction term significant, so that a good estimate of the variance of the main effect between genotypes is obtainable without further analysis.

Results:

Early embryonic death: The mean values in angles of the mortality to 6 days is shown for the two years in Table 64. Considering only the ovalbumin genotypes (within the II.AA class) it is seen that in the first year Ov.AA genotypes were inferior whereas in the second year the Ov.BB genotypes were the poorest class. The heterozygotes were intermediate in the first year but were superior to both homozygotes in the second year. The Ov.AA,II.AB class was poor in the first year and markedly inferior in the second year. However, none of these differences were significant at the 5% level as could be seen from the analysis of variance shown in Table 65. In the second year the differences just approached significance at 10% level but no reliance could be placed on this effect.

TABLE 64. Mean values in angles for early embryonic death (EED)

Year	Ov.AA,II.AA	Ov.AB,II.AA	Ov.BB,II.AA	Ov.AA,II.AB	Ov.AB,II.AB
1964	17.43	15.69	14.52	16.67	13.49
1965	16.18	15.83	17.81	20.19	16.26

TABLE 65 Analysis of variance of early embryonic death (EED)

	1964			1965		
	D.F	M.S	F	D.F	M.S	F
Between genotypes	4	112.56	1.45	4	135.8	1.91 +
Between hatches	1	100.1		1	2.38	
Genotype x Hatch interaction	4	29.2		4	49.2	
Error	259	77.45		257	71.1	

+ $P < 0.10$

Dead Germ one: The mortality during the 6th to 12th day period was very small ranging from 1.0 to 2.5 percent and analysis of variance did not reveal any significant differences between genotypes. The mortality during this period was therefore pooled with the EED and the means (Table 66) follow a similar pattern to that of EED. Again only the second year's data approached statistical significance at 10% level. (Table 67).

TABLE 66. Mean value in angles for mortality 1 to 12 days (EED + DG1).

Year	Ov.AA,II.AA	Ov.AB,II.AA	Ov.BB,II.AA	Ov.AA,II.AB	Ov.AB,II.AB
1964	18.15	17.97	16.45	17.99	15.02
1965	17.97	17.42	19.80	21.78	17.56

TABLE 67. Analysis of variance of mortality 1 to 12 days (EED + DG1).

	1964			1965		
	D.F	M.S	F	D.F	M.S	F
Between genotypes	4	53.2	1	4	153.3	1.95 +
Between hatches	1	119.9		1	16.4	
Hatch ~~X~~ genotype interaction	4	56.2		4	67.5	
Error	259	90.0		259	78.4	

+ Significant at $P < 0.10$

Dead Germs two: The completed analysis of the effects of genotypes on mortality from the 13th to 20th day of incubation is shown in Table 68. In 1964, the effect of the egg white loci was significant at the 5% level while in the second year it just approached significance at 10 percent level. From the mean values (in angles) shown in Table 69, two points ^{seem} worthy of comment, Comparison among ovalbumin genotypes (within the II.AA class) shows that the Ov.AA was superior in both years (although it was marginal in 1965) and that the Ov.BB was the poorest class/

class with the heterozygotes intermediate, indicating an additive or partially dominant effect of this locus. The other point of interest is the Ov.AA,II.AB class which proved to be the superior class in 1964 but was markedly inferior in the second year. A large part of the variance between genotypes in both years was due to this genotype.

TABLE 68. Analysis of variance of Dead Germ two (DG2)

	1964(Fitting Constants)			1965		
	D.F	M.S.	F	D.F	M.S	F
Between genotypes	4	322.8	2.60*	4	219.5	1.89 ⁺
Between hatches	1	182.6	1.47	1	738.0	6.38**
Genotype x hatch interaction	4	41.4		4	128.0	1.10
Error	250	124.1		250	115.6	

* Significant at $P < 0.05$

** Significant at $P < 0.01$

TABLE 69. Mean values in angles for Dead Germ two (DG2)

Year	Ov.AA,II.AA	Ov.AB,II.AA	Ov.BB,II.AA	Ov.AA,II.AB	Ov.AB,II.AB
1964	17.48	20.08	22.21	14.47	19.05
1965	19.78	20.18	20.51	25.70	22.09

Pips: This class includes only the embryos that pipped their shells but failed to hatch. Since a large proportion of deaths in the DG2 period occur between the 18th and 20th days, it is doubtful whether 'pips' should be treated as a separate class since quite a large proportion of 'pips' may be of the same age as the DG2 embryos. Therefore the result of 'pips' have been analysed as a separate class and/

and also after pooling the data with DG2 which gives the mortality from 13 days to the end of incubation. Analysis of deaths classified as 'pips' alone does not reveal any significant differences (Table 70). However in the analysis of mortality from 12th day to end of incubation i.e. DG2 + pips, the differences between genotypes just fail to approach significance at 5% level (Table 71). The means (in angles) shown in Table 70 follow a similar pattern to that of DG2 and it appears that those classes that had the highest mortality in DG2 also had the largest percentage of 'pips' and vice versa.

TABLE 70. Analysis of variance of pips (PIP).

	1964			1965		
	D.F.	M.S.	F	D.F.	M.S.	F
Between genotypes	4	97.9	1.37	4	89.3	1.38
Between hatches	1	12.5	1	1	703.8	100.94 ^{**}
Hatch x genotype interaction	4	116.4	1.63	4	55.6	
Error	238	71.0		223	64.3	

^{**} Significant at $P < 0.01$

TABLE 71. Analysis of variance of Dead Germ two + Pips (DG2+PIP)

	1964			1965		
	D.F.	M.S.	F	D.F.	M.S.	F
Between genotypes	4	366.4	2.10 ⁺	4	338.1	2.27 ⁺
Between hatches	1	283.6	1.63	1	1590.1	10.7
Genotype x hatch interaction	4	194.2	1.11	4	206.4	1.3
Error	250	173.7		251	248.6	

⁺ Significant at $P < 0.10$

TABLE 72. Mean values in angles for Dead Germ two + pips (DG2+PIP)

Year	Ov.AA,II.AA	Ov.AB,II.AA	Ov.BB,II.AA	Ov.AA,II.AB	Ov.AB,II.AB
1964	21.79	25.82	25.87	18.56	23.99
1965	23.71	24.11	24.71	31.16	25.75

Total embryonic mortality: The effects of the genotypes on total embryonic mortality, that is the losses throughout incubation as a proportion of fertile eggs set has also been analysed by the same method. The complete analysis of variance is shown in Table 73. The 1964 data show differences between genotypes significant at 2.5 percent while the previous year's data does not show any significant difference. The significant difference in 1965 between genotypes is almost entirely due to the high mortality of the Ov.AA,II.AB class as can be seen from the mean values (Table 74).

TABLE 73. Analysis of variance of Total Embryonic Mortality (TEM)

	D.F	1964		D.F	1965 (Fitting constants)	
		M.S	F		M.S.	F
Between genotypes	4	110.0	1	4	552	3.34*
Between hatches	1	660.7	3.07	1	1007.4	6.02*
Genotype x hatch interaction	4	153.8	1	4	250.1	
Error	259	215.1		257	165.3	

* Significant at $P < 0.05$

TABLE 74. Mean values in angles for Total Embryonic Mortality (TEM)

Year	Ov.AA,II.AA	Ov.AB,II.AA	Ov.BB,II.AA	Ov.AA,II.AB	Ov.AB,II.AB
1964	29.52	31.72	30.58	27.99	29.02
1965	29.86	30.22	32.77	39.10	31.00

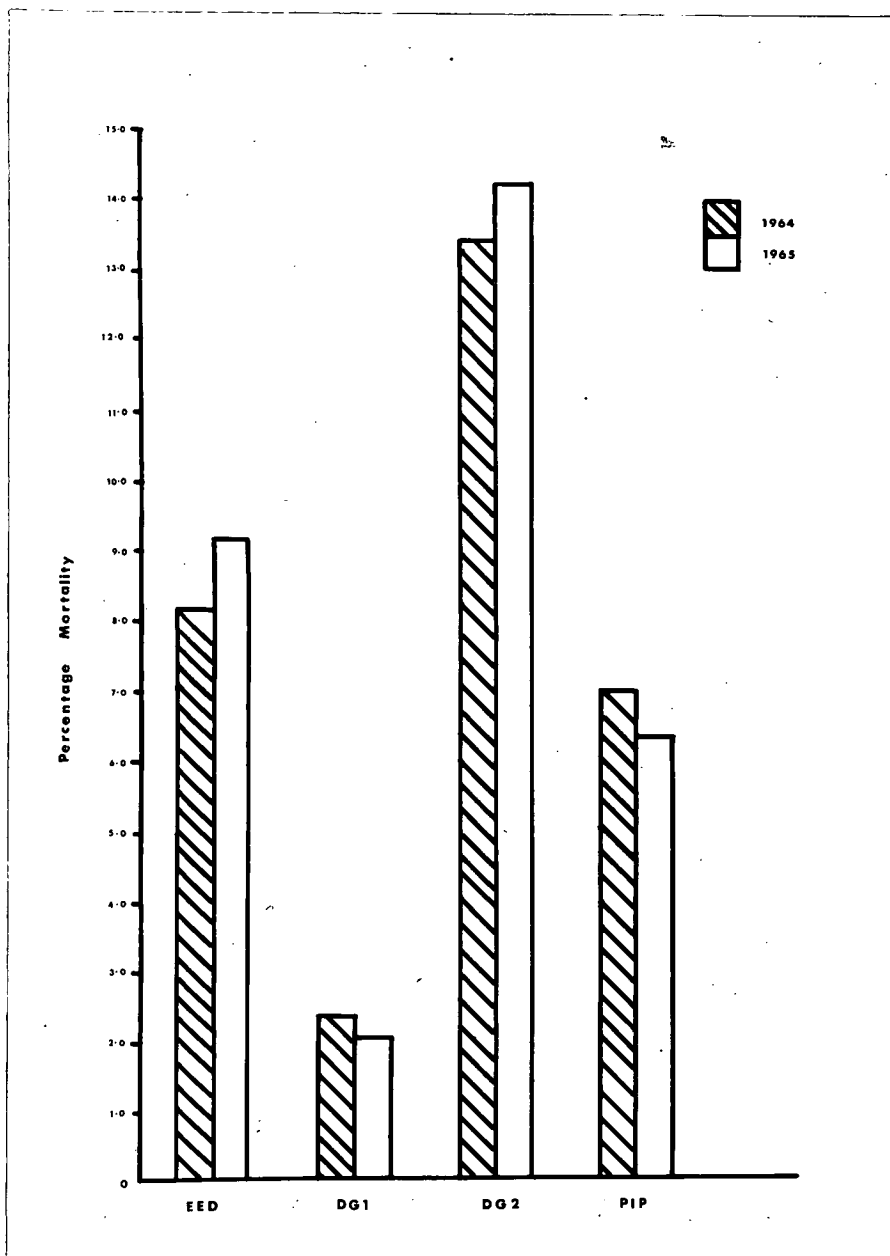


Fig 7. Embryonic mortality at the different periods of incubation during the two years in Strain 2.

Discussion

The only results to reach statistical significance were the effects on the 13th to 20th day mortality in 1964 and on total embryonic mortality during 1965. In both cases the significant effects were caused by Ov.AA,II.AB genotypes, but whereas the effect on DG2 in 1964 was due to this genotype being markedly superior to Ov.BB,II.AA, the effect on TEM in 1965 was due to the high mortality in embryos among dams of this genotype. There thus appears to be marked genotype x year interactions. The total mortality and even the mortality for the different periods was not markedly different in the two years (see Fig.7) but whereas in 1964 bad incubation conditions (improper temperature control) raised the embryonic mortality, the poor hatches in 1965 were ascribed to a rather severe Staphylococcal infection. It is thus not unreasonable to conclude that environmental conditions during the two years were dis-similar and this may account for the genotype x environment interactions. The Ov.AA,II.AB genotypes were fairly similar to Ov.AA,II.AA for total embryonic mortality in 1964 but were markedly inferior to Ov.AA,II.AA in 1965. The II.AB heterozygotes therefore, generally seemed to be inferior to II.AA under the incubation conditions used for this strain and it was therefore decided to examine rather limited data on hatchability from strain 5 birds which showed segregation at only locus II. The results of this strain shown in Table 75, support the findings made earlier in that the II.AB genotypes were again markedly inferior to II.AA at all stages of incubation. Analysis of variance reveals that the differences in EED are significant at 2.5 percent level and the TEM differences at 10 percent level. (Table 76).

TABLE 75. Percentage mortality for different periods of incubation in strain 5 genotypes.

	II.AA	Genotypes AA.AB	II.BB
No of hens	28	39	16
No fertile eggs	389	552	218
EED	4.11	10.69	11.93
DG1	1.07	1.62	1.53
DG2	5.14	12.14	13.30
PIPS	6.85	11.95	7.00
TEM	23.14	34.98	31.65

TABLE 76. Analysis of variance of strain 5 data

	EED			TEM		
	D.F	M.S.	F	D.F	M.S	F
Between genotypes	2	322.7	3.94*	2	559.3	2.5 ⁺
Error	80	81.9		79	224.4	

+ Significant at $P < 0.010$

* Significant at $P < 0.025$

Examination of the data of ovalbumin genotypes only (within the II.AA class) for strain 2 shows that in both years the Ov.AA genotypes were superior to Ov.BB for mortality from 12 days to end of incubation and for TEM though these differences were small and not significant.

The only other investigation carried out with the aim of finding associations between egg white polymorphisms and hatchability has been that of Morton, Gilmour, McDermid & Ogden (1965) with a relatively non-inbred Light Sussex strain. Their results do not deal with the Ovalbumin locus as this was not segregating in their population. The periods of mortality used in their analysis were also slightly/

slightly different. They found statistically significant effects only during the 6 to 15 day period of incubation and these effects were due to the T_f locus and interactions between T_f and III and between T_f , II and III. Their results cannot therefore strictly be used for comparison with the data given above because of lack of common ground except in locus II. In their data the II^B allele was superior (though not significant) for EED and TEM whereas in the present case the reverse was observed with the II.AA homozygote being superior to the II.AB for EED and TEM in strain 2. In strain 5, the II.AA was superior to both II.AB and II.BB genotypes. The reason for this opposite effect in the Light Sussex population of Morton et al and the present one is not clear and can only be ascribed to the different genetic constitution or incubation conditions of the populations.

The apparent failure to find any strong effect of the egg white genotype of the dam on hatchability of her eggs except for the ones mentioned above in the data of Morton et al and the present instance may be due to the fact that the effect of the genotype of the zygote on its ability to survive was not examined. Presumably any effect, if present, may involve an interaction between the genes of the zygote and the maternal albumen environment (dependent on the dam's egg white genotype). The evidence from transferrin polymorphism in cattle and pigs indicates that both these factors may be important in embryonic viability. In cattle, Ashton (1961) and Ashton and Fallon (1962) showed that whilst matings between homozygous genotypes at the T_f locus were more fertile than those involving heterozygotes, heterozygous embryos were more viable in utero than homozygotes.

The/

The latter effect however was not shown to be independent of the maternal genotype. In pigs, Kristjansson (1964) reported a similar high early embryonic mortality in matings of $T_f.BB \times T_f.AB$ genotypes but not among other classes. One may attempt to explain this on the basis of incompatibility between BB embryos and AB dams but this is not supported by data of matings of $T_f.AB \times T_f.AB$ where the embryonic mortality is very low and is in fact the lowest of all mating types. Hence this appears to be a rather specific type of incompatibility. Khattab et al (1964) also observed some disturbance in segregation ratios among progeny in matings involving the transferrin allele T_f^C in sheep.

Similarly, for the egg white loci, if the genotype of the zygote or zygote-maternal incompatibility had any effects on embryonic viability, these should appear as disturbed segregation ratios in the progeny. Therefore it was decided to examine some data available from matings made for linkage studies described earlier. These were all matings of $Ov^{AA} II^{AA} III^{BB} T_f^{bb}$ males with heterozygous females. The results are shown in Table 77. In no case was the observed distribution among the progeny significantly different from the expected ratio. In fact, the agreement between observed and expected numbers was very good. However, it is difficult to draw conclusions from data like this, because several factors may operate to minimise differences in segregation ratios. For example, selective fertilisation and embryonic viability may act antagonistically as shown by Ashton (1961) and Ashton and Fallon (1962) so that in the examination of the final segregation ratio in the progeny, no significant deviations may be observed.

TABLE 77. Distribution of progeny in homozygous δ and heterozygous φ matings.

Locus	No of progeny			χ^2	P
	AA	AB	BB		
Ov	58	61	-	0.074	>0.75
II	106	109	-	0.042	>0.75
III	-	54	42	1.520	>0.10
Tf	-	50	46	0.166	>0.50

Finally, it should be noted that the presence or absence of an effect of these genes on embryonic viability was based on the F value in the significance tests, and following convention, F values lower than those for 95 percent confidence limits were considered not significant. For all or none traits, such as the one considered here it appears that the sensitivity of the test depends on the incidence of the trait concerned. Becker and Bearse (1965) showed that for blood spot incidence in chickens, the sensitivity of the experiment i.e. the F value, was dependent on the incidence of the trait and was higher when the incidence was high. Therefore, where the F values fell just below the level of significance in some of the data considered here, raising the level of the incidence by environmental manipulation may perhaps increase the sensitivity of the tests. The detection by Morton et al (1965) of significant effects on DG1 may also be due to a similar reason, as the mortality in their population during this period was 5.3% compared to 2.2% in the ones considered here.

Summary:

An investigation was made into the association between maternal genotype/

genotype at the obalbumin locus and locus II and embryonic viability in two strains of White Leghorns. Significant effects were found for mortality during the different periods of incubation but these were not consistent in the two strains or for the two generations of the same strain.

PART VI

GENERAL DISCUSSION

It was pointed out in the introduction that this study had a two-fold purpose. Firstly, do the egg white genes have any effects on economic traits in poultry? Secondly, can anything be learnt about the mechanisms maintaining this particular genetic diversity?

The answer to the first question is that some associations were found, but these should be viewed in the light of the following two limitations.

1) Most of the observations apply to only a single generation and it is wrong to generalise from observations made during what may be an atypical season. Environmental conditions, particularly for poultry, may remain fairly uniform over a year, but considerable yearly variations often arise and interactions of these with the observed effects are possible.

2) Some of the polymorphic systems studied were found only in particular populations and not in others. In fact, except for locus II polymorphism which was observed in all three populations, the other variants were seen only in particular individual flocks. It is very probable that the populations have different gene arrays and expression of the polymorphic system may depend on the remainder of the genome (Sheppard, 1955).

Therefore the effects observed must be treated as specific to the populations considered.

The associations observed between the egg white polymorphisms and traits may be summarized as follows:

I) An association between ovalbumin locus and egg weight and body weight was found for one flock. The effect seemed to be of an additive nature since $\overset{A}{A}A$ individuals were heavier than $\overset{B}{B}B$ with the heterozygotes intermediate for both traits.

II) Loci II and Tf have effects on number of eggs laid in two populations. The effect of locus II on this character is primarily one of overdominance, since the heterozygotes are superior to either homozygote. This is however significant only in two populations. On the other hand, the Tf locus shows superiority of one homozygote over its heterozygote. Significant interaction effects were observed for egg number between the two loci and also between ovalbumin and locus II.

III) Loci II and Tf also had significant effects on sexual maturity but these were confined to the Light Sussex flock. The II.A and Tf-b homozygotes matured later than the other corresponding genotypes.

IV) Significant effects were also observed on embryonic mortality which were almost entirely due to locus II. The effect was not consistent during the two years for which this character was studied, the II^A allele being significantly superior in the first year and inferior in the second.

Egg white polymorphisms are therefore found to be associated with significant differences in economic traits, but not necessarily regularly from year to year or flock to flock.

Before answering the second question, let us first examine the mechanisms that could maintain genetic variability in populations under artificial selection and then see whether the observed effects of the egg white genes could explain this polymorphism.

The causes of stable polymorphism in natural populations could briefly be summarized as due to conflicts between (a) selection and mutation (b) Selection and segregation (c) fitness and abundance (d) selection and migration and (e) selection in two directions (Haldane & Jayakar, 1963). Selection in these cases implies natural selection only. When considering domestic populations of animals, additional factors have to be considered, in particular artificial selection. Under artificial selection, some members of the population are allowed to breed in preference to others because they exhibit some phenotypic trait desirable to the breeder. If the selection criterion remains constant from generation to generation, it would lead to fixation of genes whose effects are consistently favourable (to the breeder) and elimination of those that are unfavourable. Any remaining genetic variability is then explicable by one of the following causes.

a) Genetic variability maintained by selection for multiple objectives: Selection for multiple objectives seems to be the universal rule in domesticated populations. For example a breeder attempting to improve egg production of chickens will also select for egg size and hatchability. But the genetic correlation between some of these characters may be negative as between egg production and egg size (Schultz, 1953); large egg size and reproductive fitness (Lerner & Gunns, 1952) or egg production and albumen quality (Dickenson, 1955). This is due to pleiotropic effects of genes having a positive effect on one character and a negative effect on the other. If selection is applied simultaneously

for two or more of these negatively correlated characters the individuals selected would be those that are intermediate. These would be heterozygotes if the genes are additive in their effects (on the characters under selection). Artificial selection for multiple objectives would therefore maintain genetic variability by this 'overdominant' effect of genes controlling different traits.

b) Conflict between natural and artificial selection: - Fisher's (1930) fundamental theorem of natural selection states that the " rate of increase in fitness of any organism at any time is equal to its additive genetic variance in fitness at that time". A population in equilibrium with its present environment should thus have little or no additive genetic variance in fitness. Therefore, when artificial selection alters the mean of a population in either direction, total reproductive fitness may be expected on theoretical grounds to decline. Evidence of this effect of natural selection has been obtained by Lerner & Dempster (1951) in selecting for increased shank length in poultry. There are two stages at which natural selection might operate. First there might be differential fertility or abnormal Mendelian segregation (now called "meiotic drive") and, secondly, differential mortality where the superior phenotypes (under artificial selection) die before they can breed. The net result of these opposing selective forces would be that intermediates (ie; heterozygotes) would be selected, thus maintaining genetic variability.

c) Selection of heterozygotes by both artificial and natural selection:-

As opposed to the conflict between natural and artificial selections, natural selection may reinforce artificial selection by favouring the same genotypes which the breeder selects. This would be expected only for characters closely related to fitness (as a corollary to Fisher's theorem). But, if the favoured genotype is a homozygote, the superior allele will reach fixation, unless opposed by some other selective force. Hence for polymorphisms to persist under these conditions, both selective forces (natural and artificial) should select heterozygotes.

d) Crossbreeding: In poultry breeding, it is a common practice to cross strains or even breeds in the formation of a new strain. The purpose of crossing is to introduce new genetic variation into the original population similar to what happens when migration occurs between two genetically distinct populations. The ensuing polymorphism however is transient, since it is still not in equilibrium:

e) Environmental variation: Under continual environmental flux, the gene combinations which produce maximum total performance changes between successive environments. Under these conditions, genes whose effects are consistently favourable or uniformly unfavourable in the whole range of environments will move towards fixation or elimination. The remaining genetic variability in the population will then depend upon genes having favourable effects in some environments and unfavourable effects in others. A plausible example of this changing environment in poultry is attack by different pathogenic organisms at different times.

Let us now consider the effects of the egg white loci and attempt to explain the mechanisms maintaining genetic diversity of these loci in the light of the causes mentioned above.

a) Ovalbumin locus:- Subpopulations of two of the strains (Strain 2 and Strain 5) studied in the present work are being currently selected for egg weight and body weight (see materials & methods). But since the individuals studied are the first generation progeny of selected parents, it is very unlikely that the gene frequencies would have undergone major changes under this selection pressure. No evidence of the selection procedures used in the past (before they were random bred) is available, but since they originated from commercial strains, it is not unreasonable to conclude that some selection would have been applied for high egg number and large egg size. Since it is also a common commercial practice to 'cull' the weak and small chicks, some unconscious selection would also have been applied for large chick size. On the basis of these assumptions, three explanations of the polymorphism at ovalbumin locus may be given (i) On egg weight and body weight (and possibly chick weight) this locus has an additive effect with the AA homozygote being superior genotype. Artificial selection would therefore attempt to fix the Ov^A allele in the population. On the other hand the BB homozygote and the heterozygote are superior (though not significant) for egg number. Hence, there is a conflict of selection pressure in the two directions under selection for different objectives and this may maintain the polymorphism.

(ii) Rendel (1943) and Lerner & Gunns (1952) have shown that eggs of intermediate size hatch better and from this conclude that natural selection favours individuals producing eggs of intermediate size. This would seem to be true of body weight as well. If so, then natural selection would select the ovalbumin heterozygotes because they are intermediates (for egg size and body size). There would then be conflict of selection pressures between natural and artificial selection leading to polymorphism of this locus.

(iii) It has been shown mathematically (Kimura, 1956) that a locus closely linked to an overdominant locus will be maintained in a polymorphic state even though the ~~first~~ locus itself is additive or neutral in its effect. Since the ovalbumin locus is very closely linked to locus II, linkage itself could explain the polymorphism at Ov. locus if artificial selection makes locus II overdominant.

Locus II : This locus has an overdominant effect on egg number. Since all three populations studied here would have been selected for egg number, artificial selection itself would explain the maintenance of this polymorphism. The effect of natural selection on egg number is not clear. Some authors (Dickerson, 1955) believe that natural selection favours high egg number and explain the regression of egg number on relaxation of selection as being due simply to the break up of epistatic gene combinations (by segregation and recombination). However, if we apply Fisher's fundamental theorem, then the additive genetic variance of

fitness is zero when fitness is at a maximum. But, this does not require that the additive genetic variance for the separate components of fitness must also be zero. Therefore natural selection may not necessarily favour maximum egg production and when artificial selection forces egg production above the optimum for fitness, natural selection may oppose it. But it is doubtful whether the level of egg production in these populations is high enough for negative natural selection. It may be mentioned that in all populations examined (Table, 2, present data and Morton et al, 1965) this locus is polymorphic (except some Poultry Research Centre inbred lines). This widespread polymorphism is perhaps due to its overdominant effect on egg number which is the most important selection criterion in commercial laying flocks.

Besides its effect on egg number, this locus also influences embryonic viability, but it shows a significant interaction in the two years, the heterozygotes being significantly superior in one year and inferior in the second. This cyclic selection of genotypes, selection favouring different genotypes at different times may also maintain polymorphism of this locus. (Haldane & Jayakar, 1963).

Locus III : This locus is only polymorphic in the Light Sussex flock, the other two populations being homozygous for the III^B allele. Even in the Light Sussex flock, the III^A allele is at a low frequency of 0.16 suggesting that the III^B allele is at a selective advantage over the III^A allele and that this has led to fixation of the former in the two White Leghorn populations. A comparison of the heterozygotes with the

homozygous BB birds for egg number in the Light Sussex flock does not reveal any significant differences. The data of Morton et al (1965) on embryonic mortality also do not show any significant effect of this locus. It is therefore not possible to explain why this polymorphism exists from the meagre data available.

Transferrin Locus: This too was polymorphic only in the Light Sussex flock, the other two populations being homozygous for the Tf^b allele. The bb homozygote was superior to its heterozygote for egg number (this difference being significant). Association of this locus with other traits was not studied in this flock because no measurements were taken. However, the data of Morton et al (1965) on embryonic mortality also show only a significant additive effect of this locus, the Tf^b allele again being superior. These two effects cannot therefore explain the polymorphism at this locus, since selection for high egg number and low embryonic mortality would lead to fixation of the Tf^b allele. We have therefore to presume the existence of some balancing selective ~~forces~~ opposing the superiority of bb homozygotes at some other stages in the animal's life.

Some significant interactions between loci were also noted. An inferiority of the individuals heterozygous at both Ov. and locus II for egg number was found in strain 2. Artificial selection, selecting for high egg number, will therefore select fewer of these individuals as parents, but this will not affect the proportion of double heterozygotes

appearing in the next generation unless the two loci are linked. Selection would therefore select some mechanisms such as inversions, translocations or modifying genes to reduce crossing over between these two loci. This may explain the tight linkage between the two loci.

The data on egg white polymorphisms in natural populations of poultry such as the Jungle fowl are very meagre. Among the two Jungle fowls examined by Lush (1961), one exhibited polymorphism at locus II. This means that egg white polymorphisms (at least of locus II genes) are of great antiquity and existed before domestication of the species, began.

Let us now examine other similar studies of polymorphisms in domesticated animals where significant associations were detected, to see if they throw any light on mechanisms maintaining their variability. In poultry, many reports have appeared on relationships between blood group genes and various traits. However, several of these are in abstracts and give no details of statistical analysis or flock history (Briles, Johnson & Garber, 1953; Briles, 1954 and Briles & Krueger, 1955). In this discussion we shall consider only those reports where details of population history are given. Schultz & Briles (1953) studied the relationship of A and B blood group loci on production characters in a flock selected for egg number. They could not demonstrate superiority of genotypes at either locus for any particular trait (including egg number) but observed that the heterozygotes at the A locus occurred in higher frequency in the selected breeders than in the

total population. They concluded that this was because the A locus heterozygotes though not superior in any one special character, were above average for the totality of traits under selection. However, the increased frequency of A locus heterozygotes among the selected parents implies that there is overdominance at this locus under the objectives of artificial selection and explains the polymorphism at this locus. Briles (1956) and Briles and Allen (1962) also studied hatchability, livability and egg number in relation to the B blood group system. The populations they studied were inbred lines used in producing commercial inbred - hybrids. Studies within the different inbred lines showed a

| - superiority of heterozygotes over homozygotes for hatchability and livability while for egg number one homozygote and the heterozygote were superior over the other homozygote. These results though supporting the general idea of heterozygote superiority on fitness traits are not compatible if viewed in the light of selection procedures that may have been applied in these lines. Since the lines are used in the production of inbred-hybrids, we may expect that they would be selected for high cross performance. Then, on the basis of their results, the best crosses should be heterozygous at the B locus and this is possible only if the lines themselves are separately homozygous for the two B alleles. Hence artificial selection of the lines would attempt to make them homozygous for genes at the B locus. Therefore the continued segregation of the B locus genes in these populations against the forces of inbreeding and artificial selection towards homozygosity could only be

explained by opposing natural selection of a rather high magnitude. In a recent study, Morton et al (1965) again observed a superiority of heterozygotes at the B locus for hatchability in a 'slightly inbred' commercial strain of poultry and concluded that this was sufficient to maintain stable polymorphism of this locus in this flock. Gilmour (1960) and De Silva (1965) have also shown fertility differences between genotypes at other blood group loci in studies with the highly inbred Reaseheath lines. The continued segregation of some of these loci against the force of inbreeding must therefore be due to selection applied unconsciously (for characters like fertility) to maintain the inbred lines.

Similar studies with blood group genes in cattle are complicated by the inability to distinguish serologically, the heterozygotes from one class of homozygotes (except in two loci FV+z). Hence comparisons are only possible between one homozygote and the other two genotypes ie; - / - with + / - and + / +. It is therefore not possible to examine the forces maintaining these polymorphisms.

The other investigation of interest is that by Ashton on transferrin polymorphism in cattle. The effect of this locus on milk yield is additive, the D/D cows being superior (Ashton et al, 1964). Since artificial selection in dairy cows lays a great emphasis on milk yield, it will tend to fix the Tr^D allele in the population. But natural selection

opposes this by selecting transferrin heterozygous embryos, these being more viable in utero (Ashton & Fallon, 1962). Therefore the existence of this polymorphism can be explained by the balance between the forces of natural and artificial selection.

Finally, the possibility of using the results of this study in poultry breeding was mentioned in the introduction. It was shown that their importance could be judged by the proportion of the genetic variation between animals that they control. Though significant proportions of the variance within progeny groups were attributable to egg white loci for egg weight, body weight and egg number, these were too small and could have only a limited value in predicting the breeding value of individuals. In this respect, it is without practical importance as compared with the information obtained by more conventional methods.

SUMMARY

Four apparently diallelic loci are known to control the genetic variation in the egg white proteins of the fowl. These loci are Ovalbumin, II, III and transferrin. An examination of the genotype frequencies in a population segregating at Ov. locus and locus II showed non - random association between the two loci suggesting close linkage between them. This was confirmed by a linkage experiment and the recombination distance between the two loci was estimated at less than 1 percent. The linkage relationships of loci III and Tf were also examined. These were not linked to each other or to locus II. It is concluded that the four loci are on three autosomes.

The possible association between these polymorphic genes and five production characters was studied in three closed populations consisting of two flocks of White Leghorns (Strain 2 and Strain 5) each of about 1,000 hens and a Light Sussex flock of 388 hens. The characters studied were: body weights at different ages, egg weights at different ages, embryonic mortality, age at sexual maturity and egg number. Only the last two characters were studied in the Light Sussex flock.

In the two White Leghorn populations, the influence of environment due to time of hatch was eliminated by analysis of deviations from hatch means.

Statistically significant associations were established in the following cases.

- a) The Ov. locus had an additive effect on body weight in strain 2, the Ov.^A allele being superior. The influence on body weight was significant

only on mature body weight (after sexual maturity), the difference between the two homozygotes at 28 weeks being nearly 80 grams. However, it was shown that this effect may in part be attributable to chick weight differences and to differences in age at maturity.

b) Ov. locus had a similar influence on egg weight when the three Ov. genotypes were compared within the II.A class, but when both loci were considered simultaneously, interaction effects were found.

c) An overdominant effect of locus II on egg number was established in strain 5 and the Light Sussex flock. The difference between the heterozygote and the two homozygotes was 2.8 ± 1.11 eggs in the former strain and 12.3 ± 3.0 in the latter. Besides this overdominant effect, strong interactions were found with Ov and Tf loci.

By sampling the daughters with the highest or lowest values for these traits within each sire progeny group, these direct effects were confirmed, since the number of pullets of Ov.A was higher in the high body weight group than in the low group, while Ov.B was most frequent in the low body weight group and similarly for the other characters.

The effect on embryonic mortality was examined on two years' data in strain 2. A significant locus II effect was found affecting mortality in the latter part of incubation, but whereas in one year, the heterozygote was significantly superior, it was inferior in the second.

The variance controlled by these loci was estimated from variance components. In no case, was the proportion of variance due to these loci (as a fraction of the variance within progeny groups) large enough to be of practical importance.

Appendix Ia.

Determining optimum progeny size for linkage experiments.

In most experiments, the limit is set by the number of animals that can be studied. If T is the total number of progeny that could be studied, n the number of progeny per dam and N , the number of dams, then $nN=T$. We could either have small progeny groups from a large number of dams or vice versa for T constant. The former is desirable from a practical standpoint, since small progeny groups can be obtained from a few hatches so that brooding and rearing need not be protracted.

It is obvious that large progeny groups per dam, even though N is small, would give more information for a limited T . But the increase of information may not be linear with the increase in size of the progeny groups and hence it is desirable to obtain an optimum size from the viewpoint of experimental accuracy and minimising labour.

The method used to obtain this size is based on the method of likelihood ratio devised by Haldane and Smith (1947), for estimating linkages in matings of unknown phase in humans. It consists of determining the amount of information available in a collection of data on two loci and comparing the probability of obtaining such data if the two loci are linked with the probability if they are not. The ratio of these two probabilities gives the odds for or against linkage. If, for example, the probability of obtaining the observed distribution of two characters on a pedigree is much higher if the genes are linked than if they are not, then the odds are obviously in favour of linkage/

linkage. The probability ratio is calculated for different recombination values and the recombination value that gives the highest probability that the observed distribution will be obtained is also the best estimate of the degree of linkage between them.

From a backcross mating of AA.BB x Aa.Bb, four classes of progeny can be obtained.

- a) Ab/AB
- b) aB/AB
- c) ab/AB
- d) AB/AB

In a single mating, let

$$n_1 = a + b \text{ classes}$$

$$n_2 = c + d \text{ classes}$$

$$\text{and } n = n_1 + n_2$$

Then if the dam is Ab/AB, the probability of producing a single cross-over gamete is $\frac{1}{2} \chi$, where χ is the recombination value. Therefore the probability of obtaining n_2 cross-over progeny is $(\frac{1}{2} \chi)^{n_2}$. Similarly, the probability of obtaining n_1 non cross-over types is $\frac{1}{2} \cdot (1-\chi)^{n_1}$. Therefore the total probability of obtaining this particular sibship from a dam of the postulated type is

$$(\frac{1}{2} \chi)^{n_2} \cdot \frac{1}{2} (1-\chi)^{n_1}$$

Similarly, if the dam is of the coupling type AB/ab, the probability of obtaining this same sibship would be

$$(\frac{1}{2} \chi)^{n_1} \cdot \frac{1}{2} (1-\chi)^{n_2}$$

Therefore the total probability of obtaining this sibship from either/

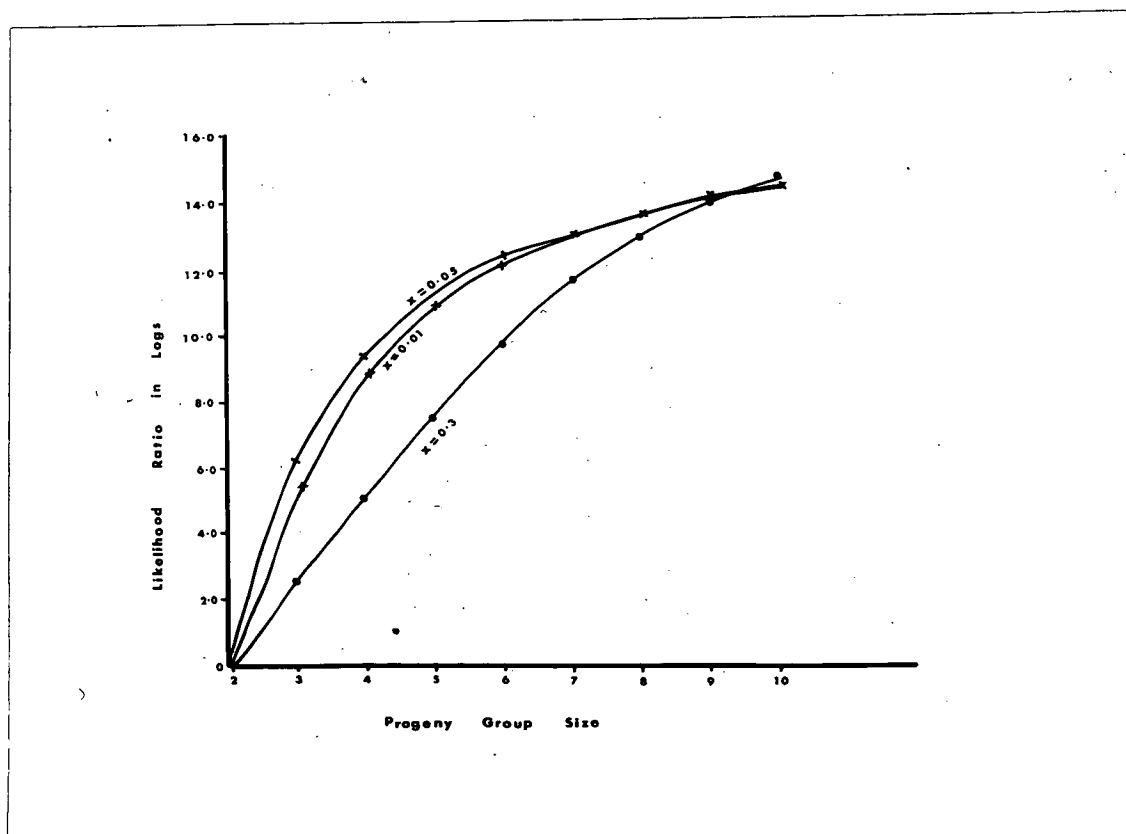


Fig 8. Graphical representation of the accuracy of linkage estimation with increasing progeny size in backcross matings of unknown phase. (X = recombination value).

either a coupling or repulsion gamete is the sum of the two probabilities, namely

$$P_1 = \left(\frac{1}{2}x\right)^{n_1} \cdot \frac{1}{2}(1-x)^{n_2} + \left(\frac{1}{2}x\right)^{n_2} \cdot \frac{1}{2}(1-x)^{n_1}$$

We now make $x=0.5$ and calculate the probability P_2 of obtaining this same sibship

$$\begin{aligned} P_2 &= \left(\frac{1}{2} \cdot 0.5\right)^{n_1} \cdot \left(\frac{1}{2} \cdot 0.5\right)^{n_2} + \left(\frac{1}{2} \cdot 0.5\right)^{n_2} \cdot \left(\frac{1}{2} \cdot 0.5\right)^{n_1} \\ &= 2 \left\{ (0.25)^{n_1} \cdot (0.25)^{n_2} \right\} \end{aligned}$$

The odds in favour of a linkage value of x as against no linkage, for this sibship is the ratio of these two probabilities

$$P = P_1/P_2$$

If we assume that all progeny groups are of equal size, then the total probability for N such sibships is equal to $(P)^N$.

The value of the total probability P^N could be calculated keeping T , the total number constant at 100 and varying n and N . This has been done for recombination values of 1%, 5% and 30%. The results are graphically plotted in Fig.8. It is apparent from the figure that for low recombination values of 1.0 to 5.0 percent, there is a clear gain in increasing from $n=2$ to $n=4$, but after this, though there is a gain with increasing n , the gain is much smaller in proportion to the earlier increase. However, with high recombination values of, say 30 percent, the gain is almost linear with n , so that it would certainly be advantageous to have large progeny groups.

Appendix I.b

Analysis of linkage data.

The details of the analytical method given below are

due to Professor D.J. Finney (personal communication).

Let x be the recombination value. Since the coupling or repulsion phase of the parents is not known, x and $(1-x)$ cannot be distinguished. Hence in the first instance one must estimate $\theta = (1-x)x$ and then take the smaller root of a quadratic equation to give a value of θ less than 0.5

$$x = \frac{1}{2} \cdot \left[1 - (1-4\theta)^{\frac{1}{2}} \right]$$

In a progeny of size n , let n_1 and $(n-n_1)$ be the numbers in the two classes. Calculate

$$y = n_1(1-n_1)$$

Two unbiased estimates of θ can be based on y .

$$\text{These are } t_1 = \sum \frac{Y}{n-1} / \sum n$$

$$\text{and } t_2 = \sum Y / \sum n(n-1)$$

Where $\sum$ denotes summation over all progeny with n greater than 1. Progenies with $n=1$ must be omitted as they contribute no information when coupling-repulsion phase is unknown. The variances of the estimators will be

$$V(t_1) = \frac{\theta(1-4\theta)}{n} + \frac{2\theta^2}{(n)^2} \cdot \sum \frac{n}{n-1}$$

$$V(t_2) = \frac{\theta(1-4\theta) \cdot \sum n(n-1)^2}{[\sum n(n-1)]^2} + \frac{2\theta^2}{\sum n(n-1)}$$

t_1 is a better estimator of θ if θ is small as it will then have the smaller variance; if θ is near to its maximum of 0.25, t_2 is preferable. As an approximate rule, t_1 will be preferable if

$$\theta < \frac{\bar{n}-1}{4\bar{n}-2} \text{ where } \bar{n} \text{ is the average size of}$$

progeny.

Using this method for the second linkage experiment involving loci II, III and Tf (Table 11) we have after omission of family/

family K39 (which has only 1 progeny), 7 families

$$n = 87 \text{ and } \bar{n} = 12.4$$

$$\frac{\bar{n}-1}{4\bar{n}-2} = 0.2448$$

Therefore t_1 will be preferable if $\theta < 0.2448$, and otherwise t_2 will be preferable. The estimates of t_1 and t_2 and their standard errors are as follows.

	t_1	t_2	s.e(t_1)	s.e(t_2)
Loci II and III	0.2324	0.2410	0.0172	
Loci II and Tf	0.2562	0.2571		0.0109
Loci III and Tf	0.2445	0.2400	0.0135	

For loci II and Tf since t_1 and t_2 exceed 0.25, the variance has been calculated on the basis of $\theta=0.25$.

The recombination values are calculated by substituting t_1 or t_2 for χ in the following formula, $\chi = \frac{1}{2} \cdot \left\{ 1 - (1-4\theta)^{\frac{1}{2}} \right\}$

The following results are obtained

	= Recombination value	s.e
Loci II and III	0.367	0.065
Loci II and Tf	0.500	-
Loci III and Tf	0.426	0.091

For significance tests it is preferable to rely on comparing t_1 or t_2 with the null hypothesis of $\theta = 0.25$ using their standard errors. When this is done, it is clear that all three values of t are fully consistent with a hypothesis of no linkage.

APPENDIX II

A search for genetic variants of Glucose - 6 - phosphate dehydrogenase and 6 - phosphogluconate dehydrogenase enzymes in poultry.

This work was prompted by the hypothesis put forward by Lyon (1961&1962) to explain the mechanism of dosage compensation of x-chromosome activity in mammals. As the evidence (Cock, 1964) did not seem to apply to the fowl, it was decided to investigate if x-chromosome inactivation occurred in the fowl using an enzyme system. To do this, it is necessary to locate some gene on the x chromosome (this is only possible if genetic Variation is present) and then measure its product in the two sexes to see if the amount produced depends on whether the individual has one or two such genes. Since it is known that the genes controlling glucose - 6 - phosphate dehydrogenase (G-6-PD) are located on x chromosome in both humans and *Drosophila* (Boyer et al 1962 and Young et al, 1964), it was decided to search for variants of this enzyme and if present, to examine if they were also sex linked in fowls.

Materials & methods : 3 ml of blood is drawn from the wing vein into a heparinised syringe or mixed with 0.05cc of saturated aqueous E D T A (Ethylene - diamine - tetra-acetic acid). The cells are spun down, washed two to three times in 0.9% saline and dispersed in about 5 ml of water. After allowing about 10 minutes for haemolysis, the haemolysate is centrifuged at 10,000 G for 30 minutes. The supernatant haemolysate is either used immediately for electrophoresis or stored

at -15°C .

The electrophoretic procedure is the same as before with slight modifications. The electrolyte concentrations are as follows:

0.076 M Tris (hydroxy methyl) Amino methane
Gel buffer 0.005 M Citric Acid
0.27 M Sodium E D T A

0.3 M Boric acid
Tray buffer 0.05 M Sodium hydroxide

The cathodic reservoir compartment nearest the gel contains in addition 10 mM N A D P.

The gel is prepared in the usual way but before pouring gel into the tray, 1.75 ml of 1mM N A D P is added and mixed thoroughly with the gel. After electrophoresis, the sliced gels are developed in the dark at 30°C for 4 hours in a solution containing

0.5 M	Tris Hcl, p^{H} 8.0	10.0 ml
0.5 M	Mg cl_2	1.0 ml
0.17 M	KCN	10.0 ml
20.0 mM	Glucose - 6 - phosphate	5.0 ml
10.0 mM	N A D P	0.5 ml
0.5%	methylene blue	0.9 ml
0.03%	Nitro BT	15.0 ml
	Water	45.0 cc.

The reaction is stopped by immersion of the gel in cold tap water. The methylene blue diffuses out during storage, leaving the reduced bands

Fig. 9. Variations in G-6-PD enzyme in a starch gel electrophoretogram.

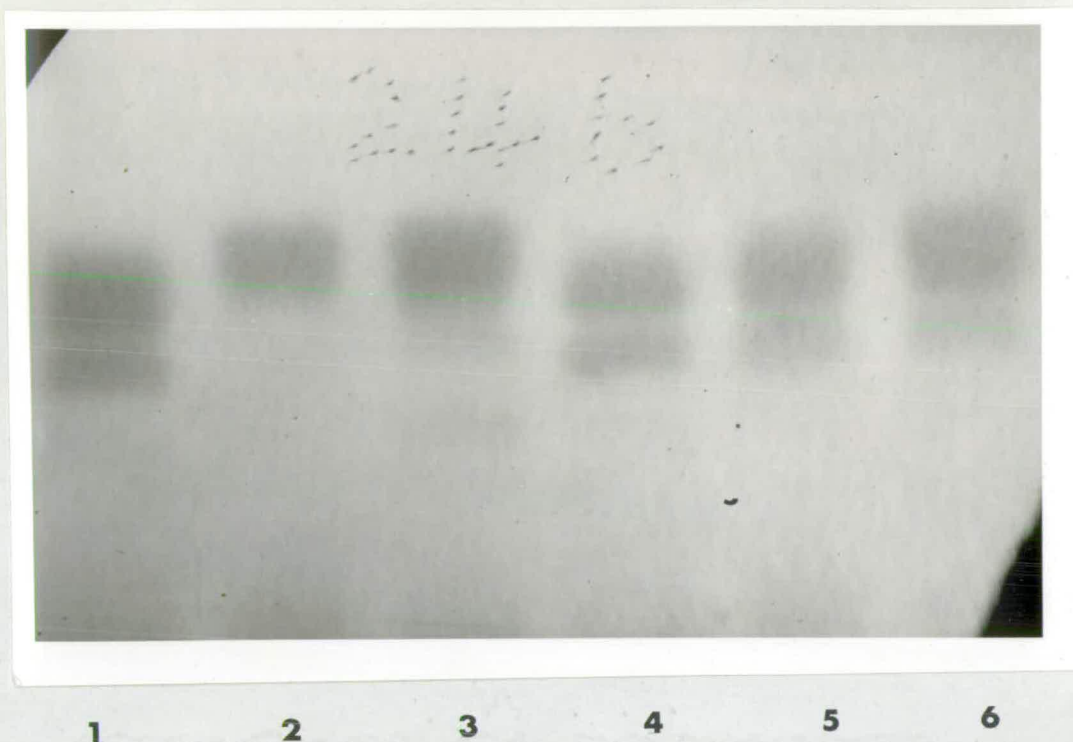


Fig 10. A typical starch gel electrophoretogram of 6 - P GD enzyme.



of purple tetrazolium against a white background.

Results:- Two distinct fractions of G - 6 - P D migrating anodically to the fast haemoglobin band are seen after electrophoresis. The faster A band is broader and slightly more intensely staining than the slower B band (Fig 9, samples 4,5 & 6).

Soon after the survey was started, electrophoretic variation in the pattern of G - 6 - PD was observed (Fig 9, samples 2 & 3). The main difference was an absence of the B band and slightly faster migration of the A band but intermediate types were also seen. However these variations were observed between as well as within individuals. The following possible causes of within individual variation were listed and each was examined by proper controlled experiments.

- a. Physiological change in the bird (ie; variation between samples taken on different days).
- b. Ageing of the samples under storage.
- c. Effect due to anticoagulant.
- d. Tissue damage while drawing blood samples.
- e. Presence of buffy coat (leucocytes) in haemolysates.

The variation was not ascribable to any one of the listed reasons and at this stage, it was decided to abandon the study due to lack of time.

While carrying out the above survey for G - 6 - PD, it was also decided to look for variants of 6 - phosphogluconate dehydrogenase, the next enzyme in the metabolic pathway. Genetic variants of this enzyme

have already been reported in the humans (Fildes & Parr, 1963).

The conditions for electrophoresis were exactly the same as before. The enzyme was located by overlaying the gel with the following mixture and incubating at 30°C for 4 hours.

0.5 M Tris Hcl, p ^H 8.0	5.0 ml
0.5 M MgCl ₂	0.5 ml
10.0 mM N A D P	0.5 ml
0.5 % Methylene blue	0.45 ml
'MTT' Tetrazolium Salt	10.0 mgs
Cyclo hexyl ammonium 6 phospho gluconate	25.0 mgs
Water	22.5 cc

In a preliminary experiment, the enzyme was located at 4.5 cms from the slow haemoglobin band, when the brown line boundary in the gel had moved 12.5 cms from the origin. For future experiments therefore, the slow haemoglobin band was used as an indicator and only a narrow band of gel, 3 cm in width was used for staining (to reduce wastage of the expensive substrate & coenzyme).

Twenty five birds were surveyed consisting of 10 Light Sussex hens (from the strain used earlier), 10 Bush crossbred cockerels (Cock, 1963) and 5 Poultry Research Centre Brown Leghorns. All showed only a single narrow band (Fig 10) with no genetic variation.

APPENDIX III

Stability of gene frequencies in strain 2 random control line.

In populations subjected to artificial selection, in order to obtain genetic gains under selection, it is necessary to have an estimate of the random yearly environmental influences. This is measured by the changes in an unselected random control line. The value of the control population, however, is based on the assumption that the frequencies of the genes in this population remain relatively stable from generation to generation. It will therefore be useful to check the frequencies of genes at a few segregating loci in the control population, to see if the random yearly changes are too large to be due to chance alone.

The strain 2 population used in this study has a control population which is maintained by random mating with a deliberate avoidance of close matings. The entire 1963 generation and 80 percent of the 1964 generation have been classified on the basis of their egg white genotypes for other studies. It therefore seemed worthwhile to investigate whether the change in gene frequencies at the Ov.locus and locus II from 1963 to 1964 are within the bounds of chance fluctuations. This is done by comparing the deviation in gene frequency (δq) between the two years with the standard error of gene frequency change ($\sqrt{V\delta q}$). Crow and Morton (1955) give

$$\sqrt{V\delta q} = \frac{p(1+p)}{2N}$$

where p is the parental gene frequency, N is the effective number of breeders and there is no inbreeding in the population. The

95 percent confidence limits for each yearly gene frequency change are $\pm 1.96 \sigma \delta q$. If these limits are wider than the observed change in gene frequency the hypothesis that the change is due to random chance is accepted.

The 1964 generation are the progeny of 70 males and 45 females (the larger number of males used being due to multiple shifts). The equivalent number of 'effective breeders' obtained by using Wright's formula (1931).

$$\frac{1}{N_e} = \frac{1}{4N_m} + \frac{1}{4N_f} \quad (N_m = \text{No of males and } N_f = \text{No of females})$$

is 110. Using this value, the standard error is calculated for each locus. The change in frequency at the two loci compared with their standard errors is shown in the following table. It is seen that both values of δq lie well within the limits.

Year	Locus I			Locus II		
	s.e		s.e	s.e		s.e
1963	0.468			0.865		
1964 [?] 1963	0.451	0.017	0.034	0.890	0.025	0.023

Since no significant change in gene frequencies is found, it appears that the maintenance procedures used with this random-bred control population have been successful in minimizing gene frequency changes at these two loci during the period considered here. However no conclusion can be drawn from this about genes affecting other traits, particularly those which are strongly influenced by natural selection.

REFERENCES

- Abplanalp, H. & Kosin, I.L. 1953. Genetic variations of fertility and hatchability in the Broad Breasted Bronze turkey. *Poult. Sci.* 32, 321.
- Allison, A.C. 1954. Notes on Sickle-cell polymorphism. *Ann. Hum. Genet.* 19, 39.
- Aschaffenburg, R. 1961. Inherited casein variants in cow's milk. *Nature (Lond.)*, 192, 431.
- _____. 1963. Inherited casein variants in cow's milk. II. Breed differences in the occurrence of beta-casein variants. *J. Dairy Res.* 30, 251.
- _____ & Drewry, J. 1955. Occurrence of different beta-lactoglobulins in cow's milk. *Nature (Lond.)*, 176, 218.
- _____ & _____. 1957. Genetics of the beta-lactoglobulins of cow's milk. *Nature (Lond.)*, 180, 376.
- Ashton, G.C. 1958a. Genetics of beta-globulin polymorphism in British cattle. *Nature (Lond.)*, 182, 370.
- _____. 1958b. Further beta-globulin phenotypes in sheep. *Nature (Lond.)*, 182, 1101.
- _____. 1959. Beta-globulin alleles in some zebu cattle. *Nature (Lond.)*, 184, 1135.
- _____. 1960a. Beta-globulin polymorphism and economic factors in dairy cattle. *J. agr. Sci., Camb.* 54, 321.
- _____. 1960b. Thread protein and beta-globulin polymorphism in the serum proteins of pigs. *Nature (Lond.)*, 186, 991.
- _____. 1961. Beta-globulin type and fertility in artificially bred dairy cattle. *J. Reprod. Fert.* 2, 117.
- _____. 1964. Serum albumin polymorphism in cattle. *Genetics*, 50, 1421.
- _____. 1965. Serum amylase (thread protein) polymorphism in cattle. *Genetics*, 51, 431.
- _____ & Fallon, G.R. 1962. Beta-globulin type, fertility and embryonic mortality in cattle. *J. Reprod. Fert.* 3, 93.
- _____ & Fergusson, K.A. 1963. Serum transferrins in Merino sheep. *Genet. Res., Camb.* 4, 240.
- _____, Fallon, G.R. & Sutherland, D.N. 1964. Transferrin (beta-globulin) type and milk and butterfat production in dairy cows. *J. agr. Sci., Camb.* 62, 27.
- Augustinsson, K. & Olsson, B. 1961. Genetic control of arylesterase in the pig. *Hereditas*, 50, 1.
- Baker, C.M.A. & Marwell, C. 1962. Molecular genetics of avian proteins. I. The egg white proteins of the domestic fowl. *Br. Poult. Sci.* 3, 161.
- Bangham, A.D. & Blumberg, B.S. 1958. Distribution of electrophoretically different haemoglobins among some cattle breeds of Europe and Africa. *Nature (Lond.)*, 181, 1551.

- Bartlett, M.S. 1937. Some examples of statistical methods of research in agriculture and applied biology. *Jl R. statist. Soc.* 4 (Suppl.), 137.
- _____. 1947. The use of transformations. *Biometrics*, 3, 39.
- Becker, W.A. & Bearse, G.E. 1965. All or none traits and the sensitivity of experiments. *Nature (Lond.)*, 205, 110.
- Bell, K. 1962. One dimensional starch gel electrophoresis of bovine skim milk. *Nature (Lond.)*, 195, 702.
- Bhattacharya, S.D., Choudhury, A.K.R., Sinha, N.K. & Sen, A. 1963. Inherited alpha-lactalbumin and beta-lactoglobulin polymorphism in Indian zebu cattle. Comparison of zebu and buffalo alpha-lactalbumins. *Nature (Lond.)*, 197, 797.
- Boyer, S.H., Porter, I.H. & Weilbacher, R.G. 1962. Electrophoretic heterogeneity of G-6-PD and its relationship to enzyme deficiency in man. *Proc. Nat. Acad. Sci.* 48, 1868.
- Braend, M. 1964. Serum types of Norwegian horses. *Nord. vet. Med.* 16, 363.
- Briles, W.E. 1954. Evidence for overdominance of the B blood group alleles in the chicken. *Genetics*, 39, 961.
- _____. 1956. The relationship between B blood group genotypes and adult performance in two White Leghorn inbred lines. *Poult. Sci.* 35, 1134.
- _____. 1958. A new blood group system, E, closely linked with the A system in chickens. *Poult. Sci.* 37, 1189.
- _____. 1960. Blood groups in chickens and their utilisation. *World's Poult. Sci. J.* 16, 223
- _____. 1964. Current status of blood groups in domestic birds. *Z. Tierz. ZuchtBiol.* 79, 371.
- _____. & Allen, C.P. 1962. The B blood group system of chickens. II. The effects of genotype on livability and egg production in seven commercial inbred lines. *Genetics*, 46, 1273.
- _____. , _____, & Millen, T.W. 1957. The B blood group system of chickens. I. Heterozygosity in closed populations. *Genetics*, 42, 631.
- _____. , Johnson, L.W. & Garber, M.J. 1953. The effect of heterozygosity at the blood group locus B on weights at 9 weeks of age in related inbred lines of White Leghorns. *Poult. Sci.* 32, 890.
- _____. & Krueger, W.F. 1955. The effect of parental B blood group genotypes on hatchability and livability in Leghorn inbred lines. *Poult. Sci.* 34, 1182.
- Brncic, D. 1954. Heterosis and the integration of genotype in geographical populations of *Drosophila pseudo-obscura*. *Genetics*, 39, 77.
- Brummerstedt-Hansen, E., Moustgaard, J. & Møller, I. 1963. Serum transferrin polymorphism in Danish cattle breeds. *R. vet. agr. Coll. Copenhagen Yrbk.* p.13
- Clark, J.R. 1963. A genetically varying minor protein constituent of chicken egg white. *J. biol. Chem.* 238, 1732.

- Cochran, W.G. 1943. Analysis of variance for percentages based on unequal numbers. *J. Am. stat. Ass.* 38, 287.
- Cochrane, D. & Annan, E. 1962. Electrophoretic differences in the egg white proteins of the hen. *Canad. J. Biochem. Physiol.* 40, 1335.
- Cock, A.G. 1956. Segregation of hypostatic colour genes within inbred lines of chicken. *Poult. Sci.* 35, 504.
- _____. 1963. Genetical studies on growth and form in the fowl. *Genet. Res., Camb.* 4, 167.
- _____. 1964. Dosage compensation and sex chromatin in non-mammals. *Genet. Res., Camb.* 5, 354.
- Crittenden, L.B. & Bohren, B.B. 1961. The genetic and environmental effects of hatching time, egg weight and holding time on hatchability. *Poult. Sci.* 40, 1736.
- Crosby, J.L. 1949. Selection for an unfavorable gene complex. *Evolution*, 3, 212.
- Crow, J.F. 1963. The concept of genetic load. A reply. *Am. J. Hum. Genet.* 15, 310.
- Crow, J.F. & Morton, N.E. 1955. Measurement of gene frequency drift in small populations. *Evolution*, 9, 202.
- Datta, S.P., Stone, W.H., Tyler, W.J. & Irwin, M.R. 1965. Cattle transferrins and their relation to fertility and milk production. *J. Anim. Sci.* 24, 313.
- Dawson, T.J. & Evans, J.V. 1962. Haemoglobin and erythrocyte potassium type in sheep and their influence on oxygen dissociation and haemoglobin denaturation. *Aust. J. Biol. Sci.* 15, 371.
- De Silva, P.L.G. 1965. Heterozygosity at red cell antigen locus L and fertility in chickens. *Genetics*, 51, 41.
- Dickerson, G.E. 1955. Genetic slippage in response to selection for multiple objectives. *Cold Spring Harb. Symp. Quant. Biol.* 20, 213.
- Dobzhansky, T. 1951. *Genetics and the Origin of Species*. New York, Columbia Univ. Press.
- Dobzhansky, T. 1965. Genetic diversity and fitness. *Genetics Today*, Vol.3 (ed. S.J.Geerts). London, Pergamon Press.
- Dunn, L.C. 1957. Evidence of evolutionary forces leading to the spread of lethal genes in wild populations of house mice. *Proc. Nat. Acad. Sci.* 43, 158.
- Erasmus, J.E. 1954. Relations of yolk, albumen and egg weight in inbred lines of Brown Leghorns. *J. agr. Sci., Camb.* 45, 32.
- Evans, J.V. & King, J.W.B. 1955. Genetic control of sodium and potassium concentrations in the red blood cells of sheep. *Nature (Lond.)*, 176, 171.
- _____, Harris, H. & Warren, F.L. 1958. The distribution of haemoglobin and blood potassium types in British breeds of sheep. *Proc. R. Soc. B.* 149, 249.

- Feeney, R.E., Abplanalp, H., Clary, J.J., Edwards, D.L. & Clark, J.R. (1963). A genetically varying minor protein constituent of chicken egg white. *J. biol. Chem.* 238, 1732.
- Fildes, R.A. & Parr, C.W. 1963. Human red cell phosphogluconate dehydrogenases. *Nature (Lond.)*, 200, 890.
- Fisher, R.A. 1930. The genetical theory of natural selection. London, Oxford University Press.
- _____. 1939. Selective forces in wild populations of *Paratettix texanus*. *Ann. Eugen.* 9, 109.
- Ford, E.B. 1940. Polymorphism and taxonomy. *The New Systematics* (ed. J. Huxley). Oxford, Clarendon Press.
- _____. 1945. Polymorphism. *Biol. Rev.* 20, 73.
- _____. 1964. *Ecological Genetics*. London, Methuen & Co. Ltd.
- Ford EHR & Woollam, D.H.M. 1964. Testicular chromosomes of *Gallus domesticus*. *Chromosoma (Berl.)*, 15, 568.
- Forsyth, R.H. & Foster, J.F. 1950. Egg white proteins. I. The electrophoretic studies on whole white. *J. biol. Chem.* 184, 377.
- Fox, T.W., Crawford, R.D. & Smyth, J.R. Jr. 1964. Rose Comb: an example of operational overdominance in the domestic fowl. *Genet. Res., Camb.* 4, 379.
- Gahne, B. 1963. Inherited variation in the post albumins of cattle serum. *Hereditas*, 50, 126.
- Gilmour, D.G. 1960. Blood groups in chickens. *Br. Poult. Sci.* 1, 75.
- Haldane, J.B.S. & Jayakar, S.D. 1963. Polymorphism due to selection of varying direction. *J. Genet.* 58, 237.
- _____. & Smith, C.A.B. 1947. A new estimate of the linkage between the genes for colour blindness and haemophilia in man. *Ann. Eugen.* 14, 10.
- Hamburger, V. 1951. A series of normal stages in the development of the chick embryo. *J. Morph.* 88, 49.
- Harris, H. & Warren, F.L. 1955. Occurrence of electrophoretically distinct haemoglobins in ruminants. *Biochem. J.* 60, 20P.
- Harvey, W.R. 1960. Least squares analysis of data with unequal subclass number. *Agr. Res. Serv. Publ., U.S. Dept. Agr. ARS-* 20-8.
- Herzenberg, L.A. 1964. A chromosome region for gamma 2a and beta 2a globulin H chain isoantigens in the mouse. *Cold Spring Harb. Symp. Quant. Biol.* 29, 455.
- Hesselholt, M. 1963. Haptoglobin polymorphism in pigs. *Acta vet. Scand.* 4, 238.
- Hiraizumi, Y. 1964. Prezygotic selection as a factor in the maintenance of variability. *Cold Spring Harb. Symp. Quant. Biol.* 29, 51.
- Horowitz, N.H. & Fling, M. 1956. Units of biological structure and function. *Enzymes* (ed. O.H. Gaebler), p.139. New York, Academic Press.

- Huisman, T.H.J., Van Vliet, G. & Sebens, T. 1958a. Sheep haemoglobins. I. Some genetic and physiological aspects of two different adult haemoglobins in sheep. *Nature (Lond.)*, 182, 171.
- _____. 1958b. Sheep haemoglobins. II. Haemoglobin types in different species of sheep. *Nature (Lond.)*, 182, 172.
- Hutt, F.B. 1964. *Animal Genetics*. New York, Ronald Press Co.
- Imlah, P. 1964. Inherited variants in serum ceruloplasmins of the pig. *Nature (Lond.)*, 203, 658.
- Ingram, V.M. 1961. *Haemoglobin and its abnormalities*. Springfield, Illinois, C.C. Thomas.
- Khattab, A.G.H., Watson, J.H. & Axford, R.F.E. 1964. Associations between serum transferrin polymorphism and disturbed segregation ratios in Welsh Mountain Sheep. *Anim. Prod.* 6, 207.
- Kiddy, C.A., Johnston, J.O. & Thompson, M.P. 1964. Genetic polymorphism in caseins of cow's milk. I. Genetic control of alpha-casein variation. *J. Dairy Sci.* 47, 147.
- Kimura, H. 1956. A model of a genetic system which leads to closer linkage by natural selection. *Evolution*, 10, 278.
- King, J.W.B., Aschaffenburg, R., Kiddy, C.A. & Thompson, M.P. 1965. Non independent occurrence of alphas₂ and beta-casein variants of cow's milk. *Nature (Lond.)*, 206, 324.
- King, J.W.B., Evans, J.V., Harris, H. & Warren, F.L. 1958. The performance of sheep with differing haemoglobin and potassium blood types. *J. Agr. Sci., Camb.* 51, 342.
- Kristjansson, F.K. 1960. Genetic control of two blood serum proteins in swine. *Canad. J. Genet. Cytol.* 2, 295.
- _____. 1963. Genetic control of two pre-albumins in pigs. *Genetics*, 48, 1059.
- _____. 1964. Transferrin types and reproductive performance in the pig. *J. Reprod. Fert.* 8, 311.
- Lamotte, M. 1959. Polymorphism in natural populations of Cepaea nemoralis. *Cold Spring Harb. Symp. Quant. Biol.* 24, 65.
- Law, G.R.J. 1963. Independence of genetic variants of egg white proteins and blood group alleles of the A and B systems of the chicken. *Genetics Today, Vol.1* (ed. S.J. Geerts). London, Pergamon Press.
- Lerner, I.M. 1951. Natural selection and egg size in poultry. *Am. Nat.* 85, 365.
- _____. 1954. *Genetic Homeostasis*. New York, John Wiley & Sons Inc.
- _____. 1958. *The genetic basis of selection*. New York, John Wiley & Sons Inc.

- Lerner, I.M. & Dempster, E.R. 1951. Attenuation of genetic progress under continued selection in poultry.
Heredity, 5, 75.
- _____ & Gunns, C.A. 1952. Egg size and reproductive fitness.
Poult. Sci. 31, 537.
- Lowry, D.C. & Schultz, F. 1959. Testing association of metric traits and marker genes. Ann. Hum. Genet. 23, 83.
- Lush, I.E. 1961. Genetic polymorphisms in the egg albumen proteins of the domestic fowl. Nature (Lond.), 189, 981.
- _____. 1963. A biochemical and genetic analysis of haemoglobin, plasma protein and egg albumen types in the fowl.
Ph.D. Thesis, Edinburgh Univ.
- Lyon, M.F. 1961. Gene action in the X chromosome of the mouse (Mus musculus L.). Nature (Lond.), 190, 372.
- _____. 1962. Sex chromatin and gene action in the mammalian X chromosome. J. Hum. Genet. 14, 135.
- Mather, K. 1955. Polymorphism as an outcome of disruptive selection.
Evolution, 9, 52.
- _____. 1956. The measurement of linkage in heredity.
London, Methuen.
- Mayr, E. 1963. Animal species and evolution. London, Oxford Univ. Press.
- McIndoe, W.M. 1962. Occurrence of two plasma albumins in the fowl. Nature (Lond.), 195, 353.
- Millson, G.C. & Pattison, I.H. 1961. Beta globulin polymorphism in goats. Vet. Rec. 73, 256.
- Morton, N.E. & Chung, C.S. 1959. Are the MN blood groups maintained by selection? Am. J. Hum. Genet. 11, 237.
- Morton, J.R., Gilmour, D.G., McDermid, E.M. & Ogden, A.L. 1965. Association of blood-group and protein polymorphisms with embryonic mortality in the chicken.
Genetics, 51, 97.
- Newcomer, E.H. 1957. The mitotic chromosomes of the domestic fowl.
J. Hered. 48, 227.
- Niemann-Sørensen, A. & Robertson, A. 1961. The association between blood groups and several production characteristics in three Danish cattle breeds. Acta agr. Scand. 11, 163.
- Ogden, A.L., Morton, J.R., Gilmour, D.G. & McDermid, E.M. 1962. Inherited variants in the transferrins and conalbumins of the chicken. Nature (Lond.), 195, 1026.
- Owen, J.J.T. 1965. Karyotype studies on Gallus domesticus.
Chromosoma, 16, 601.
- Parzen, E. 1960. Modern probability theory and its application.
New York, John Wiley & Sons.

- Poulik, M. 1957. Starch gel electrophoresis in a discontinuous system of buffers. *Nature (Lond.)*, 180, 1477.
- Rendel, J.M. 1943. Variations in the weights of hatched and unhatched ducks' eggs. *Biometrika*, 33, 48.
- _____. 1961. Recent studies on relationships between blood groups and production characters in farm animals. *Z. Tierz. ZuchtBiol.* 75, 97.
- Roberts, J.A. & Fraser, I. 1959. Blood groups and susceptibility to disease. A Review. *Br. J.Soc. Prev. Med.* 11, 107.
- Robertson, A. 1955. Selection in animals: synthesis. *Cold Spring Harb. Symp. Quant. Biol.* 20, 225.
- _____. 1961. Reviews of the progress of dairy science. Section A. Physiology. Genetics of dairy cattle. *J. Dairy Res.* 28, 195.
- _____. 1963. Influence of breeding on production efficiency. *World Conference on Animal Production. Vol.I. European Association of Animal Production.*
- Sang, J.H. 1962. Analysis of the growth of selected lines of Brown Leghorns. *Proc. XIIth World's Poultry Congr.* p.49.
- Schade, A.L. & Caroline, L. 1944. Raw hen egg white and the role of iron in growth inhibition of *Shigella dysenteriae*, *staphylococcus aureus*, *Escherichia coli* and *saccharomyces cerevisiae*. *Science*, 100, 14.
- Schultz, F. 1953. Concurrent inbreeding and selection in the domestic fowl. *Heredity*, 7, 1.
- _____. & Briles, W.E. 1953. The adaptive value of blood group genes in chickens. *Genetics* 38, 34.
- Sheppard, P.M. 1955. Genetic variability and polymorphism: synthesis. *Cold Spring Harb. Symp. Quant. Biol.* 20, 271.
- _____. 1965. Mimicry and its ecological aspects. *Genetics Today*, vol.3 (ed.S.J. Geertz). London, Pergamon Press.
- Sick, K. & Nielsen, J.T. 1964. Genetics of amylase isozymes in the mouse. *Hereditas* 51, 291.
- Smithies, O. 1955. Zone electrophoresis in starch gels: group variation in the serum proteins of normal human adults. *Biochem. J.* 61, 629.
- Snedecor, G.W. 1962. Statistical Methods. Ames, Iowa, Iowa State Univ. Press.
- Stansfield, W.D., Bradford, G.E., Stormont, C. and Blackwell, R.L. 1964. Blood groups and their associations with production and reproduction in sheep. *Genetics* 50, 1357.
- Stormont, C. & Suzuki, Y. 1963. Genetic control of albumin phenotypes in horses. *Proc. Soc. exp. Biol. N.Y.* 114, 673.
- Turnbull, A. & Giblett, E. 1960. The binding and transport of iron by unusual transferrins. *Clin. Res. Proc.* 8, 133.
- Vetukchiv, M. 1957. Longevity of hybrids between geographic populations of *Drosophila pseudo-obscura*. *Evolution*, 11, 348.

- Wallace, B. 1955. Inter population hybrids in *Drosophila melanogaster*. *Evolution* 9, 302.
- Warner, R.C. 1953. The egg proteins. The Proteins (ed. Neurath, H. & Bailey, K.), New York, Academic Press.
- Watson, J.H. & Khattab, A.G.H. 1964. The effect of haemoglobin and potassium polymorphism on growth and wool production in Welsh mountain sheep. *J. agr. Sci., Camb.* 63, 179.
- Williams, J. 1962. A comparison of conalbumin and transferrin in the domestic fowl. *Biochem. J.* 83, 355.
- Wright, S. 1931. Evolution in Mendelian populations. *Genetics*, 16, 97
- Young, J.W., Porter, J.E. & Childs, B. 1964. Glucose-6-phosphate dehydrogenase in *Drosophila*. X-linked electrophoretic variants. *Science*, 143, 140.

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ABSTRACT OF THESIS

Name of Candidate VANNITHALBY SUBRAMANIAM

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Title of Thesis Studies on the relationship between protein polymorphisms and production characters in the fowl.

SUMMARY

Four apparently diallelic loci are known to control the genetic variation in the egg white proteins of the fowl. These loci are Ovalbumin, II, III and transferrin. An examination of the genotype frequencies in a population segregating at Ov. locus and locus II showed non-random association between the two loci suggesting close linkage between them. This was confirmed by a linkage experiment and the recombination distance between the two loci was estimated at less than 1 per cent. The linkage relationships of loci III and Tf were also examined. These were not linked to each other or to locus II. It is concluded that the four loci are on three autosomes.

The possible association between these polymorphic genes and five production characters was studied in three closed populations consisting of two flocks of White Leghorns (Strain 2 and Strain 5) each of about 1,000 hens and a Light Sussex flock of 366 hens. The characters studied were: body weights at different ages; egg weights at different ages; embryonic mortality; age at sexual maturity and egg number. Only the last two characters were studied in the Light Sussex flock.

In the two White Leghorn populations, the influence of environment due to time of hatch means was eliminated by analysis of deviations from hatch means.

Statistically significant associations were established in the following cases:

- (a) The Ov. locus had an additive effect on body weight in strain 2, the Ov.^A allele being superior. The influence on body weight was significant only on mature body weight (after sexual maturity), the difference between the two homozygotes at 28 weeks being nearly 80 grams. However, it was shown that

this effect may in part be attributable to chick weight differences and to differences in age at maturity.

(b) Ov. locus had a similar influence on egg weight when the three Ov. genotypes were compared within the II.A class, but when both loci were considered simultaneously, interaction effects were found.

(c) An overdominant effect of locus II on egg number was established in strain 5 and the Light Sussex flock. The difference between the heterozygote and the two homozygotes was 2.8 ± 1.11 eggs in the former strain and 12.3 ± 3.0 in the latter. Besides this overdominant effect, strong interactions were found with Ov. and Tf. loci.

By sampling the daughters with the highest or lowest values for these traits within each sire progeny group, these direct effects were confirmed, since the number of pullets of Ov.A was higher in the high body weight group than in the low group, while Ov.B was most frequent in the low body weight group and similarly for the other characters.

The effect on embryonic mortality was examined on two years' data in strain 2. A significant locus II effect was found affecting mortality in the latter part of incubation, but whereas in one year, the heterozygote was significantly superior, it was inferior in the second.

The variance controlled by these loci was estimated from variance components. In no case was the proportion of variance due to these loci (as a fraction of the variance within progeny groups) large enough to be of practical importance.