FURTHER STUDIES ON DIPLOPODIA

III. THE RELATIONSHIP BETWEEN EXPRESSIVITY AND PENETRANCE IN SELECTED LINES AND CROSSES

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Introduction

Diplopodia occurs in chicken embryos homozygous for **dp,** an autosomal recessive lethal gene (Taylor and Gunns, 1947). Taylor, Abbott and Gunns (1959), by selecting different genetic backgrounds for **dp,** were able to develop lines of heterozygous carriers characterized by significantly different incidences of diplopod offspring. They were able to show that factors affecting gametic or very early zygotic viability were not responsible for the different phenotypic segregation ratios. Studies of comparative development of diplopod and normal embryos from the selected lines indicated that diplopod embryos were retarded and that this retardation was most marked in those from the normal-ratio line, I, (Abbott, 1959). Abbott also found the three lines to produce diplopod embryos differing significantly in length of embryonic survival.

Diplopod Morphology

The diplopod gene affects the numbers of digits on the feet and wings, the numbers of metatarsal bones, the size and shape of the long bones, and the structure of the beak. Embryos with typical expressions of the anomaly have six toes per foot, which are arranged in two sets of three each (Plate 1). The normal set consists of digits II, III and IV, while the other three replace the hallux (digit I) and are usually smaller than normal and variable in structure. Extra metatarsal bones are present as well. The femur, tibiotarsus and tarsometatarsus are shortened and thickened. The tibiotarsus is bent. There are either three or four digits on each wing replacing the normal wing finger or pollex. The pre-maxilla is shortened.

Atypical expressions of diplopodia (with less than six toes per foot) were found to be especially common in progenies of low-ratio carriers produced after an introduction of germ-plasm from the unrelated U.C. Production Flock of White Leghorns. Before this outcross 94% of all diplopod embryos had at least six toes per foot (average, 11·9 toes per embryo). After the outcross **dp**-carriers produced both normal and deficient phenotypic segregation ratios. Of diplopod embryos produced by dams with normal (3:1) segregation ratios, 77% had at least six toes per foot (average, 11·5 toes per embryo) while those from hens with deficient ratios averaged only 11·2 toes per embryo.

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Asymmetrical Expressions of Supernumerary Toes

Taylor and Gunns (op. cit.) found that asymmetrical numbers of toes occurred in about 1/6 of the diplopod embryos they examined, and that 5/6 of these had more toes on the right than on the left foot. Their data included post-outcross progenies, as well as those produced by the original carrier stock. This finding was later confirmed by Landauer (1948). Other investigators (Punnett and Pease, 1929; Warren, 1944; Landauer, 1948) have found that in cases of asymmetrical development of extra digits in other types of polydactylism, the left side is generally more affected than the right. This observation has been explained (by Landauer) as a reflection of the well known differential growth potentials on the two sides of the developing avian embryo. He further postulated that the failure of diplopodia to conform to this general rule might be due to the specific time of action of the **dp** gene; and suggested that the side of the embryo which shows the more intense growth activity at the determinative period is more resistant to a genic effect.

Procedures and Results

Changes in expressivity of diplopodia accompany changes in penetrance

The three lines of diplopod carriers established by Taylor, Abbott and Gunns included line I, in which the original 3:1 phenotypic segregation ratio was restored; line II, selected for an intermediate (12-14%) incidence of diplopod offspring but which had dropped below a 10% incidence by 1954; and line III, selected for the lowest detectable segregation ratios, and which approximated a 5% incidence of diplopod offspring in 1954. Descriptions of all diplopod specimens produced in the three lines and in line crosses detailed the numbers of extra toes and fingers, the presence or absence of the hallux and extra metatarsals and the presence of a normal or shortened pre-maxilla. Early in the period of line differentiation it became obvious that phenotypic variability in the expression of the diplopod syndrome was increasing. New effects of the mutation were discovered, especially in the normal-ratio line. The original method of describing specimens gradually became inadequate; that is to say, distinct differences in the extent of reduction of the pre-maxilla, tibiotarsus and tarsometatarsus and in the degree of tibiotarsal bending were found. The investigator conducting the post-mortem examinations was tempted more and more to make use of comparatives such as "slightly" or "greatly reduced", etc. It became clear that this variability was increasing between lines and decreasing within lines, at least within line I. Accordingly, after line separation had progressed to the point that significant differences in phenotypic ratio could be demonstrated between line I and the two lower-ratio lines a more comprehensive analysis of phenotypic expression was carried out. This paper deals specifically with the analysis of differences in the expressivity of diplopodia in the three selected lines and in line crosses from data collected during 1953 and 1954. In addition a further study of asymmetry or discordance in digit expression was made. Data on discordance are included for the period between 1947 and 1954. In 1954 the incidence and type of discordance in wing finger expression was studied as well.

The procedure used in recording data on phenotypic expression was as follows: In the case of digit number, which was amenable to a quantitative treatment, total numbers of fingers[1] and toes present on each foot and wing were recorded. This procedure allowed a consideration of embryos with normal expressions on any limb. For shortening or bending of the long bones and for reduction in the length of the premaxilla, a present or absent classification and a classification describing severity were adopted. In each case three arbitrary classes were used where 1=slight, 2=intermediate and 3=severe. Thus, in the case of disproportionate long bone reduction (micromelia) class 1 included diplopod embryos with the tibiotarsi equal in length to the tarsometatarsi and both bones approximating 2/3 of the length of the normal tibiotarsi. Class 2 included embryos with tibiotarsi shorter than the tarsometatarsi and approximately 1/2 of normal length, and class 3, embryos with tibiotarsi 1/2 the length of the tarsometatarsi and less than 1/3 of normal length. A simple present or absent classification was used for hallux, pollex and newly discovered expressions of diplopodia such as egg tooth ventral (rather than dorsal), wolf jaw and other beak abnormalities. Data relating to the pattern of expression of supernumerary digits were recorded by means of a code describing their relative size, shape and position. The observation that abnormal development in organ systems not usually diagnostic for **dpdp** appeared to be increased in diplopod embryos led to the systematic recording of the incidence of all anomalies in all embryos, whether diplopod, normal or early unidentified deads. Where possible, as in crooked toes, account of severity of the defect was taken; in this instance severity was measured in four grades from 1, slight, to 4, severe. All data were recorded on I.B.M. cards.

NEW EXPRESSIONS OF DIPLOPODIA

1. *Attenuated expressions*

(*a*) *Wing duplicates*

A new phenotype considered here to represent the most attenuated and yet identifiable form of the mutation was discovered in low-ratio progenies in 1953 and 1954. These embryos lack the diagnostic supernumerary toes but have other phenotypic expressions of the diplopod syndrome including extra wing fingers. Taylor and Gunns classified embryos as normal or diplopod according to the presence of extra toes. They found additional wing fingers in 97% of the diplopod embryos they examined. They did not find embryos with extra wing fingers but normal numbers of toes. In addition to extra digits on one or both wings, this new class of embryo typically has a slight micromelia and tibiotarsal bending and either a normal or very slightly reduced premaxilla (Plate 2). Wing duplicates (as we have called them) generally survive the incubation period; some hatch, but the majority are found alive-in-shell at hatching time.

[1]As explained in Taylor, Abbott and Gunns (1959) total finger number refers to the anterior complement in a spread wing (replacing or in addition to the pollex).

The high incidence (Table 1) of this type of embryo in line III progenies, typified also by a higher incidence of other atypical expressions of diplopodia, as well as their absence in line I, supports the suggestion that wing duplicates represent the final identifiable stage in the phenotypic modification of the diplopod mutant toward normality.

(b) Non-duplicates

A second phenotype, which may represent a further attenuation of diplopod expression, was observed in 1954. Non-duplicates (Plate 3) have no extra digits on the feet or wings but show instead some combination of the following characteristics, including at least two of the first three listed below:

(1) micromelia of the lowest grade;
(2) pre-maxilla slightly reduced;
(3) tibiotarsi slightly bent; those which hatch may be crippled;
(4) ventral position of the egg tooth;
(5) body size slightly less than normal siblings.

For all calculations of phenotypic progeny ratios both wing duplicates and non-duplicates were perforce considered as normal segregants since they lack the criteria (doubled foot structures) which has given diplopodia its name (see Taylor, Abbott and Gunns, 1959). However, in this paper, dealing as it does specifically with expressivity of the diplopod syndrome, wing duplicates are considered as attenuated forms of diplopodia; non-duplicates, which are more difficult to recognize and which may be confused easily with sporadic types of micromelia, are classified as normal embryos. Their incidence in 1954 is reported in Table 1.

Table 1. *Incidence of Diplopods (D), Wing-duplicates (WD) and Non-duplicates (ND) Among 1954 Progenies.*

Parental Line ♂♂	Parental Line ♀♀	Total Progeny Classified	Phenotype % D	% WD	% ND
I	I	1797	26·2	0·0	0·2
II	II	822	8·2	1·8	1·2
III	III	629	4·3	4·8	3·2
I*	II	690	16·7	3·2	1·4
I*	III	507	13·6	2·0	1·6
I	LC†	136	15·4	2·2	1·5
III	LC†	614	7·2	3·4	1·5

* Reciprocal cross data.
† LC—female progeny from a mating of a line III male with line I females.

2. *Severe expressions*

(a) Abnormal position of the egg tooth in diplopod embryos

The egg tooth, a horny protuberance located in normal embryos on the dorsal surface of the upper beak and used by the chick to pip the shell at hatching time, is in an ab-

normal ventral position in a large proportion of diplopod embryos. In 1953 the several lines and line crosses differed in the frequency of this defect. The egg tooth was located in the abnormal ventral position in 75·6% of the diplopod embryos of line I but in only 4·9% of those of line III. The ventral position of the egg tooth may be one of the major causes of lethality (failure to hatch) of diplopod embryos. In both 1953 and 1954, all diplopod hatched chicks had the egg tooth in its normal dorsal location.

(b) Wolf jaw (la gueule de loup)

Another anomaly affecting the beak region, the absence of the latero-ventral part of the maxilla, was found in diplopod embryos, especially those of line I, but not in their normal siblings (Plate 4). The edge of the maxilla in this region is serrated. The normally cleft palate of the chicken is widened to form a large irregular breach. The nasal orifice remains open ventrally. Occasionally the defect involves the face parts adjoining the maxilla. Expression may be either unilateral or bilateral. Unilateral or disproportionate involvement of the two sides of the face leads to asymmetrical beak development and thus to a typical crossed beak. In some cases the maxilla does not show the characteristic defect but is crossed and extremely short. This syndrome resembles one described by Ancel (1950), as *la gueule de loup* (wolf jaw), which results from a deficiency in, or faulty development of, the upper maxillary bud. Ancel reported that more severe interferences with the developing maxillary bud led to coloboma of the face, which frequently occurred with wolf jaw. Crossed beaks occurred in cases of unequal involvement of the two sides of the face in Ancel's material as well. Ancel was able to produce *la gueule de loup* and facial coloboma by arresting the development of the upper maxillary bud by the injection of trypaflavine into the early developing embryo. Such treatments also led to abnormal development of the limbs and of celosomia.

Table 2. *Incidence of Wolf Jaw (WJ) and Crossed Beak (CB) in Diplopod* Progeny, 1954.*

Parental Line ♂♂	♀♀	Diplopods Classified	Number			Percent	
			WJ	CB	WJ+ CB	WJ	WJ+/or CB
I	I	464	44	9	56	21·6	23·5
II	II	82	3	2	2	6·1	8·5
III	III	56	0	0	0	0·0	0·0
I†	II	135	5	3	10	11·1	13·3
I†	III	79	0	1	2	1·3	3·8
I	LC	23	0	2	2	8·7	17·4
III	LC	64	0	0	0	0·0	0·0

* Includes embryos with extra wing digits but normal numbers of toes.
† Data from reciprocal crosses.

In a similar fashion Landauer (1952) produced a syndrome of anomalies resembling in several respects those of the line I diplopod phenotype, complicated with wolf jaw,

by injecting boric acid into developing embryos. He found the most frequent beak and face defects to be cleft palate and bilateral facial coloboma, both found in some diplopod embryos, and a shortening of the lower beak, not found in diplopodia. Limb abnormalities accompanying these facial defects included micromelia, abnormal bending of the limbs and curled toes, all of which are characteristic of the diplopod phenotype.

In general, diplopod embryos with severe expressions of diplopodia in other organs (13 or 14 toes and 9 or more fingers) had wolf jaw as well. In line I, 23·5 percent of all diplopod embryos classified in 1954 had either wolf jaw, wolf jaw complicated with coloboma or some form of crossed beak. This syndrome was rare in embryos produced from matings of the intermediate ratio line and absent in the low-ratio line (Table 2). A high incidence of wolf jaw was characteristic also of crosses of line I.

Abnormalities not diagnostic for diplopodia

1. *Crooked toes*

Crooked toes, a foot defect expressed as a persistent flexion of the plantar digits of the foot, is influenced by both hereditary and environmental factors (Hicks, 1953) and occurs frequently in diplopod embryos. The incidence of crooked toes here reported for diplopod segregants refers only to the embryonic and day-old form of the disorder. All embryos, reaching 13 days of embryonic age, were classified as normal or crooked-toed; and if the latter, the severity of the defect was described in four grades

Table 3. *Incidence and Average Severity of Crooked Toes, 1953.*

Class of Embryo	Parental Line ♂♂	Parental Line ♀♀	Percent Crooked Toes		Right or Left Foot	Average Severity	
			Right Foot	Left Foot		Right Foot	Left Foot
Diplopod (C × C)†	I	I	57·5	52·5	66·4	2·1	2·2
	II	II	41·5	32·1	47·8	2·0	2·2
	III	III	30·0	20·0	38·7	1·6	2·0
	I	II*	50·7	45·3	59·0	1·8	1·9
	I	III*	28·6	52·4	57·1	2·0	1·6
	II	III*	25·0	25·0	25·0	1·0	2·0
	TOTAL		49·6	45·7	59·4	2·0	2·1
Normal (C × C)	I	I	6·9	6·8	12·1	1·8	1·8
	II	II	3·6	4·1	6·6	1·5	1·6
	III	III	7·4	5·1	12·8	1·6	1·6
	I	II*	7·4	8·6	14·5	1·7	1·6
	I	III*	6·4	6·2	10·3	2·1	2·0
	II	III*	4·4	6·7	10·8	1·5	1·3
	TOTAL		6·3	6·3	11·2	1·7	1·7
Normal (NC** × C)			4·9	4·4	6·7	1·7	1·6

* Reciprocal crosses.
† C=Heterozygous carrier of **dp**.
** NC=Noncarrier of **dp**.

from slight (grade 1) to severe (grade 4). Crooked toes occurred on either one or both feet, were of the same grade on both feet or showed bilateral discordance in expression (Plate 5). In diplopod embryos this defect involved both the normal and supernumerary digits at the same time or the normal or supernumeraries alone. Crooked toes were sometimes found together with a curled-toe condition. A few embryos had curled toes only; these often showed other symptoms of riboflavine deficiency.

The incidence of crooked toes in the diplopod stocks was relatively high. The incidence in normal embryos from non-carrier matings was 6·7%, and that from carrier matings was 11·2%, considerably higher than the 2% incidence reported by Hicks for the U. C. Production Flock. The incidence of crooked toes was far higher in diplopod embryos than in their normal siblings. In 1953, 59·4% of all diplopod embryos classified for this defect had crooked toes on either the right or left foot or both (Table 3). Asymmetry in both expression and severity was common. However, no significant difference in the frequency of crooked toes between the left and right foot was found. Line I and crosses of line I produced the largest proportion of affected embryos with the highest average grade of the defect. The relatively high incidence of crooked toes in normal embryos may reflect the comparatively high level of inbreeding of all diplopod stocks. The high incidence in the progeny of line crosses may reflect the level of inbreeding of the dam, influencing nutritional value or shell quality, rather than that of the offspring. χ^2 values comparing the proportion of crooked toes in normal and diplopod embryos of each line indicated that in each case, the defect was significantly more frequent in diplopod embryos than in their normal sibs. The χ^2 values for the three comparisons were all highly significant; line I, 415·7; line II, 143·1; and line III, 14·8.

2. *Non-specific anomalies*

Non-specific abnormalities were three times as frequent in diplopod embryos as in normal siblings from the same matings (Table 4). The total incidence of these abnor-

Table 4. *Incidence of Sporadic Anomalies in Normal (N) and Diplopod (D) Progeny, 1953.*

Parental Line ♂♂	Parental Line ♀♀	Embryo Phenotype	Number of Progeny	% of Progeny with Anomalies
I	I	N	1192	19·0
		D	356	67·1
II	II	N	608	19·4
		D	119	55·5
III	III	N	315	18·1
		D	31	38·7
I	II*	N	502	22·3
		D	85	63·5
I	III*	N	390	18·7
		D	44	52·3
II	III*	N	92	28·3
		D	4	25·0
TOTAL		N	3099	19·8
		D	639	61·8

* Reciprocal crosses.

malities in diplopod embryos was higher in line I matings than in line II or line III matings. The abnormalities considered under this heading included a variety of malformations, non-specific disturbances of development and chick defects. The malformations included cerebral hernia, edema, heterotaxia, microphthalmia, disproportionate retardation, dwarfing, anterior and posterior duplications and omphalocephaly. The non-specific disturbances included hemorrhage, ruptured yolk, strangulation by yolk sac, unabsorbed yolk and anemia. The chick defects included crippling, spraddled legs and defective seal of the abdomen at the umbilicus. Edema, hemorrhage and celosomia, especially, affected a large proportion of diplopod embryos, particularly those from line I matings.

The relationship between number of toes or fingers and penetrance

Both total foot and total wing digit number were related to parental line. Embryos from line I had the highest number of both foot and wing digits, line II embryos were intermediate and those of line III had the lowest number. Line crosses were intermediate between parental lines except for the cross III × I in 1953, where the numbers of digits in diplopod embryos approximated line III. Figures 1 and 2, which are based on

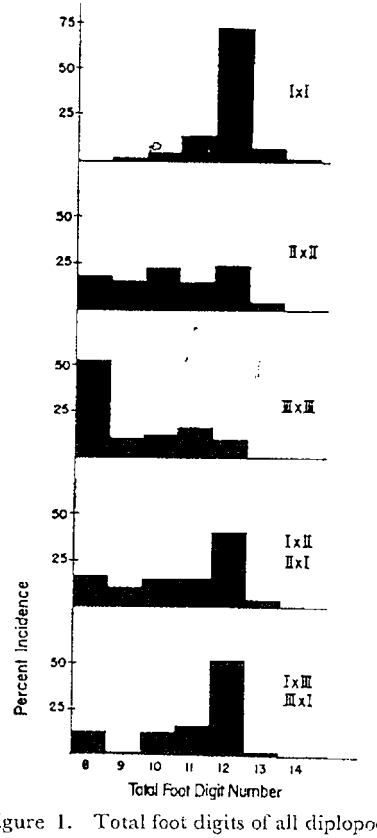

Figure 1. Total foot digits of all diplopod embryos from matings of line I, II and III and line crosses in 1954.

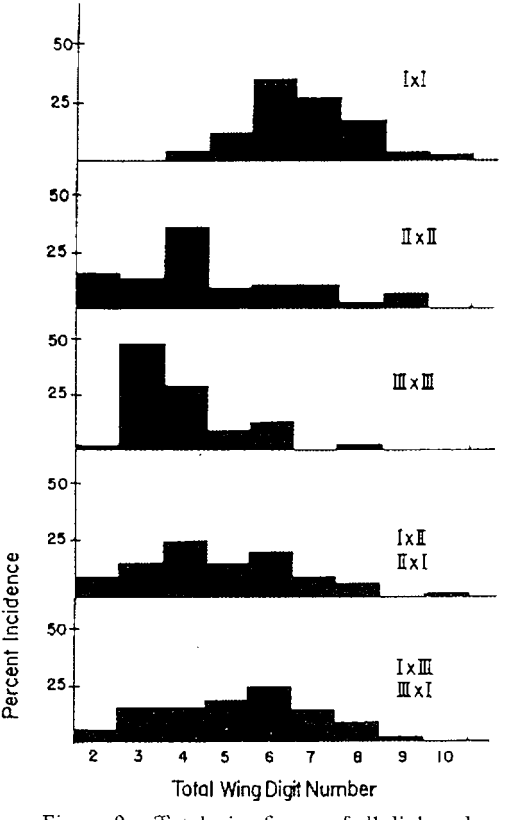

Figure 2. Total wing fingers of all diplopod embryos from matings of lines I, II and III and line crosses in 1954.

total foot and wing digit numbers respectively, illustrate this point. In this case the comparison is based on the total number of digits (both normal and supernumerary) of both feet, in Figure 1, and of both wings[2], in Figure 2. Thus, for foot digits the classes include 8, the normal complement of four digits per foot, through 14, the maximum number of foot digits observed in any embryo. These totals represent any combination of right and left foot or wing digits with the restriction that the minimum number of digits on any one foot is 4 and that on any one wing, one. That the difference in digit numbers, illustrated in Figures 1 and 2, was a real one is attested to by Table 5,

Table 5. *Analysis of Variance of Foot Digit Number, 1954.*

(*a*)

Source of Variation	df	Mean Square	F
Total	607		
Between Lines	2	234·86	150·55*
Between Sires	14	1·56	1·19
Between Dams	107	1·31	
Within Dams	484	·77	
Mean Digit Number			
Line I	11·8±·01		
Line II	10·3±·03		
Line III	9·2±·04		

Analysis of Variance of Wing Digit Number, 1954.

(*b*)

Source of Variation	df	Mean Squares	F
Total	604		
Between Lines	2	286·28	51·77*
Between Sires	14	5·53	3·95
Between Dams	107	1·40	
Within Dams	484	1·70	
Mean Digit Number			
Line I	6·6±·03		
Line II	4·6±·46		
Line III	3·9±·25		

* $P < .01$.

which summarizes the results of an analysis of variance of total foot and wing digit number for the three lines. This study also indicated that variation in progeny digit number was related to parental phenotypic segregation ratio. Embryos of line I were least variable in both foot and wing digit number and those of line II and line crosses most variable. Wing digit number was far more variable than foot digit number.

Variable expression of the diplopod syndrome

Diplopod embryos of the three selected lines differed both in the frequency with which different organs affected by the gene were altered and in the relative severity of

[2]See footnote 1, page 194.

such alterations. The majority of **dpdp** embryos produced by line I matings exhibited a phenotypic expression of diplopodia in all organs characteristically altered by the gene (Figure 3). Furthermore, the majority had either the intermediate or severe grades of expression and seldom the attenuated forms. On the other hand, diplopod embryos from line III matings often exhibited normal phenotypic expressions in a number of the organs typically altered in **dpdp** embryos; organs which were affected showed the mild or intermediate form of the disorder and seldom the severe form. Line II embryos were more variable in expression than those of either line I or line III. Diplopod embryos with typical expression of all parts of the phenotype, those with severe expression in certain organs and attenuated expression in others and those with nearly normal phenotypes were common in progenies of this line. Line crosses also produced a wide range of types of diplopod embryos; they varied in number of **dpdp** phenotypic effects per embryo and in grade of severity of these effects. The occurrence of embryos with diplopod expression limited to only certain organs, and yet present in severe degree in these, as well as those with both severe and mild expressions in progenies of the same birds, indicated that the several "pleiotropic" effects of the **dpdp** genotype may be independent of each other. Pleiotropy is not a useful term in this context, seeming, as it does, to imply some common causality.

Average expression in the several organs characteristically altered in diplopod embryos is illustrated for the three lines and the line crosses in polygonal graphs (Figure 3). These graphs do not take into account the variability in expression within different matings and between different characters, which has been exemplified by total foot and wing digit number. Seven morphological effects of **dpdp** and the incidence of embryonic death in the three lines of 1953 are represented on the eight radii of an octagon (Figure 3*a*). The point farthest from the intersection of radii in each case represents the theoretical maximum effect of the **dpdp** genotype. Some of the characters represented on the graphs were measured in grades, and for these a *severity index*, taking into account both incidence and grade of expression, was computed. For present or absent characters, the percentage point represented on the graph is equivalent to the incidence of the anomaly, expressed as a percentage of all diplopod embryos. Figure 3*b* represents foot and wing expression (severity, incidence and type of asymmetry), premaxillary expression and percent of embryos lacking the pollex from each line and linecross of 1954.

Selection for lower phenotypic ratios of diplopodia decreased the average expression in all characters measured. Several of the characters considered differed in their response to selection. Lines I and III are distinct in phenotypic expression while line II produced embryos with intermediate expressions, closer to that of line III.

Asymmetrical expression of extra toes and fingers

The incidence of diplopod embryos with unequal numbers of extra toes on the right and left foot increased after the introduction, in 1942, of germ-plasm from the unrelated U. C. Production Flock. The incidence fluctuated between 1947 and 1952 but increased after this time (Figure 4*a*). This latter trend reflected in part, at least, the relatively larger proportion of diplopod embryos coming from matings of lines II and

III and from line crosses in 1952, 1953 and 1954. Symmetrical expression of extra digits was highest in line I diplopod embryos, intermediate in line II and lowest in line III in both 1953 and 1954 (Figures 3*b* and 5). Both line crosses had concordance[3]

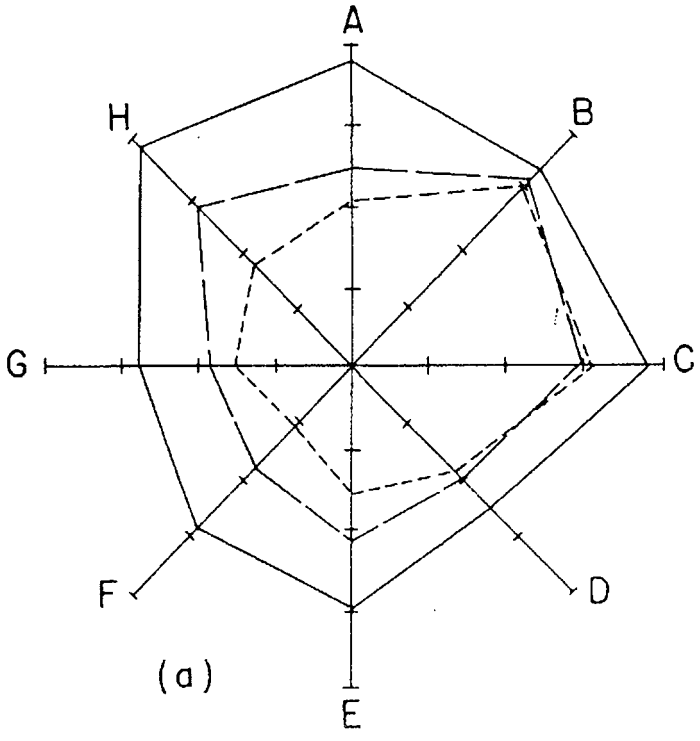

Figure 3*a*. Seven morphological expressions of diplopodia, 1953.
line I———; line II— — —; line III - - - - - -

Radii	*Character*
A	% hallux absent
B	foot digit severity index
C	% diplopod embryos dying in shell
D	wing digit severity index
E	% diplopod embryos with ventral egg tooth
F	limb curvature severity index
G	micromelia severity index
H	metatarsal severity index

approaching or surpassing the lower parental line. In line I, which produced the highest proportion of diplopod embryos with symmetrical expressions of digit number, most often symmetry was associated with the typical expression of diplopodia,

[3]Concordance is here used to denote equivalent expression on the two sides of the body in either foot or wing digit number, and discordance, the opposite expression, *i.e.* unequal involvement of the two sides.

three extra foot digits. Matings producing a substantial proportion of embryos with attenuated expressions of diplopodia also had a higher proportion with an asymmetri-

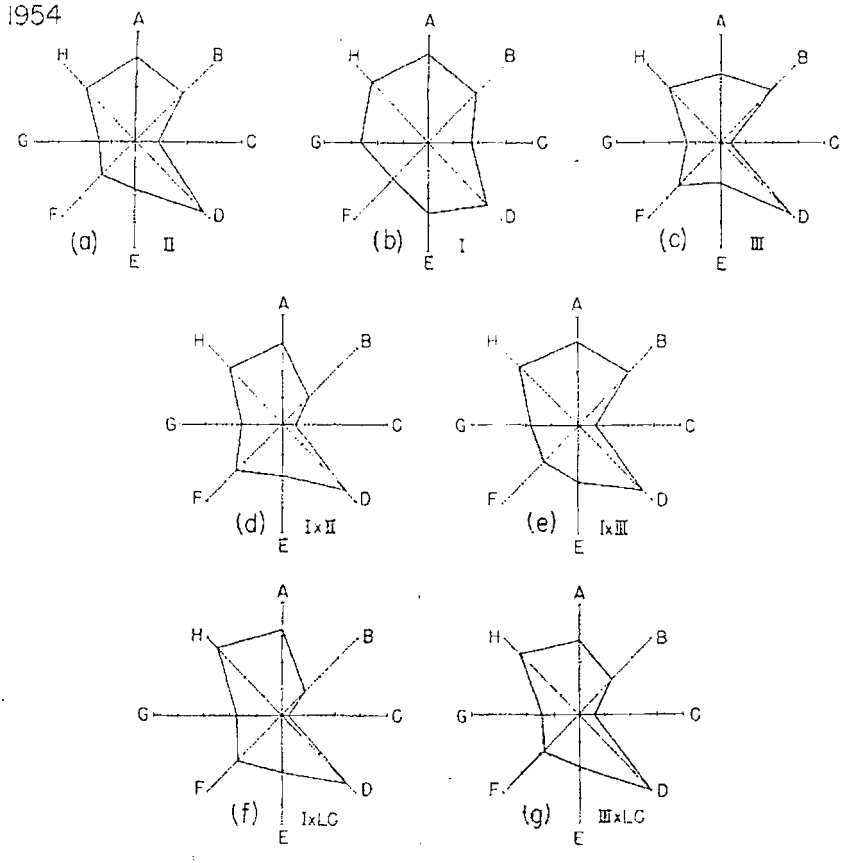

Figure 3*b*. Morphological expressions and concordance in diplopodia, 1954.

(*a*) line II
(*b*) line I
(*c*) line III
(*d*) line I × line II (reciprocal cross)
(*e*) line I × line III (reciprocal cross)
(*f*) line I × linecross♀♀ (line III × line I, 1953)
(*g*) line III × linecross♀♀ (line III × line I, 1953)

Radii	*Character*
A	foot digit severity index
B	% right asymmetry in foot digit number
C	% pollex absent
D	% right asymmetry in wing digit number
F	% wing digit concordance
G	pre-maxillary severity index
H	% foot digit concordance

cal expression of extra digits, most generally among the attenuated forms. On the other hand, a tendency toward an increase in more severe expressions (four extra digits per foot) in line I increased the proportion of diplopod embryos with discordant expressions of foot digit number in this line in 1954. Embryos with five or seven digits on

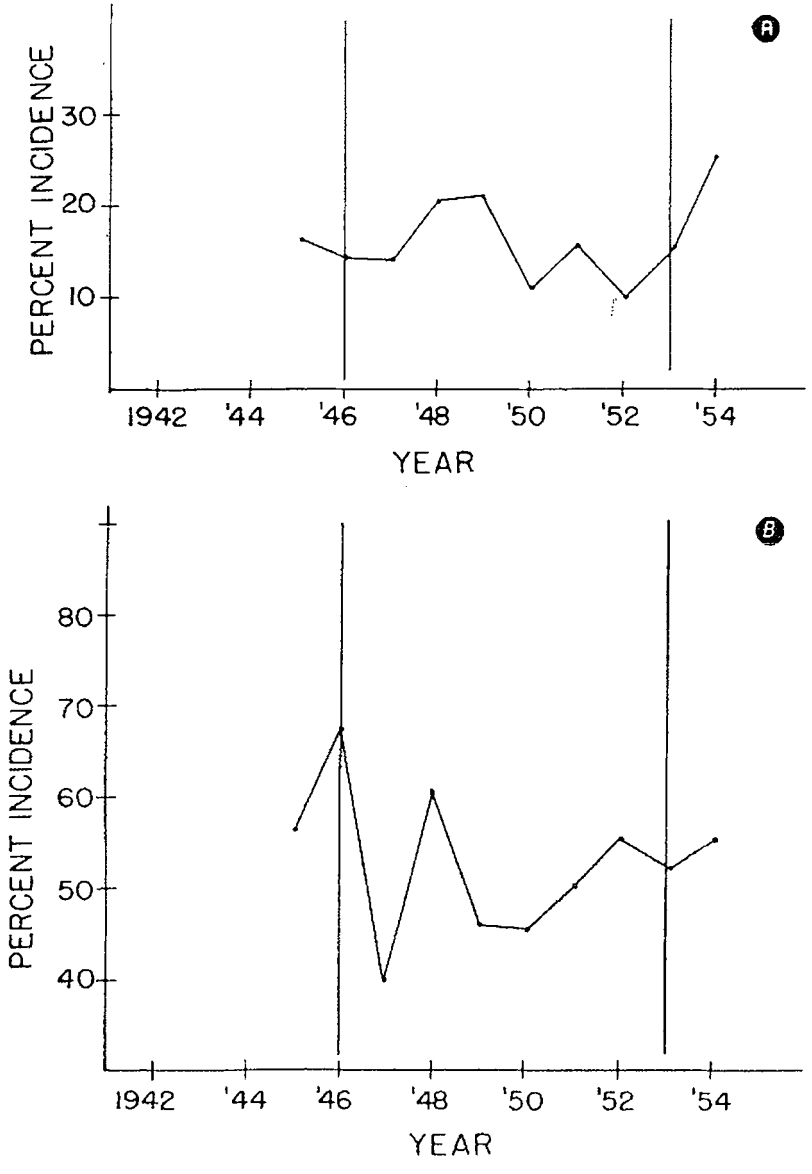

Figure 4 A Incidence of discordance in foot digit number in diplopod embryos in 1943 and from 1945-1954.

B Incidence of diplopod embryos with more digits on the right than on the left leg as a proportion of total discordant in 1943 and from 1945-1954.

one foot were more likely to have a different number on the other foot than those with four or six digits (Table 6).

Table 6. *Concordance in Several Expressions of Digit Number*

(a) Foot Digit Number

Total Digits Right Foot	Number Diplopods	% with Identical Number Left Foot
8	1	0·0
7	38	21·1
6	686	83·2
5	209	54·5
4	134	78·4

(b) Wing Digit Number

Total Digits Right Wing	Number Diplopods	% with Identical Number Left Wing
6	2	0·0
5	36	25·0
4	287	35·9
3	454	61·2
2	250	58·0
1	36	83·3

Taylor and Gunns reported that three or four wing fingers were present in diplopod embryos. They did not report on discordance in wing finger expression. Accordingly, data were collected in 1953 and 1954 comparing discordance in toe and finger expressions in the several lines and crosses. Wing digit number proved to be far more irregular in its expression than foot digit number in diplopod progeny of all lines and crosses. However, the highest proportion of embryos discordant for finger number occurred in line III or in line cross progenies. Concordance in numbers of fingers was also associated with the typical expression of diplopodia (three digits per wing) and discordance with both the more severe and attenuated expressions (Table 6). Discordance in both toe and finger expression was higher in 1954 than in 1953 in line I, where it was also associated with an increase in the number of embryos with the most extreme manifestations of diplopodia, seven foot digits and five or more wing digits.

Concordance in both foot and wing digits was studied in the following manner. Diplopod embryos with equal numbers of digits on the feet were described as 1- in a two numeral expression, while those with unequal numbers of digits on each foot were described as 0-. Expression in the wings was recorded in the same manner with the wing description following that of the legs. The proportional representation in the four classes describing symmetrical digit expression changed between 1953 and 1954

(Figure 5). In all lines and crosses the proportion of diplopod embryos falling into the 1-1 class decreased while the proportion in the 1-0 class increased. In line I progenies this change was due to an increased severity of expression (an increase in the proportion of diplopod progeny with four, five or six digits per wing). On the other hand, in line III progenies the change was due to a reduction in both total numbers of toes and fingers. A higher proportion of embryos had only the normal complement of toes but one extra digit on the right wing. A χ^2 analysis of the relationship of concordance in foot digit number with that in wing digit number revealed that in line I the two systems were dependent (related) while in lines II and III they were independent.

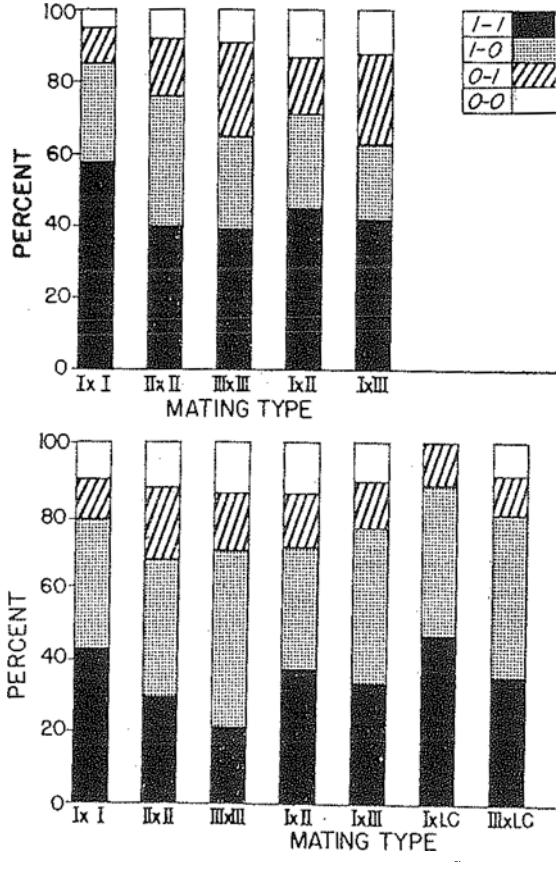

Figure 5. Concordance and discordance in foot and wing digit number in 1953 (above) and 1954 (below).

1-1 feet and wings concordant
1-0 feet concordant, wings discordant
0-1 feet discordant, wings concordant
0-0 feet and wings discordant

Discordance in digit number implies that one of the feet or wings (as the case may be) will have more digits than the other. According to Taylor and Gunns (1947), 5/6 of the embryos they found with unequal numbers of toes on the two feet had the larger

number on the right foot[4]. It should be noted that Taylor and Gunns came to this conclusion from a study of diplopod embryos produced both by the original stock, in which the mutant was found, and in progenies with varying amounts of foreign germplasm (from the U. C. Production Flock). Landauer, who received some diplopod carrier stock from this station, later confirmed Taylor and Gunns' conclusion (1948).

Right asymmetry in toe number was not found to be characteristic of all diplopod stocks. The original high incidence decreased (Figure 4B).

In 1953 and 1954 the three selected lines and the several line crosses differed in incidence of right asymmetry in both feet and wings. Wing digits showed a far higher incidence of right asymmetry than foot digits in all cases. The highest incidence of right asymmetry in foot digit expression was found in line I, the lowest in line III and in line III × LC cross while the highest incidence in wing digit expression occurred in line III progenies and in the mating of line III × LC (from III × I) and the lowest in line I. The high proportion of embryos with right asymmetry in finger number in the low-ratio line directly reflected the incidence of wing duplicate embryos. This most attenuated identifiable expression of diplopodia consists largely of embryos with two digits on the right wing (one supernumerary) and only the normally present wing finger on the left. The converse arrangement was rare.

Bilaterality in position, relative size and arrangement of extra digits.

Bilaterality in extra digit arrangement was investigated in diplopod embryos from the three lines and line crosses. Normal embryos have identical sets of four toes on both right and left feet and one finger on right and left wings. These sets of toes and fingers are identical in size, shape and position. Diplopod embryos may have the same or different numbers of toes per foot and fingers per wing. In the case of identical numbers of toes on a pair of diplopod feet, the normal group (II, III and IV) will always be identical in pattern on both feet. The supernumeraries may or may not be identical. They may differ in position, size or shape or all three (Abbott, 1959). Similarity in pattern (pattern concordance) in both foot and wing digits was greatest in line I and lowest in line II (Table 7). The most common expression of diplopodia

Table 7. *Incidence of Pattern Concordance in Digit Patterns of Diplopod Embryos*

Parental Line ♂♂	♀♀	% Concordant Foot Patterns	% Concordant Wing Patterns
I	I	70·8	47·0
II	II	55·5	46·3
III	III	52·3	61·1
I*	II	61·8	43·7
I*	III	31·8	50·0
II*	III	50·0	75·0
TOTAL		64·2	47·6

* Reciprocal cross data.

[4]For the sake of convenience a discordant expression of digit number in which the right foot or wing has the larger number of supernumerary digits is here referred to as right asymmetry. The opposite expression is described as left asymmetry.

(six digits per foot) also exhibited the highest incidence of pattern concordance. Wing digit patterns were less consistent than foot digit patterns but in this case too, embryos with three wing fingers had the highest degree of exact bilateral replication in pattern.

DISCUSSION

The studies of diplopodia (Taylor, Abbott and Gunns, 1959; Abbott, 1959) and the results reported here illustrate the interrelationship between the action of a major gene and its residual genotype in vertebrate organisms. They demonstrate the dependence of all developing parts of an embryo on the entire genotype.

The investigation of the manifestation of a major gene in a variety of genetic environments is a means of establishing the nature of whatever interactions may exist. The study of the ontogeny of such a mutant phenotype helps to clarify these relationships. As well as providing information relating to the major action of the gene, this comparative approach is useful in interpreting development in terms of what Waddington (1940, 1942) has called canalization.

In diplopodia the ease with which both the penetrance, or proportion of homozygous genotypes expressing diplopodia in a measurable form, and the expressivity, or form that this phenotypic manifestation may take, may be changed by altering the genetic milieu is impressive. The effect of a gene in a line of birds, selected from a population exhibiting decreased penetrance and expressivity, can be increased until the gene achieves a regular mendelian behaviour. This process is illustrated in the present study by the reestablishment of a line of carriers, typified by the complete penetrance and the predictable expressivity of a recessive genotype. While selection for regular mendelian behaviour may be successful, efforts to alter dominance relationships are more tedious, as illustrated by the failure of our attempts to exceed a 3:1 phenotypic ratio significantly through the selection of line I birds with especially high phenotypic ratios for diplopodia (Taylor, Abbott and Gunns, 1959).

A second type of change in gene expression, exemplified in this study by the low-ratio line, is selection in the direction of a new type of balance and expression characterized by the gradual attenuation of the deleterious effects of the mutant syndrome. Concomitantly, ill-adapted and inviable forms appear among the normal segregants of the population. The probable end point of this type of selection is the masking of a once lethal phenotype in normality.

In the case of diplopodia, the original carrier stock was at a stage where all affected individuals expressed the diplopod character in a highly regular fashion. In other words, the population was characterized by full penetrance and a relatively uniform expressivity in terms of all the organs affected and time of embryo death. The mutation undoubtedly had been present in this stock for several generations since it was fairly widespread when first discovered. The results of our selection program (from the post-outcross stock) indicate that a balance of this sort, if not present initially, could be established in as few as four or five generations under adequate selection pressure. There is no particular reason for assuming that a mutation, when it first occurs, must

conform to a specific mendelian phenotypic ratio. The particular population genotype in which it appears may undergo considerable alteration before predictable phenotypic segregations result. Flexibility in this respect may be brought about by changes in both penetrance and expressivity. Regularity in behaviour of a mutation or the apparent lack of it thus depends on the other components of the genotype. As a result the history of the population carrying a mutation and that of the mutation itself, in terms of numbers of its appearances, affect its behaviour at any given time. After an initial occurrence, its spread and eventual integration into the population depends on its effect on fitness. If low in penetrance and variable in expressivity and lethality initially, it may be found in more extreme forms guaranteed to eliminate homozygotes. If it has only slight effects, eventually modification may proceed toward the gradual disappearance of any detectable expression.

The introduction of foreign germ-plasm from the Production Flock into the diplopod carrier stock destroyed the existing balance and produced a number of birds typified by progenies varying in penetrance and expressivity. Subsequently, artificial selection favored, in one case, individuals with genotypes allowing full expression of diplopodia and in the other, those with genotypes masking the phenotype effectively. In this regard it is interesting to note that Landauer (1956), studying a second and apparently independent mutation for diplopodia in Minorca fowl, reported a broader range of phenotypic variability (toe number, complexity of hand structures and age at death) than that typical of the original diplopod mutation in Leghorns. However, the increasingly severe expressions of the syndrome now typical of line I more closely approach the Minorca mutant and, indeed, considering our three lines at once, our phenotypic variability transcends that of the new mutant. It is not clear how much of the higher expressivity reported by Landauer may be due to actual differences in gene activity and how much to differences in residual heredity. The genetic background provided in the Minorca stock may allow greater developmental lability. Thus Landauer (1953) found highly significant differences in response to boric acid treatment between embryos from Black Minorca and certain white breeds.

Two alternative explanations of the manner in which the residual genotype exerts its control over penetrance and expressivity in diplopodia may be considered. According to the original premise used in constructing the breeding program, a limited number of specific modifiers of diplopodia were introduced into the diplopod carrier stock from the Production Flock. The impact of these modifiers in inhibiting diplopod expression was detectable in the progeny of females of the second backcross generation. These atypical carriers exhibited both lower penetrance of the gene and variable and lower grades of expressivity. Subsequent selection eliminated these inhibitors from line I and established them in different frequencies in lines II and III. The highly variable behaviour of line II in both penetrance and expressivity, after selection, suggested heterozygosity for the modifiers while the strong ratio-suppressive effect of certain line III individuals, suggested homozygosity for one or more modifiers in this line. According to this interpretation, the variable phenotypic segregation ratios obtained by crossing lines, indicates heterozygosity of the intermediate- and low-ratio

lines for different numbers of modifiers. Back-crosses of linecross pullets to lines I and III indicated that an explanation of diplopod expression, based on a single dominant inhibitor of **dpdp** expression as suggested by the behaviour of certain line III birds, was not tenable (Taylor, Abbott and Gunns, 1959). Instead the data suggest that a large number of modifiers, some with at least partially dominant action, are present in different combinations in the low-ratio lines.

An alternative view is that the mode of action of the different residual genotypes selected in the three lines is based upon a series of epistatic interactions. Under this assumption, the original diplopod carrier stock had a balanced and internally integrated set of chromosomes, stabilized to produce full phenotypic expression of diplopodia according to a simple 3:1 expectation. The phenotype was highly regular in form and almost completely lethal during embryogeny. The introduction of new chromosomes, with a different internal balance from the Production Flock, resulted in the upset of this system. Both the first atypical ratios and the first atypical expressions appeared in the progeny of a backcross to the original diplopod carrier stock as a consequence of the breaking up by crossing-over of the delicately adjusted internal balance. Segregation, recombination and further crossing-over led to the appearance of low-penetrance birds homozygous or heterozygous for different numbers of these unbalanced chromosomal combinations. These birds produced progeny characterized by variable penetrance, expressivity and embryo viability.

Selection for low ratios favored these unbalanced combinations. The variable behaviour of line II, especially in crosses with line I, reflects the heterogeneity and imbalance of this line. Selection far exceeded the original objective, based on the assumption of modifiers, and continues to be effective as more and more chance combinations of chromosome parts give rise to additional "modifier effects". The end point of selection for low ratios is a diplopod carrier stock characterized by the complete absence of a phenotypic expression of diplopodia, based on a new intra- and inter-chromosomal balance. Line III may be approaching this state. However, in this instance the end defeats the means of identification. The predictable and uniform behaviour of line I suggests that the pre-outcross balance has been restored or a new balance attained by the selection of individuals largely free from foreign chromosomes and cross-over products.

According to Waddington (1940), the epigenotype of an organism can best be visualized as a branching system of developmental pathways, each leading to a different expression of one of the components of adult form. Each path is, to a greater or lesser extent, canalized or buffered. This means that the reactions determining the path are so interlocked with one another that there is a strong tendency for a normal result even after considerable environmental or genetic disturbance. Waddington has suggested that such buffering is strongest in wild genotypes and weakest in strains carrying recent mutations. Similarly, Schmalhausen (1949) has indicated that mutants and gene combinations not historically established as normal constituents of natural populations of a species may be deficient in homeostatic responses, even in their usual environments. Other investigators have been led to conclude that phenotypic

likeness does not depend upon genotypic similarity, but rather upon the balance and interrelationships between the various genes and chromosomes present and their interplay during development (i.e., Thoday, 1953; Lerner, 1954).

The most fit individuals or strains are those which give rise to the highest proportion of progeny epigenotypes able to achieve normal phenotypic expression in spite of alterations of their developmental environments. The diplopod studies provide additional evidence that mutant genotypes are impaired in their developmental canalization. Embryos carrying **dpdp,** a genetic stress, are less well buffered in development than those with only one **dp** gene or none. The loss in developmental canalization due to the **dpdp** genotype has been expressed in an increased incidence of nonspecific abnormalities of development in systems not characteristically altered in diplopodia; in bilateral asymmetries of foot and wing digit number (affecting supernumerary digits and occasionally the hallux and pollex) and of pattern or arrangement of supernumeraries; and in irregular phenotypic expressions among the different organs altered by **dpdp.** Landauer (1948) also found bilateral asymmetry in foot digits to be more common in polydactylous progeny of stocks which had not been selected for polydactyly than in progeny of well established polydactylous breeds.

Absence of the normal allele of **dp** acts as a switch mechanism, which throws development out of its normal well-defined channels into a variety of unusual developmental pathways. The residual genotype is not so adjusted that it may always cope effectively with minor environmental fluctuations under these conditions, and consequently, the new course produces a spectrum of forms.

The relative stability of characters used in taxonomy as compared with those seldom used for such purposes, suggests that organ systems also may differ in degree of developmental lability. The diplopod studies indicate that developmental flexibility differs in the several systems characteristically affected by the gene, suggesting that the concept of genotypic canalization may be broadened to include specific structural canalization.

Baumann and Landauer (1944), studying the expression of supernumerary wing fingers in polydactylous fowl, carrying the dominant gene **Po,** found that selection for increased expression of extra foot digits increased the numbers of progeny with extra fingers. However, the response was relatively minor compared to that achieved for foot digits. In addition (in cases of asymmetrical expression) they reported a strong tendency for sinistral heterodactylism in wing digits as well as in foot digits. They concluded that the development of wing fingers in polydactylous stock was in part controlled by the same system of modifiers as those affecting toe number and in part by different modifiers. The relatively low incidence of extra wing fingers at hatching time and in embryos of some advanced ages as compared to the incidence in embryos of approximately nine days of embryonic age corroborated earlier results of Barfurth (1911) and Schmalhausen (1934), who reported that wing fingers were frequently formed in polydactylous breeds and subsequently lost during embryonic development. In diplopodia extra wing fingers persist and in addition show a strong response to selection based on parental phenotypic ratio. Extra foot and wing digits have been found to be independent in the selected low lines. They are characterized by greater discordance

than that reported for polydactyly and in addition tend to have the greater number of extra digits on the right rather than on the left wing. Although the selection method of Baumann and Landauer differed from that employed here, in both instances evidence for independence of the two systems was obtained.

In diplopodia the wing digit system proved to be far more flexible (unstable) than the foot digit system. The fact that asymmetrical expression of digit number was far more common in wings than in feet leads to the inference that the wing digit system is less effectively buffered and so more likely to be influenced by varying environmental or genetic conditions. This difference may reflect the operation of historical selection pressures if it may be assumed that regularity in both the normal and supernumerary complements of digits is under similar control. A strong selective disadvantage results from irregular expression of foot digits, which have a well-defined function, while it is possible that no such selective disadvantage accrues from irregularity in wing digit expression, since the pollex of the normal wing has no known function in the chicken. For this reason natural selection may have failed to organize a system of modifiers able to control expression of this character under stress conditions. The striking asymmetry in wing digit number of all stocks, most marked in the low-penetrance line and in low-penetrance crosses, may then reflect both the lower developmental canalization of the wing digit system as well as the presence of genetic variation not yet stabilized by selection and which gives these groups a higher developmental flexibility.

Rasmuson (1955) made the interesting suggestion that characters that can be allowed some instability in development ought to respond more to artificial selection than those more rigorously determined. The demonstrably greater flexibility of the wing digit system appears to support this proposition. As well as fluctuating more with varying genetic arrangements, total wing digit number showed a greater response to selection (for penetrance) than total foot digit number. By 1954 embryos with ten wing digits were more common in the normal-ratio line and those with only three wing digits were very frequent in the low-penetrance line.

The diplopod studies emphasize the dangers besetting attempts to infer causal explanations of related or pleiotropic phenotypic effects. The original diplopod carrier stock and the selected normal-ratio line were both characterized by full penetrance and expressivity in a number of morphological characters. Accordingly, diplopodia could be described as a pleiotropic syndrome. However, the demonstration that lines could be established characterized by different incidences of these phenotypic effects and different severities of expression of each suggested that parts of the syndrome may be independent and that their relationship is largely a property of the integration of the genotype as a whole. In addition the occurrence of characters not known to be related to the action of **dpdp** as well as the general tendency for other systems to display an increased variety of non-specific developmental disturbances in the presence of **dpdp** becloud efforts to use meaningfully the term pleiotropic syndrome in reference to the diplopod case.

The tendency to abnormal development in systems or organs not diagnostic for **dpdp** also proved to be dependent to some extent on the residual genotype. If one is prepared

to consider micromelia, pre-maxillary reduction, and egg tooth position as pleiotropic effects, the incidence of all of which is lowered by selection of low-penetrance stocks, then there appears to be equal justification for considering any characteristic appearing with comparable regularity in association with the diplopod phenotype as a pleiotropic effect as well. For example, crooked toes were frequent in diplopod embryos of the normal-penetrance line, yet this character is completely disassociated from diplopodia in other strains of chickens and has indeed been shown to depend largely on genotypic balance (Lerner, 1954).

Similarly, wolf jaw appeared specifically in approximately one-quarter of the diplopod (but in none of the normal) progeny of line I and in a lower frequency in diplopod embryos produced by crosses of line I. It did not occur in progeny of line III or in diplopod embryos from line II or line crosses with mild expressions of the phenotype (as judged by foot digit number). Rather than suggest that wolf jaw belongs to the diplopod syndrome in one line and not in the others, it seems more reasonable to consider this character as an expression of a severe developmental upset, occasioned by the presence of **dpdp** unmitigated in effect by modifiers. The injection of certain chemicals into embryos during their early differentiation leads to remarkably similar phenotypic effects (Ancel, 1950 and Landauer, 1952). The absence of wolf jaw as well as the reduced incidence of other anomalies of development in diplopod progeny of the intermediate- and low-penetrance lines suggest that the three lines differ in developmental canalization. Heritable differences in buffering capacity have also been demonstrated by Landauer (1948), who found polydactylous and non-polydactylous breeds of chickens to vary in resistance to abnormalities resulting from the injection of insulin.

The line producing the highest proportion of phenotypically like individuals may be considered best canalized in its development. With respect to the diplopod character itself, line I embryos showed a higher developmental canalization than those of lines II or III. Selection of birds with low ratios of diplopodia in one sense was a selection for lower developmental canalization. Possession of the **dpdp** genotype no longer results in an automatic switch to the pathway leading to the characteristic diplopod phenotype. Instead embryos, with a **dpdp** genetic constitution may develop in several ways as follows:

a) a characteristic diplopod phenotype,
b) an attenuated form of diplopodia with less than three supernumerary digits per foot,
c) a nearly masked form of diplopodia such as that of wing-duplicate or non-duplicate individuals,
d) a normal phenotype (not yet established by progeny-test),
e) any combination of normality and severity among the several different organs affected in diplopodia.

Thus, while the kinds of genotypes present in the normal-ratio line determine precisely the kinds of phenotypes present, the kinds of genotypes present in the intermediate, and low-ratio lines give rise to a wide spectrum of phenotypes.

SUMMARY

In diplopodia both penetrance and expressivity vary according to the genetic background of the carrier parents. Selection based on a progeny test for phenotypic ratio produced lines differing in proportion of **dpdp** offspring displaying the phenotype and in kind of expression among those affected. The typical form of expression in normal-ratio stocks, including both the pre-outcross and the selected normal-ratio line, I, involved six digits per foot, three or four digits per wing and grade 2 or 3 micromelia, limb curvature and pre-maxilla reduction. In addition many embryos of line I had a ventral egg tooth, wolf jaw and an increased tendency toward abnormalities in other systems not diagnostic for diplopodia. After selection line I produced a larger proportion of forms with seven digits on one or both feet and one exceptional specimen with eight digits on one foot. Expression in lines II and III was attenuated and more variable in all characters. Both total foot and wing digit number differed significantly in the three selected lines. Line III averaged fewest toes and fingers. More than 1/2 of the diplopod phenotypes produced by Line III matings in 1954 had only the normal complement of toes, the diplopod phenotype being marked by extra wing fingers and a very slightly shortened pre-maxilla or grade I micromelia.

Phenotypic expression in other organs was more variable than that for toe number. While in typical expressions the wings possessed three or four fingers, embryos with only one finger (the normal pollex) or as many as six fingers (all supernumerary) appeared. Wings exhibited a greater tendency toward discordance both in digit number and arrangement than feet.

Degrees of severity in the several organs altered in **dpdp** were related in typical expressions of diplopodia but were not related in the low- and intermediate-ratio lines or in line-crosses. Selection for low ratios broke up the pleiotropic syndrome characteristic of the original carrier stock. While the average severity of expression in all organs increased in the line selected for normal ratios and decreased in the lines selected for intermediate and low ratios, the responses of the several organs involved were not uniform. Some organs displayed additional phenotypic variability after selection. Thus finger number, which was more variable than toe number, showed a prolonged response to selection. More severe beak effects appeared following continued selection for high ratios. Some parts of the syndrome showed little response to selection in either direction.

Right asymmetry in toe expression was not characteristic of all diplopod progeny as suggested by Taylor and Gunns and confirmed by Landauer. Fingers showed a more striking asymmetry than toes. Almost invariably the larger number were found on the right wing. Discordant digit expression appeared to be related to level of expression in other organs.

Two alternative explanations of the manner in which the residual genotype exerts its control over penetrance and expressivity are considered. Diplopod expression is discussed in terms of Waddington's (1940) concept of developmental canalization. These studies provide additional evidence of the loss in developmental canalization experienced by mutants. The lower developmental canalization due to the **dpdp**

genotype has been expressed in an increased incidence of abnormal development in systems not characteristically altered in diplopodia, in bilateral asymmetries of toes and fingers (affecting supernumeraries and the hallux or pollex) and of pattern of supernumerary expression, and in variable phenotypic expression among different organs in the same individual. The diplopod studies indicate that developmental flexibility differs among systems, suggesting that the concept of genotypic canalization may be broadened to include specific structural canalization, and emphasize the dangers besetting attempts to infer causal explanations of related or pleiotropic phenotypic effects.

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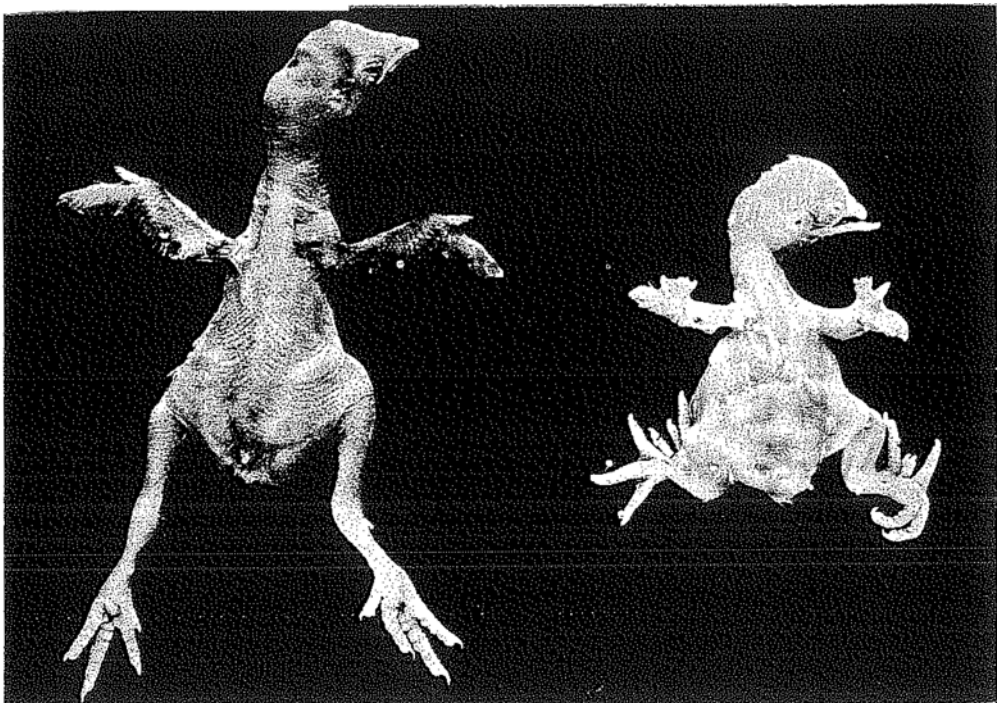


PLATE I

Living normal and diplopod siblings of 21-days embryonic age. The diplopod embryo illustrates the expression typical of line I embryos.

Total number of toes—12
Foot pattern—6011 (Halluces absent)
Total number of fingers—10
Wing pattern—56 (one wing lacks pollex)
Micromelia-grade—3
Limb curvature-grade—3
Pre-maxilla reduction-grade—3
Supernumerary metatarsals present
Curled toes and crooked toes on one foot
Egg tooth ventral
Wolf jaw
Crossed beak
Reduced body size

PLATE 2

A wing-duplicate embryo from line III. The pollex and one supernumerary digit are present. The embryo has micromelia and a reduced pre-maxilla (grade I) and a ventral egg tooth.

PLATE 3

A non-duplicate embryo from line III with the normal numbers of foot and wing digits but with micromelia, limb curvature (tibio-tarsal bending) and a reduced pre-maxilla of grade I and a slightly reduced body size.

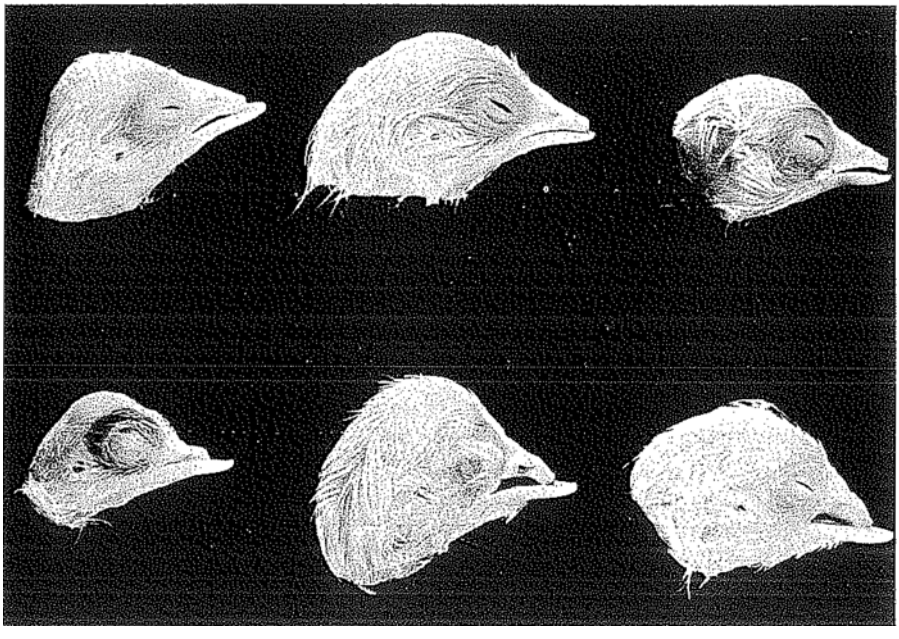
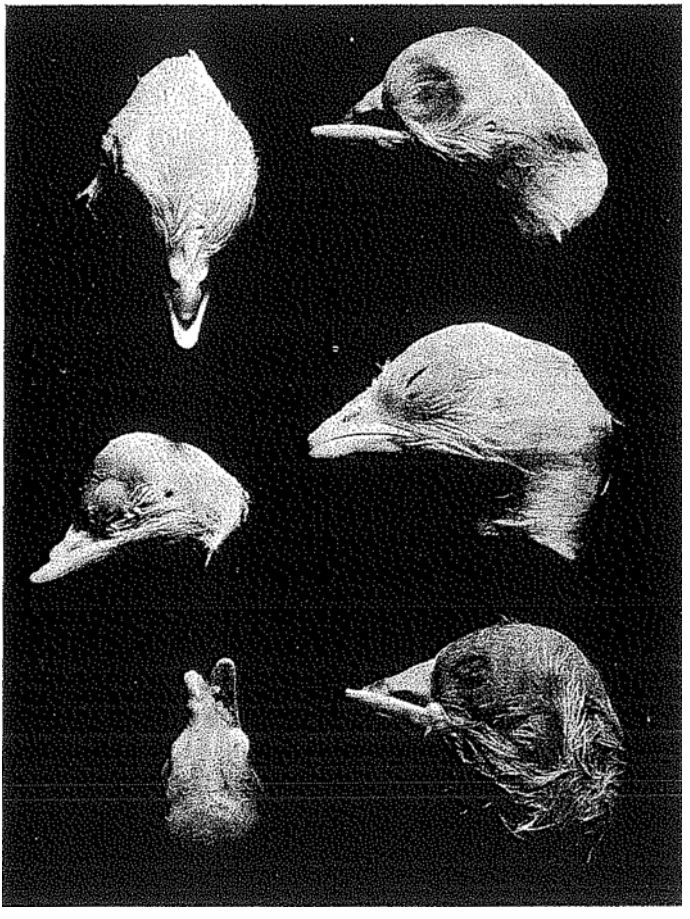


PLATE 4

(*a*) Top row from left to right—reduced pre-maxilla grades 3, 2, 1. Bottom row reduced pre-maxilla grade 3 and wolf jaw.



(*b*) Several views of line I diplopod embryos with unilateral or bilateral expressions of wolf jaw. Middle row right—a line III diplopod embryo without wolf jaw.

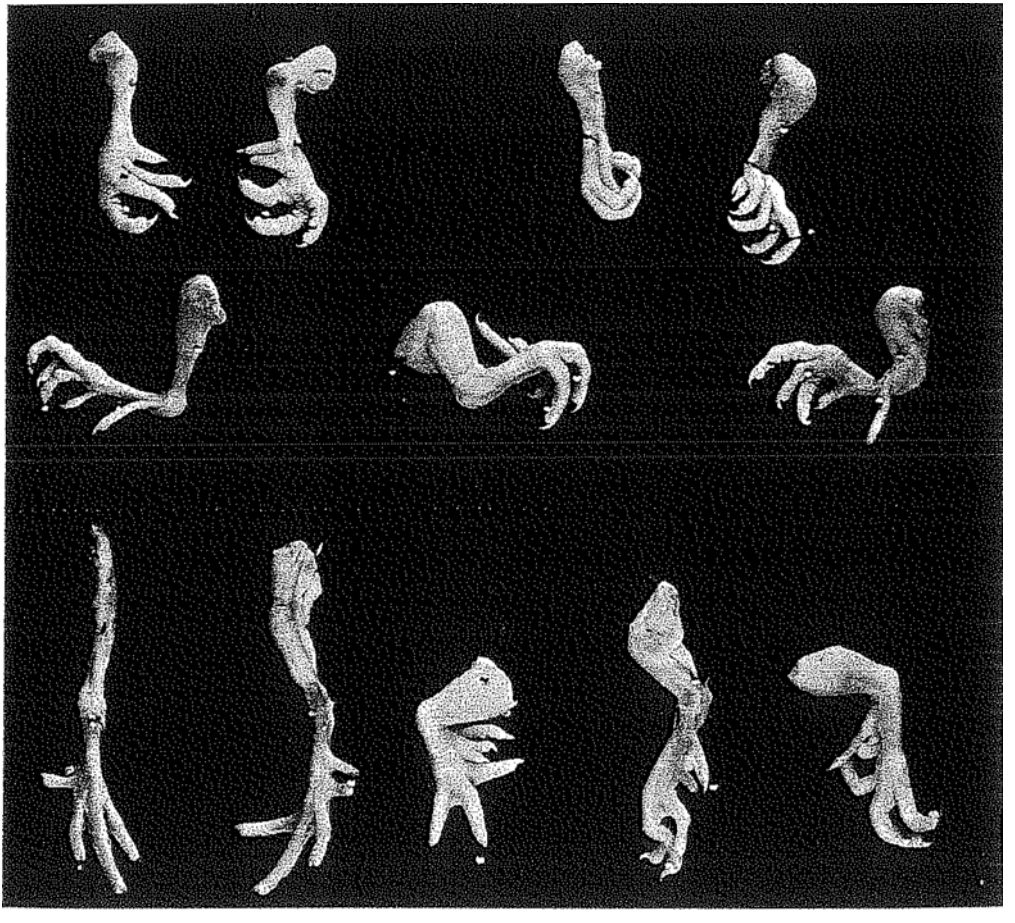


PLATE 5

Some diplopod feet illustrating several expression patterns with and without crooked and curled toes in normal and/or supernumerary toes.