HILGARDIA

THE RELATIVE EFFECTS OF INBREEDING AND OUTBREEDING ON REPRODUCTION IN THE DOMESTIC FOWL

PARTS I–V

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This study of the relative effects of inbreeding and outbreeding on reproduction in the domestic fowl is based on two years' observations of triallel matings of hens having an inbred, outbred, and crossbred origin. (P. 529.) Among the many relations established by these observations and reported in the following pages, the data with respect to inbreeding and outbreeding show that:

Fertility was influenced indirectly but not directly by the breeding system. Hens of an inbred origin were definitely less fertile than those of an outbred origin, irrespective of the kind of male to which they were mated. (P. 535.)

Hatchability decreased with inbreeding of outbred females ($F = 0$ to $F = .25$) but did not decrease with further inbreeding of inbred females. Inbred females showed lower hatchability than outbred females whatever the system of mating, but the difference was not statistically significant. (P. 547.)

Mean blastoderm diameter of unincubated eggs and *number of somites* in embryos after 38 hours incubation were smaller under inbreeding than outbreeding, and greater under outcrossing than crossbreeding. (P. 560.)

The general *fertility-hatchability relationship* observed was not altered by origin or genetic relation, or by system of mating. Closely inbred matings of birds with an outbred origin showed a negative correlation between fertility and hatchability which supports the view that fertility is distinct from hatchability. (P. 611.)

The Relative Effects of Inbreeding and Outbreeding on Reproduction in the Domestic Fowl[1]

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I. SOURCES OF STOCK AND DESIGN OF EXPERIMENT

THIS SERIES of papers will report results obtained in an experiment planned to determine some of the effects of inbreeding, outcrossing and crossbreeding on reproduction and on the performance of the offspring resulting from these mating systems. This first paper deals with the preliminary work that led to the development of stocks of chickens for the experiment and to the formulation of an experimental design which would reduce the number of unidentified or unmeasured genetic and nongenetic variables found in experiments previously reported in the literature dealing with the subject of inbreeding. Subsequent papers of this series will deal with experimental results on specific factors of reproduction and progeny performance.

INTRODUCTION

Poultrymen and investigators have long been concerned with the determination of the advantages and disadvantages involved in the use of different systems of mating poultry. The extensive literature existing on the subject is referred to later in considering the specific characters studied in this experiment. At this point it is sufficient to note that many of the results which have been obtained by different investigators on the effects of inbreeding and outbreeding are not in agreement, whereas conclusions asserting the dangers of inbreeding and the advantages of outbreeding generally have been accepted.

The experiment reported in this series of papers represents an attempt to secure specific information on the effect of mating systems as expressed in characters of economic importance to the poultry industry. In order to overcome some of the difficulties that have beset experiments previously reported in the literature and to reduce the uncontrolled variables affecting the results obtained, an improved design of experiment was used. This design included the following important features:

1. The various kinds of matings were made simultaneously and the products of the matings were treated uniformly throughout;

2. Each breeding individual was mated consecutively under each of the breeding systems compared; and

3. Other characters, particularly those of embryonic development, were added to the list of those usually reported in the literature on the subject.

Definitions

In order to establish unequivocally the meaning of terms as used in this series of reports, the following definitions are given:

Inbreeding: The mating of individuals which are more closely related to each other than the average relationship within the population concerned (Lush, 1945). The various degrees of inbreeding and of relationship have been measured by coefficients of inbreeding (Wright, 1922, 1923) calculated for the five generations of ancestors immediately preceding the individual.

Outbreeding: The mating of individuals less closely related to each other than the average relationship within the population concerned (Lush, 1945). Outbreeding within a breed or variety is termed outcrossing, whereas outbreeding involving different breeds is called crossbreeding.

To express the types of matings used in this experiment, the following terminology developed by a committee of the American Society of Animal Production (1940) has been adopted, with certain exceptions noted:

Crossbred: The progeny resulting from the mating of different breeds or, in the case of poultry, different breeds and varieties.

Inbred: The progeny resulting from the mating of closely related animals.

Incross: The progeny resulting from the crossing of individuals of inbred lines[5] within the same breed, or variety.

Incrossbred: The progeny resulting from the crossing of individuals from inbred lines[5] of different breeds, or varieties.

Outcross: The mating of relatively unrelated animals within the same breed, or variety.

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[5] To qualify as an inbred line an inbreeding coefficient of .375 was suggested by the committee of the American Society of Animal Production. Since then the Federal Trade Commission has issued a ruling that the coefficient must be more than .50. (Trade Practice Rules for the Baby Chick Industry. Promulgated September 15, 1948.) The term incrossbred is, nevertheless, used in this series of papers where the inbred parents had lower coefficients of inbreeding, since the principle of crossing inbred birds from different breeds is comparable.

Topcross: The mating of a male of a certain family to females of another family of the same breed, or variety.

Topincross: The progeny resulting from the cross of inbred sires on noninbred dams of the same breed, or variety.

Topcrossbred: The progeny resulting from the mating of inbred sires with noninbred dams of different breeds, or varieties.

SOURCES OF STOCK

All of the stocks used in this experiment were developed at the University poultry plant in Berkeley and complete pedigree records of the birds were available for more than five generations. This flock was free from pullorum disease for several years preceding the beginning of the experiment; no reactors were found in annual pullorum tests made during the course of this work. The following breeding lines were used.

1. The Small Embryo Line

This line had its origin in 1934 from stocks originally selected for high and for low hatchability. In a project designed to determine the sources of variation in the size of the unincubated blastoderms (embryos) of freshly laid eggs, it was established that hens varied greatly in the amount of development that had taken place in their fertile eggs before laying. Based on the assumption that this characteristic might be inherited, matings involving inbreeding were begun to develop a small embryo line. Since the characters developed in this line are the subject of study in succeeding papers of this series, a more detailed description of results and their validity obtained previous to this experiment is included here.

Among the factors found to influence the size of the embryo at the time the egg was laid were clutch position and individuality of the hen (Taylor and Gunns, 1935). Apparently hatchability was not correlated with embryo size or with the development of parts of the unincubated chick blastoderm with exception of the amount of overgrowth (Taylor and Gunns, 1939). The latter gave a low positive correlation with hatchability (0.31 ± 0.13).

That the size of the embryo, as determined by maximum diameter of the blastoderm, was a valid, though somewhat inexact, measure of cell development was established microscopically by cell nucleus counts in each tenth section of eight embryos selected to represent a normal range of sizes. The following data were obtained:

Maximum diameter mm	Number of nuclei
3.38	9,738
3.47	13,334
3.57	10,573
3.64	11,854
3.71	11,356
3.80	14,053
3.87	13,591
3.92	13,430

A correlation of .67 ± .16 for these limited data indicated that the relationship between maximum size and cell number was significant. Limited data also indicated that hens producing large embryos at the unincubated stage tended to produce embryos with a higher number of somites after 38 hours of incubation than the small embryo dams.

In the years between 1934 and the inception of this experiment, the mean blastoderm size of 46 daughters from 21 dams with different characteristic embryo sizes was measured. A low and positive, but nonsignificant, correlation was found to exist between dam and daughter average blastoderm sizes (.22 ± .14).

In 1940 there were available for use 31 inbred birds of both sexes with an average inbreeding coefficient of .36 and 3 females from an outcross mating of small embryo dams to a nonrelated male; of these 18♀♀ and 2♂♂ were used. this line was designated SE.

One of the anticipated advantages of the use of the SE line in this experiment was to determine whether the small embryo character was inherited. Unfortunately, due to reproductive failures in 1940, this line had to be replaced in 1941 by the outcross offspring from matings of SE♂♂ × OB♀♀ (see below). The offspring from the OB dams having characteristically small embryos were designated SEOBs and those from dams producing large embryos SEOBl.

2. The Outbred (Outcross) Stock

These birds were derived from the production-bred lines of the University flock. No inbreeding was detectable in the five preceding generations of the family of 12 full sisters and two full brothers chosen for this line. This was designated the OB line; the birds used in 1941 were from OB × OB matings made in 1940 and were designated OB^2.

3. The Crossbred Line

Preceding this experiment, reciprocal crosses had been made between Columbian Plymouth Rock and Silver Spangled Hamburg chickens. From the F_2 generation of these crosses, color segregates for predominantly white and predominantly black plumages were selected to develop two color lines which, with their descendants, were then inbred for several generations. In 1940 the white line was chosen to represent the line of originally crossbred origin and was designated the CB line. Failing to reproduce satisfactorily, this white line was replaced in 1941 by the black line which was designated XB.

DESIGN OF THE EXPERIMENT

The plan of matings and the resulting ranges of coefficients of inbreeding for the offspring of the matings are given in table 1.

Essentially the same design was used in both years of the experiment. Three sets of males, in most instances full- or half-brothers of the females representing each line, were used to head the breeding pens. The males were rotated from pen to pen after a four-week mating period, thus enabling all females to be mated in turn with each of the three sets of males. A period of two weeks elapsed from the time the males were changed from pen to pen before the new period was started, thus reducing the chances for carrying over fertility from

the preceding set of males. Since each pen contained females representing each of the lines, in any given period males of each origin were mated to females of every origin used. In other words, the various systems of mating were in operation within each pen at each period, while each female was mated in all of the systems at some period during the year.

TABLE 1

EXPERIMENTAL DESIGN FOLLOWED IN THE 1940 AND 1941 MATINGS

1940 matings

Females	Pen one			Pen two			Pen three		
	Period 1 SE males	Period 2 OB males	Period 3 CB males	Period 1 OB males	Period 2 CB males	Period 3 SE males	Period 1 CB males	Period 2 SE males	Period 3 OB males
SE	inbred .20-.51*	outcross	crossbred and incross-bred	outcross	crossbred and incross-bred	inbred .20-.51*	crossbred and incross-bred	inbred .20-.51*	outcross
OB	topin-cross	inbred .25	topcross-bred	inbred .25	topcross-bred	topin-cross	topcross-bred	topin-cross	inbred .25
CB	incross-bred	cross-bred	inbred .33-.36*	cross-bred	inbred .33-.36*	incross-bred	inbred .33-.36*	incross-bred	cross-bred

1941 matings

Females	Pen one			Pen two			Pen three		
	Period 1 SEOBs males	Period 2 OB² males	Period 3 XB males	Period 1 OB² males	Period 2 XB males	Period 3 SEOBs males	Period 1 XB males	Period 2 SEOBs males	Period 3 OB² males
SEOBs	inbred .3350	inbred .1250	topcross-bred	inbred .1250	topcross-bred	inbred .3350	topcross-bred	inbred .3350	inbred .1250
SEOBl	inbred .2514	inbred .1250	topcross-bred	inbred .1250	topcross-bred	inbred .2514	topcross-bred	inbred .2514	inbred .1250
OB²	inbred .1250	inbred .3750	incross-bred	inbred .3750	incross-bred	inbred .1250	incross-bred	inbred .1250	inbred .3750
XB	cross-bred	incross-bred	inbred .16-.30*	incross-bred	inbred .16-.30*	cross-bred	inbred .16-.30*	cross-bred	incross-bred

* Figures separated by - refer to range in coefficients of inbreeding of progeny.

This triallel mating design was conceived to have distinct advantages for detecting variability of environmental as well as genetic origin in the data obtained, besides being very well suited for the methods of statistical analysis employed.

Care of Stock and Collection of Data

So far as possible identical conditions were provided for mated birds and their offspring. The same breeding and rearing rations were used throughout the experiment. The eggs from each hen were identified by trapping and the approximate hour of the laying of the egg was recorded. Clutch position for

each egg was obtained from the total record of the bird's production. After each trapping, eggs were gathered and put in an icebox at approximately 45° F so that development of the embryo would not be prolonged by high environmental temperatures. The eggs were weighed on the morning of the next day and either used for observation of blastoderm size or returned to the icebox.

The following schedule was set for the utilization of the eggs produced: Monday and Wednesday for microscopically determining blastoderm size after fixation, sectioning and staining; Thursday and Sunday for 38 hours of incubation and determination of the number of somites in the embryo: Tuesday, Friday and Saturday for full-term incubation and determination of hatchability. Eggs were incubated up to the 18-day stage in a forced-draft machine, then transferred in pedigree sacks to a natural-draft machine for hatching. The chicks from each hatch were brooded and reared together. Production data were taken on birds housed together in the same series of pens and fed the same rations.

Data available from this experiment included time of laying, clutch position of the egg, the time interval between successive eggs, egg weight, fertility, unincubated blastoderm size, number of somites after 38 hours of incubation, hatchability, rate of growth of chicks reared, and egg production and mortality of females to 18 months of age.

SUMMARY

The design of a triallel system of matings involving inbreeding, outcrossing and crossbreeding is presented and the origins and characters of the three lines of stocks used in the experiment are described.

ACKNOWLEDGMENT

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II. FERTILITY

VARIATION IN THE FERTILITY of eggs has been attributed to the individuality of the breeding males and females as well as to many environmental factors (Parker, McKenzie and Kempster, 1942). Investigations made to establish whether or not variations in fertility have a hereditary basis have given negative results. Jull (1935) is the only investigator to have reported a low but significant coefficient of correlation of .19 ± .05 between the fertility of dams and that of their daughters. Inbreeding has been observed to result in a decrease in fertility by Dryden (1915, 1916), Goodale (1927), Hays (1924, 1929), Schleinitz (1925), Dunkerley (1931), Jull (1930, 1933), the Northern Sub-committee (1934) and Knox (1946) while Cole and Halpin (1916), Waters and Lambert (1935, 1936a, b, c), and Wilson (1948) observed no change in fertility following inbreeding.

Materials and Methods

All the eggs laid by the individual birds throughout the three different periods within each of the two years were used to determine fertility. The eggs used to measure hatchability were candled during the seventh day of incubation and the infertile eggs were removed, opened and examined to check if they were actually infertile. The eggs used to measure embryo size after incubation for 38 hours permitted the easiest determination of fertility as they were all opened at that time. Finally, the eggs used for measuring the size of the blastoderm in the unincubated eggs could also be classified easily as fertile or infertile by the size and the structure of the blastodisc. In doubtful cases the eggs were considered as fertile until certainty could be obtained after fixation, sectioning, staining, and microscopic examination.

Analysis of Observations

The data available on fertility are summarized in table 2. The weighted average fertility is the statistic $\bar{p}$ calculated by the method outlined by Hendricks (1935). It is an estimate of the average fertility of the eggs produced by a given group of birds and is thus a weighted average. This method of analysis has the disadvantage of necessitating calculation of new sums of squares for each classification in the analysis of variance. These sums of squares are therefore nonadditive and preclude a multiple classification scheme as well as an analysis of covariance. Another method of analysis was also used as stated

later. It consisted of converting the percentages into angles which were then used in a standard analysis of variance. The difference in fertility between years is significant and for this reason as well as the fact that different birds were used the data of each year were analyzed separately.

Incidence of Completely Infertile Matings

In 1940, out of a total of 85 matings, 23 were classified as completely infertile, while in 1941 only one mating out of a total of 121 was completely infertile. A statistical test gave evidence of heterogeneity in the distribution of infertile matings in the two years. The high incidence of infertile matings observed in 1940 could have entirely disrupted the picture of fertility in that

TABLE 2

DATA AVAILABLE ON FERTILITY

Year	Number of:		Number of eggs		Number of fertile eggs	Average fertility	Weighted average (p) SE
	Birds	Matings	Total	Per mating			
1940	33	85	1510	17.8	873	.5781	.5323±.0464
1941	43	121	1769	14.6	1573	.8892	.8791±.0185
Both years	76	206	3279	15.9	2446	.7460	

year and for this reason it was deemed advisable to make two separate analyses of the data obtained in that year; one analysis using all the matings, fertile as well as infertile, and the other using only the fertile matings.

The incidence of infertile matings in 1940 was studied within periods, origins of males, origins of females, mating systems, and also rates of production. The results of these studies are presented in table 3 together with the results of the tests of heterogeneity of the frequencies of infertile matings in the various classifications. These tests of heterogeneity reveal that two classifications, origins of males and origins of females, show evidence of heterogeneity in the incidence of infertile matings. The other three classifications (periods, systems of mating, and rates of production) show that infertile matings occurred at random within them. Apparently complete infertility is thus largely accounted for by the constitution of the birds mated. Examination of the results obtained with the different origins of females shows that the SE and the CB lines are the only ones in which infertile matings occurred and it is interesting to note that these two groups of females have an inbred origin. The inbred females, SE and CB, particularly the SE females, could have been ignored more frequently by the males or were possibly less sexually receptive than the OB females. It is also probable that certain conditions associated with the SE and the CB females, possibly conditions in their oviduct, did not permit the ascension of spermatozoa to the infundibulum. Support for the latter tentative hypothesis comes from observations on attempted artificial insemination of a number of completely infertile SE females; these birds remained infertile. These observations would eliminate sexual inactivity on the

part of the males and also sexual unreceptivity on the part of the females. Decreased viability of the sperm is also eliminated since the OB females mated with the same males proved fertile. The most logical assumptions are thus that oviducal conditions did not permit survival of the sperm, that the

TABLE 3

DISTRIBUTION OF THE INFERTILE MATINGS IN 1940 ACCORDING TO PERIODS, ORIGINS OF MALES, ORIGINS OF FEMALES, SYSTEMS OF MATING, AND RATE OF PRODUCTION, TOGETHER WITH TESTS OF HETEROGENEITY OF THE FREQUENCIES

Classification	Infertile matings	Fertile matings	Total	χ^2	P
Periods					
1..	7	24	31		
2..	6	23	29		
3..	10	15	25		
Total......................................	23	62	85	3.02	.2–.3
Origins of males					
SE..	13	18	31		
OB..	7	20	27		
CB..	3	24	27		
Total......................................	23	62	85	6.99	.02–.05
Origins of females					
SE..	17	20	37		
OB..	0	30	30		
CB..	6	12	18		
Total......................................	23	62	85	18.18	.001
Systems of mating					
Inbreeding....................................	10	17	27		
Outbreeding...................................	6	17	23		
Crossbreeding.................................	7	28	35		
Total......................................	23	62	85	2.25	.3–.5
Rate of production					
Less than 18 eggs or median....................	16	31	47		
More than 18 eggs or median...................	7	31	38		
Total......................................	23	62	85	1.86	.1–.2

eggs had a constitution that did not permit fertilization, or even that the eggs were fertilized but the zygotes died so early that it was practically impossible to determine whether or not they were fertilized.

Analysis of Variation in Fertility

The data available in each year for all matings and for the fertile matings only in 1940 were analyzed separately. These data were arranged according to periods, origins of males, origins of females and systems of mating as presented in tables 4, 5, and 6.

<center>TABLE 4</center>

DATA ON FERTILITY FOR ALL MATINGS IN 1940 CLASSIFIED ACCORDING TO PERIODS, ORIGINS OF MALES, ORIGINS OF FEMALES AND SYSTEMS OF MATING

Classification	Number of matings	Number of eggs	Number of fertile eggs	Unweighted average fertility	Weighted average (p) fertility and SE
Periods					
1	31	517	361	.6628	.6638±.0752
2	29	567	336	.5532	.5537±.0808
3	25	426	176	.3376	.3476±.0764
Origins of males					
SE	31	553	292	.4605	.4267±.0811
OB	27	453	258	.5158	.5180±.0816
CB	27	504	323	.6238	.6232±.0786
Origins of females					
SE	37	602	149	.2352	.2358±.0561
OB	30	664	560	.8483	.8481±.0423
CB	18	244	164	.6042	.6073± 1057
Systems of mating					
Inbreeding	27	470	230	.4551	.4561±.0821
Outbreeding	23	432	264	.5288	.5304±.0918
Crossbreeding	35	608	379	.5880	.5014±.0722
All matings	85	1510	873	.5297·	.5323± 0464

<center>TABLE 5</center>

DATA ON FERTILITY FOR FERTILE MATINGS ONLY IN 1940 CLASSIFIED ACCORDING TO PERIODS, ORIGINS OF MALES, ORIGINS OF FEMALES AND SYSTEMS OF MATING

Classification	Number of matings	Number of eggs	Number of fertile eggs	Unweighted average fertility	Weighted average (p) fertility and SE
Periods					
1	24	410	361	.8561	.8597±.0479
2	23	457	336	.6975	.6987±.0768
3	15	306	176	.5627	.5636±.1098
Origins of males					
SE	18	362	292	.7932	.7929±.0668
OB	20	351	258	.6963	.6999±.0736
CB	24	460	323	.7012	.7011±.0742
Origins of females					
SE	20	332	149	.4352	.4363±.0803
OB	30	664	560	.8443	.8481±.0423
CB	12	177	164	.9063	9167±.0505
Systems of mating					
Inbreeding	17	320	230	.7227	.7223±.0733
Outbreeding	17	334	264	.7154	.7199±.0850
Crossbreeding	28	519	379	.7350	.7357±.0654
All matings	62	1173	873	.7263	.7280±.0419

The periods in the two years did not correspond in dates. In 1940, period 1 extended from February 21 to March 17, period 2 from April 2 to April 28, and period 3 from May 14 to June 9. In 1941, period 1 extended from January 1 to February 19, period 2 from March 6 to April 7, and period 3 from April 22 to May 14. In both years, the periods extended over five months; each consisted of 25 days with an interval of 14 days between consecutive periods.

TABLE 6

DATA ON FERTILITY FOR ALL MATINGS IN 1941 CLASSIFIED
ACCORDING TO PERIODS, ORIGINS OF MALES, ORIGINS
OF FEMALES, INBREEDING AND CROSSBREEDING,
AND DIFFERENT LEVELS OF INBREEDING

Classification	Number of matings	Number of eggs	Number of fertile eggs	Unweighted average fertility	Weighted average ($\bar{p}$) fertility and SE
Periods					
1	42	569	506	.8776	.8999±.0239
2	41	635	586	.9140	.9222±.0205
3	38	565	481	.8443	.8405±.0409
Origins of males					
SEOBs	41	599	462	.9332	.9335±.0214
OB²	43	620	573	.9077	.9100±.0279
XB	37	550	438	.7871	.7863±.0417
Origins of females					
SEOBs and SEOBl	67	1076	967	.8844	.8865±.0249
OB²	12	120	88	.7356	.7158±.0685
XB	42	573	518	.9126	.9079±.0266
Systems of mating					
Inbreeding	70	1054	941	.8726	.8723±.0419
Crossbreeding	51	715	632	.8788	.8777±.0285
All matings	121	1769	1573	.8795	.8791±.0185
Levels of inbreeding of progeny					
.1250	28	439	391	.8726	.8723±.0419
.1600–.2514	26	402	360	.8956	.8931±.0404
.3000–.3750	16	213	190	.8672	.8776±.0423

Periods, origins of males, origins of females and systems of mating all appear to have influenced fertility though not all to the same degree in the two years. An analysis of variance of the weighted averages ($\bar{p}$) has given the results reported in tables 7, 8, and 9 for all matings in 1940, fertile matings only in 1940 and all matings in 1941 respectively.

The analysis shows that the variations between periods are significant only within 1940, and whether all matings or only the fertile matings are taken into consideration. The variations in fertility between periods in 1940 are thus not explained by the varied incidence of completely infertile matings as already demonstrated by the test of heterogeneity of the distribution of infertile matings in table 3. The variations in fertility between periods in 1941 are not statistically significant although a slight decrease was noted in the third period. The earlier termination of the experiment in 1941 may

explain the lack of significant differences between periods in that year. The general trend is a decrease in fertility as the hatching season progressed. In the two years the trend is an increase in fertility in February as compared to January, a decrease in April as compared to March, and a decrease in June as compared to May.

TABLE 7

ANALYSIS OF VARIANCE OF THE DATA ON FERTILITY FOR ALL
MATINGS IN 1940 USING WEIGHTED AVERAGES ($\bar{p}$)

Source of variation	Degrees of freedom	Sums of squares	Mean square	F*
Between periods..	2	8.77	4.385	4.36
Within periods...	82	82.41	1.005	
Between origins of males..............................	2	2.10	1.05	1.05
Within origins of males...............................	82	82.21	1.00	
Between origins of females............................	2	76.93	38.46	38.08
Within origins of females.............................	82	82.74	1.01	
Between systems of mating............................	2	0.77	0.385	.....
Within systems of mating.............................	82	81.99	1.00	

* F values at 5% and 1% points are 3.11 and 4.88 respectively.

TABLE 8

ANALYSIS OF VARIANCE OF THE DATA ON FERTILITY FOR FERTILE
MATINGS ONLY IN 1940 USING WEIGHTED AVERAGES ($\bar{p}$)

Source of variation	Degrees of freedom	Sums of squares	Mean square	F*
Between periods	2	10.19	5.095	5.04
Within periods..	59	59.58	1.01	
Between origins of males..............................	2	1.24	0.62	....
Within origins of males...............................	59	58.86	1.00	
Between origins of females............................	2	32.02	16.01	15.85
Within origins of females.............................	59	59.65	1.01	
Between systems of mating............................	2	0.02	0.01	....
Within systems of mating.............................	59	59.92	1.02	

* F values at 5% and 1% points are 3.15 and 4.98 respectively.

The analysis of variance of fertility between origins of males (tables 7, 8, and 9) shows that the variations are significant only in 1941. These significant variations observed in 1941 are apparently entirely explained by males P183 and P184 (XB males) which showed a fertility lower by 14.6% and 12% respectively when compared to S193–S194 (SEOBs) and S195–S196 (OB²) as reported in table 10.

The analysis of variance shows that the differences in fertility observed between origins of females are statistically significant in 1940 and in 1941, whether or not the completely infertile matings are taken into account. The origins of the females apparently account for a great part, if not the greatest

part, of the differences observed in fertility. The most significant fact is undoubtedly the lower fertility exhibited in both years by the Leghorn females with an inbred origin (SE and OB²) when compared with those having an outbred origin (OB and SEOB).

The variations in fertility between mating systems appeared to be of no significance and this was confirmed by an analysis of variance. Inbreeding,

TABLE 9

ANALYSIS OF VARIANCE OF THE DATA ON FERTILITY FOR ALL
MATINGS IN 1941 USING WEIGHTED AVERAGES ($\bar{p}$)

Source of variation	Degrees of freedom	Sums of squares	Mean square	F*
Between periods..	2	3.47	1.74	1.91
Within periods...	118	107.64	0.91	
Between origins of males................................	2	9.51	4.76	4.62
Within origins of males.................................	118	121.72	1.03	
Between origins of females..............................	2	7.57	3.78	3.94
Within origins of females................................	118	113.53	0.96	
Between inbreeding and crossbreeding...................	1	0.02	0.02	....
Within inbreeding and crossbreeding....................	119	120.46	1.01	
Between levels of inbreeding............................	2	0.17	0.085	....
Within levels of inbreeding.............................	67	70.83	1.06	

* F values at 5% and 1% points are 3.08 and 4.80 respectively.

TABLE 10

FERTILITY OBTAINED WITH THE FEMALES OF THE
TWO BREEDS WHEN MATED TO THE MALES
OF THE THREE ORIGINS (1941)

Females	SEOBs males	OB² males	XB males
White Leghorns (OB², SEOBs and SEOBl).................	.9189	.8824	.7850
Crossbreds (XB)..	.9557	.9732	.8026
Average	.9332	.9077	.7871

outcrossing, crossbreeding, and inbreeding to varying levels apparently did not affect fertility in a direct way, or, in other words, the fertilization of an egg was neither impaired nor enhanced by the presence of sperm from a related or an unrelated male.

The data on fertility were also analyzed statistically by another method than that of Hendricks (1935) as the weighted averages ($\bar{p}$) could not be used in the analysis of covariance of fertility and hatchability reported on in another part of this study (page 571). In the second method of analysis, the individual percentages of fertility were converted to angles and used in a standard analysis of variance.

The results of this second analysis, given in table 11, are in complete agreement with those obtained in the first. Significant differences in fertility between periods were observed in 1940 but not in 1941. Significant differences in fertility were observed between origins of males only in 1941 and between origins of females in both years, while the differences between systems of mating were not significant.

TABLE 11

ANALYSIS OF VARIANCE OF DATA ON FERTILITY IN 1940 AND
1941 FOR FERTILE MATINGS ONLY, USING INDIVIDUAL PER-
CENTAGES OF FERTILITY CONVERTED TO ANGLES

Source of variation	1940			1941		
	Degrees of freedom	Mean square	F	Degrees of freedom	Mean square	F
Total......................	61	659.26		107	222.01	
Between periods.............	2	2659.18	4.50*	2	17.48	....
Within periods..............	59	591.47		105	225.91	
Between origins of males.....	2	321.54	....	2	1256.07	6.21†
Within origins of males.......	59	670.71		105	202.31	
Between origins of females....	2	7286.20	16.76†	2	1097.70	5.35†
Within origins of females.....	59	434.62		105	205.33	
Between systems of mating...	2	42.46	....	1	6.01	....
Within systems of mating.....	59	680.17		106	224.05	

* Significant at the 5% level.
† Significant at the 1% level.

Relation Between Intensity of Egg Production and Fertility

In the study of the incidence of infertile matings (table 3), infertility was higher in hens laying at a rate less rapid than the average, but the differences were not statistically significant. It seemed of interest to study the relationship between rate of production and fertility in all the matings studied.

Table 12 presents a summary of the data on the relation between rate of production and percentage of fertility for each individual mating in 1940 and 1941. The fertile matings of 1940 were analyzed separately. The same table also gives the results of the heterogeneity tests of the distributions. These heterogeneity tests of the distribution of the matings according to rate of production and fertility reveal that in 1940 there was no significant relation between fertility and rate of production whether all or only the fertile matings were taken into consideration. In 1941, however, there was a significant relation between rate of production and fertility.

The lack of significant relation between rate of production and fertility in 1940 may have been the result of conditions associated with the various origins of the females. As was shown previously (table 3), the SE females showed a higher incidence of infertile matings than either the OB or the CB females but this higher incidence of infertile matings was not explained by a

TABLE 12

DISTRIBUTION OF MATINGS ACCORDING TO RATE OF
PRODUCTION AND FERTILITY

Rate of production	Percentage of fertility 1940 (all matings)		Number of matings	Average fertility
	Less than the median or 53%	More than the median or 53%		
Less than the median or 18 eggs..................	24	23	47	.4530
More than the median or 18 eggs.................	14	24	38	.6239
Total.......................................	38	47	85	.5297

$\chi^2 = 1.19$ P = .20–.30

Rate of production	Percentage of fertility 1940 (fertile matings)		Number of matings	Average fertility
	Less than the median or 90%	More than the median or 90%		
Less than the median or 18 eggs..................	20	11	31	.6868
More than the median or 18 eggs.................	13	18	31	.7898
Total.......................................	33	29	62	.7263

$\chi^2 = 2.33$ P = .10–.20

Rate of production	Percentage of fertility 1941 (all matings)		Number of matings	Average fertility
	Less than the mean or 86%	More than the mean or 86%		
Less than the median or 16 eggs..................	22	36	58	.8319
More than the median or 16 eggs.................	8	55	63	.9232
Total.......................................	30	91	121	.8795

$\chi^2 = 9.01$ P = .01–.001

decreased rate of production. It seems reasonable to venture the hypothesis that factors other than decreased rate of production are responsible for the decreased fertility observed in 1940.

The relation between rate of production and fertility was also studied within origins of females in 1940 and it remained without significance.

Relation Between Coefficients of Inbreeding of Dams and Their Fertility

The fact that the SE females, with an inbred origin, were significantly less fertile than the OB females, led to the study of the relation between the coefficients of inbreeding of the individual SE females and the fertility of their eggs regardless of the male they were mated with. The range in coeffi-

cients of inbreeding of the 14 SE females used in 1940 was from zero to .46 while the fertility varied between zero and 96%.

The results of the analysis of covariance of coefficients of inbreeding and percentages of fertility converted to angles gave a negative coefficient of correlation of −.540 which is significant at the 5% level of significance.

Another method of testing the available data on inbreeding and fertility for association was used: a test of the heterogeneity of the distribution of infertile and fertile eggs according to coefficients of inbreeding. The results are presented in table 13. This test of heterogeneity reveals that the level of

TABLE 13

DISTRIBUTION OF INFERTILE AND FERTILE EGGS ACCORDING TO THE COEFFICIENT OF INBREEDING OF THE BIRDS THAT LAID THEM. DATA FROM THE SE FEMALES IN 1940

Coefficient of inbreeding	Average fertility	Fertile eggs	Infertile eggs	Total eggs
0...	.9646	55	2	57
.19..	.1698	9	44	53
.32..	.0286	3	102	105
.34..	0.0	0	58	58
.37..	0.0	0	47	47
.38..	.2760	53	139	192
.46..	.3222	29	61	90
Total...................................	.2475	149	453	602

$$\chi^2 = 121.49, \quad P < .001$$

fertility is significantly related to the coefficient of inbreeding of the various dams. Better fertility was observed in the eggs from dams with a low (zero) and a high (.38 or more) coefficient of inbreeding than in the eggs from dams with a coefficient of inbreeding varying between .1875 and .37. The results of the analysis of covariance are thus confirmed by the test of the heterogeneity of the distribution of infertile and fertile eggs according to coefficients of inbreeding. The data presented in table 13 reveal a non-linear relationship between coefficient of inbreeding and fertility. More reliance is put on the results presented in table 13 than on the correlation coefficient previously reported in view of the fact that normality is not a prerequisite for a test of heterogeneity whereas it is for a covariance analysis.

The test of heterogeneity reported in table 13 might be criticized for lack of correction for different levels of production, but it was demonstrated previously, table 12, that there was no significant relation between rate of egg production and fertility in the SE females in 1940.

The study of the relation between coefficients of inbreeding and fertility could not be made in the females used in 1941 because of the small range of variation in the inbreeding coefficients in any one line.

DISCUSSION

The results obtained in this part of the study of the relative effects of in-breeding and outbreeding are that inbreeding to varying degrees, out-crossing, and crossbreeding do not affect fertility directly, but that they do so indirectly. In this study the fertility of hens was apparently neither im-paired nor enhanced when they were mated to either related or unrelated males. The most significant fact observed is that the SE and CB females, both with an inbred origin, showed a higher incidence of infertile matings and also had lower fertility in fertile matings than the OB females with an out-bred origin. This finding is contrary to the prevalent opinion that fertility in the domestic fowl is not hereditary. The fertility of matings appears to be a property of the parents and not of the prospective zygotes resulting from the mating. This may be the reason for the lack of agreement in conclusions of the many investigations on the heredity of fertility in the domestic fowl.

The indirect effects of inbreeding on fertility are very clearly demonstrated in the results obtained in this study. The inbred Leghorn females, in both years, were significantly less fertile than the outbred Leghorn females. The reasons for this impaired fertility of inbred females remain undetermined but the analysis made of the results obtained provides a number of hypotheses.

It is generally admitted that inbreeding results in decreased vigor of the progeny and it is not illogical to assume that a decrease in sexual receptivity accompanies such a decrease in vigor. The infertility and lowered fertility of inbred females would thus be explained by the absence of or less frequent mating. It is interesting to note that the inbred Leghorn males, in both years, did not show any significant difference in their sexual activity when compared to outbred males of the same breed. This is perhaps explained by the small number of males used in the study and the possible selection of vigorous individuals.

A significant relation was observed between origins of males and origins of females and incidence of infertile matings in 1940. In the same year, 1940, there was no significant relation between fertility and rate of production whether all the matings or only the fertile matings were considered. In 1941, the relation between rate of production and fertility was significant. Lamoreux (1940) has reported that the relation between rate of production and fertility is explained by the greater sexual receptivity of the females laying at a high rate.

As hypotheses to explain the high incidence of infertility and the reduced fertility of the inbred females, particularly the SE females, the following are suggested: First, the inbred females because of their reduced vigor show a decreased sexual receptivity. This first hypothesis is weakened by the lack of relation observed between rate of production and fertility in the inbred fe-males. A second hypothesis is that the eggs of the inbred females are fertilized but the zygotes die so early that it is impossible to determine whether or not the eggs were fertile. This hypothesis cannot be definitely rejected until better means of detecting early embryonic mortality are available. A third hypo-thesis is that the inbred females exhibit a normal relation between rate of

production and sexual receptivity but to no avail because their eggs are of such a constitution that they cannot be fertilized. A fourth hypothesis is that certain conditions in the oviduct of the inbred females do not permit the survival of the sperm or its ascension to the infundibulum of the oviduct. The third and fourth hypotheses are supported by the fact that attempts to obtain fertile eggs from certain of the inbred SE females through artificial insemination proved futile and the females remained infertile. Another fact is that the males which were in turn mated to the inbred SE and the CB females gave very good fertility with the outbred OB females so that viability of sperm was not at fault.

Substantial evidence has been presented in this study to show that genetic factors definitely influenced fertility as demonstrated by the low level of fertility in many inbred females. The actual reasons for this impaired fertility have not been determined but future investigations may establish that certain of the hypotheses suggested above apply.

In addition to the findings just discussed, the results of this study on fertility showed a striking difference in fertility between the two years which is not explained by the infertile matings observed in the first year. There was also evidence of a seasonal decline in fertility as the incubation season progressed, but the differences between periods were statistically significant only in the first year. The males used in the two years, except for the XB set in 1941, showed no significant difference in their sexual activity as manifested by the fertility of the eggs of their common mates. The SE and CB males gave a higher incidence of infertile matings in 1940 but this was not statistically significant. The females were apparently mainly responsible for the significant variations observed in infertility and fertility.

SUMMARY

The results of this study on fertility based on two years' observations of triallel matings of hens having an inbred, outbred, and inbred-crossbred origin, demonstrate that inbreeding, in itself or to varying coefficients, outcrossing, and crossbreeding, do not influence fertility directly. However, the breeding system responsible for the production of a population of females has an effect on their fertility. Thus, hens of an inbred origin are definitely less fertile than those of an outbred origin, irrespective of the kind of male to which they are mated. Birds laying at a higher rate than the average produced a higher percentage of fertile eggs in one of the two years.

The Relative Effects of Inbreeding and Outbreeding on Reproduction in the Domestic Fowl

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III. HATCHABILITY

NUMEROUS FACTORS have been found to affect the hatchability of fertile eggs in the domestic fowl. These factors are both environmental and hereditary and they have been the subject of many investigations. Fortunately the vast amount of literature has been very adequately reviewed by Jull (1940) and especially by Landauer (1941) so that it is not necessary to review the subject here as a whole except for the phases bearing directly or indirectly on the factors studied in this investigation.

The influence of genetic factors on hatchability has been demonstrated in a number of ways. Waters (1944) has reported significantly greater variation in hatchability between dams than within dams. Pearl and Surface (1909), Hays and Sanborn (1924), Jull (1931), and Landauer (1937, 1941) have all reported significant coefficients of correlation between the hatchability of dams and that of their female progeny. Hays (1926) and Waters (1944) have both used polyallel crossing, the mating of the same females to different males, to demonstrate the influence of genetic factors on hatchability.

Another way of demonstrating the influence of genetic factors on hatchability is its behavior following inbreeding and outbreeding. Dryden (1916), Cole and Halpin (1916, 1922), Dunn (1923, 1928), Goodale (1927), Warren (1927, 1934), Jull (1929a, b), Dumon (1931), Dunkerley (1931), the Northern Subcommittee (1934), Byerly, Knox and Jull (1934), Vecchi (1936), Waters (1932, 1945), Waters and Lambert (1935, 1936a, b, c) and Knox (1946) have all reported that hatchability decreases as inbreeding increases. By rigid selection, however, Waters and Lambert (1935, 1936a, b, c) and Knox (1946) successfully established inbred lines showing relatively high hatchability.

Finally, a number of lethal and semi-lethal genes resulting in decreased hatchability have been isolated in the course of investigations conducted with the domestic fowl. Jull (1940) has pointed out that these lethals cannot be responsible for any considerable portion of the embryonic mortality commonly observed in most flocks, but they are nevertheless of importance in inbreeding investigations as revealed by Waters (1945). A check-list of lethal and semi-lethal factors in the domestic fowl has been published by Lerner (1944).

Materials and Methods

The eggs collected three days each week, Tuesday, Friday, and Saturday, and set in the incubator the following Monday constituted the material used to measure hatchability. Each individual egg was identified as to parents

and hour and date of laying. The eggs were regularly candled on the seventh
and fourteenth days of incubation; all eggs removed at such candlings and
those remaining after the chicks were hatched were broken to determine
whether they had been fertile, the stage at which the embryos had died and the
determinable causes of death.

The data obtained were analyzed in the same manner as the data on fertility.
Hatchability was expressed as the percentage of fertile eggs that produced
chicks.

Analysis of Observations

In the course of the two years during which observations were made on
hatchability, a total of 72 females used in 169 matings produced a total of 937
fertile eggs from which 701 chicks hatched. The data available in each year
and for the two years together are summarized in table 14.

TABLE 14

DATA ON HATCHABILITY FOR 1940 AND 1941

| Year | Number of: | | Number of eggs | | Number of fertile eggs | Hatch- ability | Weighted average ($\bar{p}$) SE |
	Birds	Matings	Total	Per mating			
1940................	29	60	381	6.35	282	.7006	.7582±.0369
1941................	43	109	556	5.10	419	.7536	.7803±.0133
Both years....	72	169	937	5.54	701	.7481	.7801±.0218

The difference in hatchability between the two years is not very large but
the data gathered in each year are nevertheless analyzed separately since
different birds were used.

Analysis of Variation in Hatchability

The data available for each year on hatchability, arranged according to
periods, origins of males, origins of females and systems of mating, are pre-
sented in table 15.

Periods, origins of males, origins of females, and systems of mating appear
to have influenced hatchability to varying degrees. The data, weighted within
each classification, were submitted to an analysis of variance to determine the
significance of the variations observed. The results of the analysis of variance
for both the 1940 and 1941 data are presented in table 16.

The variations in hatchability did not show the same periodic trends in
1940 and 1941 (table 15). Hatchability was at a maximum during the first
period in 1940 and in the second period in 1941, but as stated previously in the
study of fertility, the periods did not coincide in the two years. The second
period in 1941 more nearly corresponded to the first in 1940 so that when the
data for both years were considered together the trend was an increase in
February and March as compared to January and a decrease in April and May
as compared to March. The analysis of variance of the data on hatchability
revealed that the variations between periods were not significant in 1940, but
were so in 1941. No explanation is offered for these periodic variations in
hatchability.

TABLE 15

DATA ON HATCHABILITY IN 1940 AND 1941 ARRANGED ACCORDING
TO PERIODS, ORIGINS OF MALES, ORIGINS OF FEMALES
AND SYSTEMS OF MATING

Classification	Number of matings	Number of eggs	Number of fertile eggs	Unweighted average hatchability	Weighted average $(\bar{p})$ hatchability and SE
1940					
Periods					
1	24	160	127	.7882	.7736±.0431
2	21	146	106	.6641	.6779±.0645
3	15	75	49	.6114	.6139±.0933
Origins of males					
SE	18	128	98	.7094	.7143±.0753
OB	19	109	77	.6877	.6929±.0632
CB	23	144	107	.7044	.7148±.0567
Origins of females					
SE	18	68	48	.6675	.6708±.0841
OB	30	244	190	.7622	.7748±.0404
CB	12	69	44	.5962	.6105±.0954
Systems of mating					
Inbreeding	17	99	58	.5743	.5664±.0762
Outcrossing	16	117	101	.8132	.8364±.0525
Crossbreeding	27	165	123	.7134	.7216±.0530
All matings	60	381	282	.7006	.7532±.0369
1941					
Periods					
1	39	170	114	.6450	.6538±.0556
2	39	208	182	.8685	.8877±.0299
3	31	178	123	.6888	.6899±.0497
Origins of males					
SEOBs	39	209	148	.6844	.6979±.0509
OB²	37	191	147	.7609	.7636±.0459
XB	33	156	124	.7739	.7696±.0581
Origins of females					
SEOBs and SEOBl	63	334	258	.7724	.7761±.0352
OB	10	38	26	.6842	.6170±.1219
XB	36	184	135	.7460	.7386±.0513
Systems of mating					
Inbreeding	62	326	228	.6756	.6848±.0400
Outbreeding	47	230	191	.8190	.8249±.0383
All matings	109	556	419	.7536	.7803±.0133
Levels of inbreeding of progeny					
.1250	26	135	102	.7191	.7289±.0722
.16–.2514	23	126	82	.6152	.6270±.0585
.30–.3750	13	65	44	.6956	.6861±.0868

The sets of males used in 1940 and in 1941 (table 15) did not apparently affect hatchability to any marked degree and this is confirmed by the analysis of variance. No set of males was definitely inferior or superior in hatchability, in either of the two years, when mated in turn to all the females.

The data arranged according to origins of females (table 15) show that, in the White Leghorns, in 1940 the inbred SE females and in 1941 the inbred

TABLE 16

ANALYSIS OF VARIANCE OF THE DATA ON HATCHABILITY
FOR 1940 AND 1941 USING WEIGHTED AVERAGES ($\bar{p}$)

Source of variation	Degrees of freedom	Sums of squares	Mean square	F	F at 5%	F at 1%
		1940				
Between periods...................	2	3.58	1.79	1.97	3.17	5.01
Within periods....................	57	51.81	.91			
Between origins of males..........	2	.06	.03	....	....	....
Within origins of males............	57	48.32	.85			
Between origins of females........	2	2.97	1.49	1.56	3.17	5.01
Within origins of females..........	57	54.23	.95			
Between systems of mating........	2	6.68	3.34	4.02	3.17	5.01
Within systems of mating..........	57	47.24	.83			
		1941				
Between periods...................	2	15.30	7.65	8.60	3.09	4.82
Within periods....................	106	93.92	.89			
Between origins of males..........	2	1.37	.69	....	....	....
Within origins of males............	106	98.27	.93			
Between origins of females........	2	1.51	.76	....	....	....
Within origins of females..........	106	101.90	.96			
Between systems of mating........	1	5.90	5.90	6.41	3.94	6.90
Within systems of mating..........	107	98.38	.92			
Between levels of inbreeding......	2	1.14	.57	....	....	....
Within levels of inbreeding........	59	57.98	.98			

OB^2 females gave inferior hatchability when compared to the outbred OB and SEOB females respectively. The analysis of variance (table 16), however, shows that the variations observed between origins of females are not significant in either 1940 or 1941. Thus no origin of females was definitely inferior or superior in hatchability, in either of the two years, when mated in turn to the sets of males of the different origins.

Inbreeding gave the lowest hatchability results in both 1940 and 1941 (table 15) and the analysis of variance (table 16) revealed that the variations between systems of mating were statistically significant for each year. In 1941, there was no significant variation in hatchability between the various levels

of inbreeding. Maximum hatchability was obtained under outcrossing in 1940 and under outbreeding in 1941.

The data on hatchability were also analyzed by another method than that of weighted averages ($\bar{p}$) as was previously done for the data on fertility (table 11). This second method was used for the analysis of covariance of fertility and hatchability using percentages converted to angles, since the weighted averages could not be utilized in the analysis of covariance. The results of this second analysis of variance of the data on hatchability for both years are presented in table 17.

The results of the two methods for analysis of variance are in complete agreement except for the discrepancy in significance of the analyses between systems of mating in 1940. The method of weighted averages ($\bar{p}$, table 16) showed the variance between systems of mating in 1940 as significantly greater than the variance within systems of mating while the method of percentages converted to angles (table 17) showed the same variance ratio not to be significant.

It was also possible to extend this second method for the analysis of variance within periods, within origins of females within periods, within systems of mating within origins of females within periods, and finally within inbreeding within origins of females within periods, as shown in the lower part of table 17. The extended analysis of variance within each origin of females within periods is also given in the same table. The results indicate significant variations between origins of females within periods and between systems of mating within origins of females within periods, but in 1941 only. The analysis of the variations in hatchability within each origin has shown that only the OB females in 1940 exhibited significant variations in hatchability between systems of mating within periods.

The extended analysis of variance has thus revealed a few facts which would have remained unknown otherwise. It is of interest to note that the discrepancy between the two methods of analysis for 1940 seems to be explained by the fact that only the females of OB origin showed significant variations in hatchability between systems of mating; the inbreeding of these females resulted in lower hatchability. In 1941, the three origins of females exhibited lower hatchability when inbred but the analysis showed that the variance ratios never reached the level of significance. When the three origins are pooled the variations in hatchability between systems of mating are significant (F value of 2.12), inbreeding showing significantly lower hatchability than outbreeding.

Relation Between Rate of Egg Production and Hatchability

The data on egg production and on hatchability were analyzed for a possible relationship. Table 18 represents a summary of the available data on the hatchability of fertile eggs laid by hens laying less and more than the median number of eggs during each period.

There is an apparent relationship between rate of production and hatchability since in both years the hens that laid more intensively also produced fertile eggs that hatched better than those laid by hens laying less intensively.

TABLE 17

ANALYSIS OF VARIANCE OF DATA ON HATCHABILITY IN 1940 AND
1941 USING INDIVIDUAL PERCENTAGES CONVERTED TO ANGLES

Source of variation	1940			1941		
	Degrees of freedom	Mean square	F	Degrees of freedom	Mean square	F
Total..	59	836.00		107	704.16	
Between periods.............................	2	1776.05	2.01	2	3785.78	5.87†
Within periods..............................	57	885.60		105	645.47	
Between origins of males....................	2	115.05	....	2	566.07	
Within origins of males......................	57	791.80		105	706.80	
Between origins of females..................	2	1104.79	1.46	2	1099.36	1.58
Within origins of females....................	57	758.25		105	696.64	
Between systems of mating..................	2	1164.11	1.54	1	4612.86	6.91*
Within systems of mating....................	57	756.24		106	667.29	
Within periods						
Between origins of females..................	6	861.38	1.19	6	1757.36	3.04†
Within origins..............................	51	721.24		99	578.08	
Within periods						
Within origins of females						
Between systems of mating..................	14	1011.51	1.64	9	1103.09	2.12*
Within systems of mating....................	37	617.03		90	524.58	
Within periods						
Within origins of females						
Within inbreeding						
Between levels of inbreeding................	..	....	....	12	604.66	1.19
Within levels of inbreeding.................	..	....	....	41	508.41	
Within periods						
Within origin	(SE	females)		(SEC	B females)	
Between systems of mating..................	6	1163.94	....	3	1295.27	2.50
Within systems of mating...................	10	1373.27	....	56	518.09	
Within inbreeding...........................	..	....	....	41	549.80	
Between levels of inbreeding................	..	....	....	6	510.40	
Within levels of inbreeding.................	..	....	....	35	556.56	
Within periods						
Within origin	(OB	females)		(OB²	females)	
Between systems of mating..................	6	1131.93	7.47†	3	602.45	2.47
Within systems of mating...................	21	151.54	....	4	244.32	
Within inbreeding...........................	..	....	....	4	244.32	
Between levels of inbreeding................	..	....	....	2	460.03	16.07
Within levels of inbreeding.................	..	....	....	2	28.62	
Within periods						
Within origin	(CB	females)		(XB	females)	
Between systems of mating..................	2	192.99	....	3	1541.54	2.69
Within systems of mating...................	6	782.40		30	574.07	
Within inbreeding...........................	..	....	....	8	572.68	
Between levels of inbreeding................	..	....	....	4	818.36	2.50
Within levels of inbreeding.................	..	....	....	4	327.01	

* Significant at the 5% level.
† Significant at the 1% level.

The tests of heterogeneity of the distribution of matings according to rate of production and hatchability have revealed, however, that the relation between rate of production and hatchability is significant only for 1941. It is interesting to recall that in 1940 there was also no relation between rate of production and fertility.

TABLE 18

RELATION BETWEEN RATE OF EGG PRODUCTION AND
HATCHABILITY TOGETHER WITH TESTS OF
HETEROGENEITY OF THE FREQUENCIES

Rate of production	Percentage of hatchability 1940		Number of matings	Average hatchability
	Less than the median or 70%	More than the median or 70%		
Less than the median or 18 eggs..................	14	15	29	.6579
More than the median or 18 eggs.................	10	21	31	.7419
All matings.................................	24	36	60	.7006

$\chi^2 = 1.00$ P = .317

Rate of production	Percentage of hatchability 1941		Number of matings	Average hatchability
	Less than the median or 78%	More than the median or 78%		
Less than the median or 17 eggs..................	29	30	59	.6502
More than the median or 17 eggs.................	14	36	50	.8410
All matings.................................	43	66	109	.7536

$\chi^2 = 4.21$ P = .04

Relation Between Clutch Position of Fertile Eggs and Their Hatchability

The data accumulated in this study permitted an analysis of the relation between clutch position of fertile eggs and their hatchability. Table 19 presents the results of the analysis.

Eggs occupying a clutch position intermediate between the first and the last hatched significantly better than the eggs occupying the first and the last clutch positions. The distribution of hatched and unhatched fertile eggs in the first and last clutch positions was tested for heterogeneity in clutches of varying sizes. The results are presented in table 20.

The hatchability of first and last eggs in a clutch is apparently not influenced by the size of the clutch to which they belong. Last eggs in a clutch show a tendency to hatch less well in shorter than in longer clutches, but the test of heterogeneity has given a χ^2 value slightly over the 5% level of significance. The hatchability of first and last eggs in a clutch is apparently independent of the rate of egg production.

TABLE 19

DISTRIBUTION OF HATCHED AND UNHATCHED FERTILE EGGS
ACCORDING TO CLUTCH POSITION FOR 1940 AND 1941 DATA

Clutch position	Fertile eggs		Number of eggs	Average hatchability
	Hatched	Unhatched		
First	136	43	179	.7598
Last	138	67	205	.6732
Intermediate	347	88	435	.7977
Total	621	198	˙819	.7582

$$\chi^2 = 11.79 \quad P = .01$$

	Hatched	Unhatched		
First	136	43	179	.7598
Intermediate	347	88	435	.7979
Total	483	131	614	.7866

$$\chi^2 = .872 \quad P = .35$$

	Hatched	Unhatched		
Last	138	67	205	.6732
Intermediate	347	88	435	.7979
Total	485	155	640	.7578

$$\chi^2 = 11.10 \quad P = .001$$

	Hatched	Unhatched		
First	136	43	179	.7598
Last	138	67	205	.6732
Total	274	110	384	.7135

$$\chi^2 = 3.10 \quad P = .08$$

TABLE 20

DISTRIBUTION OF HATCHED AND UNHATCHED EGGS IN FIRST AND
LAST CLUTCH POSITIONS ACCORDING TO SIZE OF CLUTCH

Size of clutch	Fertile eggs		Total	Average hatchability
	Hatched	Unhatched		
First clutch position				
1, 2, and 3 eggs	77	18	95	.8105
4 and more eggs	50	16	66	.7576
Total	127	34	161	.7888

$$\chi^2 = .375 \quad P = .54$$

	Hatched	Unhatched		
Last clutch position				
1, 2, and 3 eggs	80	44	124	.6452
4 and more eggs	61	19	80	.7625
Total	141	63	204	.6912

$$\chi^2 = 3.75 \quad P = .053$$

Relation Between Hour of Day Fertile Eggs Were Laid and Hatchability

The fertile eggs available in 1940 and 1941 were classified according to the hour of the day they were laid and according to whether they hatched or not. Table 21 presents a summary of the analysis for fertile eggs in all clutch positions and within first and subsequent clutch positions.

TABLE 21

DISTRIBUTION OF FERTILE EGGS ACCORDING TO
HOUR OF LAY AND HATCHABILITY

Hour of lay	Mean time interval	Fertile eggs		Total	Average hatchability
		Hatched	Unhatched		
All clutch positions					
– 8 o'clock	....	84	39	123	.6829
8–10 o'clock	....	174	39	213	.8169
10–12 o'clock	....	152	44	196	.7755
12– 2 o'clock	....	122	36	158	.7722
2 o'clock+	....	89	40	129	.6899
Total	....	621	198	819	.7582

$\chi^2 = 11.57$ P = .02–.05

First clutch position					
– 8 o'clock	41:34 hrs.	50	19	69	.7246
8–10 o'clock	42:46 hrs.	62	15	77	.8052
10 o'clock+	45:46 hrs.	24	9	33	.7273
Total	....	136	43	179	.7598

$\chi^2 = 1.53$ P = .30–.50

Subsequent clutch positions					
– 8 o'clock	23:17 hrs.	34	20	54	.6296
8–10 o'clock	24:12 hrs.	112	24	136	.8235
10–12 o'clock	25:04 hrs.	137	38	175	.7829
12– 2 o'clock	26:32 hrs.	114	33	147	.7755
2 o'clock+	27:43 hrs.	88	40	128	.6875
Total	....	485	155	640	.7578

$\chi^2 = 12.33$ P = .01–.02

Fertile eggs laid before 8 o'clock in the morning did not hatch as well as eggs laid at other hours in the forenoon while eggs laid after 2 o'clock in the afternoon did not hatch as well as those laid between noon and 2 o'clock. Eggs laid between 9 o'clock in the morning and 2 o'clock in the afternoon consequently hatched better than those laid before or after. The test of heterogeneity of the frequencies of hatched and unhatched eggs according to hour

of lay revealed that the differences are significant for eggs in all clutch positions and for eggs in clutch positions subsequent to the first. Eggs occupying first clutch position did not hatch as well when laid before 8 o'clock in the morning or after 10 o'clock in the morning but the differences were not statistically significant.

In table 21, along with the hour of the day the eggs were laid, time interval between the egg concerned and the preceding one is also reported. The later in the day the egg was laid the longer the average time interval whether the egg occupied first clutch position or a subsequent one.

TABLE 22

RELATION BETWEEN TIME INTERVAL BETWEEN
EGGS AND HATCHABILITY

| Clutch position | Time interval | Fertile eggs | | Total | Average hatchability |
		Hatched	Unhatched		
First...................................	40–41 hrs.	45	15	60	.7500
	42–43 hrs.	56	11	67	.8358
	44–48 hrs.	35	17	52	.6731
Total...............................	....	136	43	179	.7598

$$\chi^2 = 4.28 \quad P = .10\text{-}.20$$

| Clutch position | Time interval | Fertile eggs | | Total | Average hatchability |
		Hatched	Unhatched		
Subsequent to first......................	20–22 hrs.	27	9	36	.7500
	23–24 hrs.	145	40	185	.7838
	25–26 hrs.	157	43	200	.7850
	27–28 hrs.	66	24	90	.7333
	29–30 hrs.	6	9	15	.4000
Total...............................	....	401	125	526	.7624

$$\chi^2 = 12.38 \quad P = .01\text{-}.02$$

Relation Between Time Interval Between Eggs and Hatchability

The data gathered in 1940 and 1941 permitted an analysis of the relation between the length of time interval and hatchability. The results of this analysis are presented in table 22.

The eggs following the shortest and the longest time intervals did not hatch as well as those with a medium time interval, whether they occupied the first clutch position or one subsequent to the first. The tests of heterogeneity of the frequencies of hatched and unhatched eggs reveal that the differences are significant only in eggs occupying a clutch position subsequent to the first.

DISCUSSION

The most significant observation made in this study is perhaps the fact that only the females with an outbred origin, the OB females in 1940, have shown statistically significant differences in hatchability when inbred and outbred.

This observation is in accord with the reports of Jull (1929a) and of Warren (1934) to the effect that the greatest decrease in hatchability occurs when the coefficients of inbreeding range from zero to .125. The females with an inbred origin in both years have shown differences in hatchability following further inbreeding as compared to outbreeding, but these differences were not statistically significant.

Outbred females are heterozygous for a large number of genes. Some of these genes may affect hatchability. When such females are used in inbred matings the result is possibly a number of zygotes homozygous for some of the genes with a deleterious effect on hatchability. Inbred females, being already homozygous for a number of desirable genes affecting hatchability, since they are the surviving zygotes that did not carry homozygous deleterious genes, are less likely than outbred females to produce gametes with deleterious genes. Consequently further inbreeding may not lead to a significant decrease in hatchability.

Another important observation made in this study is that no significant differences in hatchability were observed in 1941 between levels of inbreeding. This observation is not in complete agreement with a report of Jull (1929b) who observed a decrease in hatchability as the coefficient of inbreeding increased.

The origins of the females influenced hatchability while those of the males did not. In both years the females with an inbred origin gave lower hatchability than those with an outbred origin but the differences were not found to be statistically significant. Hatchability was observed to be much more variable in eggs from inbred females than in eggs from outbred females.

A periodic decline in hatchability was observed in both years but it was found to be statistically significant only in 1941. This can only be explained by variations in environmental factors which remain unknown so far as this study is concerned.

A significant relation was observed between rate of egg production and hatchability in 1941 but not in 1940. In the latter year, the analysis was extended within origins of females and the relation remained without significance. Byerly, Titus and Ellis (1933) and Funk (1934, 1939) found a significant correlation between current egg production and hatchability. The relation between current egg production and hatchability is more complex than generally assumed, as has been pointed out by Byerly, Titus and Ellis (1933). While it may be explained in part by the effect of the diet on both egg production and hatchability, it may also be the result of length of interval between eggs or the length of time required for egg formation. This latter point would lead us to the relation between the stage of embryonic development reached in the egg at the time of oviposition and hatchability. This topic will be dealt with in another part of this study.

Significant differences in the hatchability of eggs from different clutch positions exist. Eggs in first clutch position hatched less well than eggs in intermediate clutch positions but the difference was not statistically significant. Eggs occupying the last clutch position gave a significantly lower hatchability than those occupying intermediate clutch positions. There was no sig-

nificant difference between the hatchability of eggs occupying the first and those in the last clutch positions although the latter hatched slightly poorer than the former. These findings are contrary to the observations of Funk (1939) that the order of eggs within a clutch has no bearing on their hatchability. Rendel (1943) also reported that in ducks the position of an egg in a clutch does not affect its chance of hatching.

The eggs laid early in the morning, before 8 o'clock, and late in the afternoon, after 2 o'clock, hatched less well than those laid between those hours. The differences were statistically significant except for the eggs occupying first clutch position. Contradictory reports are available on the relation between hour of day eggs are laid and their hatchability. Hutt and Pilkey (1930), Patterson (1930), Pritzker (1940), and McNally and Byerly (1936) have reported that eggs laid in the morning hatched better than eggs laid in the afternoon, while Funk (1934) concluded the reverse, that afternoon eggs hatched slightly better than morning eggs. Nicolaides (1933) found no statistically significant difference in the hatchability of eggs laid at different hours of the day although his data show that the eggs laid between 7 and 9 o'clock in the morning and between 2 and 6 o'clock in the afternoon did not hatch as well as those laid between 9 o'clock in the morning and noon. The data available in the present study are thus in agreement with those reported by Nicolaides. The statistical analysis in the present study, however, has revealed significant differences in the hatchability of early morning and late afternoon eggs when compared with eggs laid between 8 and 2 o'clock.

The eggs in first clutch position with a time interval of less than 42 hours and of more than 43 hours did not hatch as well as eggs with a time interval of 42 and 43 hours but the differences were not statistically significant. The eggs in clutch positions subsequent to the first with a time interval of 23 hours or less and 27 hours or more did not hatch as well as those with a time interval varying between 23 and 27 hours. The differences were found to be statistically significant. McNally and Byerly (1936) have noted that the best hatchability was observed in eggs with a time interval of approximately 27 hours. They observed an increase in hatchability as the time interval increased from 24 to 27 hours and a decrease in hatchability as the time interval increased over 27 hours. Hays (1936) obtained evidence that eggs with intervals of 24½ hours or less and of 27½ hours or more did not hatch as well as eggs with intervals of 25 to 26½ hours. Hays (1938) also reported that as the time interval between clutches lengthened there was a tendency for hatchability to decline. The results of the present study are thus in accord with these observations of McNally and Byerly (1936) and Hays (1936, 1938) on the existence of a curvilinear relation between time interval and hatchability.

SUMMARY

The results of this study on hatchability, based on observations made during two years on matings of females with an inbred, an outbred and inbred-crossbred origin to males of the same three origins, indicate that inbreeding of outbred females (from $F = 0$ to $F = .25$) resulted in decreased hatchability

while further inbreeding of inbred females did not lead to any significant decrease in hatchability. The inbred females showed lower hatchability than the outbred females, whatever the system of mating, but the differences observed were not statistically significant. In one year, the birds laying at a higher intensity produced eggs that hatched better. The fertile eggs laid before 8 o'clock in the morning and after 2 o'clock in the afternoon did not hatch as well as those laid between those hours. The eggs that required 23 hours or less and 27 hours or more for their formation did not hatch as well as those that required between those hours. Premature and delayed oviposition apparently result in decreased hatchability. Eggs occupying a clutch position intermediate between the first and the last hatched significantly better than the eggs occupying the first and the last clutch position.

The Relative Effects of Inbreeding and Outbreeding on Reproduction in the Domestic Fowl

PAUL E. BERNIER, LEWIS W. TAYLOR, and C. A. GUNNS

IV. EARLY EMBRYONIC GROWTH

THE DOMESTIC FOWL has been a favorite material for studies on growth, as can be gathered from Lerner's review of the literature (1937). As a result, much progress has been made in the analysis of the environmental factors influencing size, as well as in the study of the genetic differences that exist between and within breeds. The majority of these studies have, however, been made during the post-embryonic growth period; comparatively few, omitting the purely descriptive ones, deal with the embryonic period and these few all deal with stages beyond the 72-hour stage of incubation.

Embryologists, until the latter part of the nineteenth century, were mostly concerned with purely descriptive studies and paid little attention to variability during early development. As pointed out by Mehnert (1896) only typical observations were considered and all variations were thought to be unimportant, accidental, unusual, or even aberrations. With the improvement of technique, the adoption of standard measurements, and the observation of large numbers of specimens, it soon became evident to most investigators that variability was of great importance in embryology and in fact of much value in their studies. This became particularly evident in experimental embryology, since its object is the study of embryological processes through natural or artificial variations in the developing organisms or in their environment.

The present study was undertaken in an effort to fill a gap in our knowledge of early embryonic growth in the domestic fowl by an analysis of the relative effects of genetical and environmental factors on the variability present in the very early stages of development. The events leading to this study were related in Part I. In addition to theoretical aspects, this study has practical aspects as well, such as the relation of the rate, and of the stage of development, before and at the beginning of incubation, to hatchability or embryonic mortality. In fact these practical aspects led to the initiation of the observations reported upon here and the theoretical aspects came into consideration as the investigation proceeded.

Review of Literature

The investigations made on the variability of the early embryonic growth rate in species of birds other than the domestic fowl indicate that differences in rate of development in early stages are difficult to detect, especially in closely related species. The genetic differences accounting for the specific

differences in size would enter into play only as development attains relatively advanced stages. The outstanding investigations on the comparative rate of embryonic growth in various species of birds are those of Levi (1922, 1925), Kaufman (1927, 1929, 1930*a, b, c*), and Price (1938).

A number of investigators have studied the early embryonic development of different breeds and varieties of the domestic fowl. Keibel and Abraham (1900) and Keibel (1904) could not observe any noticeable difference in the size of embryos of Brown Leghorns and bantam hens, and they combined the data obtained from the two breeds. Byerly (1930) observed no difference in the size of embryos in two breeds varying in adult size. He later (1932) reported that the major differences in embryo size in different breeds and their hybrids could be satisfactorily explained by differences in egg size and that the inherent growth rate is probably identical for all breeds. Henderson (1930) made observations on embryonic growth in the Dark Cornish and the Single Comb White Leghorn breeds and reported little, if any, consistent difference in the weight of the embryos from the fourth day on. Rensch (1923) could not detect any difference between embryos of different breeds prior to the tenth day of incubation, but after that day the embryos of the larger breed were found to contain more and larger cells so that cell size and rate of cell proliferation were thought to be the causes of the differences in size between chick embryos in breeds differing in size. Keller (1933) found that embryo size in large and bantam breeds differed only after the first week of incubation and that there was no difference in the size of cells but only in the number of cells.

Blunn (1934) and Blunn and Gregory (1935) compared embryonic development in carefully paired eggs of Single Comb White Leghorn and Single Comb Rhode Island Red hens and observed that the embryos of the latter breed contained more and smaller cells. They concluded that there was a genetic difference in the rate of cell proliferation between the two breeds during embryonic development.

Kaufman (1936) compared the embryonic development of Polish Greenfoot and Silkie embryos as well as of their reciprocal hybrids. She observed that the pure Polish Greenfoot embryos were significantly larger than the crossbred embryos in eggs of the same size at eleven days of age. The Silkie embryos appeared to have their growth inhibited in the second week of incubation. She also observed differences in the proportion of parts to the whole in the Polish Greenfoot, Silkie, and reciprocal hybrids and interpreted these differences as evidence for the genetical control of embryonic growth and not merely as an effect of the amount of available food.

Byerly, Helsel and Quinn (1938) compared the development of Silkie, Rhode Island Red and their reciprocal hybrids. They concluded that the differences attributable to genetic factors are small and appear only after the first week of incubation. Egg size was found to be a more important factor in determining embryo size. They found no evidence of inherent differences in rate of cell division in embryos differing genetically in size although they observed that variability was greater in embryos from different matings than from those in the same mating.

Derrick (1937) observed that embryos of the White Leghorn breed were frequently smaller than those of the Barred Plymouth Rock breed, at least during the first three days of incubation, but she did not submit her data to any statistical analysis to establish whether or not the difference was significant.

Thus, there is evidence in favor of the hypothesis that genetic factors influence the early embryonic development of the chick, although this evidence is contradictory in nature. The genetic factors apparently do enter into play after development has proceeded to a certain stage, approximately the second week of incubation. There is, however, the possibility that genetic factors enter into play at an earlier period, but that variability is so great that difficulty is experienced in ascribing any portion of such variability to genetic factors. Under such conditions it would be necessary to make a large number of observations on embryos from a large number of matings and under conditions of a planned experimental design which would allow the use of statistical methods. This was done in the present investigation.

Materials and Methods

The main object of this study was to determine the influence of genetic factors on early embryonic growth, hence no steps were taken to control genetic variation except to employ suitable statistical methods to measure it as accurately as possible. The length of time that embryos were under favorable temperature conditions for development is undoubtedly the most important source of variation that had to be controlled, or at least measured accurately, in order to make statistical adjustments. In the general introduction it was stated that eggs were collected hourly and kept in a refrigerated cabinet. No significant variation can be attributed to development occurring after the egg was laid and before it was incubated. The only remaining source of variation as to the time that the eggs were under conditions favorable to embryonic development is thus the period between ovulation and oviposition. The measurement of this period of time is dependent upon the time ovulation and fertilization occur in relation to oviposition, as well as the time required for the egg to descend through the oviduct. Warren and Scott (1934) were able to establish that ovulation normally occurs within one hour after laying of the immediately preceding egg of the clutch, while Olsen (1942) has observed that the egg is normally fertilized, in the infundibulum, within fifteen minutes after ovulation. The time interval between successive eggs of a clutch can therefore be accepted as a relatively accurate measurement of the time eggs are under conditions favorable to embryonic development before laying.

The two measurements of early embryonic growth used in these investigations were maximum diameter of the blastoderm in the unincubated egg and the number of somites present in the embryo after 38 hours of incubation. The first measurement is actually the only one that can be readily used in view of the relatively undifferentiated stage of the blastoderm at that time. The use of number of somites could be questioned, however, as there is no apparent reason to assume that the number of somites, which are presumably the result of differentiation, bears a definite relation to the size of the whole

embryo. This question was important in these investigations since a satisfactory index of size, preferably one with a linear relation with size, was needed. Very fortunately a study on variability in the early development of duck embryos made by Fischel (1896) provided suitable data for verification of the assumption. Among all the data he reported on, for a total of 104 duck embryos, two measurements, total length of embryo and number of somites were used. Analysis of his data showed a correlation coefficient of .91 between number of somites and total length of embryo and the regression of total length of embryo on number of somites was found to be $82.16 + 4.65 \times$ somites. There is thus good evidence that the number of somites is a valid measurement of embryo size in the early stages of development.

Analysis of Observations

Before proceeding to a detailed analysis of the data collected in 1940 and 1941 on early embryo size as influenced by inbreeding and outbreeding, it was thought advisable to study the frequency distribution of early embryos as to size and the possible relation between early embryo size and factors such as: position the eggs occupy within a clutch, time interval or length of time elapsing between the oviposition of two successive eggs, and hour of lay. In addition to the data collected in the course of the present investigations in 1940 and 1941, Professor Taylor was kind enough to place at the disposal of the authors data he had collected on blastoderm size and on number of somites during the periods 1930–1938 and 1937–1938 respectively. Measurements on a total of 1677 blastoderms and somite counts on a total of 1163 embryos were available for analysis.

Frequency Distributions of Blastoderms as to Maximum Diameter and of Embryos According to Number of Somites

The observations on blastoderm size, as measured by their maximum diameter to the nearest hundredth of a millimeter, were grouped in classes of one-tenth of a millimeter. The measurements between .01 and .10 mm were grouped together to give classes with mid-values of .05 mm. Figure 1 gives the frequency distribution of the 1677 blastoderms as to maximum diameter. The statistics obtained in the analysis and describing the frequency distribution are:

Number.........................	1677
Mean............................	3.6242 mm.
Standard deviation.................	.32865
Coefficient of variability...........	9.07%
g_1...............................	$1.31 \pm .06$
g_2...............................	$6.65 \pm .12$

The mean and the standard deviation were used to calculate the expected frequency distribution superimposed (broken line) on the observed frequency distribution in figure 1. A test of goodness of fit gave a χ^2 value of 99.06 with a probability point of less than .0001. The observed distribution clearly

deviates from a normal one. Inspection of figure 1 reveals the presence of leptokurtosis, an excess of observations near the mean, and positive skewness, the mode being smaller than the mean. These facts are confirmed by the statistics g_1 and g_2, which are respectively measures of skewness and kurtosis, computed from the third and fourth moments, or powers of the deviations from the mean, in the observed frequency distribution. The positive skewness of the observed frequency distribution, as compared to the expected, is the result of an excessively large number of blastoderms with a large diameter. There is thus very good evidence to conclude that the very large blastoderms
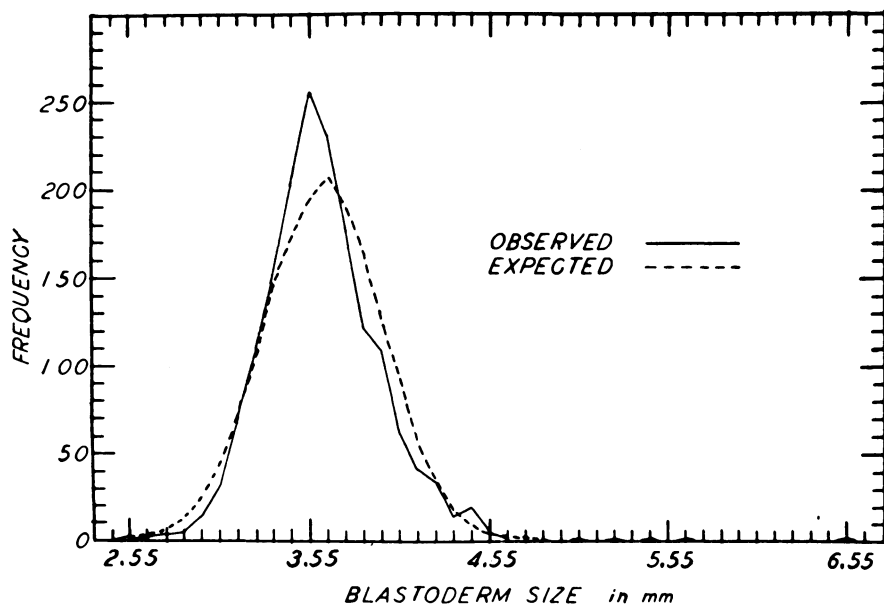
Fig. 1

were observed more frequently than would be expected. The frequency of the very large blastoderms is high enough to suspect their occurrence is due to recurring conditions.

The frequency distribution of somite counts made in 1937, 1938, 1940 and 1941 is given in figure 2. The statistics obtained in the analysis are:

Number....................... 1163
Mean......................... 11.3758 somites
Standard deviation............. 2.3631
Coefficient of variability......... 20.8%
g_1............................ — .4763 ± .07

The form of the graph indicates negative skewness which is confirmed by the statistic g_1. The frequency of low somite counts is excessive; it is logical to assume that these low somite counts were made on abnormal embryos,

dead or moribund, that probably do not belong to a normal distribution. It was therefore decided that within every bird, the extremely low somite counts (those that were four units below the mean for that bird) should be discarded; this criterion of rejection corresponds to twice the standard deviation.

Relation Between Clutch Position of Eggs and Early Embryo Size

The data on the relation between clutch position of eggs and the size of the blastoderm they contained are summarized in table 23.
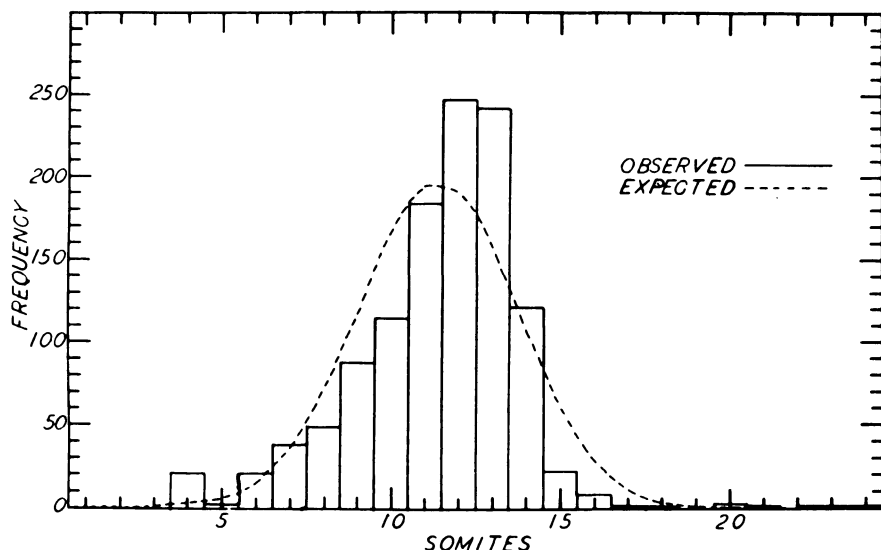

Fig. 2

The larger size of blastoderms in first clutch position is particularly strik-ing, as is also the greater variability. There is evidence that eggs occupying second clutch position also contained a larger blastoderm than eggs occupy-ing clutch positions subsequent to the second although the variability in this case is not greater than that for eggs in subsequent clutch positions.

Tests of homogeneity of the distributions of blastoderms classified accord-ing to deviation below and above the median and clutch position of eggs have given the results reported in table 24. The distribution in clutch positions one to five is heterogeneous and eggs in first and second clutch positions apparently contribute most to the heterogeneity. A comparison of the dis-tribution of eggs in first clutch position and of the total number of eggs in clutch positions subsequent to the first also shows heterogeneity. The elimina-tion of eggs occupying first clutch position gives a distribution which still shows heterogeneity. The distribution of eggs occupying second clutch posi-tion compared to that of the total number of eggs occupying clutch positions subsequent to the second also shows heterogeneity. These tests show that

TABLE 23

RELATION BETWEEN CLUTCH POSITION OF EGGS
AND BLASTODERM SIZE, DATA OF 1937,
1938, 1940, AND 1941

Clutch position	Number of blastoderms	Mean size in mm	Standard deviation
1	582	3.7761	.3825
2	462	3.5801	.2701
3	269	3.5101	.2631
4	136	3.5265	.2571
5+	227	3.5108	.2413
All positions	1676	3.6242	.3287
1	582	3.7761	.3825
2+	1094	3.5431	.2626
All positions	1676	3.6242	.3287

TABLE 24

TESTS OF HOMOGENEITY OF THE DISTRIBUTION OF BLASTODERMS
IN EGGS OCCUPYING VARIOUS CLUTCH POSITIONS AND
CLASSIFIED ACCORDING TO DEVIATION BELOW
OR ABOVE MEDIAN DIAMETER

Above or below median blastoderm size	Clutch position of eggs					Total	χ^2	P
	1	2	3	4	5+			
Below	190	257	179	83	146	855		
Above	392	205	90	53	81	821		
Total	582	462	269	136	227	1676	129.99	.001
	1	2+						
Below	190	665	....	....	....	855		
Above	392	429	....	....	....	821		
Total	582	1094	....	....	....	1676	119.25	.001
		2	3	4	5+			
Below	...	178	136	65	99	478		
Above	...	284	133	71	128	616		
Total	...	462	269	136	227	1094	11.09	.01–.02
		2	3+					
Below	...	178	300	...	...	478		
Above	...	284	332	...	...	616		
Total	...	462	632	...	...	1094	8.31	.004

blastoderms varied significantly in size in eggs occupying different clutch positions, particularly in the first and second clutch positions.

The combined data of 1937, 1938, 1940 and 1941 were analyzed to study the relation between the position of eggs within a clutch and the stage of development reached by the embryos after 38 hours of incubation as measured by number of somites. Table 25 gives the statistics of samples in clutch positions 1 to 5 with the latter position also including all the data in clutch positions subsequent to 5.

The mean number of somites varies according to the position the egg occupies in a clutch and the difference in mean number of somites is especially marked when eggs in first clutch position are compared with eggs in subse-

TABLE 25

STATISTICS ON NUMBER OF SOMITES PRESENT AFTER 38 HOURS OF INCUBATION IN EGGS OCCUPYING DIFFERENT CLUTCH POSITIONS

Clutch position	1	2	3	4	5+	Total
Number of embryos..........	324	277	160	102	203	1066
Mean number of somites.....	12.6389	11.7256	11.4875	11.3824	10.8818	11.7735
Standard deviation...........	2.0735	1.6694	1.7839	1.6771	1.6001	1.9125

quent clutch positions. Eggs occupying first clutch position also show more variability in mean number of somites than eggs in subsequent clutch positions.

Tests of homogeneity of the distribution of embryos as to number of somites in eggs occupying first clutch position as compared to eggs in subsequent positions, and in second clutch position eggs as compared to eggs in subsequent positions, have given the results presented in table 26. These tests of homogeneity reveal that the distribution of embryos according to number of somites is significantly different in eggs occupying first clutch position than in eggs occupying subsequent clutch positions. The distribution in eggs occupying second clutch position is also significantly different when compared with the distribution of embryos in eggs occupying subsequent clutch positions.

Relation Between Time Interval Between Eggs and Early Embryo Size

A study was made of the relation between the length of the time interval between eggs and early embryo size on the data available for 1937, 1938, 1940 and 1941.

The results of the analysis of the data on blastoderm size are presented in table 27. A separate analysis was made for eggs occupying first clutch position and for those in subsequent clutch positions.

Eggs in first clutch position, as already demonstrated, show more variability in blastoderm size and do so particularly when the time interval is 43 hours or less and 48 hours or more.

In eggs occupying clutch positions subsequent to the first, there is a slight tendency towards an increase in blastoderm size with an increase in length

of time interval between eggs. This trend is more marked for intervals of 26 to 30 hours. Eggs in clutch positions subsequent to the first also show less variability in blastoderm size than eggs in first clutch position.

Tests of homogeneity of the distribution of eggs classified according to first and subsequent clutch positions, time interval and deviation from the median blastoderm size gave the results presented in table 28.

TABLE 26

TESTS OF HOMOGENEITY OF THE DISTRIBUTION OF
EMBRYOS AS TO NUMBER OF SOMITES
IN EGGS OCCUPYING VARIOUS
CLUTCH POSITIONS

| Number of somites | Number of embryos | | Total |
	Eggs in first clutch position	Eggs in subsequent clutch positions	
6, 7, and 8	8	42	50
9	14	73	87
10	16	95	111
11	30	147	177
12	69	174	243
13	97	145	242
14	63	56	119
15 to 24	27	10	37
Total	324	742	1066

$$\chi^2 = 113.33 \quad P < .01$$

| Number of somites | Number of embryos | | Total |
	Eggs in second clutch position	Eggs in subsequent clutch positions	
6, 7, and 8	12	30	42
9	19	54	73
10	29	66	95
11	48	99	147
12	65	109	174
13	76	69	145
14 to 16	28	38	66
Total	277	465	742

$$\chi^2 = 30.04 \quad P < .01$$

The tests of heterogeneity reveal that eggs in first clutch position show no significant variation in blastoderm size when classified according to time interval. This lack of significant relation is probably accounted for by the inaccuracy of the time interval between clutches as an index of the actual time spent by the egg in the oviduct. Eggs in clutch positions subsequent to the first do show significant variations in blastoderm size when classified accord-

TABLE 27

RELATION BETWEEN TIME INTERVAL
AND BLASTODERM SIZE

Time interval	Number of blastoderms	Mean size	Standard deviation
Eggs in first clutch position			
		mm	
40 hours	10	3.6200	.3268
41 hours	72	3.7403	.2496
42 hours	23	3.7587	.6105
43 hours	68	3.8279	.5582
44 hours	20	3.7050	.2837
45 hours	22	3.7955	.2483
46 hours	19	3.7816	.3400
47 hours	3	3.5833	.1248
48 hours	11	3.8136	.4202
49 hours	2	3.7500	.1414
50 hours	7	3.3929	.1618
51 hours	4	3.3750	.5188
52 hours	1	3.3500	.....
53 hours	1	3.4500	.....
54 hours	1	4.2500	.....
55 hours	1	3.8500	.....
All blastoderms	265	3.7511	.4126
Eggs in subsequent clutch positions			
20 hours	1	2.9500	.....
21 hours	7	3.5071	.2494
22 hours	6	3.5000	.2121
23 hours	37	3.4338	.2467
24 hours	139	3.5104	.2125
25 hours	69	3.4558	.2345
26 hours	192	3.5193	.2418
27 hours	110	3.5291	.2378
28 hours	58	3.5586	.2319
29 hours	44	3.5727	.2587
30 hours	8	3.7000	.3817
31 hours	5	3.6300	.3033
32 hours	...	......	.....
33 hours	1	3.9500	.....
All blastoderms	677	3.5172	.1863

ing to time interval. This relation can be interpreted as evidence that, in eggs occupying a clutch position subsequent to the first, time interval between eggs is a reliable index of the actual time spent by the egg in the oviduct.

The data on number of somites present after 38 hours of incubation were also analyzed for the possible relation between time interval and number of somites. In view of the indicated relative inaccuracy of the time interval for eggs occupying first clutch position, the analysis was limited to eggs in clutch positions subsequent to the first. Table 29 gives the mean number of somites observed in embryos in eggs with different time intervals.

The relation between time interval and mean number of somites is very

TABLE 28

TESTS OF HOMOGENEITY OF THE DISTRIBUTION OF EGGS
CLASSIFIED ACCORDING TO CLUTCH POSITION,
TIME INTERVAL AND DEVIATION FROM
THE MEDIAN BLASTODERM SIZE

Above or below median blastoderm size	Time interval in hours								Total
	40–41	42	43	44	45	46	47–48	49–55	
Eggs in first clutch position									
Below..........................	28	12	21	7	4	5	5	9	91
Above.........................	54	11	47	13	18	14	9	8	174
Total........................	82	23	68	20	22	19	14	17	265

$$\chi^2 = 9.29 \qquad P = .2-.3$$

Above or below median blastoderm size	Time interval in hours								Total
	20–23	24	25	26	27	28	29	30–33	
Eggs in clutch positions subsequent to first									
Below..........................	41	89	51	125	78	34	24	5	447
Above.........................	10	50	18	67	32	24	20	9	230
Total........................	51	139	69	192	110	58	44	14	677

$$\chi^2 = 17.83 \qquad P = .01-.02$$

TABLE 29
RELATION BETWEEN TIME INTERVAL IN EGGS
OCCUPYING CLUTCH POSITIONS SUBSEQUENT
TO THE FIRST AND NUMBER OF SOMITES

Time interval in hours	Number of embryos	Mean somites
21..	3	9.33
22..	5	9.80
23..	43	10.42
24..	153	10.64
25..	97	10.94
26..	205	11.80
27..	121	11.59
28..	54	12.28
29..	54	13.00
30..	7	13.00
Total.....................	742	....
Average.....................	....	11.4443

striking. This relation is further supported by observations made on eight
eggs in first clutch position which contained embryos with a large number
of somites, i.e., from 16 to 24 somites. These observations led to the interpre-
tation that these eight eggs were really in last clutch position and had been
retained an abnormally long period of time in the oviduct. This is supported

by the following facts. These eight eggs weighed less than the egg following them, were laid very early in the morning and were each followed by an egg also laid very early in the morning, and, finally, were in a longer clutch than the preceding or following ones. The time interval of these eight retained eggs also shows a good relation with number of somites as shown in table 30.

TABLE 30

RELATION BETWEEN TIME INTERVAL AND
NUMBER OF SOMITES IN EIGHT
RETAINED EGGS

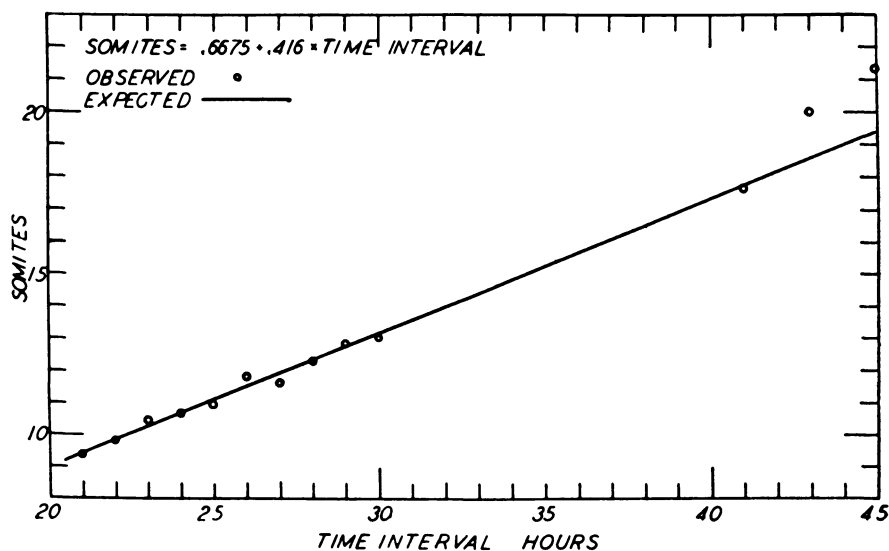| Time interval	Number of eggs	Mean somites
41 hours...............................	3	17.67
43 hours..	2	20.00
45 hours...............................	3	21.33

Fig. 3

An analysis of the regression of number of somites present in the embryos after 38 hours of incubation on time interval between eggs, including the data on the eight embryos with a large number of somites, has given the following regression equation:

Number of somites $= 0.67 + 0.416 \times$ time interval

The least square fit is illustrated by a straight line in figure 3 and the observed values (means of classes) are indicated by circles.

The analysis of the data on time interval and number if somites shows a positive relation between the two variables in eggs occupying a clutch position subsequent to the first.

Relation Between Clutch Position and Length of Time Interval Between Eggs

In view of the results of the two preceding analyses, a study was made of the relation between clutch position and time interval (table 31).

Time interval apparently decreases with later clutch positions. A test of the homogeneity of the distribution of eggs in second and subsequent clutch

TABLE 31

RELATION BETWEEN CLUTCH POSITION
AND TIME INTERVAL

Clutch position	Number of embryos	Mean time interval
1	265	43:29 hrs.
2	291	26:36 hrs.
3	149	25:54 hrs.
4	85	25:36 hrs.
5+	152	24:36 hrs.
Total	942	

TABLE 32

RELATION BETWEEN TIME INTERVAL IN EGGS
OCCUPYING SECOND CLUTCH POSITION
AND SIZE OF CLUTCH

Clutch size	Number of eggs	Mean time interval between first and second egg
Two eggs	181	28:12 hrs.
Three eggs	115	26:19 hrs.
Four eggs	75	25:55 hrs.
Five eggs	33	25:04 hrs.
Two to five eggs	404	27:00 hrs.

positions according to time interval has given a χ^2 value of 60.19 with a probability of less than .01. The distribution is heterogeneous and eggs occupying second clutch position have a longer time interval than eggs in subsequent clutch positions. This relation between clutch position and time interval, however, may be spurious. Eggs in short clutches may have a longer time interval. These eggs may also have a disproportionately large representation in the total number of eggs studied and the result may be a longer time interval for clutch positions 2 and 3 as compared to clutch position 4 and subsequent ones. This hypothesis is supported by the data presented in table 32 showing the relation between time interval in eggs occupying second clutch position and size of clutch.

Interaction Between Time Interval and Clutch Position on Early Embryo Size

The data gathered in 1940 and 1941 were submitted to a more detailed analysis of the relation between clutch position and early embryo size, taking into account length of time interval between eggs. The analysis of the data on blastoderm size is summarized in table 33.

As observed previously in the combined data for 1937, 1938, 1940 and 1941, the eggs occupying first clutch position in 1940 and 1941 contained a larger blastoderm and also showed a higher variability in blastoderm size than eggs in either last or intermediate clutch positions.

TABLE 33

RELATION BETWEEN CLUTCH POSITION, TIME INTERVAL
BETWEEN EGGS AND BLASTODERM SIZE

Clutch position	Number	Blastoderm size		Time interval		Coefficient of correlation
		Mean	Variance	Mean	Variance	
			1940			
				hrs. + min.		
First...........................	41	3.7400	.3433	44:34	7.80	−.01
Last............................	36	3.5678	.0851	26:47	1.95	.29
Intermediate...................	52	3.5629	.0542	24:35	2.60	.08
			1941			
First...........................	112	3.7080	.1450	43:10	11.04	−.18
Last............................	102	3.5458	.0597	27:10	2.68	.15
Intermediate...................	190	3.4519	.0493	25:17	2.63	.28

A very interesting finding is that eggs occupying the last clutch position in 1941 contained a larger blastoderm than eggs in intermediate clutch positions. However, when the last or second egg in two-egg clutches is eliminated, the mean size of blastoderms in last eggs for 1941 is reduced to 3.4933 mm as compared to 3.4519 for intermediate eggs. In 1940 the last eggs did not show a higher blastoderm size when compared to eggs in intermediate clutch positions.

In both 1940 and 1941, the time interval for eggs in the last clutch position was observed to be significantly higher than that for eggs in intermediate clutch positions. In 1941, even after eliminating the second or last egg in two-egg clutches, the time interval for last eggs was 26:45 hours as compared to 25:17 hours for eggs in intermediate clutch positions. The same significant difference was also observed in 1940. Since this longer time interval for last eggs in a clutch is not accompanied by a corresponding increase in blastoderm size, a delay in ovulation could very well explain part of the longer time interval between the last two eggs in a clutch.

Eggs in two-egg clutches, as already intimated, contained blastoderms larger than the mean of the whole population and this is clearly demonstrated in table 34.

In 1940 and 1941 respectively, second eggs in two-egg clutches had a time interval of 27:42 and 28:15 hours as compared to 25:49 and 25:55 hours for eggs in clutch positions subsequent to the first. Second eggs in two-egg clutches thus had a longer time interval than the average for all eggs in clutch positions subsequent to the first. The coefficients of correlation between time interval and blastoderm size in the last or second egg in two-egg clutches were found to be −.19 and −.01 for 1940 and 1941 respectively. These observa-

TABLE 34

BLASTODERM SIZE IN EGGS IN CLUTCHES OF TWO AS COMPARED
TO THE SAMPLE AS A WHOLE BEFORE AND AFTER
ELIMINATION OF CLUTCHES OF
ONE AND TWO EGGS

Size of clutch	Clutch position	1940			1941		
		Number of blastoderms	Mean size	Variance	Number of blastoderms	Mean size	Variance
Two eggs..........	First	15	3.6840	.8187	25	3.6152	.0839
Two eggs..........	Second	17	3.6576	.1113	40	3.6270	.0650
Both positions...	......	32	3.6700	.4274	65	3.6225	.0711
All eggs............	......	129	3.6205	.1588	403	3.5466	.0897
All eggs after elimination of clutches of one and two eggs............	......	88	3.5795	.0689	322	3.5238	.0888

tions can be interpreted as evidence that time interval in two-egg clutches, as was the case for eggs in first clutch position, is not an accurate indication of the actual time spent by the egg in the oviduct, even though it is expressed in a larger blastoderm than that found in eggs from clutch positions subsequent to the first. The time interval between the first and second egg in two-egg clutches, although representing an actually longer interval of sojourn in the oviduct as manifested by a larger blastoderm, is also attributable in part to a delay in ovulation after the oviposition of the first egg.

The data collected on number of somites after 38 hours of incubation, during 1940 and 1941, were also submitted to a more detailed analysis to establish the relation between clutch position, time interval between eggs and number of somites. The available data are summarized in table 35.

The eggs occupying first clutch position contain embryos with a larger mean number of somites than the eggs in either last or intermediate clutch positions. Eggs in first clutch position also have slightly higher variances for number of somites than eggs in intermediate clutch positions. The variances of time interval are also higher for eggs in first clutch position than for eggs in either last or intermediate clutch positions. This higher variance in

time interval, however, does not explain the higher variance in number of somites, as indicated by the low and nonsignificant correlation observed between time interval and number of somites in eggs occupying first clutch position.

The eggs occupying last clutch position in 1941 showed a higher mean number of somites than the eggs in intermedate clutch positions. In 1940 there was no difference in the mean number of somites between eggs occupy‑ ing the intermediate and last clutch positions. The elimination of the second or last egg of two-egg clutches in 1941 reduced the mean number of somites in last eggs from 12.43 to 12.29. The latter was still significantly higher than

TABLE 35

RELATION BETWEEN CLUTCH POSITION, TIME INTERVAL
BETWEEN EGGS AND NUMBER OF SOMITES

Clutch position	Number	Somites		Time interval		Coefficient of correlation
		Mean	Variance	Mean	Variance	
1940						
				hrs. + min.		
First..............................	28	11.79	4.6929	44:30	5.00	.11
Last..............................	39	10.51	4.2563	26:51	3.45	−.09
Intermediate.....................	57	10.56	3.5446	25:07	1.97	.20
1941						
First..............................	146	13.18	5.2646	42:45	4.15	.04
Last..............................	118	12.43	3.3928	27:02	3.26	.22
Intermediate.....................	202	11.40	2.9531	25:16	2.05	.25

the mean (11.40) for eggs in intermediate clutch posïtions. In both 1940 and 1941 eggs occupying last clutch positions had a longer time interval than eggs in intermediate clutch positions. The time interval for last eggs was reduced from 27 :02 to 26 :24 hours following the elimination of the second or last egg in two-egg clutches; the latter time interval is still higher than that for eggs in intermediate clutch positions (25 :16 hrs.). The last egg in a clutch, in 1941, apparently spent a longer period of time in the oviduct and the result was a larger mean number of somites. A positive correlation between number of somites and time interval of last eggs was observed in 1941 but not in 1940. This correlation was of the same order as that within eggs occupy‑ ing intermediate clutch positions.

The correlation coefficients between time interval and number of somites, given in table 35, are higher for eggs occupying intermediate clutch posi‑ tions than for eggs occupying first clutch position. This observation lends further support to the conclusion suggested previously, that time interval is a better indication of actual time spent in the oviduct for eggs in interme‑ diate clutch positions than for eggs in first clutch position ; this is the result of relatively better synchronization between oviposition of the preceding egg and ovulation of the one on which observations are made.

In view of the larger blastoderm and higher number of somites observed in eggs belonging to one- and two-egg clutches, it was thought that possibly a general relation existed between size of clutch and early embryo size. Measurements on blastoderms and on number of somites were classified according to size of clutches as summarized in table 36.

No appreciable difference in early embryo size could be detected except in the case, already demonstrated, of clutches of one and two eggs. An analysis of variance (table 37) was made to establish the significance of the differences

TABLE 36

SUMMARY OF DATA ON RELATION BETWEEN SIZE
OF CLUTCH AND EARLY EMBRYO SIZE

Size of clutch	Mean blastoderm size		Mean somites	
	1940	1941	1940	1941
1	3.8456	3.6894	13.0	13.0
2	3.6700	3.6225	10.3	12.9
3	3.5604	3.5404	10.5	12.3
4	3.3880	3.5760	11.0	12.3
5	3.6567	3.5490	10.4	12.2
6	3.7136	3.5200	11.8	12.2
7	3.1900	3.4758	9.0	12.2
8	3.5917	3.4580	12.5	10.7
9	3.3200	3.3609	11.0	11.8
10	3.2967	3.4550	....	11.0
11	......	......	....	....
12	3.5850	3.3667	10.3	11.5
13	......	3.3225	....	11.2
14	3.7080	3.5429	12.4	11.1
15	......	3.4250	....	11.0
16	......	......	....	....
17	......	......	....	....
18	3.8500	......	9.3	....
21	......	3.3133	....	11.0
26	3.2850	......	....	....

in early embryo size between clutches of varying size. These differences be-- tween clutches of varying size are not significant. This can be interpreted as evidence that the eggs in long clutches do not necessarily spend a shorter time in the oviduct.

The information obtained on the relation between time interval and early embryo size stresses the importance of the period of time spent by the egg in the oviduct as a factor influencing the stage of development reached by the embryo at the time of oviposition. Data gathered on eggs occupying first clutch position, in contrast to eggs occupying subsequent clutch positions, have to be submitted to a separate analysis because of the highly significant differences observed in the mean size of the blastoderms and mean number of somites they contain. The lack of a significant correlation between the time interval separating two clutches and early embryo size in eggs occupying first clutch position, makes it impossible to adjust the values on early embryo size for their regression on time interval. Such an adjustment for regression

of early embryo size on time interval is possible in eggs occupying clutch positions subsequent to the first, because of the comparatively high relation observed between time interval and early embryo size.

Relation Between Hour of Lay and Early Embryo Size

In Part III, it was demonstrated that eggs laid early in the morning and late in the afternoon did not hatch as well as those laid during the middle part of the day. In view of this demonstration, it was thought of interest to study the possible relation between hour of lay and early embryo size.

TABLE 37

ANALYSIS OF VARIANCE OF EARLY EMBRYO SIZE IN
CLUTCHES OF VARIOUS SIZES

Source of variation	Blastoderm size		F*	Somites		F*
	d.f.	Variance		d.f.	Variance	
1940						
Total..........................	120	.1653		123	4.26	
Between clutches..............	13	.1837	1.13	12	8.90	2.37
Within clutches...............	107	.1631		111	3.76	
1941						
Total..........................	403	.0895		465	4.37	
Between clutches..............	14	.1492	1.71	14	2.41	....
Within clutches...............	389	.0873		451	4.43	

* No significant F values.

The available data in 1937, 1938, 1940, and 1941, on the relation between hour of lay and blastoderm size are summarized in table 38. Eggs laid early in the morning, particularly those laid at or before 8 o'clock, contained larger blastoderms and exhibited a higher coefficient of variability than eggs laid later in the morning. The trend is for a decrease in blastoderm size at each successive hour of lay in the morning up to noon and again a decrease in eggs laid after 2 o'clock. A test of the homogeneity of the distribution of the eggs classified as to hour of lay and deviation in blastoderm size below or above the mean has given the results presented in table 39. The distribution is definitely heterogeneous; blastoderms vary in size according to the hour of day the eggs are laid. Early morning eggs contain a larger blastoderm than those laid later in the day, and late afternoon eggs contain a smaller blastoderm than those laid earlier in the afternoon.

The data on number of somites present in the embryos after 38 hours of incubation were also classified according to the hour of the day the eggs were laid (table 40). Eggs laid at or before 8 o'clock in the morning and at or after 1 o'clock in the afternoon show a tendency to produce incubated embryos with a larger number of somites than the mean for all eggs. A test of the homogeneity of the distribution of embryos according to number of somites

TABLE 38

RELATION BETWEEN HOUR OF LAY
AND BLASTODERM SIZE

Hour of lay	Number of blastoderms	Mean size	Standard deviation
8 o'clock or before............................	199	3.7565	.4317
9 o'clock....................................	105	3.7090	.3339
10 o'clock...................................	323	3.6540	.3160
11-12 o'clock................................	338	3.5609	.2951
1 o'clock....................................	278	3.5885	:3380
2 o'clock....................................	100	3.6120	.2936
3 o'clock....................................	189	3.5728	.2483
4 o'clock or after............................	102	3.5618	.3119
All blastoderms..........................	1634	3.6219	.3309

TABLE 39

TEST OF HOMOGENEITY OF THE DISTRIBUTION OF
BLASTODERMS AS TO SIZE AND HOUR OF LAY

	Blastoderm size below or above mean		
Hour of lay	Below	Above	Total
8 o'clock or before.................................	73	126	199
9 o'clock..	40	65	105
10 o'clock...	156	167	323
11-12 o'clock......................................	201	137	338
1 o'clock..	153	125	278
2 o'clock..	52	48	100
3 o'clock..	108	81	189
4 o'clock or after.................................	60	42	102
Total..	843	791	1634

$$\chi^2 = 40.95 \quad P < .01$$

TABLE 40

RELATION BETWEEN HOUR OF LAY AND
NUMBER OF SOMITES

Hour of lay	Number of embryos	Mean somites	Standard deviation
8 o'clock or before............................	172	12.2558	2.580
9 o'clock....................................	43	11.3448	1.974
10 o'clock...................................	197	11.4264	1.768
11-12 o'clock................................	253	11.5375	1.789
1 o'clock....................................	205	11.8000	1.503
2 o'clock....................................	43	11.9302	1.737
3 o'clock....................................	113	12.1947	1.874
4 o'clock or after............................	40	11.9000	1.464
All embryos.............................	1066	11.7739	1.912

TABLE 41

TEST OF HOMOGENEITY OF THE DISTRIBUTION OF
EMBRYOS AS TO NUMBER OF SOMITES AND
WHETHER EGGS WERE LAID BEFORE
OF AFTER 10 O'CLOCK

| Number of somites | Eggs laid | | Total |
	Before 10 o'clock	After 10 o'clock	
6 and 7..	7	7	14
8...	19	17	36
9...	45	42	87
10..	35	76	111
11..	61	116	177
12..	82	161	243
13..	98	143	241
14..	50	70	120
15..	6	16	22
16 and over..................................	9	6	15
Total.....................................	412	654	1066

$\chi^2 = 21.17$ $P = .01-.02$

TABLE 42

TEST OF HOMOGENEITY OF THE DISTRIBUTION OF
MORNING EGGS ACCORDING TO NUMBER
OF SOMITES AND TIME OF LAY

| Number of somites | Eggs laid | | Total |
	Before 8 o'clock	Between 8 o'clock and noon	
8 or less......................................	8	32	40
9...	20	42	62
10..	9	62	71
11..	16	96	112
12..	32	103	135
13..	49	108	157
14..	25	39	64
15..	5	6	11
16 and over..................................	8	5	13
Total.....................................	172	493	665

$\chi^2 = 35.60$ $P < .01$

and whether the eggs were laid before or after 10 o'clock gave the results
presented in table 41. The distribution is heterogeneous, and the number of
somites present in the embryos after 38 hours of incubation varies accord-
ing to the time of the day the eggs are laid. Additional tests of the homogeneity
of the distribution of embryos according to number of somites and time
of lay were made for morning and afternoon eggs separately, tables 42 and 43.

Eggs laid in the morning at or before 8 o'clock contain embryos with a larger number of somites than those laid after 8 o'clock and before noon. The distribution is heterogeneous. A large proportion (80%) of the eggs laid at or before 8 o'clock in the morning are the first of a clutch and have presumably spent a longer period of time in the oviduct.

The eggs laid between 2 and 4 o'clock show a tendency to contain a larger number of somites than the eggs laid between noon and 2 o'clock. The test has revealed, however, that the two distributions are not heterogene-

TABLE 43

TEST OF HOMOGENEITY OF THE DISTRIBUTION OF
AFTERNOON EGGS ACCORDING TO NUMBER
OF SOMITES AND TIME OF LAY

Number of somites	Eggs laid		Total
	Between noon and 2 o'clock	Between 2 and 4 o'clock	
8 or less	5	5	10
9	16	9	25
10	27	13	40
11	47	18	65
12	67	41	108
13	53	31	84
14	28	28	56
15 and over	5	8	13
Total	248	153	401

$$\chi^2 = 10.58 \qquad P = .10-.20$$

ous; eggs laid between noon and 2 o'clock and between 2 and 4 o'clock contain embryos which did not differ significantly in number of somites after 38 hours of incubation.

In view of the greater mean number of somites observed in eggs occupying the first clutch position as compared to eggs in subsequent clutch positions, a separate analysis of the relation between hour of lay and number of somites was made in the two groups of eggs, table 44. Tests of homogeneity of the distribution of embryos according to number of somites, clutch position and whether the eggs were laid before or after 11 o'clock in the morning have given the results presented in table 45.

There is no relation between number of somites and hour of lay in eggs occupying the first position in a clutch, but the relation is statistically significant in eggs occupying subsequent clutch positions. In the latter, there is a larger mean number of somites when the egg is laid later in the day.

Further tests of homogeneity of the distribution of embryos according to number of somites and hour of lay were made for eggs occupying a clutch position subsequent to the first, table 46.

The two distributions are heterogeneous. Eggs in clutch positions subsequent to the first, when laid at or before 9 o'clock in the morning, contained

TABLE 44

RELATION BETWEEN HOUR OF LAY AND NUMBER OF SOMITES
IN EGGS ACCORDING TO CLUTCH POSITION

Hour of lay	Eggs in first clutch position			Eggs in subsequent clutch positions		
	Number of embryos	Mean somites	Standard deviation	Number of embryos	Mean somites	Standard deviation
8 o'clock or before..........	137	12.88	2.401	35	9.80	1.659
9 o'clock..................	25	11.92	1.846	18	10.56	1.918
10 o'clock..................	73	12.27	1.648	124	10.93	1.649
11–12 o'clock...............	65	12.68	1.954	191	11.17	1.558
1 o'clock..................	19	13.11	1.370	183	11.64	1.452
2 o'clock..................	3	13.67	.577	40	11.80	1.728
3 o'clock..................	1	12.00	.....	112	12.20	1.884
4 o'clock or after...........	1	10.00	.....	39	11.95	1.451
All embryos..............	324	12.64	2.074	742	11.40	1.710

TABLE 45

TESTS OF HOMOGENEITY OF THE DISTRIBUTION OF
EMBRYOS BY NUMBER OF SOMITES, ACCORDING
TO TIME OF LAY AND CLUTCH POSITION

Number of somites	Eggs laid		Total
	Before 11 o'clock	After 11 o'clock	
Eggs in first clutch position			
10 or less...	28	10	38
11..	25	5	30
12..	49	20	69
13..	72	25	97
14..	46	17	63
15 and over.......................................	15	12	27
Total...	235	89	324

$$\chi^2 = 5.92 \qquad P = .3-.5$$

Number of somites	Eggs laid		Total
Eggs in subsequent clutch positions			
7 or less...	5	6	11
8..	15	16	31
9..	33	40	73
10..	25	70	95
11..	36	111	147
12..	33	141	174
13 and over.......................................	30	181	211
Total...	177	565	742

$$\chi^2 = 44.84 \qquad P < .001$$

embryos with a smaller number of somites than eggs laid between 9 o'clock and noon. The early morning eggs, in clutch positions subsequent to the first, presumably belong to long clutches in which the time interval is shorter and more regular than in the late morning eggs. The same deductions apply to the early afternoon eggs which contain embryos with a smaller number of somites than those laid later in the afternoon. The eggs laid later in the afternoon are presumably last eggs in a clutch or belong to two-egg clutches which, as shown previously, have a longer time interval expressed in a larger number of somites.

TABLE 46

TEST OF HOMOGENEITY OF THE DISTRIBUTION OF EMBRYOS ACCORDING TO NUMBER OF SOMITES, HOUR OF LAY, AND WHETHER LAID IN THE MORNING OR IN THE AFTERNOON, IN EGGS OCCUPYING CLUTCH POSITIONS SUBSEQUENT TO THE FIRST

Number of somites	Morning eggs		Total	Afternoon eggs		Total
	Before 9 o'clock	After 9 o'clock		Before 2 o'clock	After 2 o'clock	
8 or less......................	9	23	32	5	5	10
9.............................	15	33	48	16	9	25
10............................	8	48	56	27	12	39
11............................	8	76	84	45	18	63
12............................	8	65	73	61	40	101
13............................	5	70	75	46	31	77
14 and over...................	...	...	...	23	36	59
Total.....................	53	315	368	223	151	374

$$\chi^2 = 21.89 \quad P < .01 \qquad\qquad \chi^2 = 16.19 \quad P < .01$$

Relation Between Clutch Position and Hour of Lay

Both clutch position and hour of lay have been demonstrated to be factors influencing embryo size. The study of the relation between these two factors has given the results presented in table 47.

A test of the homogeneity of the distribution of eggs in first and subsequent clutch positions according to hour of lay has given a χ^2 value of 253.63 with a probability less than .001. Eggs occupying the first clutch position as a rule are laid earlier in the day than eggs occupying subsequent clutch positions.

Relation Between Hour of Lay and Time Interval

Both time interval and hour of lay have been demonstrated to be factors influencing embryo size and further the two factors are related as shown in table 48. There is an apparent increase in length of time interval with later hours of lay for eggs in both first and subsequent clutch positions, and both distributions are heterogeneous (table 49).

TABLE 47

RELATION BETWEEN HOUR OF LAY AND
CLUTCH POSITION

Clutch position	Number of eggs	Mean hour of lay
1	551	10:00 o'clock
2	451	12:02 o'clock
3	264	1:02 o'clock
4	134	12:30 o'clock
5+	219	12:02 o'clock
All positions	1619	11:24 o'clock
1	551	10:00 o'clock
2+	1068	12:15 o'clock

TABLE 48

RELATION BETWEEN HOUR OF LAY AND
TIME INTERVAL

Clutch position	Hour of lay	Number of eggs	Mean time interval
First	8 o'clock or before	121	41:50 hrs.
	9 o'clock	12	43:18 hrs.
	10 o'clock	65	43:36 hrs.
	11 o'clock	45	45:00 hrs.
	1 o'clock	12	47:48 hrs.
	3 o'clock	8	50:54 hrs.
	4 o'clock or after	2	52:00 hrs.
	Total	265	....
	Average	....	43:48 hrs.
Subsequent to first	8 o'clock or before	39	23:24 hrs.
	9 o'clock	16	24:18 hrs.
	10 o'clock	127	24:42 hrs.
	11–12 o'clock	147	25:16 hrs.
	1 o'clock	148	26:12 hrs.
	2 o'clock	29	27:00 hrs.
	3 o'clock	133	27:24 hrs.
	4 o'clock or after	38	27:24 hrs.
	Total	677	....
	Average	....	25:54 hrs.

Eggs laid later in the day have a longer time interval regardless of whether they occupy the first position or a subsequent one in a clutch. There is, however, a confounding effect of rate of lay on the relation between time interval and hour of lay. Eggs in short clutches are laid later in the day along with last eggs in longer clutches, and since both those types of eggs have been shown to have a longer time interval, the result is an apparent increase in length of time interval with later hours of lay.

Individuality of the Bird as It Influences Early Embryo Size

In the preceding analyses it has been demonstrated that clutch position, time interval between eggs, and hour of lay influence the maximum diameter of the blastoderm in unincubated eggs and also the number of somites present in the embryos after 38 hours of incubation. It was further pointed out that there are interrelations between clutch position, time interval between eggs, and hour of lay, and that these factors reflect variations in length of time

TABLE 49

TESTS OF HOMOGENEITY OF THE DISTRIBUTION OF EGGS LAID
BEFORE AND AFTER 10 O'CLOCK IN THE MORNING
ACCORDING TO LENGTH OF TIME INTERVAL

Clutch position	Time interval	Eggs laid		Total
		8–10 o'clock	After 10 o'clock	
First	Less than 44 hours	169	23	192
	More than 44 hours	29	44	73
	Total	198	67	265

$\chi^2 = 62.76$　　　P < .01

Clutch position	Time interval	Eggs laid		Total
Subsequent to first	Less than 25 hours	203	56	259
	More than 25 hours	116	302	418
	Total	319	358	677

$\chi^2 = 162.48$　　　P < .01

spent by the eggs in the oviduct. As an additional step in the analysis of factors influencing early embryo size, it was thought of interest to study the variations in early embryo size between individual birds.

Variance in Blastoderm Size. An analysis of variance was made of the data gathered on blastoderm size over a period of 11 years and the results are presented in table 50.

The total variance of blastoderm size for eggs in all clutch positions in each year is reported first. The variances or mean squares range from a low of .0506 in 1930 to a high of .1459 in 1935.

For eggs in all clutch positions, the variance between means of eggs occupying first and subsequent clutch positions is significantly greater than the variance within first and subsequent clutch positions in all years except 1937. Nine of the 11 F values are above the 1% level of significance, one is above the 5% level and the other is not significant. This result is a confirmation of a fact previously demonstrated in the analysis of the relation between clutch position and blastoderm size.

The data within first clutch position and within subsequent clutch positions were next analyzed for the significance of variation between means of

TABLE 50

ANALYSIS OF VARIANCE OF DATA ON BLASTODERM SIZE

	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1941
Number of observations	40	110	175	176	61	36	96	114	181	221	418
Number of individual females	7	10	15	14	6	5	12	12	23	23	38
Degrees of freedom	39	109	174	175	60	35	95	113	180	220	417
Total variance	.0506	.0950	.0932	.1088	.1432	.1459	.1269	.0761	.0869	.0692	.1170
Between first and subsequent clutch											
Degrees of freedom	1	1	1	1	1	1	1	1	1	1	1
positions: variance	.4001	1.3697	.8166	1.7056	.7309	1.6989	1.3889	.2200	4.2723	.7059	5.3370
Degrees of freedom	38	108	173	174	59	34	94	112	179	219	416
Within first and subsequent clutch											
positions: variance	.0414	.0832	.0890	.0996	.1232	.1003	.1134	.0748	.0635	.0663	.1044
F value	9.66†	16.46†	9.18†	17.12†	5.49*	16.93†	12.25†	2.94	67.28†	10.65†	51.12†
Degrees of freedom	11	17	28	26	10	8	21	22	44	39	73
Between individuals: variance	.1249	.3168	.2862	.2952	.3240	.2179	.3195	.1516	.1425	.0928	.2132
Degrees of freedom	27	91	145	148	49	26	73	90	135	180	343
Within individuals: variance	.0074	.0396	.0510	.0653	.0943	.0641	.0541	.0533	.0378	.0605	.0813
F value	16.88†	8.00†	5.61†	4.52†	3.44†	3.40†	5.91†	2.84†	3.77†	1.53*	2.62†
Within first clutch position											
Degrees of freedom	15	33	74	71	32	11	36	36	58	48	113
Total variance	.0255	.0745	.1145	.1418	.1321	.1715	.1475	.0750	.0953	.0844	.2430
Degrees of freedom	5	8	14	13	5	4	10	12	22	17	36
Between individuals: variance	.0582	.1561	.2890	.2960	.4633	.3548	.3598	.0067	.1845	.1322	.2843
Degrees of freedom	10	25	60	58	27	7	26	25	36	31	77
Within individuals: variance	.0091	.0484	.0738	.1072	.0708	.0668	.0658	.1039	.0413	.0582	.2237
F value	6.40†	3.23*	3.92†	2.76†	6.54†	5.31†	5.47†	.....	4.47†	2.27†	1.27
Within clutch positions subsequent to the first											
Degrees of freedom	23	75	99	103	27	23	58	76	121	171	303
Total variance	.0518	.0871	.0700	.0705	.1345	.0662	.0923	.0738	.0481	.0612	.0528
Degrees of freedom	6	9	14	13	5	4	11	11	22	22	37
Between individuals: variance	.1805	.3598	.2835	.2944	.4606	.0810	.2830	.3241	.1005	.2218	.1441
Degrees of freedom	17	66	85	90	22	19	47	65	99	149	266
Within individuals: variance	.0063	.0363	.0348	.0382	.0604	.0631	.0477	.0319	.0365	.0374	.0401
F value	28.65†	9.91†	8.15†	7.71†	7.63†	1.28	5.93†	10.16†	2.75†	5.93†	3.59†

* Significant at the 5% level.
† Significant at the 1% level.

individual birds in blastoderm size. The variance between means of individual birds is significantly greater than the variance of blastoderm size within individuals in each year. Ten of the F values are above the 1% level of significance and the other is above the 5% level of significance. Individual birds thus laid eggs containing blastoderms that differed significantly in size.

A separate analysis of variance was made for the data within first clutch position and for the data within clutch positions subsequent to the first.

Within first clutch position, blastoderm size, as already demonstrated, shows a greater variability than in subsequent positions. Even under that condition, the variance between means of individual birds is significantly greater than the variance within individual birds in nine years out of the 11. In two years, 1937 and 1941, there is no significant difference between means of individual birds in eggs occupying first clutch position.

In eggs occupying clutch positions subsequent to the first, the variance between means of individual birds is significantly greater than the variance within individuals in ten years out of the 11. In one year, 1935, there is no significant difference between individual birds probably because the data were gathered on only five birds. In the ten years showing significant differences between individuals, the F values are all above the 1% level of significance.

There is very good evidence that differences in the maximum diameter of the blastoderm in unincubated eggs exist between individual dams. These individual differences may be the reflection of characteristic variations in the time spent by the eggs in the oviduct, the result of inherent differences in the rate of embryonic growth, or the expression of slight individual variations in body temperature. In order to determine if the differences in blastoderm size between individuals are accounted for by variations in the length of the time interval between eggs, an analysis of covariance was made of blastoderm size and length of time interval for eggs occupying first clutch position and also for those occupying a subsequent clutch position. Data on length of time interval were available only for 1937, 1938, 1940, and 1941. The results of the analysis of covariance are presented in table 51 for each of the four years and separately for eggs within first and subsequent clutch positions.

In eggs within first clutch position, the correlation coefficients between blastoderm size and time interval in the total population of each year range from + .02 to + .23. The four correlation coefficients are positive but not significant. The regression coefficients of blastoderm size on time interval range from + .004 to + .026, and they indicate how small and variable is the change in mean blastoderm size brought about by a variation of one hour in mean time interval. For eggs occupying first clutch position, the correlation coefficients between blastoderm size and time interval within individual birds range from − .26 to + .11 and are all without significance. The regression coefficients of blastoderm size on time interval range from − .021 to + .009. These coefficients show that the size of the blastoderm within individual birds cannot be predicted to any accurate degree when the time interval of the first egg of a clutch is known. In fact, data on the time interval of the first

egg of a clutch for a given bird are of no more value in predicting the mean blastoderm size in the eggs of the given birds than is the mean interval of a group of eggs, without reference to individuals, in predicting mean blastoderm size.

Within clutch positions subsequent to the first, the correlation coefficients between time interval and blastoderm size for the sample of each year are all positive and range from .08 to .29 but only the latter coefficient is significant. The regressions of blastoderm size on time interval range from .011 to .036. Within individual birds, and still in clutch positions subsequent to the first, the correlation coefficients between time interval and blastoderm

TABLE 51

ANALYSIS OF COVARIANCE OF BLASTODERM SIZE
AND TIME INTERVAL

Clutch position	Year	Total			Within individual birds		
		d.f.	Regression coeff.	Correlation coeff.	d.f.	Regression coeff.	Correlation coeff.
Eggs in first clutch position........	1937	37	.0260	.230	26	.0091	.068
	1938	58	.0087	.096	36	.0068	.110
	1940	48	.0097	.104	31	−.0206	−.259
	1941	113	.0037	.021	77	−.0008	−.004
Eggs in subsequent positions......	1937	76	.0355	.185	64	.0290	.241*
	1938	121	.0135	.113	99	.0268	.192
	1940	171	.0114	.082	149	.0251	.238†
	1941	303	.0348	.287*	266	.0369	.317†
Pooled regression and correlation..	....	...	.....	....	...	.0314	.270†

* Significant at the 5% level.
† Significant at the 1% level.

size within each year range from .19 to .32 and are all positive. Three of the four coefficients of correlation are significant; two at the 1% level of significance and the other at the 5% level. The correlation coefficient for the pooled data of the four years is .27 and is significant at the 1% level of significance. The regression coefficients for each year range from .025 to .037 and for the pooled data the coefficient is .031.

The analysis of covariance has thus revealed that there is a closer correlation between time interval and blastoderm size in eggs occupying clutch positions subsequent to the first than in eggs of the first position. The correlation within individual birds is still higher than that in the sample of eggs in clutch positions subsequent to the first disregarding individuality of dams. This supports evidence previously presented (table 35, page 535), showing that the time interval between eggs in clutch positions subsequent to the first is a better measurement of the actual time the eggs spent in the oviduct than the time interval for eggs occupying first clutch position. The time interval, as a measurement of the actual time spent in the oviduct by the eggs occupying a clutch position subsequent to the first, is also more accurate within individual birds than for a group of birds as a whole.

The next step consisted of adjusting the data for the regression of blastoderm size on time interval. An analysis of variance of the adjusted values has given the results presented in table 52. This analysis was confined to eggs occupying a clutch position subsequent to the first, since they showed the only significant correlation coefficients between time interval and blastoderm size.

The variance between means of individual birds is significantly greater (1% level of significance) than that within individual birds in each of the four years. The differences between individuals are thus still significant, even after adjustments are made for variations attributed to time interval, and presumably to length of time spent by the eggs in the oviduct. These remain-

TABLE 52

ANALYSIS OF VARIANCE OF DATA ON BLASTODERM SIZE IN EGGS OCCUPYING A CLUTCH POSITION SUBSEQUENT TO THE FIRST, ADJUSTED FOR THE REGRESSION OF BLASTODERM SIZE ON TIME INTERVAL BETWEEN EGGS

Year	Total variance	Degrees of freedom	Variance between individuals	Degrees of freedom	Variance within individuals	F
1937	.0722	11	.3175	25	.0300	10.58†
1938	.0471	22	.1032	35	.0355	2.91†
1940	.0611	17	.2349	30	.0353	6.65†
1941	.0486	36	.1374	76	.0362	3.80†

† Significant at the 1% level.

ing differences between individual birds may still be the result of characteristic variations in the actual time the egg spent in the oviduct, which are not accurately measured by the time interval between eggs. These variations in actual time spent by the egg in the oviduct could be in length of time elapsed between the oviposition of the preceding egg and ovulation. Two other explanations for the individual differences in mean blastoderm size after correction for variations in length of time interval, are that rate of embryonic development and body temperature may vary significantly between birds.

Variance in Embryo Size after 38 Hours of Incubation. The data available on number of somites after 38 hours of incubation, in 1937, 1938, 1940 and 1941, were also analyzed to establish the significance of variations between individuals. An analysis of variance was made for the data of each year, and also separate analyses for eggs in first clutch position and for eggs in clutch positions subsequent to the first. The results of these analyses of variance are presented in table 53. The variance of the total population used in each year is presented first; the range in the latter is from 2.7826 to 3.6229.

The variance between means of eggs in first and subsequent clutch positions is significantly greater than the variance within first and subsequent clutch positions in all years except 1937. (It is interesting to note that in 1937 there was no significant difference in blastoderm size between the means of eggs in first and of eggs in subsequent clutch positions.) The three significant F

values have a P of .01 or less. The variance between means of individual birds within eggs in first clutch position and eggs in subsequent clutch positions is significantly greater than the variance within individual birds in each of the four years. The F values are all significant at the 1% level.

TABLE 53

ANALYSIS OF VARIANCE OF DATA ON NUMBER OF SOMITES
PRESENT IN THE EMBRYOS AFTER 38 HOURS
OF INCUBATION

	1937	1938	1940	1941
Number of observations...................................	185	230	197	465
Number of dams...	12	22	21	35
Degrees of freedom.......................................	184	229	196	464
Total variance...	3.1821	2.7826	3.6229	3.1614
Degree of freedom...	1	1	1	1
Between first and subsequent clutch positions: variance......	11.2561	67.1220	73.9243	48.8451
Degrees of freedom.......................................	183	228	195	463
Within first and subsequent clutch positions: variance.......	3.1380	2.5004	3.2623	3.0663
F value...	3.59	26.84†	22.66†	15.14†
Degrees of freedom.......................................	22	40	37	67
Between individuals: variance.............................	13.8348	5.6040	8.3451	6.2953
Degrees of freedom.......................................	161	188	158	396
Within individuals: variance..............................	1.6764	1.8401	2.0721	2.6909
F value...	8.25†	3.05†	4.03†	2.34†
Within first clutch position				
Degrees of freedom.......................................	61	75	38	137
Total variance...	3.5137	2.5689	3.3657	2.2302
Degrees of freedom.......................................	11	19	17	33
Between individuals: variance.............................	13.2881	4.2421	5.1207	3.9216
Degrees of freedom.......................................	50	56	21	104
Within individuals: variance..............................	1.3634	2.0013	1.9450	1.6935
F value...	9.75†	2.12*	2.63*	2.32†
Within subsequent clutch positions				
Degrees of freedom.......................................	122	153	157	326
Total variance...	2.9502	2.4669	3.2373	3.4176
Degrees of freedom.......................................	11	21	20	34
Between individuals: variance.............................	14.3810	6.8363	11.0859	7.2535
Degrees of freedom.......................................	111	132	137	292
Within individuals: variance..............................	1.8174	1.7717	2.0916	2.9710
F value...	7.91†	3.86†	5.30†	2.44†

* Significant at the 5% level.
† Significant at the 1% level.

A separate analysis of variance of number of somites in eggs occupying the first position in a clutch and in eggs occupying subsequent clutch positions was also made. In eggs occupying the first clutch position, the variance between means of individual birds was significantly greater than the variance within individuals. Two of the four F values were significant at the 1% level and the other two were significant at the 5% level. In eggs occupying a clutch position subsequent to the first, the variance between means of individual

birds was also significantly greater than the variance within individuals. The four F values were all significant at the 1% level.

The results of the analysis of variance of the data on number of somites are in very good agreement with those obtained in the analysis of the data on blastoderm size. They both indicate that significant differences in early embryo size, measured either by maximum blastoderm size in the unincubated egg or number of somites after 38 hours of incubation, exist between individual birds.

The individual differences in number of somites, like those in blastoderm size, may be the result of characteristic variations in the length of time the

TABLE 54

ANALYSIS OF COVARIANCE OF NUMBER OF SOMITES
AND TIME INTERVAL

Clutch position	Year	Total			Within individual birds		
		d.f.	Regression coefficient	Correlation coefficient	d.f.	Regression coefficient	Correlation coefficient
Eggs in first clutch position	1937	61	.078	.08	50	.236	.38†
	1938	75	.025	.04	56	.084	.10
	1940	38	.022	.03	21	.225	.35
	1941	137	.017	.02	104	.033	.04
Eggs in subsequent positions	1937	122	.297	.44†	111	.282	.52†
	1938	153	.194	.28†	132	.105	.11
	1940	157	.235	.23†	137	.431	.43†
	1941	326	.337	.32†	292	.260	.24†

† Significant at the 1% level.

eggs spent in the oviduct and they may also be the effect of genetic or other differences in the rate of embryonic growth. An analysis of the covariance of number of somites and length of time interval was made to throw more light on the problem. The data gathered on eggs occupying first clutch position and on eggs occupying a clutch position subsequent to the first were analyzed separately. The results of these analyses of covariance are presented in table 54.

In eggs occupying the first position in a clutch, the correlation coefficients between number of somites and time interval for the total sample of each year range from .02 to .08 and are not significant. The regression coefficients of number of somites on time interval are all low and range from .017 to .078. Within individual birds and still within eggs in first clutch position, the correlation coefficients between number of somites and time interval range from .04 to .38 and only the latter coefficient is significant. The regression coefficients range from .033 to .236. Contrary to what was observed in the analysis of blastoderm size, the correlation between time interval and number of somites is higher within individuals than when individuality is disregarded, and the regression coefficients also indicate some relation between the two variables.

In eggs occupying a clutch position subsequent to the first, the correlation coefficients between number of somites and time interval range from .23 to .44.

They are all significant at the 1% level. The regression coefficients of number of somites on time interval range from .194 to .337 and indicate an appreciable influence of time interval on number of somites. Within individual birds and still within eggs occupying a clutch position subsequent to the first, the correlation coefficients between time interval and number of somites range from .11 to .53; three of the four coefficients are significant at the 1% level of significance and the other is not significant. The regression coefficients of number of somites on time interval range from .105 to .431.

The analysis of covariance of number of somites and time interval has revealed a closer relation between the two variables in eggs occupying a

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TABLE 55

ANALYSIS OF VARIANCE OF DATA ON NUMBER OF SOMITES IN EGGS OCCUPYING A CLUTCH POSITION SUBSEQUENT TO THE FIRST, ADJUSTED FOR THE REGRESSION OF NUMBER OF SOMITES ON TIME INTERVAL

</div>

Year	Total variance	Degrees of freedom	Variance between individuals	Degrees of freedom	Variance within individuals	F
1937	2.3926	11	13.2699	110	1.3266	10.00†
1938	2.2759	21	5.5777	131	1.7640	3.16†
1940	3.0890	20	12.4109	136	1.7181	7.22†
1941	3.0754	34	5.4135	291	2.8031	1.93†

† Significant at the 1% level.

clutch position subsequent to the first than in eggs in first clutch position. The same result was obtained in the analysis of blastoderm size. A closer relation between number of somites and time interval was also observed within the eggs of individual birds than in the sample disregarding individuals. This latter relation was much more marked in eggs occupying a clutch position subsequent to the first although a slight relation was also present in eggs in first clutch position.

The data on number of somites were adjusted for the regression of number of somites on time interval and then submitted to an analysis of variance. Only the data available on eggs occupying a clutch position subsequent to the first were analyzed in view of the higher relation observed in them between the two variables. The results of this analysis of variance are presented in table 55.

The differences between individuals in mean number of somites remain highly significant even after adjusting number of somites for variations in length of time interval between eggs.

Individual Variations in Early Embryo Size Between Years. In the course of their investigations on blastoderm size, Taylor and Gunns (unpublished) gathered data on the same birds in two consecutive years. These data were analyzed to establish if any significant difference in the mean blastoderm size of the same birds was observed between years. Only eggs occupying a clutch position subsequent to the first were used in the analysis. A total of 105 observations made on eight females were available. The results of the analysis of variance are presented in table 56.

Highly significant differences were observed between individual hens but
these birds also produced eggs which contained blastoderms that varied sig-
nificantly in size in different years. Unfortunately the data on time interval
were not available, and it remains to be determined whether the differences
between years are accounted for by variations in time interval or by genetic
differences such as could be effected by mating to different males in consecu-
tive years. It is important, however, to note that the same hen will not neces-
sarily produce eggs that will contain blastoderms of the same size in different
years. For this reason any study of the influence of genetic factors on early

TABLE 56

ANALYSIS OF VARIANCE OF BLASTODERM SIZE
BETWEEN YEARS IN THE SAME INDIVIDUALS
AS MEASURED IN EGGS OCCUPYING A
CLUTCH POSITION SUBSEQUENT
TO THE FIRST

Source of variation	Degrees of freedom	Variance	F
Total...	104	.0622	
Between birds.................................	7	.3469	8.34†
Within birds..................................	97	.0416	
Within birds..................................			
Between years................................	8	.0899	2.41*
Within years.................................	89	.0373	

* Significant at the 5% level.
† Significant at the 1% level.

embryo size must be made within a period of time when rate of production
is uniform in order to make the necessary corrections for variations in time
spent by the eggs in the oviduct.

Influence of Systems of Mating on Early Embryo Size

The preceding analyses have demonstrated that, even after blastoderm size
and number of somites are adjusted for their regression on length of time
interval between eggs, there are still significant differences between dams.
As previously suggested, these may still be the result of characteristic varia-
tions in length of time the eggs of an individual hen remain in the oviduct.
The latter variations may not be measured accurately by the length of the
time interval between eggs, because of fluctuations in the length of time
elapsing between the oviposition of the preceding egg and ovulation. The
differences in mean blastoderm size and in number of somites between indi-
vidual hens may also be the result of slight individual variations in body
temperature. Finally, the differences between individuals may also be due to
genetic differences in the rate of early embryonic growth.

In order to test this last hypothesis of genetic differences in early em-
bryonic growth, the experimental design outlined in the general introduction
to these studies was followed. It consisted of mating females of three different
origins, within the same year, and in turn with males of the same three origins.

This resulted in various degrees of inbreeding, in outcrossing, and in cross-breeding. If genetic factors influence rate of early embryonic growth, the experimental design followed should allow their demonstration on blastoderm size and on number of somites.

To reduce the labor involved in the statistical analysis, as well as to profit from the facts observed in the preliminary analysis, only the data gathered on eggs occupying a clutch position subsequent to the first were used since they allowed making the necessary adjustments for regression on time interval.

A summary of the data gathered under the experimental design in 1940 and 1941, classified according to periods, origins of males, origins of females, systems of mating, and in 1941, levels of inbreeding of progeny, is given in table 57. Both blastoderm size and number of somites show apparent variations between periods, between origins of males, between origins of females, and between systems of mating. Of immediate interest are the smaller mean blastoderm size and lower mean number of somites observed in both years under inbreeding as compared to outcrossing and crossbreeding.

The preliminary analyses of variations in both blastoderm size and number of somites have revealed that clutch position, time interval between eggs, and individuality of the dams have to be taken into account in the analysis of data on early embryo size. The first factor was eliminated from the present analysis as only eggs occupying a clutch position subsequent to the first were used. An additional source of variation necessarily taken into account in view of the experimental design followed was that of the possible influence of season or periods within each year. As shown in table 57, periods have apparently influenced early embryo size.

The data on blastoderm size, for 1940 and 1941, were submitted to an analysis of their covariance with time interval and the results are presented in tables 58 and 59 for 1940 and 1941 respectively. The variations in blastoderm size between periods were significant in 1941, but without significance in 1940. The variations in time interval between periods were highly significant in both 1940 and 1941. The adjustment of the means of blastoderm size for their regression on time interval accounted for the variations observed between periods. The variations in mean blastoderm size observed between periods in 1941 were thus the result of variations in length of time interval between eggs.

The three origins of females used each year differed very significantly in mean blastoderm size within periods. In 1940 there were also significant differences in mean time interval between eggs in the three origins but not in 1941. The values on blastoderm size corrected for their regression on time interval still showed highly significant differences between origins of females. This is in agreement with a previous conclusion that the individuality of the hen affects mean early embryo size, since each origin of females was comprised of related individuals.

Significant differences in mean blastoderm size and time interval were observed between systems of mating within origins of females within periods in both years. The differences in mean blastoderm size between systems of

TABLE 57

SUMMARY OF DATA ON BLASTODERM SIZE AND NUMBER OF SOMITES
GATHERED ON EGGS OCCUPYING A CLUTCH POSITION SUBSEQUENT
TO THE FIRST, IN 1940 AND 1941, CLASSIFIED ACCORDING TO
PERIODS, ORIGINS OF MALES, ORIGINS OF FEMALES,
SYSTEMS OF MATING AND DEGREES OF
INBREEDING OF PROGENY

Classification	Blastoderm size			Number of somites		
	Number	Mean blastoderm size	Mean time interval	Number	Mean somites	Mean time interval
1940						
Periods						
1	67	3.6148	25:55 hrs.	56	10.54	26:00 hrs.
2	72	3.5779	24:34 hrs.	74	10.24	25:01 hrs.
3	33	3.5297	24:49 hrs.	28	10.79	25:08 hrs.
Origins of males						
SE	62	3.6239	24:52 hrs.	55	10.75	25:10 hrs.
OB	50	3.5542	25:07 hrs.	46	10.28	25:37 hrs.
CB	60	3.5648	25:27 hrs.	57	10.28	25:25 hrs.
Origins of females						
SE	22	3.6855	26:38 hrs.	19	11.00	27:13 hrs.
OB	123	3.6079	24:33 hrs.	115	10.49	24:52 hrs.
CB	27	3.3863	26:34 hrs.	24	9.79	26:28 hrs.
Systems of mating						
Inbreeding	46	3.4911	25:20 hrs.	40	10.05	25:37 hrs.
Outcrossing	59	3.6622	24:45 hrs.	55	10.85	25:14 hrs.
Crossbreeding	67	3.5764	25:20 hrs.	63	10.33	25:22 hrs.
Data for all embryos	172	3.5830	25:08 hrs.	158	10.44	25:23 hrs.
1941						
Periods						
1	89	3.5430	26:50 hrs.	91	11.99	27:02 hrs.
2	120	3.4690	25:39 hrs.	130	11.62	25:37 hrs.
3	95	3.4440	25:38 hrs.	106	11.79	25:12 hrs.
Origins of males						
SEOBs	112	3.4553	26:06 hrs.	193	12.00	25:56 hrs.
OB²	108	3.5242	25:53 hrs.	123	11.64	25:42 hrs.
XB	84	3.4665	25:59 hrs.	91	11.69	26:04 hrs.
Origins of females						
SEOBs and SEOBl	204	3.4669	25:49 hrs.	219	11.82	25:44 hrs.
OB²	16	3.3700	26:23 hrs.	14	10.29	25:34 hrs.
XB	84	3.5431	26:20 hrs.	94	11.90	26:16 hrs.
Systems of mating						
Inbreeding	186	3.4651	26:01 hrs.	190	11.71	25:46 hrs.
Outcrossing	...	......	....	...	.....	....
Crossbreeding	118	3.5108	25:56 hrs.	137	11.88	26:03 hrs.
Data for all embryos	304	3.4828	25:59 hrs.	327	11.78	25:53 hrs.
Levels of inbreeding of progeny						
.1250	80	3.4814	25:48 hrs.	79	11.62	25:35 hrs.
.16–.2514	74	3.4720	26:12 hrs.	70	11.67	25:40 hrs.
.30–.3750	32	3.4081	26:08 hrs.	41	11.95	26:14 hrs.

mating remained significant after adjustment for regression of blastoderm size on time interval. In both years, inbreeding thus resulted in a lower mean blastoderm size as compared to outbreeding.

In 1941, no significant difference in mean blastoderm size or in time interval was observed between the various levels of inbreeding of the progeny. They remained without significance after adjustment for regression on time interval.

Origins of females, systems of mating or inbreeding and outbreeding, as well as individuality of the females were thus the sources of significant variations in mean blastoderm size before and after adjustments were made for regression of blastoderm size on time interval.

The data on number of somites for 1940 and 1941 were also submitted to an analysis of their covariance with time interval and the results are presented in table 60 and table 61 for 1940 and 1941 respectively. No significant difference in mean number of somites was observed between periods in 1940 or 1941. The differences in mean time interval were, however, highly significant in both 1940 and 1941. The data on number of somites adjusted for regression on time interval still showed no significant difference in mean number of somites between periods.

Significant differences in mean number of somites were observed between origins of females within periods in 1941 but the differences were not significant in 1940. The differences in time interval between origins within periods were highly significant in both years. The adjustment of the values on number of somites for regression on time interval resulted in highly significant differences between origins of females in both years.

The differences in mean number of somites between systems of mating within origins of females within periods were significant in 1940 and not significant in 1941. No significant difference in time interval was observed between systems of mating. Adjustment of the number of somites for its regression on time interval changed the highly significant differences observed in 1940 between systems of mating to the barely significant level (observed F value being 1.92 as compared to 1.90 at P .05), but the differences between systems of mating observed in 1941 remained without significance.

In 1941, the differences in mean number of somites between the levels of inbreeding of the progeny were without significance before and after adjusting the values on number of somites for their regression on time interval.

Highly significant differences in mean number of somites were observed between individuals within systems of mating in both years. Significant differences were also observed in time interval between individuals in 1941 but not in 1940. The values on number of somites adjusted for their regression on time interval resulted in highly significant differences in mean number of somites between individuals in both 1940 and 1941.

Mean number of somites adjusted for regression on time interval was thus affected primarily by origins of females, individuality of the females, and in 1940 possibly by systems of mating.

These detailed analyses of covariance of blastoderm size and time interval, and number of somites and time interval, gave results that agree completely so far as origins of females and individuality of the females are concerned.

TABLE 58

ANALYSIS OF COVARIANCE OF BLASTODERM SIZE AND TIME INTERVAL IN EGGS OCCUPYING A CLUTCH POSITION SUBSEQUENT TO THE FIRST, 1940, AND ANALYSIS OF VARIANCE OF DATA ON BLASTODERM SIZE ADJUSTED FOR REGRESSION ON TIME INTERVAL

Source of variation	d.f.	Variance blastoderm size	F	Variance time interval	F	Regression coefficient	Correlation coefficient	Analysis of variance of adjusted means		
								d.f.	Blastoderm size	F
Total............	171	.0612		3.18				170	.0611	
Between periods......	2	.0817	1.34	33.89	12.02†	.012	.09	2	.0604	.....
Within periods......	169	.0609		2.82		.009	.06	168	.0511	
Within periods										
Between origins of females......	6	.2774	5.24†	22.67	10.85†	.029	.18*	6	.3190	6.19†
Within origins of females......	163	.0529		2.09				162	.0515	
Within periods										
Within origins of females										
Between systems of mating......	11	.1129	2.32*	4.44	2.31*	.029	.18*	11	.1087	2.29*
Within systems of mating......	152	.0486		1.92				151	.0474	
Within periods										
Within origins of females										
Within systems of mating										
Between individual dams......	32	.1159	3.78†	1.77	.....	.044	.35†	32	.1223	4.50†
Within individual dams......	120	.0307		1.95				119	.0272	

* Significant at the 5% level.
† Significant at the 1% level.

TABLE 59

ANALYSIS OF COVARIANCE OF BLASTODERM SIZE AND TIME INTERVAL IN EGGS OCCUPYING A CLUTCH POSITION SUBSEQUENT TO THE FIRST, 1941, AND ANALYSIS OF VARIANCE OF DATA ON BLASTODERM SIZE ADJUSTED FOR REGRESSION ON TIME INTERVAL

Source of variation	d.f.	Variance blastoderm size	F	Variance time interval	F	Regression coefficient	Correlation coefficient	Analysis of variance of adjusted means		
								d.f.	Blastoderm size	F
Total	303	.0527		3.59		.035	.29†	302	.0485	....
Between periods	2	.2443	4.74†	44.55	13.42†			2	.0480	
Within periods	301	.0515		3.32		.031	.24†	300	.0485	
Within periods										
Between origins of females	6	.1810	3.71†	4.24	1.28	.032	.26†	6	.1921	4.21†
Within origins of females	295	.0488		3.30				294	.0456	
Within periods										
Within origins of females										
Between systems of mating	7	.1145	2.43*	7.83	2.45*	.032	.26†	7	.1041	2.36*
Within systems of mating	288	.0472		3.19				287	.0442	
Within periods										
Within origins of females										
Within systems of mating										
Between individual females	83	.0673	1.72†	5.15	2.15†	.040	.31†	83	.0656	1.85†
Within individual females	205	.0391		2.39				204	.0355	
Within periods										
Within origins of females										
Within inbreeding	178	.0483	1.10	3.13	1.67	.026	.21†	177	.0464	1.15
Between degrees of inbreeding of progeny	11	.0530		5.02				11	.0529	
Within degrees of inbreeding of progeny	167	.0480		3.00		.028	.22†	166	.0460	

* Significant at the 5% level.
† Significant at the 1% level.

TABLE 60

ANALYSIS OF COVARIANCE OF NUMBER OF SOMITES AND TIME INTERVAL, 1940, IN EGGS OCCUPYING A CLUTCH POSITION SUBSEQUENT TO THE FIRST, AND ANALYSIS OF VARIANCE OF DATA ON NUMBER OF SOMITES, ADJUSTED FOR REGRESSION ON TIME INTERVAL

Source of variation	d.f.	Variance number of somites	F	Variance time interval	F	Regression coefficient	Correlation coefficient	Analysis of variance of adjusted means		
								d.f.	Number somites	F
Total..........	157	3.18		3.04		.294	.24†	156	3.01	
Between periods..........	2	3.37	1.06	16.52	5.76†			2	2.82	....
Within periods..........	155	3.18		2.87		.253	.24†	154	3.01	5.22†
Within periods										
Between origins of females..........	5	4.69	1.50	22.82	10.37†			5	13.84	5.22†
Within origins of females..........	150	3.13		2.20		.474	.40†	149	2.65	
Within periods										
Within origins of females										
Between systems of mating..........	10	9.57	3.58†	3.01	1.41			10	4.77	1.92*
Within systems of mating..........	140	2.67		2.14		.296	.27†	139	2.49	
Within periods										
Within origins of females										
Within systems of mating										
Between individual females..........	28	6.09	3.36†	2.73	1.37			28	6.40	4.24†
Within individual females..........	112	1.81		2.00		.396	.42†	111	1.51	

* Significant at the 5% level.
† Significant at the 1% level.

TABLE 61

ANALYSIS OF COVARIANCE OF NUMBER OF SOMITES AND TIME INTERVAL, 1941, IN EGGS OCCUPYING A CLUTCH POSITION SUBSEQUENT TO THE FIRST, AND ANALYSIS OF VARIANCE OF DATA ON NUMBER OF SOMITES, ADJUSTED FOR REGRESSION ON TIME INTERVAL

Source of variation	d.f.	Variance number of somites	F	Variance time interval	F	Regression coefficient	Correlation coefficient	Analysis of variance of adjusted means		
								d.f.	Number somites	F
Total............................	326	3.42	1.05	3.02	36.52†	.337	.32†	325	3.08	1.86
Between periods..................	2	3.60		90.58		.380	.32†	2	5.70	
Within periods..................	324	3.42		2.48				323	3.07	
Within periods										
Between origins of females......	6	13.29	4.11†	7.76	3.26†	.347	.30†	6	9.18	3.11†
Within origins of females.......	318	3.23		2.38				317	2.95	
Within periods										
Within origins of females										
Between systems of mating.......	7	2.88	.....	2.68	1.13	.351	.30†	7	2.81	.....
Within systems of mating........	311	3.24		2.37				310	2.96	
Within periods										
Within origins of females										
Within systems of mating										
Between individual females......	74	4.81	1.75†	3.95	2.10†	.235	.19†	74	3.92	1.48†
Within individual females.......	237	2.75		1.88				236	2.65	
Within periods										
Within origins of females										
Within inbreeding...............	181	3.65	1.51	2.26	1.98	.274	.22†	180	3.50	1.24
Between degrees of inbreeding of progeny......	9	5.39		4.25				9	4.29	
Within degrees of inbreeding of progeny.......	172	3.56		2.15		.237	.18†	171	3.45	

† Significant at the 1% level.

The only disagreement in the two analyses of early embryo size is in the significance of the differences observed between systems of mating. Significant differences in mean blastoderm size were observed between systems of mating in both years, while the differences between systems of mating in mean number of somites were found to be without significance in 1941 and barely significant in 1940.

The main purpose of these analyses was to determine the possible influence of systems of mating on early embryo size. The results indicate that their effects were significant on blastoderm size in both 1940 and 1941, and possibly on number of somites in 1940. The variance ratio in each instance indicates significance at the 5% level. The effects of systems of mating were definitely not of the magnitude of those caused by origins and individuality of the females, and this led to the further testing of the significance of the effects of systems of mating on early embryo size before accepting the aforementioned results as definite.

The analyses of covariance revealed that origins of females and individuality of the females are the main sources of variation in early embryo size before and after adjustment for regression on time interval. The experimental design followed in these investigations necessitated dividing the females from each origin into three groups which were in turn mated to the same males. It is not illogical to suppose that possibly the three subgroups within each origin of females were not as uniform as was assumed in planning the experiment in view of the highly significant differences observed between individuals within origins. The assumption of uniformity within the subgroups in each origin of females is, however, not necessary, following the demonstration that the variations between periods are all accounted for by the regression of early embryo size on time interval between eggs. The analysis of the effects of systems of mating has therefore been made disregarding periods, by proceeding immediately to the analysis of variance between systems of mating within individuals. The results of this second analysis are presented in table 62. The same table also contains the results of the analysis of variance of the data on both blastoderm size and number of somites adjusted for their regression on time interval.

The results indicate that systems of mating had a highly significant influence on blastoderm size and a significant influence on number of somites in 1940; inbreeding resulted in a smaller blastoderm and in a lower number of somites than outbreeding. No significant difference in either blastoderm size or number of somites was observed between inbreeding and crossbreeding in 1941 (last column, table 62). There is thus contradiction between the results obtained in the two analyses. Results of this last analysis parallel those of the analysis of variance of hatchability where significant differences between systems of mating were observed in 1940 and none in 1941.

A summary of the observations made on early embryo size within each origin of females within each system of mating, in 1940, may help to visualize the results obtained. This summary is presented in table 63. Inbreeding has consistently resulted in a smaller mean blastoderm and except in one instance, SE females, in a lower number of somites. The smaller embryo size observed

TABLE 62

ANALYSIS OF COVARIANCE OF EARLY EMBRYO SIZE AND TIME INTERVAL BETWEEN SYSTEMS OF MATING WITHIN INDIVIDUALS, IN EGGS OCCUPYING A CLUTCH POSITION SUBSEQUENT TO THE FIRST, 1940 AND 1941, TOGETHER WITH ANALYSIS OF VARIANCE OF DATA ON EARLY EMBRYO SIZE ADJUSTED FOR REGRESSION ON TIME INTERVAL

Source of variation	d.f.	Variance early embryo size	F	Variance time interval	F	Regression coefficient	Correlation coefficient	Analysis of variance of adjusted means		
								d.f.	Embryo size	F
Blastoderm size 1940										
Within individual females	149	.0374		3.34		.025	.24†	148	.0355	
Between systems of mating	29	.0655	2.13†	9.05	4.64†		.35†	29	.0700	2.57†
Within systems of mating	120	.0307		1.95		.044		119	.0272	
Blastoderm size 1941										
Within individual females	266	.0401		2.97		.037	.32†	265	.0362	
Between systems of mating	61	.0432	1.10	4.90	2.05†		.31†	61	.0384	1.08
Within systems of mating	205	.0391		2.39		.040		204	.0355	
Number of somites 1940										
Within individual females	137	2.09		2.08		.431	.43†	136	1.72	
Between systems of mating	25	3.35	1.85*	2.46	1.23		.42†	25	2.64	1.75*
Within systems of mating	112	1.81		2.00		.396		111	1.51	
Number of somites 1941										
Within individual females	292	2.97		2.48		.260	.24†	291	2.80	
Between systems of mating	55	3.94	1.43*	5.06	2.69*		.19†	55	3.49	1.32
Within systems of mating	237	2.75		1.88		.235		236	2.65	

* Significant at the 5% level.
† Significant at the 1% level.

under inbreeding in the OB females is particularly striking in view of the limited variability in mean time interval under the three systems of mating. An analysis of covariance of early embryo size and time interval between systems of mating within origins of females, as reported in table 64, has, however, revealed no significant difference. This is very likely accounted for by the small number of observations available within each origin.

TABLE 63

MEAN EARLY EMBRYO SIZE OBSERVED UNDER VARIOUS SYSTEMS
OF MATING WITHIN EACH ORIGIN OF FEMALES, IN EGGS
OCCUPYING A CLUTCH POSITION SUBSEQUENT
TO THE FIRST, 1940

System of mating	Measurement	Origins of females		
		SE	OB	CB
Inbreeding	Mean blastoderm size	3.4575	3.5597	3.2850
	Coeff. of variation	5.32	7.64	6.62
	Mean time interval	25:45 hrs.	24:39 hrs.	27:18 hrs.
	Mean number of somites	11.00	10.11	9.44
	Coeff. of variation	19.64	18.50	11.97
	Mean time interval	26:45 hrs.	25:00 hrs.	27:00 hrs.
Outcrossing	Mean blastoderm size	3.7010	3.6543	....
	Coeff. of variation	3.94	5.82	....
	Mean time interval	25:54 hrs.	24:31 hrs.	....
	Mean number of somites	11.11	10.80	....
	Coeff. of variation	19.80	15.37	....
	Mean time interval	26:54 hrs.	24:55 hrs.	....
Crossbreeding	Mean blastoderm size	3.78	3.5905	3.4459
	Coeff. of variation	3.28	6.14	6.46
	Mean time interval	28:00 hrs.	24:31 hrs.	26:07 hrs.
	Mean number of somites	10.83	10.38	10.00
	Coeff. of variation	22.90	17.24	15.10
	Mean time interval	28:00 hrs.	24:43 hrs.	26:08 hrs.
All systems	Mean blastoderm size	3.6855	3.6079	3.3863
	Mean time interval	26:38 hrs.	24:33 hrs.	26:33 hrs.
	Mean number of somites	11.00	10.49	9.79
	Mean time interval	27:13 hrs.	24:52 hrs.	27:00 hrs.

The complete parallel between the results of the analysis of the observations on early embryo size and those on hatchability, which is analyzed in greater detail in part V, has led to the following interpretation of the reduced mean embryo size observed following inbreeding: it may be the result of actual or impending early embryonic mortality. Some of the blastoderms and some of the early embryos, in which somites were counted, produced by inbred matings may have been retarded because of impending mortality. These observations on moribund embryos would result in a smaller mean blastoderm diameter and lower mean number of somites. This in turn would

TABLE 64

ANALYSIS OF COVARIANCE OF EARLY EMBRYO SIZE AND TIME INTERVAL BETWEEN SYSTEMS OF MATING WITHIN EACH ORIGIN OF FEMALES, IN 1940, IN EGGS OCCUPYING CLUTCH POSITIONS SUBSEQUENT TO THE FIRST, TOGETHER WITH ANALYSIS OF VARIANCE OF DATA, ADJUSTED FOR REGRESSION ON TIME INTERVAL

Origin	Embryonic stage	Source of variation	d.f.	Variance embryo size	F*	Variance time interval	F*	Variance of adjusted means		
								d.f.	Embryo size	F*
SE	Blastoderm	Total............	21	.0514		2.24		20	.0515	
		Between systems of mating......	2	.1409	3.35	1.67	1.35	2	.1164	2.63
		Within systems of mating........	19	.0420		1.24		18	.0443	
	Somites	Total............	18	4.67		2.06		17	4.78	
		Between systems of mating......	2	.14	.....	2.76	1.39	2	.05	.....
		Within systems of mating........	16	5.23		1.98		15	5.41	
OB	Blastoderm	Total............	122	.0545		2.25		121	.0519	
		Between systems of mating......	2	.0963	1.79	.24	.....	2	.1053	2.06
		Within systems of mating........	120	.0538		2.28		119	.0510	
	Somites	Total............	114	3.11		2.30		113	2.57	
		Between systems of mating......	2	4.44	1.44	.78	.....	2	4.64	1.83
		Within systems of mating........	112	3.09		2.32		111	2.53	
CB	Blastoderm	Total............	26	.0530		2.95		25	.0548	
		Between systems of mating......	1	.1630	3.35	8.81	3.20	1	.0742	3.24
		Within systems of mating........	25	.0487		2.71		24	.0239	
	Somites	Total............	23	1.91		2.43		22	2.00	
		Between systems of mating......	1	1.76	.....	4.23	1.80	1	1.83	.....
		Within systems of mating........	22	1.92		2.35		21	2.01	

* No significant F value.

result in greater variability in early embryo size in inbred matings as compared to outbred matings. The analysis of the data, as reported in table 63, shows that the coefficients of variability for blastoderm size were greater under inbreeding in all three origins and were also greater for number of somites in the OB origin. The coefficients of variability reported in the latter origin are probably the most reliable for purposes of comparison, in view of the greater number of observations on which they are based and also because of the insignificant variation in mean time interval observed under the different systems of mating. The coefficients of variability for both blastoderm size and number of somites are decidedly greater when the OB females were inbred as compared to when they were either outbred or crossbred. There is some indication that the smaller embryo size observed following inbreeding is related to early embryonic mortality or reduced growth rate because of impending embryonic mortality.

DISCUSSION

The results obtained in this part of the study of the relative effects of inbreeding and outbreeding on early embryo size, indicate that inbreeding results in a smaller mean blastoderm size at time of oviposition, and in a lower number of somites in the embryo after 38 hours of incubation, as compared to outcrossing and crossbreeding. Outcrossing results in a larger mean blastoderm size and in a higher mean number of somites than crossbreeding. Statistically significant differences in early embryo size, between systems of mating, however, were observed in 1940 and not in 1941. In the latter year, the difference observed in early mean embryo size between inbreeding and outbreeding was in the same direction, in favor of outbreeding, as that observed in 1940, but it was not statistically significant. This discrepancy in results is probably explained by differences in the genotypes of the birds used in the two years.

In 1940, the OB females with an outbred origin were probably heterozygous for a large number of genes with deleterious effects. These OB females when inbred to their full brothers were thus likely to produce a considerable proportion of zygotes homozygous for deleterious genes. Some of these genes may have influenced early embryo size, and the result was a significant difference in mean early embryo size between systems of mating.

In 1941, the SEOB females, which provided most of the data, had a topincross origin, inbred SE males on OB females in 1940, and presumably carried fewer deleterious genes than the outbred OB females used in 1940. The inbreeding in the history of the SE origin may have resulted in the elimination of a number of deleterious genes, and the surviving SE individuals may have been comparatively more homozygous for desirable alleles than the OB females. Inbreeding of SEOB females would then likely result in a smaller proportion of zygotes homozygous for deleterious genes than in the early stages of inbreeding of the OB females. As a result of this smaller proportion of zygotes homozygous for deleterious genes, mean early embryo size in the SEOB females would not be affected enough to show significant differences between systems of mating.

It must be stressed that inbreeding consistently resulted in a smaller mean embryo size in both years and in each origin of females, but the differences observed between inbreeding and outbreeding were statistically significant only in 1940 when one of the breeding lines had an outbred origin. The breeding history of the respective origins of females, as influencing their comparative heterozygosity for deleterious genes, would apparently account for the level of significance of the differences in early embryo size observed following inbreeding and outbreeding. The higher the heterozygosity of a group of birds, the greater the probability that inbreeding will bring to light a large number of deleterious genes, and conversely, the higher the homozygosity of a group of birds, the lower the probability that further inbreeding will result in the appearance of homozygotes for deleterious genes. This could very well be interpreted as indicating that with increasing homozygosity, a larger number of observations on many matings are needed to detect the effect of deleterious genes on a character such as early embryo size.

No definite information is available on the actual mode of operation of the genes with deleterious effects on early embryo size, but the following hypotheses are offered.

Inbreeding may result either in a delay in the initiation of cell proliferation after fertilization or cell proliferation may actually proceed at a slower rate in the embryos. No evidence is available to reject either hypothesis in these investigations. They receive some support from the observations of Byerly (1934) to the effect that inbred embryos tend to require more than 21 days to complete their development and hatch. A third hypothesis seems to receive very good support from the evidence obtained in this study. The smaller mean blastoderm size and lower mean number of somites observed following inbreeding may actually be the result of growth retardation, itself a manifestation of impending mortality, caused by deleterious genes. If this hypothesis were true, wholly or partly, rate of impending embryonic mortality and not embryonic growth rate proper should possibly have been measured in these investigations. This hypothesis would lead to an expected greater variability in early embryo size in inbred matings; this was demonstrated as a fact particularly in the OB origin of females in 1940. In addition, the hypothesis of impending embryonic mortality would result in some degree of correlation between early embryo size and hatchability, a point reported upon in part V.

Other Factors Affecting Early Embryo Size

Prior to the analysis of the relative effects of inbreeding and outbreeding on early embryo size, as measured by maximum diameter of the blastoderm and number of somites developed in the embryo after 38 hours of incubation, a number of factors were investigated to establish their possible influence on early embryo size. The purpose of these preliminary investigations was to determine which factors had to be taken into account in the analysis of the relative effects of inbreeding and outbreeding on early embryo size. Since a number of very interesting conclusions have been derived, it seems worth while to discuss them as they help to elucidate a number of problems which have received contradictory answers from other investigators.

Time Interval. A positive and linear correlation was observed between early embryo size and the time interval between the oviposition of the egg in which early embryo size was determined and that of the preceding egg in the same clutch. Scott and Warren (1936) and McNally and Byerly (1936) have also reported a linear relation between time interval and number of somites after 38 and 48 hours of incubation respectively. In the present investigations, the correlation between time interval and early embryo size was higher when number of somites after 38 hours of incubation was used as a measurement than when blastoderm diameter was used. This fact would in turn indicate that the number of somites is a better index of the actual length of time the embryo has been developing either *in* or *ex utero,* when the eggs have been kept under similar environmental conditions. The closer relation between length of time interval and number of somites is also relatively easy to understand from the standpoint of embryology. The blastoderm does not increase consistently in diameter at the time of gastrulation, but also differentiates in depth by delamination. Actually, the greater variability between blastoderms in unincubated eggs, or at time of oviposition, may be in degree of delamination rather than in diameter. The somites, in contrast to blastoderm size, represent primarily the result of longitudinal differentiation and hence, as reported previously from the statistical analysis of Fischel's data, the number of somites bears a definite relation to embryo length. In view of the greater accuracy of number of somites as a measure of length of period of development at an early stage, and also the simplicity and rapidity with which somite counts can be made as compared to measurements of blastoderm diameter, it is suggested that number of somites be used in any investigation in which a measurement of early chick embryo size is needed.

The high relation observed between time interval and early embryo size is also evidence in support of the observation of Warren and Scott (1934, 1935a, and 1935b) that ovulation is synchronized with the oviposition of the preceding egg in a clutch. It is also evidence in support of the conclusion of Olsen (1942) that fertilization of the hen's ovum normally takes place very shortly after ovulation.

Eggs occupying the first position in a clutch did not show any significant correlation between time interval, as measured by the length of time elapsing from the oviposition of the last egg in the preceding clutch to the oviposition of the first egg of a given clutch, and early embryo size, although there was an indication that a longer interval between clutches resulted in a larger mean embryo size. The lack of a significant relation between time interval separating two clutches and early embryo size is interpreted as evidence that the first egg of a clutch is ovulated at an irregular interval after the last egg of the preceding clutch is laid. Support for this interpretation is found in the greater variability observed in early embryo size in eggs occupying first clutch position. In addition to showing greater variability in early embryo size, eggs occupying the first position of a clutch also averaged larger in mean blastoderm size and higher in mean number of somites than eggs in clutch positions subsequent to the first. Taylor and Gunns (1935) originally made a similar observation. The larger early mean embryo size observed in

eggs occupying a first clutch position is interpreted as being caused by a longer period of retention in the oviduct before oviposition. Presumably the first egg of a clutch is often ready to be laid during the night, before the hen gets off the roost, and the egg is usually retained in the oviduct until the hen can enter a nest. Scott and Warren (1936) have obtained evidence that the first egg of a clutch spends a longer period of time in the oviduct than subsequent eggs, although they could not detect any resulting difference in the number of somites present in the embryos after 38 hours of incubation.

The second egg in a clutch was also observed to contain a larger blastoderm and a higher number of somites than eggs occupying clutch positions subsequent to the second. An extended analysis of this relation has, however, revealed that the difference was principally accounted for by the second or last egg in two-egg clutches. Atwood (1929) has demonstrated that the time interval between eggs shortens as the number of eggs in a clutch increases. In the analysis of the data available in the present investigations, this was confirmed by the demonstration that the time interval for the second egg in a clutch is shorter as the length of the clutch increases. In the total number of eggs occupying a second clutch position, there were proportionately more eggs from two-egg clutches than from longer clutches; this resulted in an increase in early embryo size in the second clutch position. The observations made in this study lead to the interpretation that the longer time interval for the second egg in two-egg clutches, and possibly for second eggs in other short clutches, is at least partly the result of a longer period of sojourn in the oviduct. The longer time interval of the second egg in two-egg clutches does not represent solely a delay in ovulation since it is expressed in a larger blastoderm and a higher number of somites. This observation is contrary to the conclusion of McNally and Byerly (1936) that eggs in two-egg clutches did not spend in the oviduct the time indicated by the length of their time interval. They based this conclusion on the inverse relation they observed between time interval and number of somites when the time interval exceeded 27 hours. The observations analyzed in the present study gave results in agreement with the conclusions of Warren and Scott (1935*b*) that the differences in length of time interval between eggs are due to variations in the time the eggs spend in the uterus.

The last egg in a clutch was observed to have a longer time interval and to contain a larger blastoderm and an embryo with a higher number of somites than eggs in a clutch position intermediate to the first and the last. Atwood (1929) and more recently Berg (1945) have reported that the last egg in a clutch has a longer time interval. Berg (1945) was also able to conclude that this longer time interval represents a longer period of sojourn in the uterus because of the comparatively thicker shell on the last egg in a clutch. The data analyzed in the present study furnish additional evidence to support Berg's conclusion since the last egg in a clutch contained a larger blastoderm and developed an embryo with a larger number of somites after 38 hours of incubation. In the preceding paragraph on eggs occupying the second position in a clutch, it was stated that the larger early embryo size observed in such eggs was accounted for by the last or second egg in two-egg

clutches. This same explanation could apply here also, since a great proportion of the total number of last eggs will be from two-egg clutches. It was found, however, that even after the elimination of the last or second egg in two-egg clutches, the last egg still developed an embryo with a significantly larger number of somites but did not contain a larger blastoderm at oviposition than eggs in intermediate clutch positions. The exact nature of the delay in the oviposition of the last egg is not known, but it could be caused by the lack of the stimulus accompanying the ovulation of a succeeding egg. It has generally been assumed that, because ovulation follows the oviposition of the preceding egg, the former takes place as a result of the stimulus provided by the latter, but the contrary also seems possible in view of the observations on the prolonged retention of the last egg of a clutch.

Time of Lay. Eggs laid early in the morning, particularly those laid at or before 8 o'clock, were observed to contain larger blastoderms and to develop embryos with a higher number of somites than eggs laid later in the day. The eggs laid at or before 8 o'clock in the morning not only developed larger embryos but these were also more variable in size than those in eggs laid later in the day. This observation is explained by the fact that 80% of the eggs laid at or before 8 o'clock were eggs occupying the first position in a clutch and hence had spent a more variable and, on the average, a longer period of time in the oviduct. McNally and Byerly (1936) have also observed that eggs laid before 9 o'clock in the morning contained embryos with a larger number of somites after 48 hours of incubation than eggs laid after 9 o'clock. In this investigation, the eggs laid later in the afternoon, after 2 o'clock, contained somewhat smaller blastoderms but developed embryos with a larger number of somites than eggs laid between noon and 2 o'clock. McNally and Byerly (1936) have reported that eggs laid in the later part of the day developed embryos with a smaller number of somites after 48 hours of incubation than those laid earlier in the day. In the present investigation the eggs occupying the first position in a clutch exhibited no significant relation between hour of lay and number of somites. The eggs in clutch positions subsequent to the first when laid early in the morning (before 9 o'clock) and early in the afternoon (before 2 o'clock) contained embryos with a lower number of somites than those laid later in the morning (after 9 o'clock) and later in the afternoon (after 2 o'clock). In the interpretation of these observations, it is well to remember that eggs in last clutch position are laid later in the afternoon. Since these eggs have been shown to have a longer time interval, representing at least partly a longer period of sojourn in the oviduct, it will not be unlikely that a larger number of somites will appear in eggs laid later in the afternoon.

This discussion has revealed that variations in the length of time the eggs are retained in the oviduct constitute a very important factor to be taken into account in the analysis of observations on early embryo size. It can be stated that in eggs occupying a clutch subsequent to the first, contrary to eggs in first clutch position, the time interval between the oviposition of the preceding egg and the ovulation of the egg in which observations are to be made is an accurate index of the actual time the egg spent in the oviduct. For this

reason, in the analysis of the relative effects of inbreeding and outbreeding on early embryo size only observations made on eggs occupying a clutch position subsequent to the first were analyzed, since they could be adjusted for their regression on time interval and the various matings hence put on a comparable time interval basis.

Individuality of the Hen. Another factor investigated was the individuality of the hen. Significant differences between individual hens were observed in both mean blastoderm diameter before incubation and mean number of somites after 38 hours of incubation, whether their eggs occupied the first position or a subsequent one in a clutch. These differences between individuals could of course be attributed to variations in length of time interval between eggs, but an analysis of variance of the data on blastoderm size and on number of somites adjusted for their regression on time interval revealed that the differences between individuals were still highly significant. The time interval elapsing between the oviposition of the preceding egg and ovulation of the one in which embryo size was measured does not entirely explain the mean size of the blastoderm or the mean number of somites developed in the eggs of an individual. This fact, however, does not eliminate entirely the possibility that the differences in early embryo size between individual birds may still be the result of variations in length of time the egg has spent in the oviduct. It is true that good evidence has been presented in support of the view that ovulation takes place shortly after the oviposition of the preceding egg, but differences may exist between individuals in the actual time interval elapsing between the two phenomena. The synchronization of oviposition and ovulation would not necessarily be affected by the presence of variations between individuals in the time relation between the two. In fact such individual variations do result as a consequence of variations in rate of egg production, as low intensity of egg production necessarily entails, at least partly, longer time intervals between successive ovulations. These variations could thus account for the significant differences observed in early embryo size between individuals.

The significant differences observed in early mean embryo size between individuals might also be explained in part by slight individual variations in body temperature. If, on the basis of a common time interval, the eggs of individual hens still vary in early embryo size, it is possible that these variations are due to differences in the environment within the hen and the most probable difference is variation in body temperature. Fronda (1925) has observed significant temperature variations between and within individual hens.

Another explanation for the significant variations observed between individual birds in early embryo size is the one offered to account for the significant variations observed between inbreeding and outbreeding. The rate of early embryonic mortality undoubtedly varies in individual birds and hence a varying proportion of the blastoderms and early embryos within individuals would be moribund and retarded in development at the time the observations were made. These varying proportions of moribund or retarded blastoderms and early embryos could result in significant differences in mean early embryo size between individual birds.

Finally, there is also the possibility of genetic factors which regulate early embryonic growth rate; these would be different from the deleterious genes referred to previously.

SUMMARY

Inbreeding, when compared to outbreeding, resulted in a smaller mean blastoderm diameter in unincubated eggs and in a lower mean number of somites in embryos after 38 hours of incubation. Outcrossing resulted in a larger mean blastoderm diameter and a higher mean number of somites than crossbreeding. Statistically significant differences in early embryo size between inbreeding and outbreeding were observed in 1940 in a group of birds with an outbred origin that were presumably highly heterozygous. The effects of inbreeding on early embryo size are tentatively explained as the result of homozygous deleterious genes which retard embryonic development and may eventually lead to embryonic mortality. Inbreeding may also result in a delay in the initiation or the rate of embryonic development.

Eggs in first and last clutch positions, laid early in the morning and late in the afternoon respectively, contained embryos comparatively more advanced in development presumably because of the longer period of time these eggs were retained in the oviduct. A linear relation was observed between length of time interval separating the oviposition of two successive eggs and early embryo size.

Significant differences in early embryo size were demonstrated between individuals and these differences remained signficant even after adjustment for regression of early embryo size on time interval. These individual differences were interpreted as possibly due to individual variations in the synchronization between oviposition of the preceding egg and ovulation, slight individual variations in body temperature, genetic factors which may affect early embryonic growth rate, and also individual variations in incidence of early embryonic mortality preceded by a retardation in development.

The Relative Effects of Inbreeding and Outbreeding on Reproduction in the Domestic Fowl

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V. INTERRELATIONSHIPS OF CHARACTERS STUDIED

ONE OF THE OBJECTIVES of the present investigations was to ascertain the presence of possible interrelationships between the characters studied; between fertility and hatchability, between blastoderm size in the unincubated egg and number of somites after 38 hours of incubation, and between early embryo size and hatchability. After the completion of the analysis of the variation in blastoderm diameter and in number of somites, it was clearly evident that these two measurements were very highly correlated and it seemed superfluous to analyze their covariance. Only two interrelationships thus remained to be studied, that between fertility and hatchability, and that between embryo size and hatchability.

Relation Between Fertility and Hatchability

Contradictory evidence has been reported on the relation between fertility and hatchability of fertile eggs. It was thought that the experimental design used in these investigations, permitting a detailed analysis of the relation between the two characters in females of three different origins mated in turn to males of the same three origins within three successive periods, could possibly shed some light on the problem.

Review of Literature

Pearl and Surface (1909) observed a small but sensible correlation between fertility and hatchability in Barred Plymouth Rocks, but they concluded that the two characters were biologically independent, a view which has since been held by most investigators in poultry husbandry. Hyre and Hall (1932) reported that hatchability and fertility behave as individual characteristics in hens and that both are fairly constant from year to year. Hays and Sanborn (1924), and Jull (1928) reported that fertility and hatchability were independent. Blyth (1945) commenting on these investigations of Hays and Sanborn, Jull, and Hyre and Hall, expressed the view that any small correlation present in their data may have been obscured by the facts that the observations were made over a short period of time, with relatively large numbers of birds mated to many males, and without knowledge or account of completely infertile matings.

Knox (1927) and Munro (1938, 1940, 1942, 1946) have observed a posi-
tive relation between fertility and hatchability. Landauer (1941) in sum-
marizing studies made on the relation between fertility and hatchability drew
attention to the apparent lack of correlation between the two characters in
the White Leghorn and Rhode Island Red breeds, but the suggestion of a
positive relationship in Barred Plymouth Rocks.

Munro and Kosin (1945), in studies on the relationship between fertility
and hatchability over a period of six years in a total of 72 pens of Barred
Plymouth Rocks and of 66 pens of White Leghorns, observed correlation
coefficients between mean fertility and mean hatchability of pens ranging
from .067 to .515 and from .014 to .636 in the Barred Plymouth Rocks and
White Leghorns respectively. The net and significant correlation coefficients
observed were .332 and .265 for the Barred Plymouth Rocks and the White
Leghorns respectively. The relation was observed to be particularly evident
between the means of mass-mated pens within single hatches and between
the means of different settings from the same pen or groups of pens at dif-
ferent times. As an explanation for the observed relation between fertility
and hatchability, Munro and Kosin (1945) suggested a preoviposital em-
bryonic mortality which might ordinarily be misinterpreted as "infertility"
and which they assumed to be correlated with postoviposital embryonic mor-
tality. They reported having secured evidence of cellular proliferation in
approximately 50% of so-called "infertile" blastodiscs. The conclusion of
Munro and Kosin was thus that there is a relation between embryonic mor-
tality during the preoviposital period, approximately 26 hours elapsing be-
tween fertilization and oviposition, and embryonic mortality during the 21
days of postoviposital embryonic development.

Blyth (1945) observed similarities in the seasonal trends of hatchability
and fertility over a period of six months in two consecutive years. She ob-
tained significant correlation coefficients of .9376 between month means, .6651
between bird means, and .2739 between all observations. The variances for
embryonic mortality and for infertility corrected for their regression on
fertility or embryonic mortality, respectively, remained significant for both
month and bird means. Blyth concluded that infertility and embryonic mor-
tality are associated, behave as inherent characteristics of individuals, exhibit
similar seasonal changes, and that each variable shows additional significant
variations peculiar to itself which tend to obscure the fundamental relation-
ship between them. According to her, seasonal variations in infertility indi-
cate that this infertility may really be undetected embryonic mortality. She
added that if true infertility exhibits seasonal trends then it can only occur
through parental sources such as variations in the sexual activity of males
and cyclic changes in the physiological status of the females.

Materials and Methods

The data reported on in part II dealing with fertility and in part III
dealing with hatchability were used to study the relation between the two
variables. In parts II and III the data were weighted within each classifica-
tion for variation in egg production; unfortunately these figures, being non-

additive, could not be used in an analysis of covariance. It was therefore necessary to work on the individual percentages of fertility and hatchability converted to angles expressed in degrees and then submitted to a regular analysis of covariance.

Analysis of Observations

It is not necessary to repeat here the analysis made on fertility and hatchability as the results can be consulted by referring to parts II and III. Essentially the same classifications were used in the analysis of covariance of fertility and hatchability as can be gathered from table 65.

TABLE 65

ANALYSIS OF COVARIANCE OF FERTILITY AND
HATCHABILITY, 1940 AND 1941

Source of variation	Degrees of freedom	Correlation coefficient	Regression coefficient	t value
1940				
Total..	59	.27*	.2914*	2.17
Between periods.......................................	2	.81	.6588	1.36
Within periods..	57	.21	.2354	1.65
Between origins.......................................	6	.24	.1496	0.56
Within origins..	51	.22	.3021	1.62
Between systems of mating............................	14	−.20	−.3126	0.74
Within systems of mating.............................	37	.43†	.5633†	2.95
1941				
Total..	107	.16	.2706	1.59
Between periods.......................................	2	.87	12.7800	1.75
Within periods..	105	.16	.2890	1.77
Between origins.......................................	6	.25	.5030	0.57
Within origins..	99	.15	.2429	1.48
Between systems of mating............................	9	.19	.2446	0.56
Within systems of mating.............................	90	.14	.2422	1.31

* Significant at the 5% level.
† Significant at the 1% level.

The correlation coefficient observed between fertility and hatchability when all matings were considered, irrespective of classification, was .27 and .16 for 1940 and 1941 respectively. The former, .27, observed in 1940, was the only significant one at the 5% level. The regression coefficients observed were .2914 and .2706 for 1940 and 1941 respectively. The former, as was the case for the correlation coefficient, is the only one that gave a t value significant at the 5% level of significance.

Seasonal Variation. The analysis of covariance of fertility and hatchability between periods gave correlation coefficients of .87 and .81 in 1940 and 1941 respectively but both coefficients are nonsignificant. The regression coefficients observed were .6588 and 12.78 for 1940 and 1941 respectively, and both also were nonsignificant.

The correlation coefficients within periods remained practically the same as in the total, .21 and .16 as compared to .27 and .16 for 1940 and 1941 respectively. The same observation applies to the regression coefficients, .2354 and .2890 for 1940 and 1941 respectively as compared to .2914 and .2706. Neither the correlation nor the regression coefficients observed within periods reach a level of significance.

Origins of Females. The next step in the analysis of covariance was to ascertain whether the three origins of females showed differences in the relation between fertility and hatchability. The correlation coefficients observed

TABLE 66

ANALYSIS OF COVARIANCE OF FERTILITY AND HATCH-
ABILITY WITHIN ORIGIN OB IN 1940

Source of variation	Degrees of freedom	Correlation coefficient	Regression coefficient
All OB females.............................	29	.20	.1991
Between subgroups or pens..................	2	−.10	−.2551
Within subgroups or pens..................	27	.20	.2014
Between systems of mating.................	6	−.44	−.5543
Within systems of mating..................	21	−.05	−.0350

were .24 and .25 for 1940 and 1941 respectively while the regression coefficients were .1496 and .5030. None of the coefficients reached the level of significance.

Systems of Mating. The analysis of covariance of fertility and hatchability between systems of mating, within origins of females and within periods, gave correlation coefficients of −.20 and +.19 in 1940 and 1941 respectively. The regression coefficients observed were −.3126 and +.2446. Again none of these coefficients reached the level of significance. The remaining figures, termed "within systems of mating" and which could be designated between individuals, showed correlation coefficients of .43 and .14 in 1940 and 1941 respectively while the regression coefficients were .5633 and .2422. The correlation and regression coefficients observed in 1940 were both significant at the 1% level.

Covariance within OB Origin. In the analysis of variance of hatchability it was established that the OB origin was the group of birds which showed very significant differences in hatchability between systems of mating. In view of this observation it was thought advisable to study further the relation between fertility and hatchability in this group of birds. An additional argument in favor of more detailed analysis of the data obtained with this group of birds is that their production was much more uniform. A first analysis, which is reported in table 66, dealt with the covariance of hatchability between and within three subgroups of OB birds, which were in separate pens, and between and within systems of mating. The overall correlation between fertility and hatchablity in the OB group of birds was .20 while the regression coefficient was .1991; both nonsignificant. No significant difference in the correlation coefficient was observed between the three subgroups

(–.10), or within them (.20). When the data for each subgroup were analyzed for the covariance of fertility and hatchability between systems of mating, the correlation coefficient observed was –.44 while the regression coefficient was –.5543. Within systems of mating, or, in equivalent terms, between individual birds, the correlation coefficient observed was –.05 and the regression coefficient –.0350. None of the correlation or regression coefficients reached a level of significance.

An additional method of analysis of the data obtained with the OB origin was applied. It consisted of dealing with each bird directly and determining the covariance of fertility and hatchability within birds. The latter term has however the inconvenience of covering both systems of mating and seasonal

TABLE 67

ANALYSIS OF COVARIANCE OF FERTILITY AND HATCH-
ABILITY WITHIN INDIVIDUAL OB BIRDS IN 1940

Source of variation	Degrees of freedom	Correlation coefficient	Regression coefficient
All OB females............................	26	.15	.1543
Between individuals........................	8	.06	.1177
Within individuals or between systems of mating and periods.....................	18	.21	.1607

variation. The results of this method of analysis are presented in table 67. The correlation coefficient observed in the total was .15 while the regression coefficient was .1543. The analysis of covariance between individual birds gave a correlation coefficient of .06 and a regression coefficient of .1177. The remainder, within individual birds, which as stated above also covers between systems of mating and between periods, gave a correlation coefficient of .21 and a regression coefficient of .1607. Again none of the correlation or regression coefficients were significant.

DISCUSSION

The most striking feature in this analysis of covariance of fertility and hatchability is undoubtedly the similarity between the correlation coefficients reported here on all observations (.27 and .16) and those reported by Munro and Kosin (1945) (.27 for White Leghorns) and by Blyth (1945) (.27 for Brown Leghorns). The second striking feature is also a similarity between the correlation coefficients observed in the present study between periods (.81 and .87) and the value reported by Blyth (1945) (.94) between six-month means. The prevalent opinion of hatcherymen and practical poultrymen as to the existence of a relation, seasonal in character, between fertility and hatchability, seems supported by the investigations of Munro and Kosin (1945), Blyth (1945), and to a certain degree by the present study. It is necessary to emphasize that the correlation coefficients reported here between periods, notwithstanding their magnitude, did not reach a level of significance.

The present investigations presented some advantages over previous studies

in that further analysis could be made on the basis of genetic relationship and also of systems of mating in an effort to elucidate the general relationship observed between fertility and hatchability. Embryonic mortality could be both genetical and environmental in origin and presumably the hypothesis of preoviposital mortality, suggested by Munro and Kosin (1945), could hold for both types of mortality. Should the hypothesis of preoviposital mortality hold for embryonic mortality of genetic origin, then it might be possible to detect differences in the relationship between fertility and hatchability in groups of birds of different origins and genetic constitution or in birds submitted to different systems of mating.

The results obtained in the present study show that origin or genetic relationship and also systems of mating do not change or alter the general relationship between fertility and hatchability. Two noticeable exceptions to this conclusion were observed. In 1940 a negative correlation coefficient was noted between systems of matings; this is explained by the OB birds which when inbred showed no noticeable difference in fertility, but a significant decrease in hatchability, hence a negative correlation. This negative correlation instead of supporting Munro and Kosin's hypothesis negates it. The other significant exception was observed within systems of mating in 1940. The significant correlation coefficient reached a value of .43. As stated previously the "within systems of mating" classification is equivalent to "between individual birds." Hence in 1940 within a given mating system, dams with better fertility also gave better hatchability. This was not the case in 1941. Since the population of 1940 had a greater number of individuals of inbred origin than that of 1941, it is possible that this difference may be responsible for the higher correlation coefficient between fertility and hatchability observed in the former year.

The relationship observed between fertility and hatchability, significant at the 5% level in one year only, is apparently not explained by genetic variability but possibly by environmental variability peculiar to that year or to the birds mated that year. The physiological condition of both males and females apparently shows seasonal variations resulting in variations in fertility. Seasonal variations also affect the hatching quality of eggs laid by the females.

Relation Between Early Embryonic Growth and Hatchability

In the analysis of the data on hatchability obtained in the present investigations, the hypothesis that the smaller mean blastoderm size and the lower mean number of somites observed under inbreeding are the results of growth retardation, itself a manifestation of impending embryonic mortality, was proposed. This hypothesis could be tested by an analysis of the relationship between early embryo size and hatchability.

Review of Literature

Interest in the relationship between early embryo size and hatchability stems from Stockard's (1921) hypothesis that nonhereditary monstrosities result from arrests in development. Since the development of birds is discontinuous, being interrupted at about the gastrulation stage, Stockard was

led to suggest that the abundant occurrence of double chick embryos and the well known high embryonic mortality are due to this natural arrest of development. The variations in development of the blastoderm in hens' eggs at the time of laying would account for the double individuals and twins among chick embryos as well as for their apparent frequency. Riddle (1923) failed to obtain double embryos by cooling premature eggs of ring doves, but he observed that eggs with fully formed shells retained in the uterus cannot obtain enough oxygen to satisfy the demands of the embryo during the post-gastrula stage and consequently die. Hutt and Greenwood (1929) expressed the view that variation in time required by the egg to traverse the oviduct results in differences in development at the time the egg is laid. Hutt and Pilkey (1930) observed a significantly higher (3.89%) mortality rate of embryos in eggs laid in the afternoon. They explained that among eggs laid in the morning there would be a greater proportion which have been held in the body longer than the normal period and are therefore safely past the critical gastrulation stage. Nicolaides (1933) reported that eggs further advanced in development at time of laying generally hatch better than others regardless of hour laid. Hays and Nicolaides (1934) observed that the most common embryonic stages in eggs at time of laying are pregastrula and early gastrula for hens with low hatchability and well advanced gastrula for high-hatching hens. Taylor and Gunns (1935, 1939) were unable to detect any significant correlation between blastoderm characteristics in unincubated eggs and hatchability of other eggs from the same hens with the exception of a positive relation between extent of overgrowth and hatchability. Bronkhorst (1933) observed that the maxima of growth generally occurred earlier among the embryos of the high-hatching hens than among the low-hatching hens. Landauer (1941) pointed out that it is impossible to judge from Bronkhorst's data whether the observed differences indicate that retarded growth was a causal factor in reduced hatchability or whether the reduced size of the embryos is an expression of retardation preceding death from unanalyzed causes, such as would occur as a result of nutritional deficiencies. McNally and Byerly (1936) reported that hens whose eggs contained embryos with 20 somites after 48 hours of incubation gave the maximum hatchability, while there was a gradual decrease in hatchability as the embryos had from 20 to 14 somites and a rapid decrease in hatchability as the number of somites increased from 20 to 23. Neel (1942) reported a correlation coefficient of +.77 ± .08 between rate of development and percentage of hatchability for individual hens, but it may be suspected that the correlation reported by him is a spurious one since his "rate of development" probably represents the average developmental stage not only of normal living embryos but also moribund and dead ones. Sturkie and Williams (1945) obtained premature eggs from hens and subsequently incubated them. The blastoderms of eggs 13½ hours after fertilization were very susceptible to the development of abnormalities, while blastoderms only one hour older were not as susceptible, as a higher percentage resumed normal development when under normal incubation. Sturkie and Williams conclude that eggs laid several hours prematurely showed a high degree of resistance to changes in milieu and that in view of this

fact it is not surprising to find that the variations observed in the state of development of normally laid eggs are not significantly related to degree of hatchability.

Materials and Methods

The data reported in part III dealing with hatchability and in part IV dealing with early embryonic growth were used to study the relation between these two variables. As was the case in the analysis of the relationship between fertility and hatchability, it was necessary to work on individual percentages of hatchability converted to angles expressed in degrees and then submitted to a regular analysis of covariance.

TABLE 68

SUMMARY OF DATA ON HATCHABILITY AND EARLY EMBRYO SIZE CLASSIFIED ACCORDING TO CLUTCH POSITION, TIME INTERVAL BETWEEN EGGS, AND HOUR OF LAY, SHOWING THEIR RELATIONSHIP

Classification	Mean blastoderm size of unincubated eggs in mm	Mean number of somites in eggs incubated 38 hrs.	Mean percentage hatchability of fertile eggs
Clutch position			
First..	$3.7761 \pm .0158$	$12.6389 \pm .1152$	75.98
Subsequent to first................................	$3.5431 \pm .0079$	$11.3956 \pm .0618$	79.79
Time interval between eggs in clutch positions subsequent to the first			
20–22 hours..	3.4643	9.6238	75.00
23–24 hours..	3.4943	10.5917	78.38
25–26 hours..	3.5025	11.5238	78.50
27–28 hours..	3.5393	11.8029	73.33
29–30 hours..	3.5923	13.0000	40.00
31–33 hours..	3.6833	....	.....
Hour of lay			
Before 8 o'clock...................................	3.7565	12.2558	68.29
9–10 o'clock.......................................	3.6675	11.4118	81.69
11–12 o'clock......................................	3.5609	11.5375	77.55
1– 2 o'clock.......................................	3.5947	11.8226	77.22
3 o'clock and later................................	3.5689	12.1177	68.99

Analysis of Observations

The pertinent data on hatchability and early embryonic growth are grouped together in table 68. Eggs occupying the first clutch position contain a larger blastoderm and a 38-hour embryo with more somites than eggs occupying subsequent clutch positions. These eggs in the first clutch position also do not hatch as well as those in subsequent clutch positions. Eggs with a time interval of less than 23 hours contain a smaller blastoderm and an embryo with less somites and do not hatch quite as well as eggs with a time interval of 23 to 29 hours. Eggs with a time interval of 29 hours or more contain a larger blastoderm and an embryo with more somites and do not hatch as well as eggs with a time interval of less than 29 hours, but more than 22 hours. Eggs laid before 8 o'clock in the morning contain a larger blas-

toderm and an embryo with more somites than those laid later in the day while eggs laid after 3 o'clock in the afternoon contain a smaller blastoderm than those laid earlier in the day. Both these groups of eggs do not hatch as well as those laid after 8 o'clock and before 2 o'clock. This evidence points to a curvilinear relationship between early embryo size and hatchability.

The next step consisted of an analysis of covariance of hatchability and early embryo size, as measured by blastoderm diameter in the unincubated egg and number of somites in the embryo after 38 hours of incubation (table 69). First, the data gathered in 1940 and 1941 were pooled irrespective of clutch position. A significant correlation coefficient was obtained between number of somites and hatchability (.18). In the pooled data and irrespective of clutch position, but under inbreeding, a significant correlation coefficient was again observed only between number of somites and hatchability (.24). In the first clutch position no significant correlation was observed between either of the measurements of early embryo size and hatchability. In clutch positions subsequent to the first, a significant correlation coefficient was observed between number of somites and hatchability under inbreeding only.

A parallel analysis of covariance was then separately conducted for the data collected in each of the two years. None of the correlation coefficients observed reached a level of significance.

It was observed earlier that the relationship between early embryo size and hatchability appeared to be curvilinear in nature and for that reason a test of linearity was made. The data gathered in 1940 were used for this analysis. No significant departure from linearity was found.

It is recalled that the only significant differences in hatchability and in early embryo size between systems of mating were observed in 1940. In that year the OB origin of females was the only group of birds with significant differences in hatchability between systems of mating. In view of these facts, the OB origin of females seemed to provide excellent material for testing the hypothesis previously enunciated: that the smaller mean blastoderm size and the lower mean number of somites observed under inbreeding are the results of growth retardation or impending embryonic mortality.

The mean blastoderm size and mean number of somites observed in each mating (each hen in turn mated to three sets of males) were correlated with the percentage of hatchability observed in the same mating. Three sets of observations on blastoderm size and three on number of somites were available. A mean was determined for all eggs irrespective of clutch position, another was obtained from observations made on eggs occupying first clutch position, and finally, another was based on the measurements made on eggs occupying a clutch position subsequent to the first.

The results of the analysis of covariance are presented in table 70. The relation between early embryo size and hatchability is positive in the individual matings of the OB origin of females in 1940. Large mean blastoderm size and large mean number of somites were indicative of high hatchability in a given mating. The most interesting fact is undoubtedly the increase in the value and significance of the correlation coefficients as the embryos increase

in age. In eggs occupying a clutch position subsequent to the first, and in which embryonic development has been under way in the oviduct for approximately 26 hours, the correlation is .10, not significant but indicative of a trend. In eggs occupying first clutch position, which are known to have remained

TABLE 69

ANALYSIS OF COVARIANCE OF HATCHABILITY AND EARLY
EMBRYO SIZE, DATA OF 1940 AND 1941

Source of data	Embryonic stage	Number of matings	Correlation
1940 and 1941			
All clutch positions...	Blastoderm	153	.038
	Somites	153	.181*
Under inbreeding...	Blastoderm	73	.074
	Somites	74	.244*
First clutch position...	Blastoderm	107	−.027
	Somites	105	.160
Under inbreeding...	Blastoderm	54	.144
	Somites	56	.196
Clutch positions subsequent to first................................	Blastoderm	148	−.091
	Somites	140	.133
Under inbreeding...	Blastoderm	70	−.078
	Somites	70	.471†
1940			
All clutch positions...	Blastoderm	52	−.023
	Somites	50	.217
Under inbreeding...	Blastoderm	16	−.197
	Somites	16	−.073
First clutch position...	Blastoderm	31	.258
	Somites	26	.302
Under inbreeding...	Blastoderm	12	−.060
	Somites	8	−.031
Clutch positions subsequent to first................................	Blastoderm	52	−.104
	Somites	46	.243
Under inbreeding...	Blastoderm	16	−.081
	Somites	16	.150
1941			
All clutch positions...	Blastoderm	101	.092
	Somites	103	.119
Under inbreeding...	Blastoderm	57	.144
	Somites	58	.237
First clutch position...	Blastoderm	76	−.154
	Somites	79	.083
Under inbreeding...	Blastoderm	42	.111
	Somites	48	.136
Clutch positions subsequent to first................................	Blastoderm	96	−.044
	Somites	94	.029
Under inbreeding...	Blastoderm	54	−.012
	Somites	54	.083

* Significant at the 5% level.
† Significant at the 1% level.

in the oviduct longer than those occupying a subsequent clutch position, the correlation is .29 and still not significant. In eggs occupying clutch positions subsequent to the first and incubated for 38 hours, the correlation between mean number of somites and hatchability is .50 which is significant at $P = .01$. Finally, in eggs occupying first clutch position and incubated for 38 hours, the correlation between mean number of somites and hatchability is .63 which

is significant at P = .05. The older the embryos from a given mating, the higher the correlation observed between their mean size and hatchability in the same mating, so far as the OB females were concerned in 1940.

The positive and significant correlation coefficients, observed between mean early embryo size and hatchability in the OB origin of females in 1940, are interpreted as evidence that inbreeding resulted in homozygosity for deleterious genes in many of the zygotes produced by these OB females. These deleterious genes started expressing themselves shortly after fertilization and their outward manifestation was a retardation in embryonic development. The higher the frequency of deleterious genes in a particular mating, the larger the proportion of zygotes affected, the greater the average reduction

TABLE 70

ANALYSIS OF COVARIANCE OF EARLY EMBRYO SIZE AND HATCHABILITY IN INDIVIDUAL MATINGS OF OB FEMALES IN 1940

Clutch position	Embryonic stage	Number of matings	Correlation
All eggs irrespective of clutch position................................	Blastoderm	30	.14
	Somites	29	.47
Eggs in first clutch position...	Blastoderm	15	.29
	Somites	13	.63*
Eggs in clutch positions subsequent to the first......................	Blastoderm	30	.10
	Somites	28	.50†

* Significant at the 5% level.
† Significant at the 1% level.

in blastoderm size or number of somites and in the percentage of hatchability. Hence, there was here a significant positive correlation between mean early embryo size and hatchability.

DISCUSSION

Eggs in first and last clutch positions contain embryos comparatively more advanced in development at time of oviposition and do not hatch as well as eggs occupying clutch positions intermediate to the first and the last. Eggs laid early in the morning and late in the afternoon contain embryos comparatively more and less advanced in development respectively and do not hatch as well as eggs laid in the later part of the morning and early part of the afternoon. Eggs with long time intervals contain embryos more advanced in development at time of oviposition and do not hatch as well as eggs with time intervals between 23 and 27 hours. On the other hand, eggs with abnormally short time intervals contain embryos comparatively less advanced in development and do not hatch as well as eggs with time intervals varying between 23 and 27 hours. These observations point towards a curvilinear relation between early embryo size and hatchability. McNally and Byerly (1936) have previously drawn attention to the curvilinear relation between early embryo size and hatchability. They observed an increase in hatchability as the mean

number of somites, after 48 hours of incubation, increased from 14 to 20, and a rapid decrease in hatchability as the number of somites increased from 20 to 23. The existence of a curvilinear relation between early embryo size and hatchability is also supported by the observations of Taylor and Gunns (1935, 1939) who could not detect any significant difference in mean blastoderm diameter between the eggs of hens with poor, medium and high levels of hatchability. Taylor and Gunns (1939) have, however, observed a positive relation between extent of overgrowth in the unincubated blastoderm and hatchability, a relation which approached significance, but was not high enough for purposes of accurate prediction of individual hatchability.

In the data gathered in the present investigations the correlation observed between blastoderm diameter and hatchability was not significant (.04) but the correlation between number of somites and hatchability reached a level of significance (.18). Under inbreeding the correlation between number of somites and hatchability reached a higher level and was still significant (.24). This observation led to the analysis of the covariance of early embryo size and hatchability in the OB origin of birds used in 1940. In this instance the relationship between early embryo size and hatchability appeared to be linear and of increased magnitude as the embryos measured became older.

SUMMARY

Origin or genetic relationship and also systems of mating do not alter the general relationship observed between fertility and hatchability. Closely inbred matings of birds with an outbred origin show a negative correlation between fertility and hatchability which supports the view that fertility is distinct from hatchability.

The normal relation between early embryo size and hatchability appears to be curvilinear as demonstrated by the fact that eggs in first clutch position, laid early in the morning and with unusually long time intervals all contain embryos relatively more advanced in development, while eggs with short time intervals and laid late in the afternoon contain embryos relatively less advanced in development; but that all of these classes of eggs do not hatch as well as those in intermediate clutch position, laid in the middle part of the day, and with a time interval of 23 to 27 hours.

Following close inbreeding of birds with an outbred origin, the relationship between early embryo size and hatchability is linear and increases in magnitude and significance as the embryos measured become older.

LITERATURE CITED

AMERICAN SOCIETY OF ANIMAL PRODUCTION
    1940. Report of committee on investigations. Proc. 33d Annual Meeting of the American
        Society of Animal Production, Chicago: 378.

ATWOOD, HORACE W.
    1929. A study of the time factor in egg production. W. Va. Agr. Expt. Sta. Bul. 223:3–
        10.

BERG, LAWRENCE R.
    1945. The relationship of clutch position and time interval between eggs to eggshell
        quality. Poultry Sci. 24:555–563.

BLUNN, CECIL THOMAS
    1934. The relation of adult body size to the rate of cell proliferation in the chicken
        embryo. Berkeley, California. 32 p. University of California, Thesis (Ph.D).

BLUNN, CECIL THOMAS, and PAUL W. GREGORY
    1935. The embryological basis of size inheritance in the chicken. Jour. Expt. Zool. 70:
        397–414.

BLYTH, JANET S.
    1945. Infertility and embryonic mortality in the domestic fowl. Roy. Soc. Edinb., Proc.
        62 B(2:23):191–201.

BRONKHORST, J. J.
    1933. A physical, chemical and physiological study of high and low hatching lines of
        single comb White Leghorns. Ithaca, New York. 110 p. Cornell University, Thesis
        (Ph.D.).

BYERLY, THEODORE C.
    1930. The effects of breed on the growth of the chick embryo. Jour. Morph. and Physiol.
        50:341–359.
    1931. Time of occurrence and probable causes of mortality in chick embryos. London,
        England. Proc. 4th World's Poultry Congress, 1930, Section A:174–181.
    1932. Growth of the chick embryo in relation to its food supply. Jour. Expt. Biol. 9:
        15–44.
    1934. Some factors affecting the length of the incubation period. Atti del V cong. mond.
        di pollicoltura, Roma, 1933, 2:373–379.

BYERLY, THEODORE C., G. W. HELSEL and J. P. QUINN
    1938. Growth in weight and cell number. Genetic effects in the chick embryo and chick.
        Jour. Expt. Zool. 78:185–203.

BYERLY, THEODORE C., C. W. KNOX and M. A. JULL.
    1934. Some genetic aspects of hatchability. Poultry Sci. 13:230–238.

BYERLY, THEODORE C., H. W. TITUS and N. R. ELLIS
    1933. Effect of diet on egg composition. II. Mortality of embryos in eggs from hens on
        diets containing protein supplements of different origin. Jour. Nutr. 6:225–242.

COLE, LEON J., and J. G. HALPIN
    1916. Preliminary report of results of an experiment on close inbreeding in fowls. Jour.
        Amer. Assoc. of Instr. and Invest. in Poultry Husbandry 3:7–8.
    1922. Results of eight years of inbreeding Rhode Island Reds. Anat. Rec. 23:97.

DERRICK, G. ETHEL
    1937. An analysis of the early development of the chick by means of the mitotic index.
        Jour. Morph. 61:257–284.

DRYDEN, JAMES
    1915. Inbreeding, its effects on vigor and egg laying. Jour. Amer. Assoc. of Instr. and
        Invest. in Poultry Husbandry 1:19.
    1916. Poultry Breeding and Management. 402 p. New York. Orange Judd Company.

DUMON, A. G.
    1931. The effects of inbreeding on hatchability. London, England. Proc. 4th World's
        Poultry Congress, 1930, Section A:1–5.

DUNKERLEY, J. S.
    1931. The effect of inbreeding. London, England. Proc. 4th World's Poultry Congress Section **A**:46–72.

DUNN, L. C.
    1923. Experiments on close inbreeding in fowls. Connecticut (Storrs) Agr. Expt. Sta. Bul. **111**:139–172.
    1928. The effect of inbreeding and crossbreeding on fowls. Berlin. Verhandl. d. V Intern. Kongr. f. Vererbungswissenschaft:609–617.

FISCHEL, ALFRED
    1896. Über Variabilität und Wachstum des embryonalen Körpers. Morph. Jahrbuch **24**:369–404.

FRONDA, F. M.
    1925. Some observations on the body temperature of poultry. Cornell Vet. **15**:8–20.

FUNK, E. M.
    1934. Factors influencing hatchability in the domestic fowl. Mo. Agr. Expt. Sta. Bul. **341**:3–22.
    1939. The relation of size of clutch and position of the egg in the clutch to hatching results. Poultry Sci. **18**:350–353.

GOODALE, HUBERT D.
    1927. Six consecutive generations of brother to sister matings in White Leghorns. A preliminary report on studies in inbreeding in poultry. Poultry Sci. **6**:274–276.

HAYS, F. A.
    1924. Inbreeding Rhode Island Red fowls with special reference to egg production. Amer. Nat. **58**:43–59.
    1926. The relation of the male to hatchability of hen's eggs. Sci. **64**:408.
    1929. Inbreeding in relation to egg production. Mass. Agr. Expt. Sta. Bul. **258**:256–302.
    1936. Time interval between eggs of Rhode Island Red pullets. Jour. Agr. Res. **52**:633–638.
    1938. Time interval between clutches in Rhode Island Red pullets. Jour. Agr. Res. **57**:575–581.

HAYS, F. A., and C. NICOLAIDES
    1934. Variability in development of fresh-laid hen eggs. Poultry Sci. **13**:74–90.

HAYS, F. A., and RUBY SANBORN
    1924. The inheritance of fertility and hatchability in poultry. Mass. Agr. Expt. Sta. Tech. Bul. **6**:20–42.

HENDERSON, EARL W.
    1930. Growth and development. XVI. The influence of temperature and breeding upon the rate of growth of chick embryos. Mo. Agr. Expt. Sta. Res. Bul. **149**:5–47.

HENDRICKS, WALTER A.
    1935. The statistical treatment of hatchability data. Poultry Sci. **14**:365–372.

HUTT, F. B., and A. W. GREENWOOD
    1929. Studies in embryonic mortality in the fowl. III. Chick monsters in relation to embryonic mortality. Roy. Soc. Edingb., Proc. **49**:145–155.

HUTT, F. B., and A. M. PILKEY
    1930. Studies in embryonic mortality in the fowl. IV. Comparative mortality rates in eggs laid at different periods of the day and their bearing on theories of the origin of monsters. Poultry Sci. **9**:194–203.

HYRE, H. M., and G. O. HALL
    1932. The constancy of hatching power in hens. Poultry Sci. **11**:166–171.

JULL, MORLEY A.
    1928. Studies in hatchability. I. Hatchability in relation to antecedent egg production, fertility, and chick mortality. Poultry Sci. **7**:195–215.

1929a. Studies in hatchability. II. Hatchability in relation to the consanguinity of the breeding stock. Poultry Sci. **8**:219–229.

1929b. Studies in hatchability. III. Hatchability in relation to coefficients of inbreeding. Poultry Sci. **8**:361–368.

1930. Studies in hatchability. IV. The effects of intercrossing inbred strains of chickens on fertility and hatchability. Poultry Sci. **9**:149–156.

1931. Studies in hatchability. V. The inheritance of hatchability. London, England. Proc. 4th World's Poultry Congress, 1930, Section **A**:167–173.

1933. Inbreeding and intercrossing in poultry. Jour. Hered. **24**:93–101.

1935. Studies in fertility in the domestic fowl. Poultry Sci. **14**:37–41.

1940. Poultry Breeding. 2d edition. 484 p. John Wiley and Sons, New York.

KAUFMAN, LAURA

1927. Recherches sur la croissance du corps et des organes du pigeon. Biol. Gen. **3**:105–128.

1929. Les différences du taux d'accroissement des oiseaux sont-elles manifestes pendant la vie embryonnaire. Arch. d'Anat. Micros. **25**:325–335.

1930a. Researches on the phaenogenesis of the differences of body-size, partly grounded on a new method of *in vitro* cultures of fowl and pigeon eggs. Acta Biol. Expt. (Varsovie) **5**:33–46.

1930b. L'origine des différences de la taille du corps des oiseaux. C. R. Assoc. Anatomistes. 25ème réunion, Amsterdam. (21):183–188.

1930c. Innere und äussere Wachstumsfaktoren. Untersuchungen an Hühnern und Tauben. Arch. f. Entwickl. Mech. der Organ. **122**:395–431.

1936. Die Entstehung von Unterschieden in der Körpergrösse bei Hühnern. Arch. Geflügelkunde **10**:374–377.

KEIBEL, FRANZ

1904. Ueber den Entwickelungsgrad der Organe in den verschiedenen Stadien der embryonalen Entwicklung der Wirbeltiere. *In* Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbeltiere, herausg. von Dr. Oskar Hertwig. 3(3:9):131–148. Jena. Gustav Fischer.

KEIBEL, FRANZ, and K. ABRAHAM

1900. Normaltafeln zur Entwicklungsgeschichte des Huhnes (*Gallus domesticus*). 132 p. Jena. Gustav Fischer.

KELLER, C.

1933. Vergleichende Zellen- und Kernmessungen bei grossen und kleinen Hühnerrassen zur Prüfung der genetisch bedingten Wuchsunterschiede. Ztschr. f. Zellforsch. u. Mikros. Anat. **19**:510–536.

KNOX, C. W.

1927. Correlation studies of certain characters upon hatchability and their interrelationships. Poultry Sci. **6**:110–117.

1946. The development and use of chicken inbreds. Poultry Sci. **25**:262–272.

LAMOREUX, W. F.

1940. The influence of intensity of egg production upon infertility in the domestic fowl. Jour. Agr. Res. **61**:191–206.

LANDAUER, WALTER

1937. The hatchability of chicken eggs as influenced by environment and heredity. Conn. (Storrs) Agr. Expt. Sta. Bul. **216**:5–84.

1941. The hatchability of chicken eggs as influenced by environment and heredity. Conn. (Storrs) Agr. Expt. Sta. Bul. **236**:5–124.

LERNER, I. MICHAEL

1937. Relative growth and hereditary size limitation in the domestic fowl. Hilgardia **10**:511–560.

1944. Lethal and sublethal characters in farm animals. A checklist and proposed numbering system. Jour. of Hered. **35**:219–224.

Hilgardia

LEVI, GIUSEPPE

    1922. Per la migliore conoscenza del fundamento anatomico e dei fattori morfogenetici della grandezza del corpo. L'accrescimento dei somiti mesodermici e di altre individualità morfologiche. Archiv. ital. di Anat. e di Embriol. 18 (Suppl.) :316–433.

    1925. Wachstum und Körpergrösse. Die strukturelle Grundlage der Körpergrösse bei vollausgebildeten und im Wachstum begriffenen Tiere. Ergeb. der Anatomie und Entwicklungsgeschichte 26:87–342.

LUSH, JAY L.

    1945. Animal breeding plans. 3d edition. 443 p. Ames, Iowa. The Collegiate Press.

MCNALLY, E. H., and THEODORE C. BYERLY

    1936. Variation in the development of embryos of hens' eggs. Poultry Sci. 15:280–283.

MEHNERT, ERNST

    1896. Die individuelle Variation des Wirbelthierembryo. Morphologische Arbeiten (Schwalbe) 5:386–444.

MUNRO, STERLING S.

    1938. The effect of dilution and density on the fertilizing capacity of fowl sperm suspensions. Can. Jour. Res. Section D, 16:281–299.

    1940. The relation of specific gravity to hatching power in eggs of the domestic fowl. Sci. Agr. 21:53–62.

    1942. Further data on the relation between shell strength, potential hatchability and chick viability in the fowl. Sci. Agr. 22:698–704.

    1946. Relative influence of heredity and environment on fertility and hatchability in Wyandottes. Empire Jour. Expt. Agr. 14:25–30.

MUNRO, STERLING S., and IGOR L. KOSIN

    1945. Proof of the existence of pre-oviposital embryonic deaths in chickens and their bearing on the relation between 'fertility' and hatchability. Can. Jour. Res. Section D, 23:129–138.

NEEL, J. K.

    1942. A calibration of the development of the chick under improved conditions of incubation and the relationship of developmental rate to age of hen and hatchability record. Poultry Sci. 21:294–300.

NICOLAIDES, COSTAS

    1933. Relation of time of laying and embryonic mortality. Poultry Sci. 12:274–276.

NORTHERN SUB-COMMITTEE OF THE NATIONAL POULTRY INSTITUTE ADVISORY COMMITTEE

    1934. Experiments on inbreeding of poultry. G. Brit. Min. Agr. and Fisheries Bul. 83: 1–59.

OLSEN, M. W.

    1942. Maturation, fertilization, and early cleavage in the hen's egg. Jour. Morph. 70: 513–533.

PARKER, JESSE E., F. F. MCKENZIE and H. L. KEMPSTER

    1942. Fertility in the male domestic fowl. Mo. Agr. Expt. Sta. Res. Bul. 347:3–50.

PATTERSON, C. F.

    1930. Good morning eggs are best for hatching. Poultry Item. March 1930.

PEARL, RAYMOND, and F. M. SURFACE

    1909. Data on certain factors influencing the fertility and hatching power of eggs. Maine Agr. Expt. Sta. Bul. 168:105–164.

PRICE, J. B.

    1938. The embryology of the cormorant (*Phalacrocorax penicillatus*) during the period of somite formation. A comparison with the chick (*Gallus domesticus*) and the quail (*Lophortyx californica*). Amer. Jour. Anat. 63:409–447.

PRITZKER, I. J.
  1940. Difference in weight and hatching capacity of eggs laid during different parts of the day. (Trans. title.) Proc. Lenin Acad. Agr. Sci. USSR (6) 41–44. [Cited after Landauer (1941).]

RENDEL, J. M.
  1943. Variations in the weights of hatched and unhatched ducks' eggs. Biometrika 33: 48–58.

RENSCH, B.
  1923. Über die Ursache von Riesen- und Zwergwuchs beim Haushuhn. Ztschr. indukt. Abstamm. u. Vererbungslehre 31:268–286.

RIDDLE, OSCAR
  1923. On the cause of twinning and abnormal development in birds. Amer. Jour. Anat. 32:199–252.

SCHLEINITZ, GRÄFIN VON
  1925. Experimente über nahe Inzucht beim Geflügel. Deutsche Landwirt. Geflügelzeitung 28. See also abst. in Internatl. Rev. Sci. and Pract. Agr. N.S. 4:909.

SCOTT, H. M., and DON C. WARREN
  1936. Influence of ovulation rate on the tendency of the fowl to produce eggs in clutches. Poultry Sci. 15:381–389.

STOCKARD, CHARLES R.
  1921. Developmental rate and structural expression: an experimental study of twins, 'double monsters' and single deformities, and the interaction among embryonic organs during their origin and development. Amer. Jour. Anat. 28:115–277.

STURKIE, PAUL D., and A. G. WILLIAMS
  1945. Studies on pregastrular development, early embryonic development, and hatchability of prematurely laid eggs of the hen. Poultry Sci. 24:546–554.

TAYLOR, LEWIS W., and C. A. GUNNS
  1935. Size of unincubated embryo in relation to hatchability. Poultry Sci. 14:294.
  1939. Development of the unincubated chick embryo in relation to hatchability of the egg. Cleveland, Ohio. Proc. 7th World's Poultry Congress: 188–190.

VECCHI, ANITA
  1936. Osservazioni sugli effetti della consanguineità nelle selezione dei polli. Berlin and Leipzig. VI Weltgeflügelkongress. Kongressbericht 2:86–89.

WARREN, DON C.
  1927. Hybrid vigor in poultry. Poultry Sci. 7:1–8.
  1934. The influence of some factors on the hatchability of the hen's egg. Kans. Agr. Expt. Sta. Tech. Bul. 37:5–42.

WARREN, DON C., and H. M. SCOTT
  1934. Ovulation in the domestic hen. Sci. 80:461–462.
  1935a. The time factor in egg formation. Poultry Sci. 14:195–207.
  1935b. Physiological factors influencing the rate of egg formation in the domestic hen. Jour. Agr. Res. 51:565–572.

WATERS, NELSON F.
  1932. Inbreeding in White Leghorn fowl. Ithaca, N.Y. Proc. 6th Inter. Congress Genetics 2:205–206.
  1944. The hatchability of chicken eggs as influenced by diallel crossing. Poultry Sci. 23:495–496.
  1945. The influence of inbreeding on hatchability. Poultry Sci. 24:329–334.

WATERS, NELSON F., and W. V. LAMBERT
  1935. Ten years of inbreeding in the White Leghorn fowl. Poultry Sci. 14:296.
  1936a. A ten-year inbreeding experiment in the domestic fowl. Poultry Sci. 15:207–218.
  1936b. Inbreeding in the White Leghorn fowl. Iowa Agr. Expt. Sta. Res. Bul. 202:5–55.
  1936c. Inbreeding in the White Leghorn fowl. Berlin and Leipzig. VI Weltgeflügelkongress. Kongressbericht 2:91–93.

WILSON, W. O.
  1948. Egg production rate and fertility in inbred chickens. Poultry Sci. **27**:719–726.
WRIGHT, SEWALL
  1922. Coefficients of inbreeding and relationship. Amer. Nat. **56**:330–338.
  1923. Mendelian analysis of the pure breeds of livestock. 1. The measurement of inbreeding and relationship. Jour. Hered. **14**:339–348.