# FURTHER STUDIES ON DIPLOPODIA

## I. MODIFICATION OF PHENOTYPIC SEGREGATION RATIOS BY SELECTION

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In recent years the integrated action of the genotype during development has received increasing emphasis. While it is most common to think primarily of quantitative characters in this regard, it is also more and more evident that we cannot divorce our consideration of "simple mendelian" cases from the context of the particular genotype in which they are studied. Possibly there are some characters which are completely determined by one pair of alleles and which behave in precisely the same manner irrespective of both their residual genotype and environment. But these must be the exceptions rather than the rule or, expressed in another way, such behaviour may indicate a specific action relatively late in the developmental sequence. Simple mendelian cases are now more commonly being found to involve a primary pair of alleles deciding a general developmental direction and, at the same time, other genes which modify this direction by their arrangement as well as their presence. If a few genes are of primary importance, the term modifiers (inhibitors or intensifiers) may be used; or, if an unspecified number prove to be involved, the general terms residual genotype or genetic background may be appropriate. Thus an allelic substitution affecting the availability of a metabolic product required relatively early in development will have far-reaching consequences affecting all organs and systems normally making use of this product (cf. Grüneberg, 1952). The consequences themselves, in terms of phenotypic result, will depend on the particular genotype in which the substitution occurred; for in some genotypes alternative metabolic pathways leading toward normality will be available and in others they may not. A gene or pair of genes may behave a certain way in one background and in a startlingly different way in another. Landauer (1948, 1955) has reported differences in gene expression in chickens after breed crossing or selection. In some cases genes apparently lying latent in one breed (with their action inhibited in one type of genetic background) may be expressed in another (Landauer 1956*a*). There are many examples from human clinical genetics of genes described as fully penetrant in one pedigree and variably penetrant in others, or dominant in one family and recessive in another (Gates, 1946). In some cases investigators have suggested that different genes are involved but usually no unequivocal experimental proof has been available. Dwarfism in beef cattle has been given several alternative genetic explanations by different investigators and a definitive explanation of the variable expressivity observed is still lacking (cf., Gregory, et. al., 1951, 1953).

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The present paper is the first of a series of reports concerning the variable behaviour of a pair of genes in chickens. Taylor and Gunns (1947) reported the discovery in White Leghorn chickens of diplopodia, a form a polydactyly which appeared to display simple mendelian behaviour. Lethal phenotypes from matings of heterozygotes were found in 22·7% of the offspring studied. This value did not differ significantly from the expected 3 : 1 phenotypic ratio for an autosomal recessive gene (symbol **dp** assigned by Hutt, 1949). Landauer (1950) confirmed this finding. Two atypical-carrier full sisters, however, were found by Taylor and Gunns to produce only 13·6% diplopods. These birds appeared in the second generation following an outcross to the unrelated U. C. Production Flock. Taylor and Gunns suggested that the deficiency of lethal embryos might be produced either by a higher mortality of diplopod than of normal embryos during the first week of incubation, before phenotypic classification was possible, or by suppression of the phenotype in some homozygous recessive progeny by genetic or environmental factors.

A further study of the cause of these deficient ratios was undertaken. In this paper the results of a selection program that was successful in forming lines characterized by significantly different phenotypic ratios for diplopodia are presented.

## Data and Classification

Data from the paper of Taylor and Gunns on the performance of the original stock have been re-analyzed separately from that secured after an outcross to the University production-bred flock. A few records not reported on by Taylor and Gunns from the breeding farm, which provided the first diplopod carriers, are also included. New data have been secured from matings made from 1947 through 1954. During the latter period, three lines of diplopod carrier stock were formed and reciprocal crosses between them made. $F_1$ linecross progenies have been tested to determine their phenotypic ratios.

All embryos produced during the study were classified as normal, diplopod or unidentified. The latter category includes all fertile eggs containing zygotes, which died before the sixth day of incubation. Prior to the sixth day the diplopod phenotype is not clearly distinguishable. Classification of embryos as diplopods has been made on the basis previously established by Taylor and Gunns, i.e., the possession of doubled foot structures. In the last two years of this study embryos possessing the normal complement of four toes on each foot but with extra wing fingers were found. These embryos are here considered as normals so that the results of the selection program may be on a basis consistent with the classifications of previous years[1]; they are discussed

[1]It will be evident that the absence of this phenotype previous to 1953 may reflect either a failure to examine the wings of all normal phenotypes for presence of supernumerary fingers or the lack (complete or almost complete) of this phenotype prior to this time. In the third paper of this series (Abbott, 1959b) evidence suggesting that the latter interpretation may play the larger role is presented. However, it is likely that this phenotype gradually increased after 1950 and was not either detected or present in appreciable numbers until the especially comprehensive investigation of diplopod phenotypic expression in 1953, 1954.

more fully in the second and third papers of this series (Abbott, 1959*a*; Abbott, 1959b) as attenuated expressions of diplopodia.

## Procedures and Results

*Homogeneity of ratios in original stock*

Progeny data secured from matings between carriers of the original breeder source from 1941 through 1943 were re-examined to determine if any significant deviations from the expected 3 normal (*N*) :1 diplopod (*D*) ratio occurred during that period. $\chi^2$ tests applied to all individual mating ratios as well as the total ratio failed to show a significant difference from 3N : 1D distribution even at the 5% probability level.

*Variable ratios following the outcross*

The first backcross of the outcross $F_1$♀♀ to a male of the original carrier stock was made in 1944. A slight, but nonsignificant, deficiency of diplopods was obtained in this generation (Table 1). The two atypical-ratio sisters (hens 26 and 27 originally identified as A 1151 and A 1156) as well as four other carriers (hens 22-25) were produced in this backcross.

Table 1. *Pre-outcross and post-outcross segregation of normal (N) and diplopod (D) embryos, 1941-1947*

| Matings | Year | N | D | D | $\chi^2$ test for 3 : 1 ratio |
|---|---|---|---|---|---|
| | | No. | No. | % | |
| Pre-outcross .. | 1941-42 | 100 | 33 | 24·8 | 0·002 |
| Original stock .. | 1943 | 253 | 94 | 27·1 | 0·81 |
| from breeding farm) | | | | | |
| | 1941-43 | 353 | 127 | 26.5 | 0·55 |
| Post-outcross .. | 1944 | 99 | 25 | 20·2 | 1·55 |
| (Following cross .. | 1945 | 507 | 110 | 17·8 | 16·93** |
| with U.C. .. | 1946 | 848 | 179 | 17·4 | 31·39** |
| production-bred stock) .. | 1947 | 1232 | 332 | 21·2 | 11·87** |

** $P < .01$

In 1945 hens 22-27 were mated in a second backcross to another male from the original stock. A significant reduction in the proportion of diplopod embryos was obtained. Hens 26 and 27 were largely responsible for the deviation from 3 :1 since only 11·3% of their 230 offspring were diplopods.

Fifteen daughters of hens 22-25 mated to first generation backcross carrier males in 1946 also produced a deficiency of diplopods (650*N*:140*D*; $\chi^2$=22·32). Six of the fifteen ♀♀ produced individual ratios significantly different from 3:1.

Since deficiences of diplopods did not appear in the original stock but only after the outcross to the U. C. Production Flock, it seems certain that the latter was responsible for some contribution affecting the segregation of diplopod phenotypes.

*Variable segregation ratios from atypical-ratio hens*

Hens 26 and 27 were mated to carrier males over a five-year period; at any given time they were always mated to the same male. Both atypical and normal phenotypic ratios were produced by each hen (Table 2). In 1945 and 1948 both produced a deficiency of diplopods and in 1949 both gave approximately normal ratios. Hen 27 in 1946 produced a significantly reduced proportion of diplopods while the other sister gave a normal ratio. Not only did these full sisters give different phenotypic segregation ratios when mated to different males, but they once produced unlike ratios when mated to the same male.

Table 2. *Incidences of unidentified (U), normal (N) and diplopod (D) offspring in progenies os two full sister, atypical-ratio hens mated with the same males within each year from* 1945-1949 *inclusive*

| | Hen 26 | | | | | | Hen 27 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | U | N | D | U | D | $\chi^2$ test for 3N: 1D Ratio | U | N | D | U | D | $\chi^2$ test for 3N: 1D Ratio |
| | No. | No. | No. | % | % | | No. | No. | No. | % | % | |
| 1945 | 5 | 61 | 8 | 6·8 | 11·6 | 6·61* | 13 | 143 | 18 | 7·5 | 11·2 | 16·40** |
| 1946 | 1 | 52 | 20 | 1·4 | 27·8 | 0·30 | 7 | 66 | 9 | 8·5 | 12·0 | 6·76** |
| 1947 | 1 | 41 | 7 | 2·0 | 14·6 | 2·78 | 9 | 71 | 18 | 9·2 | 20·2 | 1·08 |
| 1948† | 3 | 41 | 4 | 6·3 | 8·9 | 6·56* | 8 | 23 | 1 | 25·0 | 4·2 | 4·15* |
| 1949 | 1 | 52 | 13 | 1·5 | 20·0 | 0·87 | 1 | 11 | 4 | 6·3 | 26·7 | 0·02 |
| All years | 11 | 247 | 52 | 3·5 | 17·4 | 9·23** | 38 | 314 | 50 | 9·4 | 13·7 | 24·63** |

* $P < .05$

** $P < .01$

†In 1948 hens 26 and 27 produced a limited number of eggs which were injected with insulin for other purposes. High early embryonic mortality occurred with this treatment. 7·7% diplopod embryos was obtained from matings of each hen in this year if these data are included.

*Formation of lines characterized by different segregation ratios*

A selection program was initiated in 1947 by placing in line I birds having incidences of diplopod offspring approaching or equaling 25% and in line II the atypical-ratio hens and their offspring. Interchanges of birds were made between lines I and II on the basis of phenotypic ratios until 1950, when the lines were closed. Several individuals of line II produced only 5 or 6% diplopods in 1950. These birds and their carrier

offspring were used to start line III, selected for incidences of 8% or less. Line II thereafter was selected for intermediate levels of 12-14%. In 1953 and 1954 some matings were made in line I between individuals producing more than 25% diplopods.

Of the three lines, inbreeding was most intense in III, through a combination of parent-offspring and sib matings used to establish this line. Line II has been relatively much less inbred than III. Line I, having the greatest number of carrier individuals with the desired segregation ratios, was the least severely inbred.

Results of the breeding program are presented in Table 3 and Figure 1. From 1948 through 1952, phenotypic ratios of line I varied; those of 1949, 1951 and 1952 were significantly deficient in diplopods. By 1954 the percentage of diplopods had risen to 26·2%, closely approximating the 26·5% incidence in the original stock from 1941 through 1943 (Table 1). No matings, however, had segregations of diplopods significantly above the 25% level, despite the attempt to produce such by the mating of carriers with highest incidences.

Table 3. *Unidentified (U), normal (N) and diplopod (D) embryos produced after formation of selected lines and significance of segregation ratio of diplopods between lines and from the* 3:1 *ratio expected*

| Line | Year | U | N | D | U | D | $\chi^2$ test for 3: 1 ratio | |
|---|---|---|---|---|---|---|---|---|
| | | No. | No. | No. | % | % | | |
| I .. | 1948 | 130 | 430 | 123 | 19·0 | 22·2 | 2·24 | |
| | 1949 | 172 | 396 | 70 | 27·0 | 15·0 | 24·75** | |
| | 1950 | 36 | 135 | 45 | 16·7 | 25·0 | 0·00 | |
| | 1951 | 164 | 957 | 245 | 12·0 | 20·4 | 13·67** | |
| | 1952 | 388 | 3070 | 878 | 8·9 | 22·2 | 16·05** | |
| | 1953 | 349 | 2839 | 868 | 8·6 | 23·4 | 4·97* | |
| | 1954 | 111 | 1327 | 470 | 5·8 | 26·2 | 1·28 | |
| | | | | | | | | $\chi^2$ test for line II = line I |
| II .. | 1948 | 72 | 288 | 55 | 17·3 | 16·0 | 14·70** | 5·12* |
| | 1949 | 171 | 1302 | 221 | 10·1 | 14·5 | 89·37** | 0·07 |
| | 1950 | 303 | 1520 | 300 | 14·3 | 16·5 | 70·40** | 8·32** |
| | 1951 | 140 | 824 | 173 | 12·3 | 17·4 | 31·10** | 3·25 |
| | 1952 | 156 | 1117 | 174 | 10·8 | 13·5 | 91·41** | 46·53** |
| | 1953 | 118 | 996 | 151 | 9·3 | 13·2 | 85·69** | 86·92** |
| | 1954 | 51 | 755 | 67 | 5·8 | 8·2 | 124·45** | 112·16** |
| | | | | | | | | $\chi^2$ test for line III = line II |
| III .. | 1951 | 48 | 439 | 34 | 9·2 | 7·2 | 80·03** | 27·39** |
| | 1952 | 44 | 573 | 44 | 6·7 | 7·1 | 105·00** | 16·62** |
| | 1953 | 99 | 682 | 58 | 11·8 | 7·8 | 116·24** | 13·00** |
| | 1954 | 40 | 602 | 27 | 6·0 | 4·3 | 143·85** | 8·76** |

* $P < .05$
** $P < .01$

Line II, on the other hand, showed a decline in diplopod incidence which became pronounced from 1951 to 1954. In each of the 8 years of existence of this line, a significant deficiency of diplopods occurred. Differences between the performance of this line and that of line I steadily increased in significance after 1951. Despite the selection of individuals for re-mating that had given intermediate segregation ratios, by 1954 the average incidence for this line matings declined to 8·2%. Individual mating results in this line still varied widely and the line did not stabilize at the level of the phenotypic ratios consistently selected.

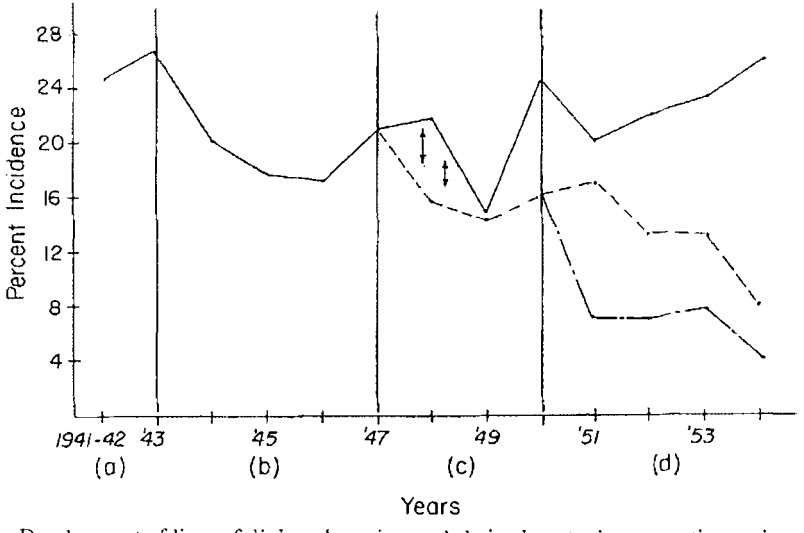


Figure 1. Development of lines of diplopod carriers and their phenotypic segregation ratios. (*a*) before the outcross; (*b*) after the outcross; (*c*) line selection with interchange between lines I and II; and (*d*) selection in closed populations—line I; · · · · line II; and · · · · line III.

The average incidence of diplopod segregants from line III declined rapidly in the first year, 1951, remained relatively constant in 1952 and 1953, then declined in 1954 to 4·3%. Individual matings have produced as low as 1-2% and proven carrier females have given 0% in progenies with as many as 57 identified embryos. Considerable difficulty is involved in testing birds of this line; as many as 100 embryos are required before classification of some dams as carriers can be established. Comparison of phenotypic ratios between lines II and III for the respective years 1951 to 1954 produced significant $\chi^2$ values each year. Line III was apparently differentiated from line II in the first year of selection; however, the actual difference between them declined in subsequent years.

Since all three lines produced significantly different phenotypic ratios from 1952 through 1954, selection of individuals with ratios typical of their respective line for crossing was justified.

*Segregation in line crosses*

Data from reciprocal line crosses made in 1953 and 1954 are presented in Tables 4 and 5 respectively, along with pureline results for comparison.

Table 4. *Segregation of diplopods from pureline and linecross matings*, 1953

| Mating type | Parental line ♂♂ ♀♀ | U No. | N No. | D No. | U % | D % |
|---|---|---|---|---|---|---|
| Pureline | I × I | 349 | 2839 | 868 | 8·6 | 23·4 |
| | II × II | 118 | 996 | 151 | 9·3 | 13·2 |
| | III × III | 99 | 682 | 58 | 11·8 | 7·8 |
| Linecross | I × II | 32 | 401 | 74 | 6·3 | 15·6 |
| | II × I | 33 | 342 | 57 | 7·6 | 14·3 |
| | I × III | 4 | 37 | 10 | 7·8 | 21·3 |
| | III × I | 32 | 360 | 31 | 7·6 | 7·9 |
| | II × III | 2 | 37 | 2 | 4·9 | 5·1 |
| | III × II | 1 | 57 | 0 | 1·7 | 0·0 |

Table 5. *Segregation of diplopods from pureline and linecross matings*, 1954

| Mating type | Parental line ♂♂ ♀♀ | U No. | N No. | D No. | U % | D % |
|---|---|---|---|---|---|---|
| Pureline | I × I | 111 | 1327 | 470 | 5·8 | 26·2 |
| | II × II | 51 | 755 | 67 | 5·8 | 8·2 |
| | III × III | 40 | 602 | 27 | 6·0 | 4·3 |
| Linecross | I × II | 28 | 353 | 66 | 6·3 | 15·8 |
| | II × I | 22 | 222 | 49 | 7·5 | 18·1 |
| | I × III | 17 | 342 | 60 | 4·1 | 14·9 |
| | III × I | 5 | 96 | 9 | 4·5 | 8·6 |

Reciprocal I × II crosses gave substantially the same percentage of diplopods within each year and also between the two years. The range of 14·3% to 18·1% diplopods represented approximately the midpoint between the parental pureline averages. Reciprocal I × III crosses, on the other hand, gave more variable results. In both years, the cross of I♂♂ × III♀♀ produced much higher percentages of diplopods than the reciprocal cross. Although intermediate between parental levels, the segregation ratio in these reciprocal crosses was closer to that of the sire's line. Reciprocal II × III crosses were made only in 1953; very inadequate numbers of offspring were obtained. No diplopods were obtained from the III♂ × II♀♀ cross, although the parents were proven carriers by matings within their line. The reciprocal cross gave a segregation ratio even more deficient in diplopods than line III matings of that year.

Table 6. *The relative fertility and proportions of unidentified (U), normal (N) and diplopod (D) embryos in progenies of nine line III pullets mated with both a line I and a line III male*

| ♀♀ Carrier | Line III♂ J163 Total Eggs | Fertile No. | Fertile % | Progeny Ratios U | N | D | %U | %D | Line I♂ J184 Total Eggs | Fertile No. | Fertile % | Progeny Ratios U | N | D | %U | %D |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K 1738 | 45 | 25 | 55·6 | 4 | 21 | 0 | 16·0 | 0·0 | 41 | 28 | 68·3 | 2 | 23 | 3 | 7·1 | 11·5 |
| K 1758 | 69 | 47 | 68·1 | 2 | 43 | 2 | 4·3 | 4·4 | 42 | 41 | 97·6 | 0 | 33 | 8 | 0·0 | 19·5 |
| K 1780 | 13 | 12 | 92·3 | 1 | 11 | 0 | 8·3 | 0·0 | 34 | 34 | 100·0 | 0 | 29 | 5 | 0·0 | 14·7 |
| K 1782 | 39 | 25 | 64·1 | 0 | 22 | 3 | 0·0 | 12·0 | 63 | 62 | 98·4 | 3 | 50 | 9 | 4·8 | 15·3 |
| K 1787 | 51 | 38 | 74·5 | 2 | 35 | 1 | 5·3 | 2·8 | 33 | 25 | 75·8 | 6 | 15 | 4 | 24·0 | 21·1 |
| K 1820 | 51 | 42 | 82·4 | 1 | 39 | 2 | 2·4 | 4·9 | 66 | 56 | 84·8 | 1 | 41 | 14 | 1·8 | 25·5 |
| Total | 268 | 189 | 70·5 | 10 | 171 | 8 | 5·3 | 4·5 | 279 | 246 | 88·2 | 12 | 191 | 43 | 4·9 | 18·4 |
| *Non-carrier* | | | | | | | | | | | | | | | | |
| K 1702 | 17 | 6 | 64·7 | 4 | 7 | | 36·4 | | 29 | 2 | 93·1 | 1 | 26 | | 3·7 | |
| K 1703 | 38 | 10 | 73·7 | 0 | 28 | | 0·0 | | 36 | 1 | 97·2 | 2 | 33 | | 5·7 | |
| K 1747 | 61 | 19 | 68·9 | 5 | 37 | | 11·9 | | 33 | 5 | 84·8 | 2 | 26 | | 7·1 | |
| Total | 116 | 35 | 69·8 | 9 | 72 | | 11·1 | | 98 | 8 | 91·8 | 5 | 85 | | 5·6 | |

| | $\chi^2$ | P |
|---|---|---|
| (a) Deviation of line III carrier♂ × line III carrier♀♀ from 3:1 | 40·31 | ·001 |
| (b) Deviation of line I carrier♂ × line III carrier♀♀ from 3:1 | 5·48 | ·019 |

(c) Independence of line III carrier♀♀ ratios with line I carrier♂ and line III carrier♂

| *Source* | *df* | | |
|---|---|---|---|
| Total | 11 | 24·92 | ·009 |
| Males | 1 | 18·11 | ·001 |
| Females | 5 | 4·13 | ·53 |
| Interaction | 5 | 2·68 | ·75 |

*Reciprocal matings of line III females*

In 1954, 9 line III pullets, some mated with a line I♂ and some with a line III♂, were reciprocally transferred and mated with the male from the other line. Six of the 9 ♀♀ were carriers of the **dp** gene (Table 6). With the line I♂ they produced 18·4% diplopods; with the line III♂ a significantly lower level of 4·5% was obtained. Both incidences were significantly below expectation on a 3:1 basis.

Table 7. *Performance of line III♂ G159 in matings with ♀♀ from lines III and I*

| Dam's Genotype | Dam's Line | Year | No. Eggs | % Fertile | U | N | D | %U | %D |
|---|---|---|---|---|---|---|---|---|---|
| + dp | III | 1951, 1952 | 631 | 79·6 | 27 | 440 | 15 | 5·6 | 3·3 |
| + + | III | 1952 | 278 | 41·4 | 10 | 105 | — | 8·7 | |
| + dp | I | 1953 | 552 | 60·3 | 26 | 290 | 17 | 7·8 | 5·5 |

Table 8. *Incidence of diplopod embryos in backcrosses of FF III♂ × I♀ linecross ♀♀ to respectively a line III son of G159 and to line I ♂♂. All ♀♀ were daughters of ♂ G159*

| | Line III♂ | | | | | Line I♂♂ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Linecross | U | N | D | U | D | U | N | D | U | D |
| | No. | No. | No. | % | % | No. | No. | No. | % | % |
| K1705 | 3 | 66 | 5 | 4·1 | 7·0 | | | | | |
| K1707 | 10 | 45 | 4 | 16·9 | 8·2 | | | | | |
| K1711 | 2 | 76 | 3 | 2·5 | 3·8 | | | | | |
| K1740 | 2 | 21 | 1 | 8·3 | 4·5 | 4 | 30 | 7 | 9·8 | 18·9 |
| K1751 | 6 | 94 | 9 | 5·5 | 8·7 | | | | | |
| K1769 | 5 | 55 | 6 | 9·1 | 9·8 | | | | | |
| K1772 | 0 | 11 | 0 | 0·0 | 0·0 | | | | | |
| K1775 | 1 | 26 | 0 | 3·7 | 0·0 | 1 | 9 | 2 | 8·3 | 18·2 |
| K1801 | 0 | 41 | 2 | 0·0 | 4·7 | 2 | 32 | 5 | 5·1 | 13·5 |
| K1830 | 5 | 85 | 7 | 5·2 | 7·6 | | | | | |
| K1833 | 2 | 50 | 7 | 3·4 | 12·3 | 3 | 44 | 7 | 5·6 | 13·7 |
| Total | 36 | 570 | 44 | 5·5 | 7·2 | 10 | 115 | 21 | 6·8 | 15·4 |

The experimental design permitted a partitioning of a total $\chi^2$ for the difference in performance between the two matings into its component parts: $\chi^2$ due to males, to females, and to an interaction between mates respectively. Only the male contribution proved to be significant.

*Performance of line III ♂ G 159*

One line III♂ mated to carrier females of his line gave exceptionally low incidences of diplopodia in two successive years. The next year he was mated to line I ♀♀ and produced diplopod offspring at a rate but slightly higher than that from matings within his line (Table 7). Pullets from this linecross were backcrossed to a line III son of G 159 and to line I♂♂ in 1954 (Table 8). Eleven identified carrier females gave percentages of diplopods ranging from 0-12·3% with the line III♂, the incidence in 614 identified progeny being 7·2%. Four of the eleven hens mated to the line I♂♂ gave a range from 13·5% to 18·9% for an incidence of 15·4% in 136 progeny. Backcrossing to line III produced individual mating results characteristic of lines II or III, whereas backcrossing to line I♂♂ gave intermediate phenotypic ratios similar to those of reciprocal I×III linecrosses.

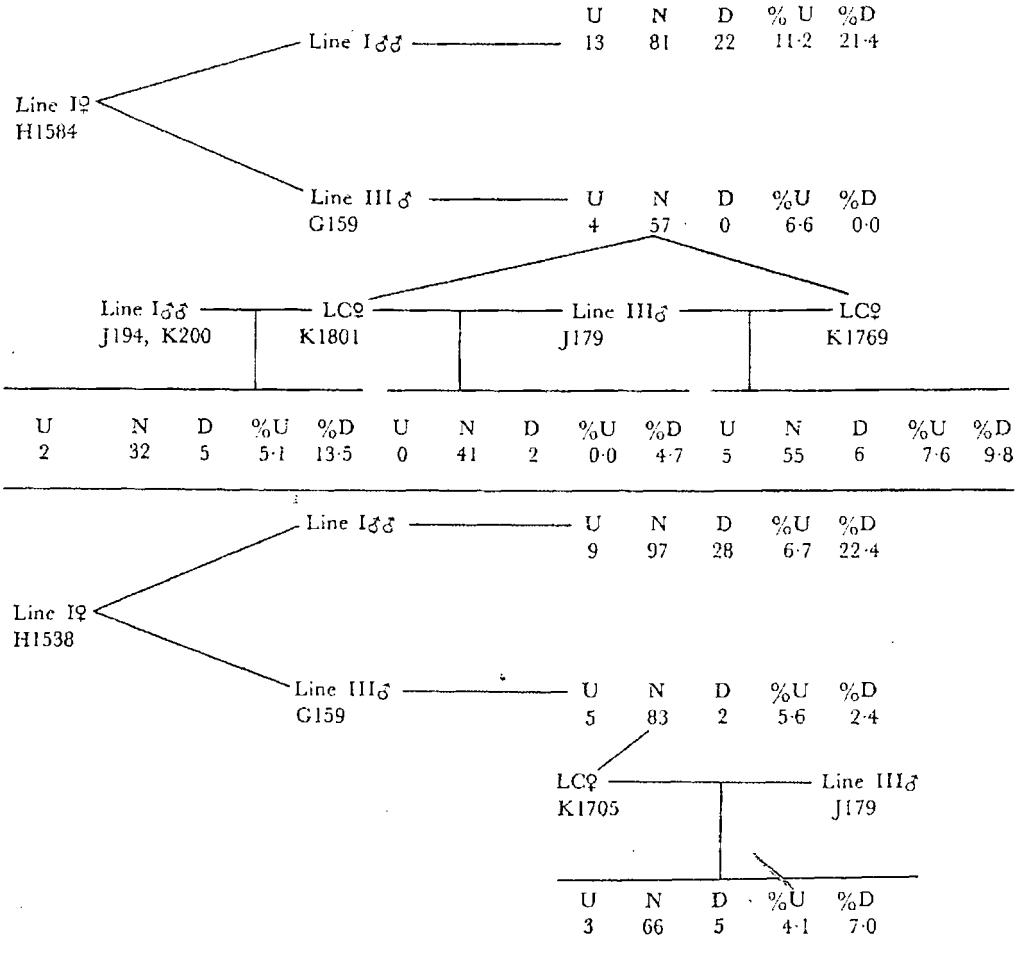


Figure 2. Influence of ♂ G159 in suppressing diplopod segregation from line I mates.

Figure 2 presents two illustrations from the linecross mating of the ability of G 159 to decrease the incidence of diplopod embryos from line I dams. These hens produced respectively 21·4% and 22·4% diplopods in matings with line I ♂♂, but with G 159 the incidences were respectively 0 and 2·4%. One linecross III × I daughter, K 1801, was reciprocally backcrossed to both the line III son of G 159, and to the two line I ♂♂. Both backcrosses produced deficiencies of diplopod offspring (4·7% and 13·5% respectively). Two other linecross daughters backcrossed to J 179 produced 9·8% and 7·0% diplopods respectively. Male G 159, therefore, exhibited a dominant tendency to decrease the incidence of diplopodia in his offspring. His daughters tested in matings with line I ♂♂ did not show the same dominant effect (Table 8), whereas in matings with his line III son more than one-half gave typical line III ratios.

*Lack of evidence for differential early zygotic mortality*

Unidentified early embryonic death rates were relatively high in diplopod carrier stocks until the period of 1952 to 1954. There was no trend toward an increase of *U* (unidentified) embryos in either lines II or III while the deficiency of diplopod segregates increased (Table 3). In fact, line I had a higher early mortality rate than the other lines.

Even if all unidentified embryos were considered to be diplopods, phenotypic segregation ratios in the later years would still show $D+U$ as percentage of total fertile eggs significantly below those expected from a 3 : 1 ratio. In 1954 for example, such percentages calculated from data in Table 3 are respectively 13·5 ($\chi^2=61{\cdot}40$) for line II and 10·0 ($\chi^2=80{\cdot}12$) for line III matings. Data included in Tables 4-8 offer further instances of the same nature.

Evidence that the percentage of *U* embryos was not associated with the segregation of diplopods was also derived from pullets tested in 1949 (Table 9). Matings of carriers × carriers did not produce higher levels of *U* embryos than either reciprocal crosses of carriers × noncarriers or noncarriers × noncarriers. Further, a line II ♂, J 157, produced 75 progeny when mated with line III carrier ♀♀; all of these were identified

Table 9. *Unidentified (U) embryos in line I and II pullet progenies, 1949, according to parental genotype classification*

| Parental Sire | Genotype Dam | Line I Progeny | | | | | Line II Progeny | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | U | N | D | U | D | U | N | D | U | D |
| | | No. | No. | No. | % | % | No. | No. | No. | % | % |
| + **dp** | + **dp** | 49 | 321 | 56 | 11·5 | 14·9 | 138 | 1039 | 171 | 10·2 | 14·1 |
| + + | + **dp** | 29 | 283 | — | 9·3 | — | 11 | 91 | — | 10·8 | — |
| + **dp** | + + | 47 | 315 | — | 13·0 | — | 4 | 73 | — | 5·2 | — |
| + + | + + | 24 | 248 | — | 8·8 | — | | | | | |

phenotypically and only four were diplopods. Apparently, diplopods are no more likely to die in early developmental stages than normal embryos of this stock. Accordingly, phenotypic ratios deficient in diplopod segregants are not to be accounted for by differential mortality of **dpdp** zygotes before identification can be made.

*Lack of association of fertility with diplopod segregation ratios*

Munro and Kosin (1945) have shown that chick embryos may die at such early stages of development that an egg would appear infertile after a period of incubation. In our work, all eggs removed as infertile were carefully examined for evidences of development and any trace of cellular proliferation in the blastodisc was given a classification as an unidentified embryo.

Although line III matings were especially prone to have a lower fertility and ♂ G 159 (Table 7) was very poor in this respect, data from individual females mated with him showed absolutely no relationship between the level of fertility and the phenotypic ratio. Furthermore, male infertility with noncarrier females was substantially the same as that with carriers.

When mated to the same group of females, line III♂ J 163 had a substantially lower fertility rate than line I♂ J 184 (Table 6). However, the fertility obtained from noncarriers was not higher than that from carrier dams segregating for diplopodia. Among the carrier dams, both K 1787 and K 1820 had almost equal fertility levels with the two males, although their phenotypic ratios for diplopodia were quite different. These hens also produced a low proportion of unidentified embryos in matings with the line III ♂. Within individual male matings, fertility and incidence of diplopodia were not related as would be expected if differential **dp** gametic viability were responsible for deficient phenotypic ratios.

The establishment of lines of diplopod carriers characterized by significant deficiencies in the proportion of diplopod progeny but not by an excessive early embryo mortality suggests that some diplopod genotypes appear phenotypically normal. If the viability of such homozygous **dp**-carriers approximates that of other normal birds, they should be represented in both the hatched population and in the adult carrier population. The demonstration of an excess of **dp**-carriers in the adult populations of line II, and especially of line III, would thus verify the results of the breeding and selection program. To date it has not been possible to establish that the expected proportion of carriers in the low lines is exceeded significantly. Data on the relative proportions of proven carrier and noncarrier birds in each line were collected throughout the study. It must be recognized, however, that all data bearing on the relative proportions of carriers and noncarriers represents only a sample of each population. It consists specifically of the birds hatched during a four week period in the spring, which lived to maturity and which, in the case of females, laid sufficient fertile eggs for a positive progeny test classification and, in the case of males, were selected for progeny test and produced viable sperm. Identifications of the genotypes of all birds saved in each line were not available in any year due to one or more of the following causes: 1) mortality during the growing period, 2) mortality before sufficient fertile eggs for

progeny test had been secured, 3) non-production, or 4) complete infertility. In addition the number of male birds tested represented a smaller sample of the population than of females due to breeding space limitations. The proportion of carrier to noncarrier females identified during the course of this study was 458 : 193 respectively. The excess of carriers is of borderline significance; $\chi^2=3{\cdot}98$, $P=0{\cdot}046$, based on the 2 carrier : 1 noncarrier expectation for the segregation of a single autosomal gene.

A search for individual birds producing a significant excess of diplopod progeny and whose normal progeny were all **dp** carriers was disappointing. Most birds with ratios excessive for their line were extremely poor in reproduction. In the case of females, both fertility and egg production were unsatisfactory, while high-ratio males were poor in fertility. In general the numbers of progeny produced were never sufficient for meaningful results save for one case. In 1953, male J 167, from line II, produced $128\mathcal{N}$: $57D$ (30·8%) diplopod embryos in matings within his line. Previously his mates had given characteristic line II ratios (13-14% **dpdp**) with other line II males. The progeny-tested offspring of J 167, however, included noncarriers as well as carriers, proving that he was heterozygous for **dp.** Furthermore, mated with ten line II females the following year (two being his mates of 1953 as well) this male gave a total ratio of $266\mathcal{N}$: $28D$ (9·5% diplopods). His average with the two mates of 1953 was $59\mathcal{N}$: $15D$ (20·3% diplopods), again considerably higher than expected for this line.

Since we could not find evidence of homozygous carriers in adult populations, an investigation of the fate of these postulated individuals was undertaken. If we assume that homozygous diplopods with normal phenotypes occur in the low-ratio lines and that in general a sufficient number of individuals of this type do not appear in the progeny-tested adults of any given generation to alter significantly the expected distribution of carriers : noncarriers, then we might expect one of the following evidences of their presence :

1. a higher post-hatching mortality of chicks from lines II and III than from line I;
2. a larger proportion of adults with inadequate tests due to late maturity, low egg production, or infertility in lines II and III than in line I.

Chicks from lines II and III had a higher post-hatching mortality than chicks from line I. Of 300 chicks reared in the 1953 season, 52 died before the fifth month. The incidence of deaths by lines was as follows : line I, 11·6%; line II, 19·0% and line III, 22·7%. Line III pullets showed a significant difference in age at sexual maturity when compared to pullets of either lines II or III in 1953 (Table 10). Carrier pullets showed a slight but non-significant earlier maturity than noncarrier pullets. Line III pullets were more variable in age at sexual maturity than pullets from lines I and II. An analysis of egg production of all 1953 hatched pullets from the three lines during November, December and January of their first year of production indicated that line III pullets laid fewer eggs than pullets from lines I or II. However, the observed differences were not significant. Egg production of carrier and noncarrier pullets during the three-month period considered was very similar. Line III, however, exhibited greater variability in this respect than either of lines II or I.

Table 10. (*a*) *Age at Sexual Maturity of* 1953 *Pullets from the Three Lines of Diplopod Carriers*

| Line | Mean Age of † All Pullets | Mean Age of Carriers | Mean Age of Noncarriers |
|---|---|---|---|
| I | 160·3 | 163±2·9 | 165±12·7 |
| II | 161·8 | 159±2·7 | 173± 5·6 |
| III | 200·1 | 192±9·8 | 195±14·0 |

| | df | Mean Square | Variance Ratio |
|---|---|---|---|
| Between Lines | 2 | 20293·97 | 38·10* |
| Between Sires Within Lines | | 532·69 | |

(*b*) *Three-Month Egg Production of* 1953 *Pullets from the Three Lines of Diplopod Carriers*

| Line | Mean Egg Production† of all Pullets | Mean Egg Production of Carriers | Mean Egg Production of Noncarriers |
|---|---|---|---|
| I | 35·9 | 42±3·2 | 42±7·9 |
| II | 34·6 | 41±4·3 | 40±6·5 |
| III | 27·5 | 28±5·4 | 30±6·1 |

| | df | Mean Square | Variance Ratio |
|---|---|---|---|
| Between Lines | 2 | 1624·63 | 2·13 |
| Between Sires Within Lines | 6 | 762·17 | |

† includes pullets with too few eggs for classification as either carrier or noncarrier.
P=·01

## Discussion

A stock of White Leghorn chickens segregating homogeneously in a 3 : 1 phenotypic ratio for diplopodia produced offspring characterized by significant deficiencies of diplopods after an outcross to the unrelated U. C. production-bred flock. Three lines with significantly different phenotypic ratios were developed. Line I was restored to the 3:1 ratio characteristic of the original stock, but attempts made to exceed this proportion of diplopods were not successful. Line II, intermediately deficient in the ratio of diplopods produced, continued to show a wide range of ratios in individual matings and a tendency to drift toward line III ratios. Line III, very deficient in diplopods, exhibited greater stability than line II, but gave rise to individuals producing only 1-3% diplopod phenotypes in their offspring. Phenotypic ratios of matings in lines I and III were more predictable than those in line II.

Reciprocal crosses between lines I and II produced ratios intermediate between those of the parental lines. On the other hand, reciprocal crosses between lines I and III produced intermediate ratios that more closely approached those of the sire's line. Females from the III ♂ × I ♀ linecross tested in backcrosses to parental lines produced only intermediate ratios. Reciprocal II × III linecrosses produced ratios even lower than the line III matings of the respective year. No explanation involving simple dominance or sex linkage suffices to explain these results. Only a complex polygenic system could offer a genetic explanation for the variety of ratios obtained in linecross and backcross matings.

Landauer (1956*b*) reported the discovery of a new recessive mutation for diplopodia in Black Minorca fowls. The locus of this new gene (here termed **dp**$^2$) was not closely linked with our diplopod gene and may have been on a different chromosome. Although varying in some respects, the expression of the new gene was substantially similar to **dp**. Matings of double heterozygotes for **dp** and **dp**$^2$ produced incidences of diplopodia considerably in excess of 25%. Since we found neither an excess of carriers or evidence of consistently higher than normal ratios, there is no reason for assuming that we are dealing with two genes for diplopodia in the present instance. We have had individual matings that have significantly exceeded a 25% incidence of diplopodia and a line II ♂, J 167, produced 30·5% diplopods in one year's progeny. Diplopod incidences consistently above 25% have not been developed in any of our lines as would be expected if more than one **dp** gene were segregating.

The failure of some proven carriers to give diplopod offspring in matings with other known carriers might be attributable either to heterozygosity for **dp** genes at different loci or to an extreme suppression of the diplopod phenotype by modifying genes. Such carriers should give only normal (3 :1) ratios when mated to carriers of the same **dp** gene, if no modifiers of diplopodia were involved. In our work the failure to produce diplopods in progenies totaling approximately 60 identified embryos has always involved at least one parent from line III whose characteristic phenotypic ratio in other matings has been very deficient in diplopods. These results are explicable as due to the action of a masking residual genotype but not as due to the segregation of different genes for diplopodia.

Early zygotic mortality, occurring before the development of embryonic limbs, cannot account for extremely deficient diplopod ratios. In fact we have no evidence indicating that mortality occurring prior to the time when classification can be made accurately is higher in diplopods than in normal embryos.

Also there has been no consistent association of infertility with the production of deficient diplopod ratios. Although line III has produced the lowest levels of fertility, evidence from individual matings within the line indicated that the fertility level was determined as a characteristic of one or both parents rather than by a factor associated with the deficiency of diplopods. Inbreeding is known to affect the fertility of chickens adversely (Bernier, Taylor and Gunns, 1951). Since line III was relatively the most severely inbred of the three lines, the seeming association of low fertility with the low incidence of diplopods here may be considered as fortuitous.

While we are aware that there are several types of gametic abnormalities of behaviour such as preferential fertilization, directed segregation, or the segregation ratio abnormality (Dunn, 1954) that can lead to abnormal genotypic, and so also phenotypic, ratios, we feel that our failure to find evidence of an association between fertility and low phenotypic ratios in either males or females tends to discount explanations of this general category. We realize, however, that this type of negative evidence can not exclude the possibility of unequal **dp** and **Dp** gamete production by low-ratio males. Because an abundance of sperm are available in an oviduct for fertilization of ova, such an effect would be very unlikely to lower fertility.

Our data suggest that the basis of the inherited tendency to produce progenies deficient in the expected proportion of diplopods is not due to a reduced proportion of **dpdp** genotypes in the low ratio lines. Neither is it attributable to selection favouring a high death rate of **dpdp** zygotes before accurate phenotypic identification. Rather the available evidence indicates that we have selected birds with an inherited tendency to produce a larger and larger proportion of their **dpdp** progeny appearing phenotypically normal. Clearly, such a trend should result in an increasing proportion of carriers in the progeny of the low ratio lines unless a normal-appearing diplopod is so poor in vigor that death occurs before the progeny test necessary for identification can be completed. We feel that our failure to date to discover a significant excess of carriers of **dp** in our tested populations must be explained on this basis. All evidences of vigor or reproductive fitness examined in the selection lines have indicated that line III (4·3% identified diplopod progeny) is considerably poorer than line I (26·2% identified diplopod progeny). Viability to five months, three-month egg record, and proportion of birds with no progeny test because of low or complete infertility or poor egg production, were all least satisfactory in line III. In addition the numbers of identified embryos required to establish a bird as a noncarrier increased steadily in line III as the phenotypic ratios of diplopods decreased. Accordingly many females of rather poor production characteristics, who may have been carriers, never produced enough fertile eggs to be classified as either carriers or noncarriers. Females from line I could be classified on the basis of 18 identified embryos; thus, females of this line with exceptionally low egg production were easier to classify accurately. Unless the fitness of the postulated homozygous **dp** carriers of line III, and to a lesser extent those of line II, was very nearly equal to heterozygous carriers and normals, the chance of obtaining an adequate record for classification would be low.

All the evidence available at the present time suggests that we are dealing with a case of the suppression of the phenotypic expression or penetrance of **dpdp** mediated by modifier systems selected in our two low-ratio lines. We have evidence that the modifier system involved is not a simple one involving one or two genes. We prefer to use the general term residual genotype rather than attempt to define the number of factors playing a role in diplopod suppression. Although our linecross results support a quantitative type of action our line distinction has been relatively very rapid. In the case of line I purification of the line from residual genotype modifier effects has been fairly fast. In line II the interactions are still complex and the results considerably

less predictable than in either line I or line III. It seems quite possible that line III could be developed to the point that no recognizable diplopod segregants were produced in carrier matings within the line. In further tests of this line carried out in 1956 an increasing number of matings have given less than 2% diplopod phenotypes.* However, our obligatory reliance on the progeny test for carrier identification makes this task extremely difficult if not impossible, although we can identify line III carriers by mating them with line I birds.

In the second and third papers of this series (Abbott, 1959*a*; Abbott, 1959*b*) embryonic development and expressivity of diplopodia from the three selected lines are discussed. Many of the most cogent evidences supporting our hypothesis of suppression of the diplopod phenotype will appear in these publications.

## Summary

Following an outcross to a nonrelated production-bred strain, a White Leghorn stock no longer segregated for diplopodia in the normal 3:1 phenotypic ratio but produced significant deficiencies of diplopod phenotypes. A program of selection over a period of seven years was successful in creating three lines producing significantly different ratios. Line I was restored to the normal 3:1 ratio (26·2% diplopods); line III produced an average incidence ranging from 7·8% to a low of 4·3%; and line II gave intermediate levels ranging from a high value of 17·4% to 8·2% in the last year's progeny.

The deficiency of diplopods could not be accounted for on the basis of early zygotic mortality and resulting unidentified embryos, or by differential fertility levels in the lines or in individual matings.

Data obtained from crosses between the lines and from backcrosses of linecross progeny could not be explained by the assumption of a simple genetic modifying factor. Occasional evidences for simple dominance or sex-linkage were not verified by more critical tests. A new mutant gene for diplopodia, discovered by Landauer, did not appear to be present in this flock.

A polygenic system of modifying factors derived from the production-bred flock offers the best explanation of the heritable suppression of diplopod phenotypes.

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