Resumen por el autor, C. H. Danforth.

Pruebas de que las células germinativas están sujetas a selección,
basadas en sus potencialidades genéticas

El autor ha emprendido una serie de experimentos para probar la posible existencia de diferencias funcionales en las células germinativas dependientes o relacionadas con la naturaleza de los "determinantes" transportados por el plasma germinativo. La existencia de tales diferencias se demostró por la acción de un agente selectivo actuando sobre las células germinativas. Este agente fué el vapor de alcohol, que al inhalarse por los pulmones se cree que pasa directamente al torrente circulatorio, y desde este a los tejidos germinativos; se administró a gallinas de la constitución genética escogida. Como índice de cualquier selección que pudiera ocurrir la proporción relativa de ciertos rasgos, braquidactilia, polidactilia y color blanco, que aparecieron en la prole producida durante los periodos de tratamiento, se comparó con la proporción de los mismos rasgos producidos durante periodos en los cuales dicho tratamiento fué suspendido. Los resultados obtenidos indican que por lo menos en el caso de ciertos rasgos la selección es posible y que es mas rigurosa cuánto mas severo es el tratamiento.

Translation by José F. Nonidez
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EVIDENCE THAT GERM CELLS ARE SUBJECT TO SELECTION ON THE BASIS OF THEIR GENETIC POTENTIALITIES

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INTRODUCTION

A great deal of evidence goes to show that individual differences among adult animals are commonly due to differences in the germ cells from which the animals have developed. In other words, peculiarities in the adult are, in general, indications of peculiarities in the germplasm. It would seem to follow as an obvious corollary of this that the germ cells of a species must be of almost as many classes as are the adult individuals. This being the case, it is perhaps not unreasonable to expect that germ cells should differ more or less widely in their responses to varying conditions and in their ability to function in the production of zygotes. If such an assumption is shown to be justified, it might be invoked to explain several obscure evolutionary tendencies, as well as certain constant departures from normal Mendelian expectations.

As a first step in determining whether or not germ cells carrying factors for a given unit character differ in their physiological responses from germ cells carrying factors for an allelomorph of that character, it seems desirable to work with the sperm or eggs of a heterozygous animal in which the normal proportion of each class of germ cells produced has already been determined. With such an animal all the germ cells are presumably subjected to the same influences, except as those influenced are varied by the experimenter, and consequently a much better control is possible than could be had by comparing results from different homozygous individuals.

The choice of a method of procedure presents a number of difficulties. Attempts to bring special influences to bear on cells within the living body are attended by many uncertainties and, on the other hand, the treatment of suitable germ cells outside the body in most cases requires the development of a special and difficult technique. The desideratum would seem to be the possession of a means of administering definite amounts of reagents to the germ cells without otherwise disturbing their normal environment. Despite the fact that it does not lend itself readily to quantitative investigations, the inhalation method devised by Stockard seems to be the most satisfactory thus far employed. A considerable number of reagents are suitable for use by this method, but ethyl alcohol is the one that has been most completely tested (Stockard, '13; Cole and Davis, '14; Stockard and Papanicolaou, '16; Nice, '17; Pearl, '17¹). For these reasons ethyl alcohol administered by the inhalation method was employed in the experiments reported in this paper.

Pearl ('17) has interpreted results obtained by himself and others as indicating that inhaled alcohol affects germ cells differently according to their vitality, destroying some, injuring some permanently, and producing no more than a transient effect on others. In the case of the fowl, he believes that practically maximum treatment with alcohol and certain other vapors results in an elimination of weaker germs with a consequent rise in the average vigor of the chicks actually produced. In other words, treating the parent with alcohol vapor results in a selection of germs in favor of those which produce the most vigorous young. It was not shown in his experiments that any characteristics other than those dependent on general vigor were affected by the treatment, the Mendelian ratios for the few characters involved being essentially the same in both experiment and control.

At the time when Pearl's papers appeared the writer was just concluding a breeding experiment with poultry in which several

¹ An extended list of titles relating to the whole subject may be found in the paper by Pearl, '17.

Mendelian characters were being investigated. Since some of these characters were not represented in Pearl's material, and the writer was at the time casting about for a method to test for germ-cell selection, it seemed worth while to continue breeding from the same birds with former conditions maintained except for the introduction of alcohol treatment. The work was planned in no sense as a repetition of Pearl's experiments, but rather as an attempt to extend his methods to a few characters not known to be directly dependent upon inherent vitality. The writer takes this opportunity of acknowledging his indebtedness to the valuable and suggestive paper mentioned above.

The first experiment, 1, was made in the spring of 1917 with results that seemed to point to a selective action of the alcohol vapor. The matter is one of such importance, however, that a repetition of the work seemed desirable before publication of the data. Consequently three further experiments, 2, 3, and 4, were carried out in the spring of 1918. As will appear below, the results obtained in the latter work proved to be essentially consistent with the findings in the first experiment, which fact seems to justify their publication.

GENERAL NATURE OF THE EXPERIMENTS

In each experiment a cross was made between normal, pure-bred stock on the one hand and hybrid, heterozygous birds on the other. The hybrid individuals were subjected for certain periods to two daily treatments with alcohol vapor, during which time as far as possible all eggs laid were incubated (table 2). Since the original purpose of the work was to test the possibility of selecting germ cells rather than of modifying them through influences brought to bear on the soma, each experiment is brief and intensive.

A control was obtained in each case by saving the eggs from the same flock, kept under as nearly identical conditions as possible, during a period before or after the alcohol experiment. All eggs that did not hatch were opened and the character of

the contents recorded. Since the eggs were candled frequently many embryos were obtained only a few days after death and consequently in a good state of preservation. Very few of the dead embryos were found to be too macerated to afford the desired data. Some living embryos were purposely taken. For the sake of brevity, data obtained from eggs laid during the periods of alcohol treatment will be referred to as A, those from eggs laid in the control periods as C. Each experiment, therefore, has an A and a C subdivision. In experiments 1, 2, and 3 the heterozygous individuals were males, in 4 they were females.

The characteristics tested for their possible response to alcohol vapor were brachydactyly, polydactyly, and color. The presence or absence of booting (feathers on the tarsi) and the apparent form of the comb were also recorded, but the former has been found to be only another manifestation of the factor which produces brachydactyly, while chicks that have been allowed to develop have shown that comb form cannot, in the material used, be accurately determined in embryos and young specimens. Sex was not recorded. The nature of the peculiarities under investigation may be briefly indicated.

Brachydactyly is a condition described by Danforth ('19) which manifests itself in a more or less pronounced shortening of digit IV of the foot. This shortening involves the length, and in extreme cases the number, of bones in the digit. Nearly all brachydactyl birds are booted, but there is a small percentage that has unfeathered tarsi. Conversely, there is an occasional individual with feather tarsi which is not brachydactyl. Breeding experiments have seemed to establish the fact that these conditions are interchangeable from the point of view of heredity or, in other words, that they merely represent different expressions of one and the same underlying cause. Consequently, a single term may be used to cover all of these phenotypic manifestations, although it must be borne in mind that in a certain small number of cases the word 'brachydactyl' used in this sense is not strictly literal. Brachydactyly may be recognized after about the tenth day of incubation. The

index of brachydactyly² was computed for all chicks that hatched and each booted individual was assigned to one of three grades based on the number of feathers present on the shanks and toes.

Polydactyly, a well-known condition, seems to be the final manifestation of an early disturbance in the developing rudiment of digit I of the foot. The character is quite variable, ranging, in the present material, from a single enlarged hallux to a condition in which the hallux is replaced by two digits with even the occasional indication of a third. The cases may be arbitrarily grouped into three grades, dependent upon the degree to which the peculiarity manifests itself. Rarely polydactyly was found to extend to the wings. The term as here used is a misnomer to the extent that a certain number of individuals which clearly manifest the fundamental character in question really have only the normal number of toes. Polydactyly can be recognized with certainty and probably in all cases after about the seventh day of incubation.

Color in the present paper refers to only two grades, 'black' and 'white'. While the pure-bred birds used were either clear snowy white (Leghorns) or uniform glossy black (Minorcas), a large percentage of the young of mixed ancestry showed some color indication of their hybrid origin. This was particularly true of the white chicks, many of which had one or more small spots of black down. By 'white' may be understood pure white down or feathers or white plumage with only a little black pigment in the form of a few dark spots. 'Black' covers all other shades, even though some individuals classified as black subsequently turned out to be barred or mottled. From the twelfth day of incubation (or even earlier) every chick could be put unhesitatingly in one or the other of these categories.

² The index of brachydactyly is an arbitrary value obtained by dividing the sum of the lengths of the two fourth toes by the sum of the lengths of the two second toes. With brachydactyl chicks this gives a value equal to 1 or less, while with normal chicks the value obtained is more than 1. The division is carried to the second decimal place and the quotient then multiplied by 100 to eliminate fractions.

METHOD OF GIVING THE ALCOHOL TREATMENT

Alcohol was administered by the inhalation method devised by Stockard ('13) in his work with guinea-pigs and subsequently employed by Pearl ('17) in the experiments with poultry. Pearl's technique was followed in its essentials, except that owing to the conditions of the experiments it was possible to use a somewhat simpler inhalation chamber. For experiments 1, 2 and 3 a glazed earthenware crock 20 inches high and 15 inches in diameter, with a measured capacity of 1.98 cubic feet, or about 56 liters, was fitted with a tight cover and a galvanized-iron false bottom. The false bottom was perforated by seventy-three $\frac{5}{8}$ -inch round holes and raised on legs $2\frac{1}{2}$ inches high. The cover was made of thick matched boards subsequently soaked in paraffin and padded along the surface of contact with the crock. A window 8 inches square was cut out of the center and covered by a piece of glass set in paraffin. When a treatment was to be given, the false bottom was tipped on edge and pieces of cotton soaked in 95 per cent ethyl alcohol were placed in the crock. A little additional alcohol was poured in, the false bottom replaced and the cover fitted over the top. At the end of from fifteen to twenty minutes, when the atmosphere was found to be saturated with alcohol vapor, the bird was quickly placed inside on the false bottom, the cover being raised as little, and for as short a time, as possible. Although frequent tests failed to reveal any odor of alcohol on the outside, the whole chamber was generally covered by heavy cloths except when observations were being made through the window. Between each two treatments the crock was cleaned and aired.

From 60 cc. to 70 cc. of 95 per cent alcohol were used for each treatment. The amount that vaporized was roughly determined in the following manner. Fresh cotton was soaked in alcohol and then squeezed as dry as possible; 66 cc. of alcohol were then poured over the cotton and on the bottom of the crock. At the end of an hour and thirty minutes, during which time a treatment was given, the alcohol on the bottom was sopped up and the pieces of cotton squeezed as dry as before.

In this way it was possible to recover 42 cc., leaving a balance of 24 cc., as the approximate amount that had evaporated. The absolute alcohol vaporized into the tank therefore probably ranged between 20 cc., and 25 cc. for treatments lasting about an hour. There was always a rise in temperature amounting to about 3.5°C. during an hour treatment (e.g., on May 21, from 26° to 29.5°), and this of course facilitated evaporation of the alcohol. The treatments were usually begun about 9 A.M. and 5 P.M. Their duration varied considerably, as will be noted below.

With the hens used in experiment 4 a slightly different container was employed for administering the alcohol. Enamelled specimen jars about 15 inches square and approximately 12 inches high, with close-fitting covers, were found to have a capacity of 1.6 cubic feet. These jars were used as inhalation chambers, and one or two hens placed in each after an excess of alcohol had been allowed to evaporate in them for from twenty to thirty minutes.

REACTIONS OF THE ALCOHOLIZED FOWLS

The degree of alcoholization obtained is perhaps best indicated by the observed responses of the birds to this treatment. While a certain amount of resistance or accommodation was acquired as the treatment progressed, it was apparent that the males were throughout less affected than were the females. The alcohol absorbed on each occasion was sufficient to cause at least a mild degree of intoxication. Since the reactions were very constant, the details of a single treatment may be given as illustrative of the typical behavior of one of the males following the inhalation of alcohol vapor.

On May 29, 1917, the inhalation chamber was prepared as usual and male no. 8 treated from 10 A.M. to 11.15 A.M. During the whole time he sat on the false bottom turning his head only when disturbed by objects seen through the window. When undisturbed the front of the head rested against the side of the jar. The nictitating membranes passed over the eyes at the rate of thirty times per minute. Notes on respiration were made as follows:

<i>Time</i>	<i>Rate</i>	<i>Remarks</i>
10.15	40 per minute	Shallow.
10.25	25 per minute	Deeper.
10.50	22 per minute	Deep, regular.
11.15	22 per minute	Deep, but irregular.

At the end of the treatment the bird was breathing noisily and with difficulty. The mouth was open.

11.15. Removed from the tank. Quite passive. Did not struggle.

11.17. Returned to the breeding pen. Stepped very high, but not unsteadily. Showed indications of hyperexcitability. Shied at a feather and jumped out of the way of a chick that approached from behind. The labored breathing improved rapidly.

11.20. Picked up pieces of grass and called hens. Very gallant.

11.22. Crowed repeatedly but abnormally.

11.25. Ate heartily when the flock was fed.

This behavior was quite characteristic of the males following both morning and afternoon treatments. The ability to crow was always affected. Occasionally one was unsteady on his feet and they frequently misjudged distances in attempting to jump or fly. Repeated rubbing of the eyes on the back was characteristic, but despite the severity of the treatment, no whitening of the conjunctiva was noted.

As a control for the above-described behavior, on the afternoon of the same day the inhalation chamber was again thoroughly cleaned and aired and the same male treated exactly as before except that no alcohol was used. He was kept in the chamber from 3.10 to 3.45 P.M. During the first half of this period he sat quietly on the bottom of the container, but during the latter half he stood, possibly owing to the gradual exhaustion of oxygen. Respiration fell from 42 to 30 per minute. The moment the cover was removed from the tank he made violent attempts to escape, struggling and squawking. (Nothing of this sort ever happened following an actual treatment.) Returned to the breeding pen he displayed none of the customary excitability, high-stepping and gallantry towards the hens. After a regular alcohol treatment given an hour later he again showed the usual reactions indicative of intoxication.

It will be seen from the foregoing that the immediate physiological effect of the alcohol upon the males, while unmis-

takable, was nevertheless remarkably slight considering the strength of the treatment. It should be noted in passing that there is the possibility of a mild degree of asphyxiation, but this probably is of no importance in the present connection.

The five hens used in experiment 4 were affected much more severely. They were commonly completely overcome and entirely unable to stand for several minutes after being released from the inhalation chamber. Staggering, which was rarely noticeable in the males, was of regular occurrence with these females. Despite precautions taken to the contrary, all but one of them ultimately died from the effects of the treatment. The one remaining individual developed, or had inherently, a considerable power of resistance.

Aside from the immediate but transient effects of the alcohol, nothing was noted except a slight loss in weight. For example, in the fifty-seven days from February 9 to April 7 no. 28 (treated), with an initial weight of 1803 grams lost 133 grams, while in the next fifty-seven days no. 28 (untreated) gained 160 grams, and no. 27 (treated) fell from 1825 grams to 1695 grams, a loss of 130 grams.

DESCRIPTION OF THE EXPERIMENTS

The method of administering the alcohol and the general character of the obvious physiological responses have already been indicated. It remains only to outline the special features of the individual experiments, of which a condensed summary is given in tables 1 and 2. The control period preceded the period of treatment in experiments 1, 2 and 4, but in experiment 3 this relation was reversed. The surroundings and care of all the flocks during both A and C periods were made as constant as possible. Eggs were put in the incubator in all cases, except 1-C, twice weekly and on definite days, so that no egg had been laid more than four days when incubation commenced.

Experiment 1. (February 15, 1917 to June 2, 1917). The heterozygous parent (male no. 8) was raised at the laboratory from mongrel ancestors. His mother (no. 6), was a monster of the

Pygopagus parasiticus type, which, however, produced only normal young. She was a small hen with a rose comb and the plumage of a barred Plymouth Rock. She was not brachydactyl, polydactyl, nor booted. The father (no. 5) was white with a pea comb and unusually heavy feathering on the neck, suggesting Asiatic blood. He was of moderate size, polydactyl, and booted. This is all that can be said of the ancestry of no. 8, the individual which supplied the traits studied in this paper, but the nature of the experiments is such that a more complete pedigree would be of little additional value. Male no. 8, was hatched in 1914. His coloring was approximately that of a barred Plymouth Rock, but with the breast somewhat spotted and with more or less white in the hackle, saddle feathers,

TABLE 1
Comparative statement of the conditions of each experiment

EXPERIMENT NUMBER	DURATION IN DAYS		DAILY DOSAGE (PART A)		
	Part A	Part C	Maximum	Minimum	Average
1	36	63	2 h. 25 m.	1 h. 30 m.	1 h. 45 m.
2	65	34	2 h. 37 m.	2 h. 0 m. ¹	2 h. 13 m.
3	58	63	4 h. 0 m.	2 h. 0 m.	2 h. 27 m.
4	43	64	2 h. 42 m.	1 h. 8 m.	2 h. 5 m.

¹ This is exclusive of one day when, in the absence of the writer, only a single alcohol treatment was given.

and tail. His comb was large and corrugated, intermediate between rose and walnut. He was polydactyl, grade 1 (left foot only); booted, grade 2, and with a brachydactyly index of 88. In view both of his ancestry and his descendants, it is clearly apparent that this bird was heterozygous for brachydactyly and for polydactyly.

The homozygous parents were thirteen pure bred white Leghorn hens of a standard strain. There can be little doubt as to the purity of this stock, especially as regards the characters under investigation.

In this experiment it was to be expected that the germ cells produced by the females would all be of the same class, while those produced by the male would fall into several classes

depending on whether or not they contained determiners for brachydactyly, polydactyly, or both. Such a situation supplies the necessary conditions for a test of germinal selection. Since Leghorn white is dominant, and all germ cells of the females necessarily carried determiners for this trait, no critical data bearing on color selection were to have been expected from this experiment.

TABLE 2
General summary of data from the four experiments

EXPERIMENT		EGGS			EMBRYOS		CHICKS
Number	Part	Number laid	Incubated	Non-fertile	Killed as embryos	Died in the shell	Number hatched
1	A	150	150	5	39	51	55
	C	362	300	34 ¹	108	72	86
2	A	191	180	2	11	153	14
	C	106	104	1	19	69	15
3	A	151	150	35	14	60	41
	C	166	155	49	12	60	34
4	A	39	39	0	6	13	20
	C	151	150	2	45	76	27
Totals: A and C separately	A	531	519	42	70	277	130
	C	785	709	86 ¹	184	277	162
Totals: A and C added	A + C	1316	1228	128 ¹	254	554	292

¹ As explained in the text, this figure is probably too large, owing to the method of recording in 1-C.

C. The control part of the experiment, running from February 15 to April 18, was not originally intended for this purpose, but rather to supply data for the study of the normal heredity and embryology of several traits, including those considered here. Consequently some of the data, especially those relative to fertility, were not entered in strict accordance with the form adopted in the other experiments. However, this set of data is easily comparable with the other sets and, except for fertility, undoubtedly furnishes a reliable control. Of the 362 eggs laid

only 300 were incubated. The 62 unused eggs were discarded from time to time in small numbers whenever the capacity of the incubator was exceeded, the incubator always being filled from the most recently laid eggs. This method leaves no room for unconscious selection as to size, shape, etc.

A. The alcohol treatment began on April 27 and continued until June 1. Beginning May 1, there were two daily treatments averaging a little over an hour in the morning and half an hour in the afternoon. In this period of thirty-six days male no. 8 was kept in the alcohol vapor a total of 63 hours and 11 minutes (table 1).

Eggs were saved from May 3 to June 2. One hundred and fifty were laid, all of which were used (table 2).

Experiment 2. (March 6, 1918, to June 19, 1918). The heterozygous parent in this case was a male (no. 27) hatched in March, 1917, from an egg used in experiment 1-C. He had the general bearing and many of the characteristics of a Leg-horn. His color was white, his comb large and walnut-rose. He was polydactyl, grade 3; booted, grade 2, and had an index of brachydactyly equal to 75. It will be apparent by reference to experiment 1 that this bird was heterozygous for the three dominant characteristics with which we are concerned, i.e., brachydactyly (including booting), polydactyly, and white color.

The homozygous parents were six single-combed black Minorca hens purchased from a local dealer who gave assurance that they were pure bred and of a stable strain. Their somatic appearance as well as their racial purity showed them to be homozygous for the absence of the three above-mentioned traits, or, in other words, they exhibited the corresponding recessive characters, normal length of toes, normal number of toes, and black color.

In this experiment, therefore, all the germ cells produced by the females were necessarily of one class, that tending to give black chicks with normal number and length of toes, whereas the germ cells of the male were expected to fall into eight classes, viz.:

Those tending to produce chicks that were

1. Brachydactyl, polydactyl, white.
2. Brachydactyl, polydactyl, black.
3. Brachydactyl, not polydactyl, white.
4. Brachydactyl, not polydactyl, black.
5. Not brachydactyl, polydactyl, white.
6. Not brachydactyl, polydactyl, black.
7. Not brachydactyl, not polydactyl, white.
8. Not brachydactyl, not polydactyl, black.

It will be apparent that by the inspection of any chick it could be determined at once to which of these eight classes the sperm cell involved in its production had belonged.

C. The hens began laying on March 6 and eggs were saved for the control experiment from that date to April 8, during which period 106 eggs were produced (table 2). Two of these were accidentally cracked and therefore discarded. One, laid April 3, was non-fertile.

A. The period of alcohol treatment followed immediately upon the control period. The first treatments, of 1 hour and 2 minutes and 1 hour and 4 minutes, were given on April 8, and the final treatment on June 11. This bird was kept in the inhalation chamber for two periods of at least an hour each every day except May 5, when in the absence of the writer an attendant misunderstanding directions gave only a single one-hour treatment. In the period of sixty-five days no. 27 was kept in the alcohol vapor for a total of 143 hours and 56 minutes. He showed the usual responses: hyperexcitability, occasional weakness in the legs, and crowing ability invariably impaired for many minutes. He was removed from the breeding pen on June 12.

Eggs were saved from April 9 to June 19. During this period 191 were laid, of which eleven, unfortunately, were lost through breakage or otherwise (table 2). The two recorded as non-fertile were laid on April 21 and June 14, respectively.

Experiment 3. (February 9, 1918, to June 12, 1918). The heterozygous parent (no. 28) hatched in June, 1917, from an egg used in experiment 1-A. He was very similar to male no. 27 described above; in fact, these two males were selected

because they were more nearly alike than any other two raised in the 1917 flock. They were at least half-brothers, possibly full-brothers. It may be noted in passing that no. 27 was produced before the father had been alcoholized, no. 28 after the alcohol treatment had begun. No. 28 was white with a large walnut-rose comb. He was polydactyl, grade 3+, showing a trace of a sixth toe on the left foot; booted, grade 2, and with an index of brachydactyly equal to 70. It may be observed that brachydactyly and polydactyly were more pronounced in this specimen than in any of the other individuals used in the four experiments.

The homozygous parents were six black Minorca hens similar to those used in experiment 2 and from the same source. In so far as the stock was concerned, the material for experiments 2 and 3 was as nearly identical as it was possible to make it. The two flocks were kept in the same room separated from each other only by a wire partition from floor to ceiling.

A. Alcohol treatment of no. 28 began on February 9 with two one-hour periods and continued till April 7. The length of the treatments gradually, but rather irregularly, increased till on April 7 two periods of two hours each were administered. Throughout the whole time this bird was much more severely affected than either of the other males, even when the treatments were of the same intensity. 'Weak', 'aimless,' 'walked in circles,' etc., are among the notations. As another indication of the somewhat different reaction of this bird it may be mentioned that no. 8, no. 27, and the alcoholized hens showed a tendency not to defecate during the treatments, at least after the first few days. This was not the case with no. 27. No. 8 defecated only once during the whole period and no. 27 only three times, while no. 28 defecated thirty-six times. During a period of fifty-eight days he was kept in the alcohol vapor for a total of 141 hours and 32 minutes (table 1).

The hens began laying February 19, and eggs were saved from that date till April 8, during which time 151 were produced. Of these, one laid February 26 was cracked, the other 150 were incubated (table 2).

C. The control period followed immediately, extending from April 9 to June 12, during which time 166 eggs were laid, eleven of which were lost for purposes of the experiment. The data relative to the remaining 155 are given in table 2.

Experiment 4. (February 20, 1918, to June 12, 1918). The heterozygous parents were five hens (nos. 21, 22, 23, 24, and 26) from the 1917 chicks, and sisters or half-sisters of males nos. 27 and 28. Unfortunately, they were not uniform in type. All were white and all were brachydactyl and booted, but they varied in comb form and in polydactyly. Nos. 21, 24, and 26 were derived from 1-C, nos. 22 and 23 from 1-A. Nos. 23 and 24 were polydactyl, the others were not. The average index of brachydactyly was 91.

The homozygous parent was an exceptionally fine black Minorca cockerel from the same source as the hens used in experiments 2 and 3.

Experiment 4 is a reciprocal of experiments 2 and 3. In this case the male produced only one class of germ cells, while the polydactyl females should have produced the eight classes mentioned above and the non-polydactyl hens the classes numbered 3, 4, 7, and 8. Since individual records were not kept, this experiment yields critical data for only brachydactyly and color.

C. For the control, eggs were saved from February 20 to April 24; 151 were laid, of which 150 were incubated (table 2).

A. Alcohol treatment administered as described in an earlier paragraph was begun on April 27. It was planned to give about three hours a day, but this proved to be more than the hens could stand, and individual treatments were frequently cut down to less than an hour. May 5, by an error, only one treatment was given and on May 31, the morning treatment having been later than usual, it was not thought safe to risk an afternoon treatment. May 7, no. 24, which had recently laid, died immediately after being removed from the inhalation chamber. May 22, no. 22, died in the chamber, and a few days later no. 26 was overcome beyond recovery. Finally, on June 8, no. 23, which had been saved with difficulty on two previous

occasions, was lost. With only one hen left, the experiment was discontinued after the morning treatment of that day. Eggs were saved till June 12, the last four being laid by the sole survivor, no. 21.

Of the six hens originally intended for this work one (no. 25) died February 20 at the beginning of the control experiment and four others were lost as a result of alcohol treatment. While an adequate number of eggs had been secured for C, there were, owing to these accidents, only thirty-nine available for A (table 2). These were all incubated and yielded enough data to justify the inclusion of this, the least satisfactory of the four experiments.

RESULTS OF THE EXPERIMENTS

In the fourth column of table 2 is shown the number of eggs incubated in each subdivision of the experiments. In the following columns the results from these eggs are indicated. Numbers in the fifth column, headed 'non-fertile,' refer to eggs in which no development whatever took place. At the end of three weeks of incubation such eggs were clear and full with a firm yolk; indeed on being broken they presented a more attractive appearance than the average 'store' egg. Only such eggs were counted as non-fertile, except in the case of 1-C where the data were originally secured for another purpose. Here, in a column headed 'infertile or dying during the first few hours,' were entered data that did not differentiate between early death and actual infertility. The other figures in this column, those for 1-A and for 2, 3, and 4, are believed to be strictly accurate.

The sixth column indicating embryos killed needs a word of explanation. The capacity of the incubators was not at all times adequate to care for the available eggs. In consequence, since the necessary data can be secured as readily from a fifteen-day embryo as from a hatched chick, many eggs were opened between the fifteenth and twenty-first days. A few additional embryos were taken in earlier stages.

The next column, 'died in the shell,' shows the number of embryos that failed to hatch. This included embryos of from the first to the twenty-first day, but a considerable number of them represent late stages, many having pipped without being able to escape from the shell. Since fresh eggs were put in one or the other of the incubators every day, the temperature could not be varied to meet the requirements of embryos in late stages, and in consequence many chicks which were doubtless originally strong did not hatch. It will be appreciated that the purpose of these experiments was not primarily to produce viable chicks nor to test the vitality of eggs, but rather to test the transmission of certain traits. To that end the main effort was concentrated on bringing as many embryos as possible to a stage where their peculiarities could be determined, the number that actually hatched (last column) being a mere incident.

As indicated in table 2, a total of 1228 eggs were used, of which 1100 proved fertile. From these 1100 fertile eggs there were obtained in the manner just explained 808 embryos and 292 chicks. One hundred and ninety-four of the embryos died before the end of the seventh day and were therefore useless for present purposes. This leaves 906 embryos and chicks which yielded data of value. For all of these the presence or absence of polydactyly was recorded. Eight hundred and thirty-three of them reached at least the tenth day and furnished data on brachydactyly. Finally, 721 developed sufficient down so that their color could be determined. The distribution of these traits in the subdivisions of the several experiments is set forth in table 3.³

Table 3 summarizes all the pertinent data and calls for only a brief explanation. It will be understood that the sum of the

³ It seems unnecessary to extend this paper by the inclusion of the detailed protocols which would fill a number of pages. Data for eggs laid each day were recorded as well as the date and (known or estimated) age of every embryo that failed to hatch. For the chicks that did hatch the measurements of each toe were recorded, the index of brachydactyly determined and the grades of polydactyly and booting estimated. The data will gladly be put at the disposal of anyone who may wish to make use of them.

numbers under 'polydactyly present' and 'polydactyly not present' is always greater than the sum of the numbers under 'brachydactyly present' and 'brachydactyly not present' in the same series because of the fact that polydactyly can be determined at an earlier age than brachydactyly, and in all cases some embryos have died between the two critical stages. In like manner, color cannot be determined till a still later period, and in consequence the numbers under this caption are still further reduced.

TABLE 3
Distribution of characteristics in embryos and chicks

EXPERIMENT		BRACHYDACTYLY		POLYDACTYLY		COLOR	
Number	Part	Present	Not present	Present	Not present	White	Black
1	A	56	64	37	89	88	0
	C	69	107	73	147	129	0
2	A	52	68	48	79	55	55
	C	26	48	30	47	38	35
3	A	52	45	52	48	53	37
	C	38	51	37	53	48	37
4	A	18	14	(2)	(31)	12	16
	C	48	77	(28)	(105)	63	55
Totals: A and C separately	A	178	191	139	247	120 ¹	108
	C	181	283	168	352	149 ¹	127
Totals: A and C added	A + C	359	474	307	599	269 ¹	235

¹ These totals are exclusive of the figures from 1-A and 1-C.

Since in experiment 4 some of the hens used were not heterozygous for polydactyly, the data entered under that head are not comparable to the corresponding data from the other experiments. They are included here, but in brackets.

In table 4 the data presented in table 3 are converted into such a form as to enable more ready comparison between different parts of the experiments. There is some question as to how this could best be done, but the following method was adopted.

First, as to brachydactyly: it was found that upon adding all the control data together there were 464 individuals of which 39 per cent were brachydactyl. Since this is about the frequency of booting in comparable crosses recorded in the literature, it is assumed that 39 per cent represents the normal incidence of brachydactyly under conditions such as obtain in the control experiments. Next the total number of cases in each group is found and the probable error calculated on the assumption that 39 per cent represents the true incidence. This gives the fourth column in the table—headed 'brachydactyl

TABLE 4
Percentage distribution of characteristics. Compare table 3

EXPERIMENT		BRACHYDACTYL		POLYDACTYL		WHITE	
Number	Part	Observed	Expected	Observed	Expected	Observed	Expected
1	A	46.7 $\pm$ 3.1	39 $\pm$ 3.0	29.4 $\pm$ 2.7	36 $\pm$ 2.9	100	100
	C	39.2 $\pm$ 2.5	39 $\pm$ 2.5	33.2 $\pm$ 2.1	36 $\pm$ 2.2	100	100
2	A	43.3 $\pm$ 3.1	39 $\pm$ 3.0	37.9 $\pm$ 2.9	36 $\pm$ 2.9	50.0 $\pm$ 3.2	50 $\pm$ 3.2
	C	35.1 $\pm$ 3.7	39 $\pm$ 3.8	39.0 $\pm$ 3.7	36 $\pm$ 3.7	52.1 $\pm$ 4.0	50 $\pm$ 3.9
3	A	53.6 $\pm$ 3.4	39 $\pm$ 3.3	52.0 $\pm$ 3.4	36 $\pm$ 3.3	59.9 $\pm$ 3.5	50 $\pm$ 3.6
	C	42.7 $\pm$ 3.5	39 $\pm$ 3.5	40.1 $\pm$ 3.5	36 $\pm$ 3.4	56.5 $\pm$ 3.6	50 $\pm$ 3.7
4	A	56.3 $\pm$ 5.9	39 $\pm$ 5.7			42.8 $\pm$ 6.3	50 $\pm$ 6.4
	C	38.4 $\pm$ 2.9	39 $\pm$ 2.9			53.4 $\pm$ 3.1	50 $\pm$ 3.0
Totals	A	48.2 $\pm$ 1.8	39 $\pm$ 1.7	36.0 $\pm$ 1.7	36 $\pm$ 1.7	52.7 $\pm$ 2.2	50 $\pm$ 2.2
	C	39.0 $\pm$ 1.5	39 $\pm$ 1.5	36.0 $\pm$ 1.6	36 $\pm$ 1.6	53.9 $\pm$ 2.0	50 $\pm$ 2.1

expected.' The values in the third column represent the percentages actually observed in each case with the probable errors calculated for the respective percentages and magnitudes. Thus in 1-A, for example, the chances, as it is generally expressed, are even that in a random sample of this magnitude the percentage of brachydactyly would fall between 36 and 42 (39 $\pm$ 3). The observed percentage is 46.7 with a probable error of 3.1, giving a range of from 43.6 to 49.8. In other words, in the hypothetical case the chances are 7.5 : 2.5 that the value would fall below 42, while in the actual case observed the chances are

the same that the true value (to be obtained from an infinite number of chicks produced under identical circumstances) would fall above 43. This may be taken to mean that the chances against the observed discrepancy being simply a chance occurrence are more than 16 : 1. In 1-C on the other hand the correspondence between observed and expected results is extremely close.

Polydactyly and color are treated in the same manner as brachydactyly, it being assumed on the basis of the controls that the normal incidence of polydactyly is 36 per cent, and on the basis of genetic literature that the incidence of white color should be 50 per cent.

DISCUSSION

In the foregoing sections the purpose and conditions of the experiments have been set forth and the data that they yielded have been presented. We may now examine these data and attempt to interpret their significance. The three characteristics especially investigated were brachydactyly, polydactyly, and color. These will be discussed first.

Brachydactyly. Table 4 brings out the fact that in each instance the percentage of brachydactyly in A is considerably in excess of that in C, the differences in the four experiments being 7.5, 8.2, 10.9, and 17.9, respectively. The results of either of these experiments taken separately would point strongly to the conclusion that alcohol is capable of influencing the percentage of brachydactyly, and the fact that all four of them show such close agreement gives strong assurance that such is the case. If alcohol were without effect, the departures from the normal distribution would not be all in the same direction and the sum of the data from the four A's should show a percentage approximating that from the four C's. Such does not prove to be the case. In the C experiments there were 464 individuals of which 39 per cent were brachydactyl, while in the A experiments there were 369 individuals of which 48.2 per cent were brachydactyl. These figures are sufficiently

large and the difference between the two percentages is sufficiently great to warrant the conclusion that treating parents which are heterozygous for brachydactyly with alcohol vapor results in an increase in the number of brachydactyl offspring produced.

It is perhaps significant in this connection that in the first three experiments, those in which the males were treated, the increase in brachydactyly is roughly proportional to the average daily dosage, the greater the dosage (table 1) the higher the percentage produced. In experiment 4, while the actual dosage was less, it was very apparent that the general physiological effects were much greater, and this fact seems to be mirrored in the more pronounced increase in brachydactyly in 4-A.

It is also of interest that in the three experiments in which the males are involved there is a correlation between the magnitudes of the C's and A's. In other words, if the percentage of brachydactyly is relatively high in one part of the experiment it is also relatively high in the other part, and vice versa. For example, in 3-C the percentage is several points higher than for all of the C's combined, and in 3-A a similar condition obtains in reference to the total A percentage. In 2, A and C are both below their respective averages while 1-A is intermediate between 2-A and 3-A, and 1-C likewise intermediate between 2-C and 3-C. This point will be reverted to in a later paragraph.

The conclusion in reference to brachydactyly that seems justified is that *by treating a heterozygous parent with alcohol vapor of sufficient strength the proportion of brachydactyl to normal offspring can be increased.*

Polydactyly. Three experiments are available for the study of polydactyly. The results of these experiments are not uniform, and when the percentages for all the C's and for all the A's are computed there is found to be an exact coincidence. Such a group of data might well serve to illustrate fluctuations of percentile values in individual samples, and the tendency of these, in a sense provisional, values to approximate the true values as the magnitude or number of samples increases. Such

an interpretation here would imply that the alcohol was without effect on the transmission of polydactyly. While this seems to be the most probable conclusion, there are two points brought out in table 4 that deserve attention. One of them is a phenomenon similar to that mentioned in the discussion of brachydactyly, namely, a positive correlation between the percentage magnitudes of the A and C divisions of each experiment. In 1 are found the lowest values, in 2 intermediate values, and in 3 the highest values. This looks very much as if each of the three males had his own peculiar capacity for producing polydactyl offspring.

The other point mentioned concerns experiment 3, where in A the percentage of polydactyl chicks was 52 which is a most unusual percentage in a cross of this sort. Here an effect of the treatment seems to be indicated, and such a supposition is strengthened by the fact that when the treatments were stopped the percentage dropped to forty. It has been pointed out that in the case of brachydactyly there is some evidence that the amount of rise in the percentage is dependent upon the strength of the treatment. It is possible that the same is true of polydactyly, but that the level required to produce results—the threshold—is higher. Experiment 3-A, which had the highest daily dosage and in which the treated male was most affected, seems to have been the only one that was sufficiently rigorous to produce an effect on the percentage to polydactyl chicks.

For polydactyly it may be said by way of conclusion that the evidence is possibly negative, but that there is some indication that when the alcohol treatment of the heterozygous parent is sufficiently intensive the relative number of polydactyl young is increased.

Color. In experiment 1 the female parents were homozygous for the dominant white characteristic of the Leghorns, while the treated male parent was a homozygous dark recessive. Since each parent could produce only one kind of germ cell, there was no chance for selection and the expectation of 100 per cent of white chicks was realized in the 217 individuals whose color was determined. In the other three experiments

the treated birds were all white and consequently, since they were derived from the cross made in experiment 1, heterozygous for color. These individuals should have developed two kinds of germ cell with reference to their color-producing potentialities, thus affording material for selection. Tables 3 and 4 show the results obtained. They do not reveal any obvious effect of the alcohol unless it be in experiment 3, where the percentage in A is considerably above what one would expect. 3-C also shows a high percentage, and when it is recalled that C followed immediately upon A, the probability that the high percentage in both cases is due to a common cause rather than to chance is somewhat increased.

Except in 4-A, where the total number of individuals involved is only 28, the percentage of white chicks does not fall below 50 in any of the six separate experiments, the average being over 53 per cent. This is rather close to expectation, but the constant upward tendency of the white is at least noticeable and suggests a possible inherent superiority of the white producing germ cells.

No very certain conclusion seems warranted in regard to the effect of alcohol treatment upon the transmission of color but in the experiment in which the treatment of the male was most severe there is some indication that the germ cells bearing determiners for the dominant character functioned more commonly.

In reviewing the four experiments, no. 3 will be seen to have yielded the most striking results throughout. In this experiment, not only did the three characters, brachydactyly, polydactyly, and color, show indications of the action of alcohol vapor upon the germ cells, but the fertility of the eggs was also markedly affected (table 2). This latter point is of interest in connection with Pearl's results. One of the most constant features reported in his paper is a regular elevation of the percentage of infertile eggs in his various alcohol series. When the present work was begun similar results were expected, but as will be seen by reference to table 2, they were realized only in experiment 3.

There are several considerations that may serve to explain this discrepancy. Pearl studied the effects of alcohol acting over long periods, while in the present work it was desired to have the treatments extend over the shortest possible periods consistent with securing a statistically sufficient number of eggs. Since Arlitt and Wells ('17) have shown that in the rat testis cells in different stages of spermatogenesis are affected differentially by alcoholic poisoning, it may be surmised that injury done to cells in early stages of gametogenesis may not show any effects in breeding tests for relatively long periods. It is possible on this assumption that Pearl's data were taken mostly after the full effects of the alcohol had been established, while most of my experiments were stopped too soon to get the later results. The data from experiment 3 are in harmony with this supposition, for in C, which followed immediately after A, the percentage of infertile eggs was actually higher than it was during the time when alcohol was being administered.

Another possibility that may be mentioned is differential susceptibility to alcohol. It might be inferred from Pearl's paper that one hour is practically the maximum time that a fowl can live in an atmosphere saturated with alcohol vapor. The males used in experiments 1, 2, and 3 were able to endure much more than that, but of these three males no. 28 showed unmistakable signs of being most severely affected. It may be, therefore, that although stronger dosages were administered, the actual physiological reactions were less except in experiment 3.

That male no. 28 was not naturally infertile is shown by his subsequent history. During July and till the 10th of August he was mated to no. 21, and this hen gave no infertile egg till August 26, sixteen days after having been separated from the male. Aug. 10, he was mated to eight black Minorcas, the remnants of experiments 2 and 3. Eggs from this mating were saved beginning Aug. 19. Despite the fact that during much of the time the weather was unfavorable and the male was moulting, out of the 101 eggs laid only three were infertile, in marked contrast to the more than 27 per cent of non-fertile eggs during the experiment. Since the number of hens in the

original experiment was smaller than that used in the supplementary test, it does not seem likely that there was a dearth of sperm at any time during the experimental periods, but more probable that some of the eggs were entered by sperm too badly injured to develop a pronucleus or at least a viable conjugation nucleus. An egg 'fertilized' by such a sperm would very likely give no other reaction than an egg that had been reached by no sperm whatever, and would be recorded as non-fertile.

Pearl's statements as to the non-effect of alcohol on the transmission of Mendelian characters are clearly meant to apply only to the data he presents, and those data were derived from experiments obviously neither intended nor adapted for the solution of the problem attacked in this paper. Since he dealt with crosses between pure-bred homozygous strains in which all the Mendelian characters were in stable equilibrium, there was no chance for selection except on the basis of such characters as vitality and vigor. With reference to these characters, Pearl found selection to be possible. In the experiments now being reported the material afforded a chance for selection between other traits, in this case Mendelian, and the results are believed to show that here also selection is possible.

The question still remains as to the precise nature of the selection that takes place. Cole and Davis ('14) have produced evidence that with rabbits the sperm of one male may have greater fertilizing capabilities than that of another, even when conditions would seem to be more favorable for the latter. They have also shown that the fertilizing power of the sperm can be influenced by poisons administered through the male soma. But in their work also, homozygous males seem to have been used and no evidence is presented as to whether or not two kinds of sperm produced by the same male could be differentially affected. These and other results obtained by Cole and his collaborators, Stockard's findings, and the conclusions reached by Pearl, all tend to suggest that the effect of poisonous reagents is lethal rather than stimulating, if such is the case, we may assume that a germ cell or nucleus bearing a determiner for brachydactyly is more resistant to the effects of alcohol treatment than one not bearing such a determiner.

On this assumption it might be expected, although it need not necessarily follow, that germ cells carrying determiners for two or more characters of selective value would be more favorably circumstanced than those carrying only one such determiner. The available data is not sufficient to throw much light on this question, but so far as it goes it would seem to indicate that such may be the case. For example, in 3-A the combination brachydactyl-polydactyl-white is represented by sixteen individuals where the expectation is thirteen on the basis of observed percentages and only six on the basis of expected percentages.

The question as to the time in gametogenesis at which the selection takes place must also be left unsettled. The purpose of these experiments was to try to select between mature germ cells, and this seems to have been accomplished, but there is also some indication that the effects of the treatments have persisted for a period greater than the probable life of such cells. Moreover, there is some evidence, too meager perhaps to be given much weight, that ova, or their nuclei, are likewise selected. This may mean that the alcohol is effective as a selective agent as far back as the first maturation division and possibly determines at that time which nucleus will remain in the egg of the female or which will be the more effective sperm produced by the male. In this connection may be recalled the finding of Arlitt and Wells (*loc. cit.*) that, in the rat at least, the alcohol affects stages of spermatogenesis in the reverse order, attacking late stages first, and early stages last.

There is one interesting by-product of these experiments which should perhaps be further emphasized, the indication of individual idiosyncracies in the transmission of traits. Among breeders a belief in 'prepotency' has occasionally had currency, but the possibility of this supposed phenomenon being real is often discounted by geneticists. When the data for these experiments were being tabulated it was observed with some surprise that they might be interpreted as furnishing evidence of something analogous to prepotency. It would

not be inconsistent with the conclusions of this paper if it should turn out that within certain limits heterozygous individuals may produce functional germ cells of reciprocal classes in different proportions, the average ratio for all individuals being in most cases about 50 : 50, but in brachydactyly, for example, 39 : 61. Such a tendency, unless very marked, would be masked in ordinary breeding experiments since individual departures would be attributed to chance and lost in the totals. The data in the present paper happen to be so arranged that there are eight opportunities to note the correlation between percentages produced by the same male parents in two different tests, and in all of these cases the correlation is positive. This is a matter that seems to be worth investigating and these data suggest a favorable method for attacking the problem. To test the question properly, many matings between pairs or small groups of animals of proper gametic constitution should be made, and the data from these matings broken up into blocks that could be compared with each other and with similar blocks from other matings.

SUMMARY AND CONCLUSIONS

These experiments were planned to test the possibility of selection between the different classes of germ cells produced by a heterozygous parent. For a selective agent alcohol vapor, which, inhaled through the lungs, is believed to pass directly into the circulation and thence to the fluids surrounding the germinal tissues, was administered to fowls of the desired genetic constitution. As an index of any selection that might occur, the relative proportion of certain traits, brachydactyly, polydactyly, and white color, appearing in the offspring produced during periods of treatment was compared with the proportion of those traits produced during control periods. The results indicate that with at least some traits selection is possible and and that it is more rigorous the more severe the treatment.

Since, under certain conditions, here artificially produced, it appears that germ cells with different genetic potentialities

react differently, a possibility of far-reaching importance is suggested: that, even under normal conditions, the genes which determine the genetic potentialities of a germ cell may have a real survival value for that cell and, moreover, that the prevalence of certain traits appearing in the adult may be in the final analysis largely regulated by the advantage or disadvantages that the determiners for such traits confer upon the germ cells in which they chance to be lodged.

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