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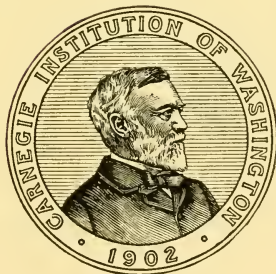
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## DEPARTMENT OF EXPERIMENTAL EVOLUTION.\*

C. B. DAVENPORT, DIRECTOR.

The principal advances of the year have been in demonstrating a chemical basis for sex-differentiation, a subject to which we have paid special attention during the past few years; in opening up new types of inheritance-behavior in the cenotheras; in completing the demonstration of duplicate determiners for a single character in *Bursa*; in discovering the first case of sex-limited heredity in plants; in demonstrating the method of inheritance, in man, of tendency to periodic "fits" of temper and certain other elements of the hysteria-complex.

### STAFF.

The Director divided his time for research between breeding experiments with poultry, canaries, sheep, goats, and cats, and the study of data secured by trained field workers on certain points in human heredity. On the occasion of attending a meeting of the American Breeders' Association at Columbia, South Carolina, he was able to make additional studies on the heredity of skin pigmentation in negro-white crosses, the results of which are incorporated in a memoir on this subject about to appear as Publication No. 188. Dr. G. H. Shull has found that the time demanded for his experimental work has prevented him from completing the Burbank manuscripts and it has been arranged that he shall devote a season to the completion of that manuscript. He will probably be absent from the Station for twelve months. Dr. Shull's green-house assistant, Mr. Leo Macy, is to be given leave of absence for further study, and Mr. C. W. Crane, who has been in training during the present year, will take his place. During this summer, Dr. G. H. Shull also was assisted in his fertilizations and records by Mr. William F. Friedman. Dr. J. A. Harris was absent during several months of the winter working at the Desert Laboratory. Meanwhile, his three assistants and computers were preparing data from the harvest of the preceding season. His work is largely of a statistical nature and has included the rearing and observation of scores of thousands of seedlings. Dr. R. A. Gortner has had charge of the biochemical laboratory, and besides continuing his studies in melanin has cooperated in various researches with Doctors Banta, Blakeslee, and Harris. Dr. A. M. Banta has had charge of the experiments with the cave organisms and has made other studies on the relation of organisms to light, notably the production, by selection, of races that differ in responsive-

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\*Situated at Cold Spring Harbor, Long Island, N. Y. Grant No. 860. \$91,854 for investigations, maintenance, and construction during 1913. (For previous reports see Year Books Nos. 3-11.)

ness to light. Dr. A. F. Blakeslee, of the Connecticut Agricultural College, Storrs, has worked at this Station, as Research Associate, on sex and mutations in molds, in part in collaboration with Dr. R. A. Gortner.

Of the more temporary appointments, Mr. H. D. Goodale resigned in January to accept a position as research biologist in the Massachusetts Agricultural Experiment Station, Department of Poultry Husbandry. Dr. G. C. Bassett began in July, as a guest of the Laboratory, his work on alcoholism and mental degeneracy in rats.

To relieve the Director of details of accounts, supervision of workmen, and purchase and care of supplies, the office of Superintendent was created and Mr. G. H. Clafin began work in that capacity in February 1913.

#### REPORTS ON INVESTIGATIONS IN PROGRESS.

##### CHEMICAL BASIS AND HEREDITY OF SEX.

###### *Sex in Molds, by A. F. Blakeslee.*

The principal new work upon sex has been with molds, by Dr. A. F. Blakeslee, and with *Lychnis*, by Dr. Shull. Dr. Blakeslee's efforts to discover which of the sexual races of mucors, provisionally designated respectively as plus (+) and minus (-), is actually male and which female met with success. He had previously discovered that among the white molds the majority of the species are dioecious, with the male and female races indistinguishable from each other except by a frequently greater vegetative luxuriance of the one race provisionally called plus (+) over the other provisionally called minus (-). Since the gametes formed by these plus and minus races are morphologically equivalent and the offspring are borne and nourished equally by the two sexes, no means were at hand to decide which of the two sexual races was actually to be considered male and which female. However, certain hermaphrodite species were known in which the gametes are regularly unequal. It was thought that if one of the two sorts of races could be induced to show a sexual reaction with the larger or female gamete of the hermaphrodite and the other race with the smaller or male gamete it would be possible to replace the provisional signs + and - by male ( $\sigma$ ) and female ( $\varphi$ ) or *vice versa*. Only two species have been found that show the sexual reaction desired. The difficulties in technic involved are great, but these have been satisfactorily overcome and it has been definitely determined that the plus (+) race shows a sexual reaction with the smaller or male ( $\sigma$ ) gamete, while the minus (-) race shows a reaction with the larger or female ( $\varphi$ ) gamete. The vegetatively more luxuriant race, therefore, may be considered female and the less luxuriant race male. Additional facts obtained by growing cultures in large quantities seem to warrant formulating the general law that whenever

a difference exists in vegetative luxuriance between the two sexes in these forms it is always the female ( $\varphi$ ) that is the more luxuriant.

The general results of the foregoing investigation were presented in a paper read by Dr. Blakeslee at the Cleveland meeting of the American Association for the Advancement of Science and are summarized in the issue of *Science* for June 6 by Dr. Blakeslee. The detailed results are to be incorporated in a paper now in preparation on "Sexual reactions between unlike species."

The argument in the foregoing publications is based upon the sexual character of unequal gametes in heterogamic hermaphroditic species. Since the sexual nature of these gametes has been recently called in question by Gruber and Atkinson, Dr. Blakeslee found it necessary to make a careful study of all the heterogamic forms available, following the process of sexual reproduction in living material—a procedure that apparently has not been satisfactorily attempted before. The results are given in a paper by Dr. Blakeslee published in the April issue of the *Mycologisches Centralblatt* and seem amply to warrant the line of argument already mentioned.

Dr. Blakeslee had previously shown that if a given dioecious species (*Mucor* V) be taken as a standard, its male ( $\sigma$ ) race will show a sexual reaction with the female ( $\varphi$ ) races of all the other dioecious forms under cultivation, but not with the other male ( $\sigma$ ) races. Conversely, its female ( $\varphi$ ) race shows a sexual reaction with male ( $\sigma$ ) races only. The conclusion seems unavoidable that all the female ( $\varphi$ ) races have something in common which elicits a similar response from the male ( $\sigma$ ) race of *Mucor* V and *vice versa*. That this common element is of a chemical nature and therefore demands a chemical study is a natural inference. There are no other forms known that seem to offer a suitable subject for such a chemical investigation. Other available organisms in which there are separate male ( $\sigma$ ) and female ( $\varphi$ ) individuals show a sexual division of labor in that the female ( $\varphi$ ) has the feeding function predominately developed, either furnishing food to the egg or to the developing offspring. Any chemical differences, therefore, between male ( $\sigma$ ) and female ( $\varphi$ ) individuals or between sex-cells found in these forms would have no necessary connection with the sex differences, but rather would be connected with the one-sided nutritional function of the female ( $\varphi$ ) sex. In the *mucors*, however, the sex-cells are morphologically equivalent and the male and female plants take an equal share in feeding the developing offspring.

*Chemical Differences between the Sexes, by A. F. Blakeslee and R. A. Gortner.*

Starting with the hypothesis that sex difference might be a difference in the proteins of the two sexes, a beginning was made with the blood tests for proteins. As yet work has been done only with

the precipitin reaction. Considerable time has been consumed in acquiring proper technic for the preparation of the mold proteins and in overcoming the difficulties due to the fact that the "press-saft" (expressed juices) which they wished to inject is more or less toxic. However, rabbits were immunized to the plus (+) and minus (-) races of two species of mucors. In both cases, the plus (+) "press-saft" when mixed with the plus (+) immune serum gave a stronger precipitate than the minus (-) "press-saft" with the plus (+) serum, and *vice versa*. The difference shows only in the higher dilutions, but this is what one would expect upon assumption of the presence of species proteins common to both sexes of a given species in addition to a separate sex protein or proteins—the large amount of precipitate caused by the species proteins making the difference due to the sex proteins. While the results so far obtained will not yet warrant the assertion that Dr. Blakeslee and Dr. Gortner have found a protein responsible for sex, still the tests, in so far as they go, are all consistent with such a conclusion. Furthermore, there is some slight evidence that the same sex proteins occur in different species. This would be a natural inference from the fact that the plus (+) races show sexual reaction with minus (-) races of other species and *vice versa*.

*Sex in Lychnis, by George H. Shull.*

In his studies on the heredity of sex in *Lychnis* Dr. Shull has discovered a sex-limited character—the first that has been described for plants. This character is broad-leaf, the determiner for which is, it is concluded, associated with the determiner for femaleness. In making this study 60 pedigreed families of *Lychnis* were grown to test completely the genotypic character of individuals making up the second generation descended from a cross between a normal broad-leaved female and a narrow-leaved male. The F<sub>2</sub> family in question consisted of 32 broad-leaved females, 11 broad-leaved males, and 6 narrow-leaved males. The analysis has had the expected result and has shown that there were really four kinds of individuals in the F<sub>2</sub> generation, namely, 16 homozygous broad-leaved females, 16 heterozygous broad-leaved females, 11 heterozygous broad-leaved males, and 6 homozygous narrow-leaved males.

These results accord exactly with expectation on the basis of an assumed association of the determiner for broad leaves with the determiner for femaleness and of a double sex- (femaleness) determiner in the female and a single one in the male. The result thus serves to confirm Dr. Shull's conclusions derived from the breeding of hermaphrodites, that the female is homozygous, while the male and also the hermaphrodite are heterozygous for the sex-determiner.



One result expected by Dr. Shull has failed of realization, so that a perplexing situation has been created which, however, in no wise invalidates the conclusions to be derived from the facts given above. It was predicted that in the crosses between heterozygous females and narrow-leaved males there would appear a new type—narrow-leaved females. This prediction has not been fulfilled. Although in the past Dr. Shull's cultures of *Lychnis* have contained an average of about 62 per cent females, the 37 matings between broad-leaved females and narrow-leaved males whose offspring have been grown this year have produced only 14 females in nearly 2,700 individuals, or a little over 0.5 per cent. Eight of these females occurred in a single family in which no narrow-leaved plants were to be expected, because the mother was homozygous for broad leaves. Only two females occurred in families which also contained narrow-leaved plants, and these two chanced to be broad-leaved. One narrow-leaved female did appear, but in a family in which it was not due theoretically to appear. This plant differed so profoundly from all his other specimens of *Lychnis* that it is considered likely that it will be found to be a mutant of an interesting nature. To offset the 31 families in which not a single female occurred, in four families there was not a single male or hermaphrodite. Three of these families were the total progeny of a single hermaphrodite crossed upon three unrelated females.

A mating between a heterozygous, broad (narrow) female and an unrelated broad-leaved hermaphrodite has produced a progeny consisting of 67 broad-leaved females, 15 broad-leaved hermaphrodites, and 9 narrow-leaved hermaphrodites, showing that this unrelated hermaphrodite parent was a heterozygote in the broad-leaf character—an independent origin of this interesting mutation. The males of any race of *Lychnis* might have the broad-leaf determiner lost from the male determiner, and such a condition could be discovered only when a female is found in which there has been a similar mutation in a female determiner. Appreciating the importance of this possibility in interpreting the origin of the first narrow-leaved male, Dr. Shull has made crosses between the males of all his independent races of *Lychnis* and heterozygous females to ascertain how generally the determiner for maleness is lacking the broad-leaf determiner.

*Dependence of Secondary Sex-Characters on the Germ-gland in Poultry,*  
by H. D. Goodale.

Dr. Goodale continued the work described in the last Year Book, page 87, both at this Station and at Amherst. Castrated drakes do not assume the summer plumage. All males which assumed the summer plumage, although previously castrated, have been dissected and, in each case, a greater or less amount of testicular material has been found. It has not been deemed desirable, at the present, to kill those which

have not assumed the summer plumage. Among the females there was one individual, castrated late last fall when four weeks of age, that is particularly interesting for two reasons. First, she has never developed the duck's voice. Her usual voice is quite like that of the drake, and even when especially stimulated, she only succeeds in producing a sort of half quack. This bird did not assume the entire plumage of the male, a few brown feathers remaining in the breast, but after a molt early in the summer assumed the *summer* plumage of the drake. Just at present she is undergoing a second molt and this time is assuming the normal breeding-plumage of the male. This is the first case that Dr. Goodale has found to behave in this way. Another duck developed the plumage of the male perfectly, but, although molting, did not take on the summer plumage of the male.

In respect to his experiments with fowl Dr. Goodale reports as follows:

Altogether, I have had eight pullets which have developed the male plumage to a greater or less extent. One individual which was castrated last summer was reported as having started to develop the male's plumage, and then after a time was found to have begun reverting to the female's. An examination was made, and it was found that there was a bit of ovarian tissue attached to the posterior side of the oblique septum. There were also three ova, each about 10 mm. in diameter, lying on the original site of the ovary. The ovarian tissue attached to the transverse ligament was removed, but the other left in place. During the present summer it was observed that this bird again began to develop the male plumage, and, to all appearances, the complete male plumage would have eventually replaced the female. On autopsy it was found that all trace of ovarian tissue was lacking.

Two other females developed the *complete* plumage of the male, but the combs and wattles still remain small. This plumage includes a cock's tail which was lacking in the first bird in which these results were obtained. Except for the small comb and wattles, these birds are much better specimens than the original.

Observations have been continued on the two capons which were castrated in the spring of 1911. Their combs and wattles remained small for 18 months, but after this period they grew considerably, and now have reached a size about that of a Brown Leghorn pullet just before she commences to lay. Early in the spring, when 2 years old, they were observed to crow, although this reaction occurred only a few times. In the absence of normal males, they tread the hens. It is important to note that these responses developed late in life and that externally the birds were capons for over 18 months, or half again the usual age for keeping capons.

#### MUTATIONS AND ABERRATIONS.

No student of evolution by experimental methods can doubt the importance of mutations. Our work has shown that they are sometimes due to irregularities in the behavior of the chromosomes during maturation, and the hypothesis seems warranted that they are always

so due. However, through lack of a cytologist, we have not been able to test this hypothesis upon all of our mutants. We have merely been able to observe their origin and inheritance but not yet to induce them nor to study their cytological basis.

*Variation in Lychnis and the Chlorophyll Factors, by G. H. Shull.*

In 10 families derived from crosses between sibs, albinos have constituted almost exactly one-fourth of the offspring, the total numbers being 713 green to 240 albino. As many other sib-crosses in the same stock have produced only green offspring there can be no doubt that this result is due to the existence of a fundamental Mendelian factor for chlorophyll-production.

Two light-green races of *Lychnis dioica* have been discovered, each of which is recessive to the ordinary dark-green strains. When members of these two light-green strains are crossed together their  $F_1$  offspring are uniformly dark green. In the  $F_2$ , which has been grown extensively this year, the light-green types reappear in approximately the expected ratio, 9 dark : 7 light. It is not certain what should be the result when the determiners for both of these pale races are absent, and it is possible that this ratio should be 9 : 6. Extensive back-crossing upon the pale types must decide this question, and the necessary crosses have been made for this purpose.

The study of several kinds of variegation has been continued, and material has been secured for testing the results of breeding from variegated males possessing a type of variegation which has proved to be readily transmissible through the seed-parent, but whose effect through the pollen-parent is as yet unknown. A golden variegation discovered two years ago was found to be slightly inheritable through the male as well as strongly inherited through the female.

*Ascidia in Fraxinus, by G. H. Shull.*

In continuation of his experiments recorded at page 88 of Year Book No. 11, with the pitcher-leaved ash, Dr. Shull reports that the two pedigrees representing crosses between a normal and a pitcher-leaved ash, and between two abnormal (*i. e.*, pitcher-leaved) ash trees, have together produced about 40,000 leaflets this year, each of which has been carefully examined for pitchers, and its position and the character of the ascidium, if present, recorded. The cross between normal and abnormal, which produced no abnormal leaflets during the first season following the germination of the seeds, has this second season displayed ascidia on 26.9 per cent of the trees, and in the family produced by crossing two ascidiate trees pitchers have developed on 58.7 per cent of the trees. A still larger percentage of abnormal trees may be expected as they grow older and become more vigorous, and it will not be surprising if in the end there should appear



approximately the 50 per cent and 75 per cent, respectively, which might be expected on the assumption that the ascidia are produced by a dominant Mendelian determiner.

*Abnormalities in Seeding Beans and in Fruits of Passiflora*, by J. A. Harris.

These studies are being continued as outlined in previous reports and are being lined up with the selective elimination, fertility, and physico-chemical studies. Material progress in the experimental control of the occurrence of certain of these phenomena by physical and chemical means has been made during the year.

*Mutations in Mucors*, by A. F. Blakeslee.

The chief work planned by Dr. Blakeslee was to induce the production of mutations in mucors by chemical or other forms of stimulation. He was thus led to make extended preliminary studies and to devise new methods. His report on this part of the work is as follows:

A standard medium was considered necessary that could be taken as representing a favorable or normal substratum, and some 308 different combinations of nutrients (natural and synthetic) were tested. It was discovered that the mucors were unlike the green molds (*Penicillium*) in that they would not grow well on liquid media nor upon synthetic media in which the necessary elements were supplied in relatively simple chemical substance. An agar nutrient with 2 per cent dry malt extract, 2 per cent dextrose, and 0.1 per cent meat peptone was eventually adopted as a standard medium sufficiently near the optimum value for most forms investigated and as nearly constant in chemical composition as any tested upon which the molds would thrive.

Sexual reproduction in the mucors demands in general the optimum cultural conditions, and the abundance of zygosporangium formation on a given substratum has been used accordingly as an index of its cultural value. Phycomyces is one of the most demanding forms so far as its requirements for zygosporangium production is concerned and in consequence is the species which has been most used in the nutrient tests. Peptone, although the usual form in which nitrogen is supplied in culture media, was found to have a slightly inhibitory action on zygosporangium formation. Meat peptone seemed to be less toxic than either peptone from egg-albumen or Witte's peptone. Nutrients containing peptone which would allow only a poor growth with entire suppression of zygosporangia could be changed into optimum substrata with luxuriant zygosporangium production by inoculating them with bacteria either before or at the very time of planting the sexual races of the mold. The subject needs further investigation before publication. It is not unlikely, however, that by tryptic action of the bacteria the peptone is further broken down into a condition less toxic and more available to the mold. This seems to be the case in the preparation of media upon which the leprosy organism has recently been successfully cultivated. The action of bacteria may explain why a decoction of horse dung has been found to be one of the best substrata for zygosporangium production.

A standard medium having been decided upon, it became necessary to provide against contamination from germs of foreign species in the pedigree cultures. An isolated culture room containing a pure-culture chamber,

within which was placed an inoculating box—all of which were sterilized before using—reduced contamination during inoculation to a minimum, as shown by the presence or absence of growths in Petri-dish cultures kept exposed while all inoculations were in progress. A record of two hours' inoculating, followed the next day by one hour's inoculating, without a single viable germ falling upon an exposed Petri dish 10 cm. in diameter, shows the value of the precautions taken. Contamination is likely to occur in Petri dishes *after* inoculation, and, to avoid this, "roll tubes" were devised from 0.5 gallon cylindrical specimen jars and were used throughout the investigation whenever "separation cultures" were made to obtain growths from individual spores. A note on this new method of separation cultures is being prepared for the journal "Phytopathology."

The preliminary work in formulating a standard medium and in devising means to prevent infection from outside sources has consumed considerable time, but has been necessary to insure accurate and credible results.

Before testing the influence of external stimuli upon the possible formation of mutations, all of my 24 mated dioecious species as well as 10 hermaphroditic forms (a total of 58 cultures) were separated out, so that each strain investigated could have a single spore as its origin. A preliminary investigation of the growths resulting from single spores of these forms showed that species in particular—an hermaphroditic form—that gave such a relatively great variability in its progeny that it has received most attention. I have cultures of this species from four different localities and have examined separately the growths from individual spores to the numbers indicated from the following sources:

Cambridge, Massachusetts.....	13,740
Geneva, Switzerland.....	1,925
Daytona, Florida.....	364
Waverly, Massachusetts.....	6,943
Total colonies examined.....	22,971

Of these 22,000 and more individual colonies examined, those that seemed different in appearance from the normal type have been isolated to the number of some 25 or 30. Among the aberrant characters observed are absence and increase or decrease of zygosporangium production, peculiarities in color, density, and rapidity of mycelial growth, differences in height of sporangial filaments, the almost exclusive production of yeast-like cells in place of a filamentous mycelium, and the production of a filamentous mycelial growth devoid of sporangia. Some of these variants are surely temporary conditions, for they eventually tend to revert to the normal type. Others may be more permanent, but have not been sufficiently investigated. All, however, tend, partially at least, to reproduce the new characters and some have for several sporangial generations kept their peculiarities in gross cultures during the few months it has been possible to propagate them. Many of them would undoubtedly be described as distinct species by specialists in the group. Of these and other molds, several hundred test-tube cultures have been made up with nutrient, to which various poisonous chemicals have been added, but time has not yet admitted a test of the effect of these chemical stimuli on the offspring. Enough has been done, however, to show the extreme lability of at least a single species under normal conditions of cultivation.

*Mutations in Poultry, by C. B. Davenport.*

While mutations hitherto observed in mammals (Castle) and insects (Morgan) are usually due to the loss of a determiner, so that the new condition is "recessive" to the former, in poultry the reverse is frequently the case. Thus all modifications of the feet—extra toes, syndactylism, absence of toe-nails—are dominant over the typical condition. It is as though some extra determiner had entered the mechanism and caused an irregularity of the developmental processes in the feet. Also the absence of tail is a positive character; there is an inhibitor to the development of this organ. One of the most instructive instances of this sort of mutation is seen in the abnormalities of feathering in poultry. Apparently all parts of the outer covering of a bird had primitively the potentiality of producing feathers. But secondarily this power has been lost in specific areas. The shanks are devoid of feathers in many races, and when an individual of any such race is mated with one of a shank-feathered race the offspring are clear-shanked or nearly so; the inhibitor is derived in the progeny from the clear-shanked parent, but the inhibition is not perfect. I was able to get some bare-necked poultry and found, on crossing them with the ordinary type, that half of the offspring have naked necks. Here again the absence of feathers is due to a specific inhibitor to the growth of feathers on the neck. Indeed, it is probable that there are at least two inhibitors (for different areas of the neck), since two types of "bare neck" occur.

## BIOCHEMICAL PROCESSES IN HEREDITY.

The internal factors that direct the course of development are the determiners; and it is by virtue of the fact that we can—through crossing—combine determiners at will, that we are able to make new and improved breeds. But between the determiners in the germ-cells and the adult characters is a great gap and it is this gap that chemical studies carried on here seek to fill.

*Chemistry of Ontogeny, by R. A. Gortner.*

Dr. Ross A. Gortner has planned an extensive investigation of the chemistry of ontogeny. For this work the adjacent State fish-hatchery affords excellent facilities, for the use of which we are indebted to the State Commissioners and to Mr. Charles H. Walters, foreman. Trout eggs in five stages of development were used to ascertain whether the chemical compounds that are present in the egg enter the growing tissues in the form in which they are laid down in the egg or whether there are synthetic changes taking place so that the material which is present in the egg is used, not in its original form, but in a modified condition. It seemed to Dr. Gortner that synthetic action must take place, for otherwise we must think of

the egg proteins, the ovalbumen, ovomucoid, ovolobulin, etc., as containing not only all of the amino acids necessary for the formation of such complex proteins as the hemoglobin, but also that each amino acid is present in the egg in *exactly the quantity which will be later needed by the growing organism.*

His results show conclusively that synthetic action *does* take place, that the simpler mon-amino acids are in some manner transformed into the more complex constituents of the cell nucleus. Aside from this synthetic action it was found that there was a selective utilization of the nitrogen fractions which were "burned" to furnish the energy of development (*Entwicklungsarbeit*). Only 25 per cent of the expected "amide nitrogen" was utilized, only 50 per cent of the expected "arginine nitrogen," only 75 per cent of the expected "lysine nitrogen," none of the "cystine" or "histidine nitrogen," while the deficit caused by the non-utilization of the basic nitrogen was filled by the elimination of the mon-amino nitrogen far in excess of the expected quantity (88.30 per cent, expected 57.65 per cent).

*Pigment-forming Processes and their Control, by R. A. Gortner.*

Dr. Gortner has continued his study of the melanins. He reports:

In the study of the melanins considerable additional work has been done, but only one paper has been completed. Inasmuch as it is practically a certainty that the melanins are formed by the action of tyrosinase on some chromogen, the nature of the chromogen involved becomes of paramount importance. The melanin which I have isolated from black wool is of a protein nature, and in this instance the chromogen must involve a protein or a peptide. In order to test whether this chromogen is a part of the normal keratin structure or whether it is a special body, elaborated solely for the purpose of pigment formation and not utilized in the hair structure when there is a lack of enzyme action (albinos) or when there is an inhibition of oxidase action (dominant whites), I have made a complete analysis of the nitrogen ratios in both black wool and in white wool taken from the same animal. The results of the analyses do not absolutely prove either hypothesis, but they tend to support the belief that the chromogen is not present in the unpigmented wool structure, but that it is introduced in an oxidized form into the black wool solely as a foreign substance.

Aside from this conclusion the work was of interest as being the first analysis on record of pigmented and unpigmented hair structures taken from the same animal, and also as furnishing confirmatory evidence for my previous contention that hydrolysis with strong mineral acids breaks down the melanin molecule.

*Relationship between Morphological Characters and Chemical Composition, by J. A. Harris and R. A. Gortner.*

The analysis of the physiological causes of variation has been one of the chief phases of Dr. Harris's work since coming to the Station. Studies of fertility and of selective elimination have both furnished the most convincing evidence of the association of physiological with morphological variations. Of course, the suggestion follows at once



that these physiological differences can be ultimately reduced to physico-chemical terms. One is led, therefore, to expect an association between the structure of organs and the physico-chemical properties of their cellular juices. These problems, which have up to now been treated at this Station only from the physiological standpoint, are now being studied from the chemical side in collaboration with Dr. Gortner. It is as yet too early to justify any generalizations, but the results are most encouraging.

Studies of osmotic pressure, electrical conductivity, and mean molecular weight in the juices of normal and structurally aberrant *Passiflora* fruits seem to show conclusively that there the distinguishing morphological features are associated with differences in the physico-chemical properties of the cell-sap. Thus, the juice of abnormal fruits has a higher osmotic pressure than that of normals; and the average molecular weight of the substances in solution in the plant-sap is, apparently, lower in the abnormal fruits. This study has an especial value as a first step in the analysis of the factors involved in morphological characters of the fruit.

Dr. Harris and Dr. Gortner also made a joint study of the records furnished by various experiment stations as to the relation of weight of the sugar beet and the composition of its juice. They find that as the weight of the sugar beet increases the total solids, sucrose, and percentage purity (*i. e.*, the ratio of sucrose to 100 parts of total solids) fall rapidly.

*Inhibition of Pigmentation, by A. M. Banta and R. A. Gortner.*

Dr. Banta and Dr. Gortner have continued their experiments with those phenols that inhibit the reaction tyrosinase + tyrosin *in vitro* upon eggs and larvæ of the little salamander, *Spelerpes*, reported in the last Year Book, page 84.

The effects of the inhibitory compounds on pigmentation are still visible in the few larvæ which are still alive, after having been removed from the influence of the drugs for nearly a year. Dr. Gortner draws the interesting conclusion that it seems probable, were it possible to secure compounds which are at the same time non-toxic and which inhibit the action of tyrosinase or tyrosin, that permanent "dominant whites" could be produced. Dr. Banta repeated last year's experiments on *Spelerpes*. With the aid of last year's experience the proper strength of solution for *Spelerpes* was closely determined and correspondingly more striking results were obtained. When the pigmentless larvæ are placed in 0.025 per cent orcinol or resorcinol three days before the pigment normally begins to form, pigmentation is almost entirely inhibited for as long a time as the larvæ retain the inhibiting substances in their pigment-producing tissues. Unfortunately the larvæ can not be kept in these solutions



with safety for more than 12 or 15 days. After removal the larvæ develop more or less pigment, although up to that time they remain almost without pigment; but they do not assume the normal type of pigmentation.

Dr. Gortner and Dr. Banta attempted to modify the pigmentation of the vinegar fly in the same way as that of *Spelerpes*. *Drosophila* offers the advantage that the inhibitors may be placed directly into the food (crushed banana) and thus gotten into the animal's digestive tract and presumably into its tissues during all stages of its active life. Some of these flies have been reared, carefully isolated, through twelve generations on banana containing known percentages of orcinol and resorcinol. The third generation showed marked reduction in pigment in many of the orcinol and some of the resorcinol "lines," and later generations have shown a slight further reduction. The modified forms show reduction in intensity of color of the areas pigmented with black pigment and a reduction in the extent of the pigmented areas, particularly in the extensive black region at the end of the abdomen in the male. Further breeding-tests ought to show whether this is a modification produced ontogenetically in each generation or one which may have hereditary significance. It has not arisen through selection, as selection has been avoided in the experiment.

*Reactions of Rabbits to Spores of Molds, by A. F. Blakeslee and R. A. Gortner.*

In their investigation of the main problem of the chemical nature of sex, Dr. Blakeslee and Dr. Gortner have obtained results in three side-problems—the effect of injection of mold spores into an animal, the influence of a vegetable protein in the food upon the serum of an animal, and the toxicity of expressed mold juices.

They found that spores of *Cunninghamella* when injected intravenously will kill a rabbit (four instances). Post-mortem examination demonstrated the presence of germinated spores in the lungs. This mold is a tropical form growing readily at rabbit temperature and its growth may have caused death by mechanically interfering with the functions of the organs infected. *Mucor V* is incapable of growing at body-temperature and its spores were used in a new series of injections. Rabbit No. 5 received intravenously, at appropriate intervals, doses averaging about 500,000,000 spores of the minus (–) race of *Mucor V*. Rabbit No. 55 was similarly treated with plus (+) spores of the same species. For the twenty-fifth injection, rabbits Nos. 5 and 55 received 800,000,000 spores respectively of the minus (–) and plus (+) spores, and at the same time two control rabbits previously untreated were similarly injected with like doses of spores of the plus (+) and minus (–) sexes respectively. Separation cultures made from loops of blood taken from these rabbits at

half-hourly, hourly, and eventually at less frequent intervals showed that viable spores disappeared from the blood-stream in about 48 hours, but no more rapidly in No. 5 and No. 55 than in the control animals that had not previously been treated. A similar experiment was performed with mixtures of spores with the sera of the treated rabbits *in vitro* and confirmed the conclusion that rabbits are incapable of forming an antibody in the blood which will dissolve these mold-spores. They are capable, however, of producing cytolytic antibodies that will dissolve red blood-corpuscles and certain other animal cells, and it was hoped a similar cytolysin might be developed for spores and that the action might be sexually specific.

In attempting to use the ordinary bread mold (*Rhizopus nigricans*) in getting immunity reactions with rabbits they discovered that this common species is extremely poisonous. The extract from 0.04 gm. of dried filaments is sufficient to kill a 3-pound rabbit. The toxin which has been obtained by Dr. Gortner by precipitation with alcohol resists peptic digestion, also the action of boiling temperature at least for 5 minutes. It is therefore not a tox-albumen. It is soluble in water, but is non-dialyzable. The minimum fatal dose intravenously is about 1 part to 225,000 parts of rabbit by weight. The enormous strength of the *Rhizopus* toxin may be shown by a comparison with some recently investigated organic toxins. White and Avery have found that the minimum fatal dose of the poisonous substance from the tubercle bacillus is one part to 15,000 parts of body-weight, while the toxic cleavage product from edestin kills in doses of 1 part to 40,000 of body-weight. Alsberg's penicillic acid, isolated from *Penicillium*, has a minimum fatal dose in subcutaneous injections of 1 part to 4,700 of body-weight. The toxicity of the *Rhizopus* poison appears, therefore, to be 5.5 times that of the tubercle bacillus, 15 times that obtained from edestin, and 45 times that of penicillic acid. Dr. Blakeslee has previously made a special study of the distribution of *Rhizopus* and finds it occurs throughout the world and is almost certain to appear as a spontaneous infection of bread and similar substrata rich in carbohydrates, whenever the proper temperature and moisture requirements are observed. Its possible relation to those diseases of unknown origin, such as pellagra and the horse-disease and the corn-stalk disease of the middle west, which have been attributed to infected food, is not being overlooked.

#### THE MODIFYING EFFECTS OF CAVE CONDITIONS.

Dr. A. M. Banta's experiments with cave material are being continued. The mud minnows (*Umbra limi*) are showing some further depigmentation; *Cambarus bartoni*, a non-cavernicolous crayfish, likewise shows a progressive loss of pigment; some of the salamander larvæ in the cave during the present year have less pigment than

those reported on a year ago; and a lot of wood-frog larvæ reared in the cave have developed very little pigment. But the most interesting material is the amphipod *Eucrangonyx gracilis*, an almost pigmentless form of which lives in caves in central Indiana, while the normally pigmented form is abundant in the surface streams in the same region. Series of these two forms have been placed both in the cave and in the vivarium and interesting results are hoped for.

During the past winter a short time was spent in the cave region in Indiana and numerous experiments were made on the light and tactile reactions of the two forms of the above-mentioned amphipod. In accord with the results of earlier experiments with an eyeless cave isopod and its near outdoor relative, the cave amphipod was found to be less responsive to light and more responsive to tactile stimulation than its outside relative.

#### VARIOUS PROBLEMS IN HEREDITY.

##### *Inheritance in Shepherd's Purse, by George H. Shull.*

The discovery by Neilsson-Ehle at Svalöf of duplicate determiners, for some of the characters of certain grains, has led to the discovery of a similar sort in animals and plants and even in man. Dr. Shull reports another case in a character upon which he has been at work for some time and whose behavior appeared at first erratic. The cultures of the present year have fully confirmed his view that there are duplicate determiners, each of which, independently of the other, is capable of producing the triangular type of capsule characteristic of *Bursa bursa-pastoris*. In support of this proposition the following facts have been demonstrated:

(a) All individuals of the  $F_1$  families formed by crossing *B. bursa-pastoris* with *B. heegeri*, have triangular capsules.

(b) In all  $F_2$  families there is an approximation to the ratio of 15:1 between plants having triangular capsules and those having top-shaped capsules.

(c) When  $F_2$  families having triangular capsules are self-fertilized, three kinds of  $F_3$  families are produced, namely, (1) those in which all of the individuals have triangular capsules; (2) those in which the individuals having the two kinds of capsules occur in the ratio 15:1; and (3) those in which the two kinds of plants appear in the ratio 3:1.

(d) In the  $F_4$  the results differ according to the type of  $F_3$  family from which plants of the dominant type are used for breeding: (1) The members of those  $F_3$  families which contained only plants with triangular capsules produce only triangular capsules again; (2) when the parents are triangular-capsuled plants from an  $F_3$  family in which a 15:1 ratio occurred, the  $F_4$  families fall into the same three groups as the  $F_3$  families; (3) from the dominant indi-

viduals of an  $F_3$  family in which a 3:1 ratio occurs, only two kinds of  $F_4$  families arise, namely, with triangular capsules only, or with the two kinds of capsules in the ratio 3:1.

(e) Extracted dominants in an  $F_3$  family displaying a ratio 3:1 are not the same genotypically as the original type used in the crosses, though indistinguishable from it by simple inspection. Crosses between these extracted dominants and *heegeri* have yielded only  $F_2$  families with a ratio 3:1, instead of 15:1, as found in the original  $F_2$  families.

A new and unequivocal case of duplication of determiners is thus added to the very few which have been found in other plants, as the results here summarized are supported by about 140 pedigreed families and can find no other logical interpretation. There is some indication that duplicate determiners exist for one of the rosette characters also in *Bursa* as well as for the distribution of sterile flowers on the racemes, but these cases require further investigation.

*Hybrids in Oenothera, by G. H. Shull.*

Dr. Shull has continued his hybridization of *Oenothera lamarckiana* and the several biotypes of *Oe. cruciata*, and the progeny are truly remarkable in their complexity.

The first generation hybrids between *lamarckiana* and the biotypes of *cruciata*, and between the several possible pairs of the biotypes of *cruciata* which were reported last year as consisting of polymorphic progenies and strikingly unlike reciprocals, have been repeated, together with parallel series in which another strain of *lamarckiana* and a *rubrinervis* were used respectively in the place of the *lamarckiana* involved in the original series. The 22 types found last year have all been confirmed in this year's cultures and several new types have been added, the corresponding forms from the two new series being indistinguishable from those originally secured. The second generation has been grown extensively and more than half of the possible combinations of  $F_1$  types with the parents and related hybrids have been studied, and additional crosses have been made to fill partially the gaps still remaining. In the crosses between the biotypes of *Oe. cruciata* the results are relatively simple, the most striking features of the situation being the facts: (a) that more than 100  $F_2$  combinations have added only one new type in addition to the occasional aberrant individuals which one is apt to meet in pure-bred as well as hybrid stocks; (b) that in most cases the results in the  $F_2$  are what would be expected if it be assumed that the hybrids produce only the eggs of their mothers and only the sperms of their fathers. There are several rather striking exceptions to the last proposition, but the exceptions occur with a degree of consistency that promises to make possible some rather simple interpretation.



When *lamarckiana* enters into the hybrid combination the situation becomes extremely complex. At many points the same principle which works out with precision among the pure *cruciata* crosses can be detected with greater or less clearness, but in most cases it is obscured or completely hidden or destroyed by the more independent movement of characteristics of *lamarckiana*, including the broad petals, long style, redness of the bud-cones, and various features of foliage and habit of branching, all of which may be recombined in various ways with the cluster of egg-borne or sperm-borne characters of any biotype of *cruciata* with which *lamarckiana* may be crossed. These independent characters of *lamarckiana* do not readily take a Mendelian interpretation, however, the same character being in one combination dominant, in the other recessive, and almost never approximating an expected ratio, except perhaps 1: 1 in several cases. Broad and narrow petals not infrequently occur on the different branches of the same plant.

*Heredity in Butterflies, by John H. Gerould.*

Professor John H. Gerould has been cooperating, as non-resident collaborator, in researches on heredity in butterflies. He makes the following report:

My principal work with butterflies this season has been with *Colias eurytheme*, in which I have studied the inheritance of albinism; I have material for the study of other color varieties, as *keewaydin* and *ariadne*; and I have again crossed this species with *C. philodice*. I have mated the species-hybrids together and with individuals of the original species, and have secured a few layings of fertile eggs from these matings. I expect this fall to raise butterflies from these eggs, or to bring them into the pupal stage before I put the stock entirely into winter quarters. The albino female of *C. eurytheme*, as in *C. philodice*, is a color-hybrid, a Mendelian heterozygote for color. Thus, from two white females of *eurytheme* received from Arizona early in June 1913, I raised family *a*, containing 29 white females, 24 orange-yellow females, and 57 males + 1 gynandromorphic male; *b*, containing 27 white females, 20 orange-yellow females, and 49 males.

One-half of the sons of a white female are presumably heterozygous for color, though yellow; so I have attempted to determine the proportions derived by mating white (heterozygous) females with heterozygous yellow males, whether 3 white females to 1 orange female, or 2 white females to 1 yellow female, as my experiments on *C. philodice* seemed to indicate; but my broods of this generation that have just been emerging do not settle the question. The families from white females mated to their own brothers (or other sons of white females) in which an excess of white female offspring appeared were small. In one such, *e* (from  $a^{59}$ , white female  $\times$   $a^{27}$  male), 9 white females and no orange females (+15 males) have thus far appeared. Possibly  $a^{27}$  male was a heterozygote for white, *y* (*w*), though, of course, yellow. In family *m* (ex  $b^{43}$  white female  $\times$   $b^{48}$  male, *i. e.*, son and daughter of a wild white female from Arizona) there are 38 males, 16 white females, 11 orange-yellow females;  $b^{48}$  male, the father, was presumably pure orange-yellow, not carrying the white latent.



The mother of this brood ( $b^{53}$  female) could not roll up her tongue. Of her 65 offspring about one-half are normal, one-half can not roll up the tongue, *i. e.*, 28 normal, 37 uncoiled. In the males the equal ratio obtains: 18 normal, 20 uncoiled; in the orange females, 5 normal, 6 uncoiled; in the white females, however, 5 normal, 11 uncoiled. These numbers indicate on the whole a regular heterozygous condition as regards abnormality of tongue.

Two white *eurhytheme* females ( $a^{94}$  female and  $b^{78}$  female) were crossed with *philodice* male, which bears no orange (pale sulphur yellow). This family, *o*, is as follows: males 72, females 71 (white 36, orange yellow 35). The hybrids are somewhat intermediate in color between the two species, showing dominance of orange, though the orange suffusion upon the yellow background is reduced in extent in the hybrid. The other family, *p*, consists of 11 males, 7 females (4 white, 3 orange-yellow).

The white species-hybrids are almost or quite indistinguishable from the albinic *C. eurhytheme*, but I have not yet studied them in detail.

The male gynandromorph that appeared in family *a* ( $a^{81}$ ) is the son of a white female  $\times$  wild male. The 57 other males of this family and 53 females are normal. Three wings of this individual bear the male color-pattern, but the right anterior wing has the color-pattern of the female (wide, spotted marginal band) with a ground-color of orange and of white in a mosaic, thus showing the influence of *both* of the female color varieties. The male external genitalia are perfect, but crowded to the left side of the median plane. The testis was well developed. No ovaries were found, but there is an imperfect development of the female external genitalia on the right of the posterior end of the abdomen.

I had obtained in 1912 (October to December) almost exclusively females from the cross between *eurhytheme* female  $\times$  *philodice* male—20 females, 2 males. Repeating this work shows (family *o*) exact equality between the sexes (72 males, 71 females). However, in the hybrid broods of 1913 the usual precocity of the male is seen to be upset by the crossing of the two species; 7 out of the first 9 to emerge (first two days of emergence) were males. I have thought that this upsetting of the usual precocity of the males may be connected with the apparent partial sterility of the species-hybrids. The females, after mating with male species-hybrids, have in many cases not shown an inclination to lay, though they are invariably full of mature eggs. The males may be in some cases sterile. I am still studying this matter.

I raised a brood of pure *eurhytheme* from a wild female; it consisted of 214 males, 206 orange-yellow females. Many pupæ of this brood were iced, and I tried to mate the resulting adults, but without success, to determine what effect cold might have upon the germ-plasm. There were few males that emerged from the iced pupæ, 15 pupæ having been killed by the conditions in the refrigerator (excessive cold and perhaps dryness). I shall try this again. This large family ought to throw some light on the matter of variation within the species, since it contains examples of varieties *ariadne* (pale orange) and *keewaydin* (deep orange) regarded by some entomologists as seasonal variations.

I want to extend my experiments eventually by breeding *C. edusa* and *hyale* from Europe and the American species, especially *C. interior* of Canada, the nearest species to *C. philodice* and *eurhytheme* geographically. *C. interior* has not been bred, and its food plant is said to be unknown, though I should be surprised if it would not lay on white clover. Species of the genus *C. coli*, although very susceptible to disease and hence difficult to raise, are full of interesting possibilities in the study of the origin of species and heredity.

*Inbreeding and Degeneration of Rats, by G. C. Bassett.*

Dr. G. C. Bassett, who has been working, under the direction of Professor John B. Watson, at Johns Hopkins University, upon the consequences of inbreeding on the intelligence of rats, is temporarily attached to this Station and continuing his work here. His early investigation showed that after four generations of closest inbreeding (brother and sister within the litter) there was a loss in brain weight of from 7 to 10 per cent on the average and a loss in ability to form habits of about 30 per cent on the average; but no further important degeneration of the stock occurred, even to the tenth generation of inbreeding. Dr. Bassett's conclusion that on the average inbreeding is accompanied by deleterious effects upon the brain and intelligence accords with results obtained at this Station on the deterioration in the yield of maize upon self-pollination. Dr. Bassett proposes now to establish in the white rat a condition analogous to that of imbecility in man; to produce through inbreeding and selection a strain of rats of inferior intelligence, and to use this, in hybridization experiments, to test the inheritance of such mental inferiority. He proposes to study, at the same time, the effects of chronic parental alcoholism upon the progeny. This will comprise a study of brain and cord weight, blood and kidneys, and the intelligence.

The foregoing experiments are closely related to those begun at Goose Island by the Wistar Institute. Those experiments were discontinued by the Wistar Institute, as the evidence showed, first, that the albino rats could not survive alongside of the brown rats of the island, and, second, that the mere trapping of brown rats on Goose Island may not exterminate them, as there is evidence that the island is subject to invasion by rats from larger islands and those nearer the mainland. In the early spring (April) rats are absent or rare, but they have been trapped in large numbers during June every year since we have had the island.

*Studies in Human Heredity, by C. B. Davenport.*

That part of the Director's studies relating to the results of experiments in heredity that man is making on himself is comprised under the name Eugenics Record Office. This aspect of the work has received continued generous support from Mrs. E. H. Harriman and Mr. John D. Rockefeller. Its board of scientific directors includes Alexander Graham Bell, William H. Welch, Lewellys F. Barker, Irving Fisher, E. E. Southard, and C. B. Davenport.

The principal investigations now being conducted are: (1) the consequences of human inbreeding in an island population; (2) the consequences upon the physical and mental traits of a blood line, formerly highly inbred and very neurotic, but which for a generation

has been widely scattered, necessitating outbreeding; (3) the establishment of a norm, or control, by the complete study of a small New England township; (4) the inheritance of chronic chorea; (5) the inheritable basis of pellagra; (6) the inheritance of hare-lip in man and animals; (7) the continued study of the families of institutional cases of the insane, feeble-minded, and emotionally uncontrolled.

The Director has studied all available cases of close inbreeding and their progeny. While the product of the interbreeding of members of the same fraternity is usually neuropathic, it is hard to deny that this is due to the neuropathic condition of parents whose emotions are so uninhabitable. The progeny of mating between more remote relatives (cousins) frequently show no deterioration. These studies form the basis for a criticism of the numerous State laws limiting marriage selection.

The study has been made of strains to which belong a number of cases of eroticism and hysterical temper. Both traits appear clearly as Mendelian dominants. In this respect they are like chronic chorea, a condition characterized by uncoordinated movements apparently due to an inability to inhibit muscular contractions. Extensive data secured by one of the field workers of the Eugenics Record Office enable us to trace back the more prominent lines carrying chronic chorea to about six immigrants to North America over 250 years ago.

The inheritable basis of so many elements of behavior having been demonstrated, it seemed desirable to call attention in a popular article (*Popular Science Monthly*, July 1913) to the consequences of the fact, in the hope that unnecessary suffering and abuse, through a wrong interpretation of their behavior, might be saved to those whose reactions are atypical.

#### ISOLATION OF BIOTYPES, AND SELECTION.

*Results of Selection of Color Pattern in Rats, by W. E. Castle and J. C. Phillips.*

The work of the animal or plant breeder who seeks to create improved stock is largely done by selecting as breeders those individuals that reveal most nearly those characters that he is looking for. But since inheritance is from the germ-cells and since the soma gives only an inadequate and partial indication of the potentialities of the germ-cells, it is clear that selection on the basis of breeding capacity must ever be a better method than selection of the appearance, composition, or behavior of the soma of the individual. Historically it appears that progress has, nevertheless, been made by pure somatic selection; and it is important to know the details and results of this method. Our associate, Professor W. E. Castle, has just completed, in collaboration with Dr. John C. Phillips, and

turned in for publication a study of a somatic selection of the color pattern of rats, extending over seven years, in the course of which over 20,000 rats were raised and studied. The results, they believe, demonstrate the efficacy of mass selection; and they are led to conclude that selection is, in animal breeding, a more important agent than mutation, partly because it is controllable and its results therefore more certain, and partly because it may even determine the occurrence of mutations of a particular sort.

*Quantitative Studies of Selective Elimination, by J. A. Harris.*

During the year, Dr. Harris's studies on natural selection have been carried forward along the lines laid down in previous reports.

The results already published for differential mortality with respect to seed weight in field cultures of *Phaseolus vulgaris* have been fully confirmed and somewhat extended by planting about 46,000 individually weighed seeds in the green-house. There is unquestionably a reduction in variability in weight due to differential mortality. The data also seem to show that in some strains the higher mortality falls upon the heavier and in other strains upon the lighter seeds.

The results for *Phaseolus* have been confirmed on an extensive though smaller scale by field experiments on *Pisum*. Some progress has been made in the physiological phases of these investigations in that a correlation between weight of seed and time required for germination has been demonstrated in *Phaseolus*.

The selective elimination studies for seedling variants in *Phaseolus* have been continued on about double the scale and in greater detail than last year. The results can not be announced until the data are secured from the crops still in the field.

The studies of fertility and fecundity instituted to check the findings for the differential mortality of ovaries have been carried forward and in part published. So far as completed they confirm the results announced in Year Book No. 9.

Thus it was demonstrated in papers published from the Station in 1910 that the chances of development to maturity of the ovaries formed by a plant are conditioned upon the structure of these ovaries. It was proved, for example, that ovaries of *Staphylea* have a death-rate roughly proportioned to the number of carpels which are bilaterally asymmetrical with respect to the arrangement of their ovules. During the year results have been published showing that in *Phaseolus* such bilaterally asymmetrical ovaries are not only less capable of maturing their seeds, but that the seeds which they do produce are lighter than those borne in symmetrical ovaries. Thus the selective elimination and the physiological studies fully check and supplement each other. The information gained from them is now furnishing the basis for the study of asymmetry in the individual.



## PURE LINES.

*Influence of Weight of Seed upon the Character of Plants Produced,  
by J. A. Harris.*

The question of the relationship between the weight of the seed planted and the characteristics of the plant produced—a problem which has necessarily been taken up at this Station because of its bearing upon questions of pure-line inheritance—is one of considerable practical importance. Extensive studies have shown that in *Phaseolus* there is regularly a positive correlation between weight of seed planted and number of pods per plant—yield increasing with weight of seed planted. But the correlation for weight and character of pods, that is, number of ovules formed and number of seeds matured, is much lower and is reduced practically to zero when correction for number of pods is made. Thus the yield in number of pods but apparently not the character of the pods produced is directly influenced by weight of seed planted.

*Selection within the Pure Line, by J. A. Harris.*

The studies on pure-line inheritance for seed weight have been continued, but publication has been delayed because of the many difficulties of analysis introduced by various physiological correlations. The number and magnitude of these difficulties has never been fully enough taken into account by those who have written on these questions. For example, the work at this Station has shown that there is a sensible correlation between the weight of the seed and the number of pods produced by the plant. There is also a correlation between the number of pods on the plant and the weight of the seeds which it produces. As a resultant of these correlations some relationship must be expected between the weight of the parent and offspring seed in the population. Again, our experiments on differential mortality with respect to seed weight show that physiologically considered this character is by no means a simple one. To distinguish relationships between parental and offspring seeds which are purely physiological from those which are genetic is a problem requiring further experimentation.

Selection for seedling abnormalities was begun in 1912 and carried out on a larger scale this year. It is too early to report results, although it may be noted in passing that some abnormal parents threw all abnormal offspring this year.

## CLONAL.

*Heredity in Parthenogenetic and Clonal Reproduction.*

While the term "pure line" is strictly applicable only to self-fertilizing species, it is reasonable to expect that parthenogenetic and non-sexually reproducing species shall follow the same laws of heredity; and several studies have been made here with such species.



Dr. A. M. Banta has, as stated in the last report, selected inside of one "pure-line" of daphnids the most and the least sensitive to light. The species reproduces parthenogenetically. The data, including the reaction times of over 10,000 individuals, covering in some cases 50 generations, are now being reduced.

The Director, working with Miss Annie P. Henchman, studied the variation in the statoblasts of the fresh-water bryozoan *Pectinatella magnifica*. These seed-like buds carry marginal hooks whose number varies from 11 to 26. By budding, compound individuals or colonies are produced, each of which may contain from 100 to 900 statoblasts. All of the statoblasts of one colony are produced from the same germ-substance without a sexual process to complicate matters. There is a great range of variation inside of any one colony; *i. e.*, one colony has a mode at 15 hooks, but there are a few statoblasts with as few as 12 hooks and some with as many as 20. But when we compare colonies that are probably or certainly unrelated the mode and the range in the number of hooks are both quite different. Thus, in another colony, the mode is at 18 hooks and the range is from 15 to 22. Thus variation inside the non-sexually produced colony is less than the variation between colonies. As yet attempts at breeding from given statoblasts have not succeeded.

#### METHODS.

##### *Statistical Analysis of Data.*

Dr. J. A. Harris has given much time to improving statistical methods and has prepared a memoir on the calculation of inter-class and intra-class coefficients of correlation from class moments when the number of possible combinations is large.

The Director has published a third edition, slightly revised from the second, of "Statistical methods with special reference to biological variation."

*Critique of Variation due to Heterogeneity of the Substratum, by J. A. Harris.*

Dr. J. A. Harris had his attention called to the failure of one series of plantings of beans to give results in harmony with his conclusion that the larger the seed planted the greater the mean number of pods per plant. The series in question gave precisely opposite results. A study of conditions of the planting shows that the seeds of each weight-group had been planted in order across a garden plot that was selected for its apparent uniformity of soil yet there was a demonstrable loss of productiveness from one side of the row to the other, and the larger seeds were planted at the least fertile end. So great was the heterogeneity that the larger seeds actually produced fewer pods than the smaller. In statistical studies on relative productiveness, homogeneity of substratum is clearly of primary importance.

*Methods of Rearing Chicks.*

In rearing chicks we have until recently suffered from a mortality of over 75 per cent. By applying the methods worked out at the Storrs Agricultural Station for reducing white diarrhea (namely, by keeping chicks in the incubator three days after hatching) mortality from white diarrhea has been reduced to about 5 per cent. We still had a large number of deaths at about 4 up to 8 weeks, apparently from coccidiosis. By increasing the area of range per chick the density of the infection of the ground is diminished and the death-rate vastly decreased. Dr. H. D. Goodale employed this method here in 1912 and has continued its use at Amherst in 1913, with good results.

## STATISTICAL SUMMARY.

*Poultry.*—Of chicks, 655 were hatched.

*Finches.*—Of canaries, 76 were hatched; 28 survived infancy.

*Sheep.*—Twenty-six sheep were born.

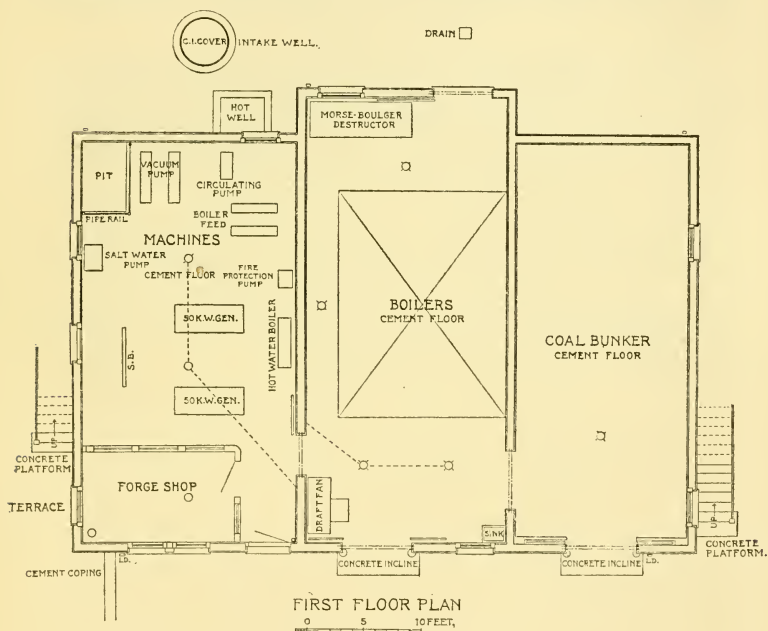
*Plants.*—No estimate has been made of the many thousand plants examined by Dr. Harris. The number and extent of cultures grown in connection with Dr. Shull's investigations are indicated by the accompanying table.

Name of species.	No. of families.	No. of individuals.	Name of species.	No. of families.	No. of individuals.
Bursa bursa-pastoris.....	13	2,200	Oenothera scintillans.....	1	20
Bursa bursa-pastoris×heegeri hybrids, F <sub>2</sub> .....	4	1,099	Oenothera traeyi.....	1	156
Bursa bursa-pastoris×heegeri hybrids, F <sub>3</sub> .....	7	1,987	Oenothera sp.....	1	13
Bursa bursa-pastoris×heegeri hybrids, F <sub>4</sub> .....	12	3,696	Oenothera cruciata-lamarckiana hybrids.....	145	15,348
Bursa bursa-pastoris×heegeri hybrids, F <sub>5</sub> .....	58	16,482	Oenothera cruciata - rubrinervis hybrids.....	6	711
Lychnis dioica.....	142	11,847	Oenothera lamarekiana - rubri- calyx hybrids.....	2	240
Lychnis dioica×coronaria.....	1	15	Oenothera lamarekiana - rubri- nervis hybrids.....	2	80
Oenothera cruciata.....	3	544	Oenothera nanella×lamarckiana	1	44
Oenothera gigas.....	1	74	Oenothera nanella×rubrinervis	1	45
Oenothera lamarekiana.....	17	944	Oenothera rubricalyx - rubriner- vis hybrids.....	2	357
Oenothera grandiflora.....	2	382			
Oenothera nanella.....	1	78			
Oenothera rubricalyx.....	1	119			
Oenothera rubrinervis.....	10	2,404	Total.....	434	58,885

## CONSTRUCTION AND EQUIPMENT.

## POWER-HOUSE.

To simplify the work of heating the buildings, including the green-houses and bird-house, it was decided to erect a power-house near the harbor. This building, of hollow tile and concrete, is 55 by 37 (or 41) feet. It comprises a boiler-room, a room for machines, including space for a pair of future 50 k. w. electric generators, a small forge shop 8 by 13.5 feet, a coal bunker, a carpenter shop, and two rooms for workmen. It is expected to install one electric generator in 1914.



Power House.

## ANIMAL HOUSE.

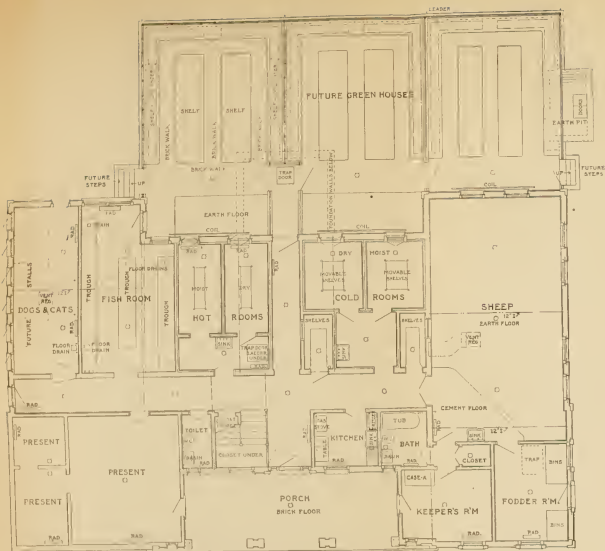
To relieve the crowded condition of the main building, to free it from the dirt incident to caring for animals in it, and also to remove the chemical laboratory to safe quarters, the Trustees provided for building a new "animal house." The building is 80 feet long by 50 feet wide; it is of hollow-tile construction covered with stucco. The roof is flat and of the Barrett specification type. The floors are of reinforced concrete with terazzo surface. The stairs are of steel

with slate treads. The first floor comprises four rooms for small mammals and one for sheep; also two suites of rooms whose walls are all insulated with cork, to be used for heat and cold experiments. This floor also has a room for aquatic animals, a kitchen for preparing food and sterilizing small dishes, and a small suite of rooms for an animal caretaker. The second floor comprises the chemical laboratory, 24 by 20 feet, with a weighing-room; a bird-room, 30 by 20 feet; a suite for operating, an adjacent hospital and laboratory, all together 40 by 18 feet; two laboratories, each 18 by 19 feet; a museum room, 14 by 11 feet; a dark room 22 by 8 feet; a fire-proof vault for records.

#### MISCELLANEOUS.

Our frame carpenter shop and the lumber shed were moved from their old sites to make room for the power-house, and set against the side hill. A retaining wall about 80 feet long and 6 feet high was built against the face of the slope. The excavated material was deposited upon the salt marsh and a full half acre has been thus reclaimed, which will be added to the garden.

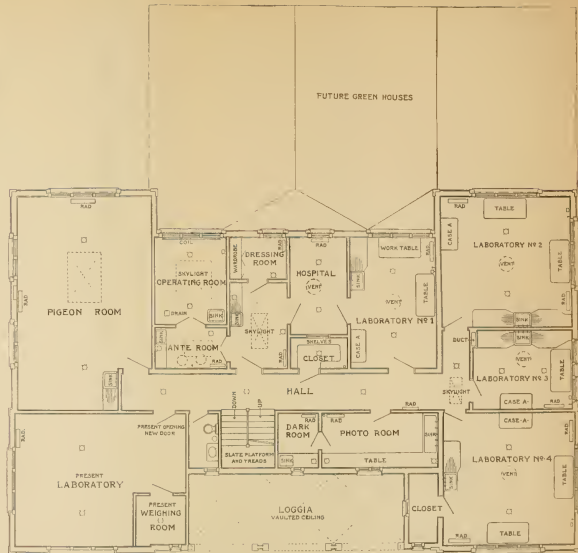
A water tower 50 feet high and a tank of 10,000 gallons capacity were installed and pipes laid to and from them. Two hydrants were placed and provided with hose. A stream of water can be thrown to the ridge of the laboratory and the animal house. The tank is kept filled by the ram at an expense of less than \$10 a year, including new rubber valves and labor in replacing them.



FIRST FLOOR PLAN

0 5 10 FEET

Animal House, Cold Spring Harbor.



SECOND FLOOR PLAN

0 5 10 FEET

Animal House, Cold Spring Harbor.



BIBLIOGRAPHY OF PUBLICATIONS RELATING TO WORK ACCOMPLISHED  
DURING FISCAL YEAR BY GRANTEES AND ASSOCIATES.

Under this heading it is sought to include titles of all publications bearing upon work done under grants from the Carnegie Institution of Washington, exclusive of the regular publications. A list of the latter which have appeared during the year will be found in the President's Report (pp. 33-34). The following list has been made as complete as possible, and in some cases titles may be included which have only an indirect connection with grants from the Institution.

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