

LELAND STANFORD JUNIOR UNIVERSITY PUBLICATIONS  
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No. 1

# INHERITANCE IN SILKWORMS, I

BY

VERNON L. KELLOGG

Professor of Entomology, and Lecturer in Bionomics

with the partial collaboration of  
RUBY GREEN SMITH  
former Instructor in Entomology

STANFORD UNIVERSITY, CALIFORNIA  
PUBLISHED BY THE UNIVERSITY  
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LELAND STANFORD JUNIOR UNIVERSITY

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[Signed] COMMITTEE ON PUBLICATIONS,

*Per* GEORGE T. CLARK, Librarian.

Stanford University, California,  
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Since 1900 the writer has given attention to the general biology of the familiar mulberry silkworm. This attention has taken the form of considerable experimental work on such problems as the causes and time of sex differentiation, regeneration of larval parts, influence of the

primary reproductive organs on the secondary sexual characters, reflexes of the moths, artificial parthenogenesis, etc. The results of much of this work have already been published as papers in various scientific journals. A list of these papers follows and an abstract of each one of them may be found in the Appendix to the present paper.

(with R. G. Bell) Notes on Insect Bionomics, in *Jour. Exper. Zool.*, v. 1, pp. 357-367, August, 1904.

(with R. G. Bell) Variations Induced in Larval, Pupal and Imaginal Stages of *Bombyx mori* by Controlled Varying Food Supply, in *Science*, N. S. v. 18, pp. 741-748, Dec., 1904.

Regeneration in Larval Legs of Silkworms, in *Jour. Exper. Zool.*, v. 1, pp. 593-599, 10 figs., Dec., 1904.

Influence of Primary Reproductive Organs on Secondary Sexual Characters, in *Jour. Exper. Zool.*, v. 1, pp. 601-605, Dec., 1904.

Some Silkworm Moth Reflexes, in *Biol. Bull.*, v. 12, pp. 152-154, Feb., 1907.

Sex Differentiation in Larval Insects, in *Biol. Bull.*, v. 12, pp. 380-384, 8 figs., May, 1907.

Artificial Parthenogenesis in Silkworms, in *Biol. Bull.*, v. 14, pp. 15-22, Dec., 1907.

At the same time that these miscellaneous studies in silkworm biology were begun, a series of planned and controlled rearings was started (one generation a year) to test the behavior in heredity of fluctuating and sport variations of larvæ, cocoons and adults. Also experiments and rearings were carried on to test structural and physiological modifications which might be induced by varying food supply (both as to character and quantity) and the possible inheritance of these modifications. From 1904 on the work has been turned chiefly to a study of the modes of inheritance of various racial characters of eggs, larvæ and cocoons, involving controlled pure and hybrid matings of individuals of some fifteen races. This study of heredity has served to test, for the silkworm, the Mendelian principles of inheritance, as well as the actuality of the potency in heredity of vigor, of sex, and of special characters. Finally the hypothesis of individual and race idiosyncrasies in matters of inheritance has been tested. The present paper is a first contribution of data and results derived from this general study of silkworm inheritance. Any discussion of a possible practical application of these results in connection with commercial silk culture is reserved for a future paper.

From 1900 to June, 1905, Mrs. R. G. Bell (now Mrs. R. G. Smith),

at that time Instructor in Entomology, was associated with the present writer in all of the silkworm work, and fully deserves therefore the title of collaborator. Certain data also have been obtained from the careful and extensive studies of Instructor McCracken, who has given special attention during the last three years to the inheritance of the *moricaud* larval sport and to the behavior of bivoltinism as a heritable character.

I am indebted to Professor E. Verson, director of the royal silk culture station at Padua, Italy, to Mr. S. I. Kuwana, entomologist of the imperial agricultural station at Nishigahara, Tokyo, Japan, and to others, for eggs of various races. Dr. L. O. Howard, Chief of the Bureau of Entomology of the U. S. Dept. of Agriculture, helped out the work in one of the years by an appropriation for assistance. Mrs. Carrie Williams and Miss E. L. Story of San Diego, California, rendered very efficient and faithful help in the 1907 rearings. Drawings for the present paper were made by Mary Wellman and Maud Lanktree as indicated on the respective plates. To all of these, and to numerous helpers, especially Isabel McCracken and R. W. Doane, in the arduous and exacting labor of rearing, observing, and tabulating through the past six years, the writer expresses his obligation and gives his sincere thanks.

## RACES AND CHARACTERISTICS.

During the course of the work fifteen different silkworm races have been bred pure and used in hybridization, but a few of these have been used much more than the others. These various races (Bagdad, Istrian, Japanese White, Japanese Green, Chinese White, Italian Yellow, French Yellow, Persian Yellow, Turkish and French Yellow, etc.) are distinguished from each other by characteristics of the egg, the larva, the cocoon and, to some degree, of the adult. The varying egg characters are size, shape, color and degree of adhesiveness. The larval characters are size, external appearance and, chiefly, color and pattern. The cocoon characters are size, shape, character of silk as to tenacity, diameter, length, etc., of the thread, and, most conspicuously, color. The adult characters are size, and degree and character of patterning of wings.

These characteristics are all of course affected by fluctuating variation and by occasional sport (reversional or mutational) variation, but for cocoon colors, larval colors and patterns, adhesiveness of egg and size of egg, and certain "commercial" characters of the silk, as tenacity, diameter and length of the thread, the races are well separated and have long been bred pure.

The mulberry silkworm has been domesticated and ameliorated by man for about five centuries. The exact feral species from which it is derived is not certainly known. It seems most probable that the home of the wild progenitor was (perhaps still is) in the mountains of northern India.

As with poultry, cattle, horses, dogs, sheep, swine, pigeons, many races have been established in many lands, and much careless and useless hybridization and selection has been indulged in. Out of it all there has been of course, unconsciously and consciously, a steady increase in the output and in the betterment in quality of the silk produced by the silkworm individual. Commercially valuable characteristics of the silk, and behavior, resistance to disease, and "tameness" of the larva have been the points striven for by breeders. But along with these, other characteristics, correlated or independent, have become fixed in various races and are useful to the experimental student of inheritance.

For the purposes of our studies the nature and distinctness of the varying distinguishing characteristics of the races and their steadfast-

ness in transmission (in pure matings) were the important matters of silkworm differentiation rather than the geographical or historical or commercial relations of the various races. Therefore no list of the races with their particular characters will be given, but instead will be given a catalogue and description of the various characteristics of eggs, larvæ, cocoons and adults. These descriptions can be made brief because of the careful illustrations (see Plates I and II) which will readily give a clear understanding of the character conditions.

#### **Egg characters.**

Adhesive (i. e., sticking, when oviposited, tightly to the object on which they are deposited). Characteristic of all races used except the Bagdad race.

Non-adhesive (i. e., eggs loose, unattached to the paper of the mating box). Characteristic of the Bagdad race.

No other egg characters have so far been made use of in my studies.

#### **Larval characters (last larval instar).**

White without darker pattern. Characteristic of several races, as Chinese White, and others. (Pl. I, figs. 1, 7; Pl. III, figs. 1, 5.)

White with certain regular but few markings, as Bagdad, etc. (Pl. I, fig. 3.)

White with well marked darker pattern. Characteristic of Japanese White and others. (Pl. I, fig. 4; Pl. III, figs. 3, 7.)

Tiger-banded (i. e., black or black-brown transverse segmental bands). Characteristic of a sub-race of Italian Salmon race. (Pl. I, fig. 2; Pl. III, fig. 2.)

Moricaud (i. e., a close pattern of black-brown lines all over the body so as to make the whole larva a "darky"). A sport which has appeared in several races in our laboratory, as Italian Yellow, Bagdad, etc., and which has been established in our laboratory as a nearly pure sub-race of Bagdad. (Pl. III, figs. 4, 8.)

#### **Cocoon characters.**

White; characteristic of Bagdad, Japanese White, Chinese White and other races. (Pl. IV, figs. 1, 2, 3, 4.)

Yellow, of various shades from lemon to golden; characteristic of Istrian, Italian Yellow, and other races. (Pl. IV, figs. 8, 9.)

Salmon, or pale yellowish pink; characteristic of Italian Salmon race. (Pl. IV, figs. 13, 14, 15.)

Green; characteristic of Japanese Green race. (Pl. IV, fig. 7.)



**Characteristics of shape.**

Several, as constricted, broad-ended, tapering, etc. (see Pl. IV), but not used in the present studies.

**Adult characters.**

Patterning of the wings (see Pl. II), venation of the wings, darkness of wings and body, etc., but no character found to be distinctive of a race.

In addition to these differences still other racial ones occur in connection with properties of the silk, but with these I have nothing to do in this paper, as they have not been used by us in the inheritance studies.

Other characters not racial but occurring as individual variations have been noted and some have been made use of to some extent in the studies. For example, melanism of the moths (darky moths) and the degree of patterning of the wings, variations in the wing-venation of the moths, various teratologic sports, the phenomenon of double and triple cocooning, the flight capacity of the moths, etc., have all been subjects of more or less observation and experiment.

As the characteristics used will be described in more detail in connection with the particular accounts of studies in which they are used, this general statement of the variety of characters available to the student of silkworm variation and heredity may suffice. It should be stated at once, however, that among these various silkworm characteristics or variations some are distinctly alternative or discontinuous in character while others are continuous or fluctuating in variational character. Thus in this one species of animal, opportunity is well afforded for studies of the behavior in inheritance of both types of variations.

## DIFFERENT KINDS OF INHERITANCE BEHAVIOR.

The silkworm is a very convenient animal with which to experiment in matters of inheritance. The matings can be made with ease and certainty. Many of the races have been bred pure for hundreds of generations and are very stable and reliable. The characters available for observation are well-marked and easy to describe and illustrate, and represent inheritance in different well-marked life-periods of the animal so that the inheritance of characters peculiar to one life-period can be compared with that of characters of another life-stage. Finally the animals can be reared in large numbers in comparatively limited space, and thus extensive series and many repetition lots be obtained for a basis for generalizations.

This last point is one on which I wish to lay stress. *My conclusions as to the behavior in inheritance, especially as regards its uniformity or non-uniformity, of various silkworm characteristics would have been quite different from what they are at present if I had not made use of numerous repetition lots. It is on the basis of these repetition lots that my conclusions as to strain and individual idiosyncrasies in silkworm heredity are based.*

It is perfectly plain from the results of my experiments (as well as from those of Coutagne and Toyama, to be referred to in a moment) that different silkworm characters behave differently in inheritance. (At least this is perfectly plain unless some ingenious analyst like Bateson by a combination of real analysis with added hypotheses of "determiners," or what not, undertakes to make it not perfectly plain.)

These different characters are those of various life-stages, as larval, pupal, or adult, but they are not necessarily like or unlike each other in their inheritance behavior on the basis of any distinction of life-stage. They differ in inheritance behavior simply on the basis of difference in characteristic. These inheritance behavior differences consist in some characteristics being alternative (and usually essentially Mendelian) in inheritance, as larval pattern (white, patterned, tiger-striped and moricaud), cocoon color, etc., while others are particulate or blend in inheritance. The former are discontinuous or non-intergrading variations or differences, the latter are fluctuating or continuous, as wing pattern of adults, richness of silk in cocoon, adhesiveness of egg, etc.

Some of these latter characters cannot be controlled even by most careful and persistent selection, and in this give a strong negative (as do some of the characters of *Leptinotarsa* experimented with by Tower) to the familiar declaration of the selectionist that there is no limit to the quantitative modification of characteristics by means of selection. "Tell me what you want made out of this plant or animal and I'll make it," exclaims the selectionist breeder. But most times he can't, and in those times that he can he will most often do it by hybridization, not pure selection. And this hybridization he will find necessary despite the start in any direction he ought to get from "infinite fortuitous variation."

*Coutagne and Toyama.*—Before setting out any of the data and conclusions derived from my own work with silkworms I must call attention to two previous studies, those respectively of Coutagne (Recherches Experimentales sur l'Hérédité chez les Vers a Soie, published as No. 422, Serie A, Thésés présentées a la Faculté des Sciences de Paris, pp. 1-194, plates I-XI, 1902), and Toyama (Studies on the Hybridology of Insects: I, On some silkworm crosses, with special reference to Mendel's law of heredity, published in Bulletin of the College of Agriculture, Tokyo Imperial University, vol. VII, pp. 259-393, plates VI-XI, 1906). The work of Coutagne was done and his thesis written without knowledge on his part of the experiments and conclusions of Mendel and of Mendel's discoverers, De Vries, Correns and Tschermak, but a part of the work done by the French student and some of his results are distinctly in line with the Mendelian or alternative inheritance principles of heredity. Coutagne, however, gave his principal attention and effort to the modification of fluctuating characters, especially those of quantity and quality of silk, by persistent selection. His work has been recognized and estimated pretty fairly by such thorough-going Mendelian students as Bateson, and needs no particular exploitation or summing up by me.

Toyama's work, in contrast with Coutagne's, has been conducted in the light of a full knowledge of Mendel's work and of that of his successors, and has indeed been directly carried on to test the application of Mendelian principles to silkworm inheritance. It is of interest—of very lively interest, indeed, to me—to note how closely parallel Toyama's work and that part of mine devoted to the same end have been going on, each of us presumably without knowledge of the other's work. We began at practically the same time—Toyama in 1900, I in 1901—and have used the same characteristics in the same way with

readily comparable although (as will be pointed out) not wholly identical results. The differences in the actual work of crossing and rearing seem chiefly to be that Toyama has brought larger proportions of the individuals in each of his experimental lots safely through to maturity (or cocooning time), while I have used a larger number of what may be called repetition lots; that is, lots of exactly similar parentage to serve as checks on each other. The differences in results and conclusions reached by Toyama and myself will be found, I believe, to rest largely on these differences in actual rearing methods.

Toyama has published his results first, and has put into admirably well organized and lucid arrangement his statements of data, results and generalizations. He finds and brings out clearly the indisputable alternative (or Mendelian) character of the inheritance behavior of certain characteristics. He finds a few exceptions to this kind of inheritance, both as to characteristics and as to individual cases of the usually Mendelian characteristics. On the whole he stands as a strong exponent of the generally Mendelian character of inheritance in the silkworms.

In those respects, which are many, in which my own experiments, carried on simultaneously with Toyama's, confirm his published conclusions it will be sufficient for me to do away almost entirely with any exposition of data and details of rearing, and to give simply summary statements of the results of a great deal of work. It is unnecessary to remind any experimental student of heredity of the laborious, exacting and anxiety-breeding character of this kind of work. The results of the expenditure of much energy, time and money can be stated in a few sentences. And especially where these sentences take on the character of simple confirmation of another man's already stated results and conclusions they may be fewer still. Such is my position in the present writing concerning that part of my seven years' work which has absorbed most time and attention. But this confirmation is of course worth while. Our science of heredity, based on experimental study, is too new not to welcome gladly independent confirmation of results already once attained. Such confirmation shows us that we are working on sure ground.

It is where my results disagree with Toyama's, or, perhaps, better expressed, where by circumstance of a considerable recourse to repetition some conspicuous exceptions have been noted, indicating a less rigorously controlled or rigidly regular behavior of inheritance, that I shall use more words than are used in discussing the cases of clear

confirmation. These cases of disagreement or of modification are mostly to be found referred to in the section entitled "Strain and Individual Idiosyncrasies."

## ALTERNATIVE INHERITANCE.

### LARVAL COLOR—PATTERN.

The larval color pattern types that show alternative inheritance are four: (1) moricaud or "darky" (Pl. III, figs. 4, 8); (2) tiger-band or zebra (Pl. I, fig. 2, Pl. III, fig. 2); (3) patterned (Pl. I, fig. 4, Pl. III, figs. 3, 7), and (4) white (Pl. I, figs. 1, 7; Pl. III, figs. 1, 5). The white type shows several sub-types which are, however, of the nature of fluctuating variations (see p. 40). Of these types the moricaud is a melanic sport which has appeared in three different races in our laboratory; the tiger-banded is a dimorphic (or better, dichromatic) form of the Italian Salmon race; the patterned is characteristic of the Japanese White and other races, and the white is characteristic of the Bagdad, the Chinese White, the Istrian and other races.

*Moricaud type.*—In 1904 the first examples of moricaud larvæ appeared in the laboratory. Two moricaud individuals appeared in a lot of Italian Salmon race (eggs received from Sondrio, Italy). One of these died as larva; from the other a male moth was obtained. This was mated with a female of Chinese Cross race (white larval type). The offspring were, as to larval character, equally divided between the paternal (moricaud) type and the maternal (white) type. There were no intergrades. In the second generation rearings all the larvæ derived from mating moths of white larval type together were white, while in cross matings, i. e., moricaud larvæ with white larvæ, lots were obtained composed of moricaud larvæ and white larvæ without intergrades. On account of disease the lots were too small to give the numbers of each kind of larva any value as revealing the true numerical relation of the two types.

In 1905 a single moricaud larva appeared in a Bagdad (white larva) lot. This larva produced a female moth, which was mated with a male Bagdad (from white larva). The young (1906) of this mating were 154 white larvæ and 153 moricaud larvæ with no intergrades. Ten second generation lots were reared (in 1907) by making the following inbred pure and cross matings:

(536) moricaud X moricaud, producing all moricaud larvæ.

(444) white X white, producing all white larvæ.

(470) white X moricaud, producing 11 white larvæ and 5 moricaud. (Lot so reduced by disease as to make the numerical proportions of no significance.)

(564) white X Galbin Italiano race, white larva, producing all white.

(440) moricaud X Japanese Green race, white larva, producing 5 moricaud and 3 white. (Lot so reduced by disease as to make numbers of no significance.)

(563) white X Italian Salmon race, tiger-banded larva, producing 135 tiger-banded, 62 white and 2 moricaud.

(343) white X Italian Yellow race, white larva, producing all white.

(441) moricaud X Japanese White race, patterned larva, producing 45 moricaud and 46 patterned.

(468) moricaud X Istrian race, white larva, producing 120 moricaud and 154 white.

(475) moricaud X Persian Yellow race, white larva, producing 17 moricaud and 19 white.

These few rearings show that moricaudness in larvæ is a dominant Mendelian character, and whiteness a recessive. In all the outmatings with other races than the Bagdad (Nos. 564, 440, 563, 343, 441, 468 and 475) the Bagdad moricaud must have been a cross-bred (heterozygote) individual.

I have had a single moricaud larva appear in a lot of white Chinese Cross race, and a single one in a white Galbin Italiano race.

In 1904 a single larva in a lot of 100 (race unknown) appeared of a "remarkable warm tawny brown clouding over the whole body, the skin being everywhere strongly dotted and finely lined, the spots and lines being a warm brown instead of a blackish brown or blackish lead color characteristic of other moricaud sports."

In some lots of larvæ reared (experimentally) under conditions of extreme humidity from time of hatching to pupation, a marked tendency toward an abundant fine dotting, aggregating into short curved lines was shown, so that the bodies of the worms had a very noticeable blackish or moricaud appearance.

A detailed study on extensive scale of the inheritance behavior of moricaudness is being made in our laboratories by Miss McCracken.

*Tiger-banded or Zebra type.*—The tiger-banded or zebra larval type (Pl. I, fig. 2, Pl. III, fig. 2) is a perfectly distinct and strongly marked type and appears as a regular dimorphic, or better, dichromatic, larval variant in the Italian Salmon race. In relation to the unstriped or white type it is dominant in the Mendelian sense and usually behaves with almost perfect regularity in conformity with Mendelian

principles. Hundreds of examples of this could be adduced from my rearings, both from pure and crossed matings (with reference to larval pattern) within the Italian Salmon race and to outbred matings with various other races, as Bagdad, Istrian, Chinese White, etc., etc., having larvæ of white type. A few cases out of these hundreds will suffice. In all of the scores of matings in the past five years within the Italian Salmon race, testing the Mendelian behavior or relation in inheritance of the two larval types, tiger-banded and white, the two characters behaved in rigorous Mendelian manner, tiger-banded being dominant, white recessive. Never did intergrades appear, never did a tiger-band larva appear in a white X white mating, and wherever the reared cross-bred lots were carried through in something like their full strength the proportions of the two types called for by Mendelian inheritance were closely approximated.

In outbred matings, with races of white larval types, the results may be summed as follows:

Italian Salmon crossed with Bagdad. Tiger-band characteristic is dominant in matings of tiger-band Italian Salmon larvæ with Bagdad larvæ (always white). In second generation rearings from hybrid matings the parental characters segregate according to Mendelian proportions, in many cases the 3 to 1 proportions being almost exactly followed. White larvæ mated together either in  $F_1$  race crosses or in  $F_2$  and succeeding hybrid generation crosses never produce a tiger-band larva. Reciprocal crosses (as to sex) in both  $F_1$  and succeeding generations behave similarly; i. e., show no dominance of sex.

Italian Salmon crossed with Istrian; Italian Salmon crossed with Galbin Italiano; Italian Salmon crossed with Chinese White, and other crosses of Italian Salmon with white larva races. In these race crosses tiger-bandedness of the larvæ behaved regularly as a Mendelian dominant and whiteness as a recessive, and the various familiar 3 to 1, 2 to 1 and 1 to 1 proportions dependent upon the assumed germ cell character of the dominant-carrying member of the pair were all closely approximated in the many lots bred.

*Patterned type.*—The "patterned" type of larva (Pl. I, fig. 4; Pl. III, figs. 3, 7) is shown characteristically by the Japanese White, Italian White and certain other races. Although subject to considerable fluctuating variation (see p. 43) it behaves in inheritance usually as a unit characteristic and is alternative in transmission. It is recessive to tiger-bandedness but dominant to whiteness. But it seems not to be really a unit character in that in cross matings with tiger-bandedness



(as in Japanese White X Italian Salmon, and Italian White X Italian Salmon) not only tiger-banded larvæ and patterned larvæ appear but also tiger-banded-patterned larvæ (Pl. I, fig. 5) and pure white larvæ. And this in the first generation as well as in later ones. In matings of patterned larvæ with white ones (race crosses) pattern is regularly dominant, and follows Mendelian proportions. Occasionally a pure white larvæ or two appear in a pure Japanese White race lot (patterned larvæ). For example, in a 1905 pure race crossing of Japanese White, two white (unpatterned larvæ) appeared, and these mated together (they were fortunately male and female) produced a lot of uniformly white larvæ.

*White type.*—White is regularly recessive to all of the other larval color-pattern types. And white larvæ mated with white never produce any but white larvæ.

#### COCOON COLORS.

The various cocoon colors represented by the races being reared in my laboratory are white, green, pale pinkish yellow (or salmon), lemon yellow, and golden yellow (see Plate IV). To these colors, which are race characteristics, I have added as the result of "break-downs" after hybridizations a long series of mid-shades connecting any pair of members of the racial series. The facts and results of these "break-downs" are to me the most interesting data, perhaps, that the silkworm work has revealed, for I seem to see in them a significance of prime importance. The pointing out of this significance and the facts of the breaking down of the racial color types may be passed for the moment, however, to attend to what phenomena of alternative and Mendelian inheritance may be discovered in these cocoon types.

Mating gold yellow (Istrian race) with pure white to faintly greenish white (Bagdad race) produces sometimes an all gold-yellow first generation with splitting in Mendelian proportions in the second generation lots as in the following example:

F<sub>1</sub> ♂ Istrian (gold-yellow) X ♀ Bagdad (white); produced all gold-yellow cocoons.

F<sub>2</sub> Hyb. yellow X hyb. yellow; produced 64 yellow, 24 white cocoons.

F<sub>2</sub> Hyb. yellow X hyb. yellow; produced 61 yellow, 28 white cocoons.

But this is not always the result of a gold-yellow X white mating, even using the same races. As an example:

F<sub>1</sub> ♂ Bagdad (white) X ♀ Istrian (gold-yellow); produced all white cocoons.

F<sub>2</sub> Hyb. white X hyb. white; produced 77 white, 17 yellow cocoons.

F<sub>2</sub> Hyb. white X hyb. white; produced 62 white, 15 yellow.

This latter example is also a Mendelian type of inheritance, but the difficulty comes when it is compared with the former example. The dominancy in one is with yellow; in the other with white. Note that the two are reciprocal crosses. The dominancy has followed the male. But necessarily so? For answer take another example from this same Bagdad-Istrian series of crossings:

F<sub>1</sub> ♂ Bagdad (white) X ♀ Istrian; produced 31 white cocoons, 21 gold-yellow cocoons.

F<sub>2</sub> Hyb. white X hyb. white; produced 57 white, 31 gold-yellow cocoons.

F<sub>2</sub> Hyb. white X hyb. white; produced 51 white, 18 gold-yellow cocoons.

F<sub>2</sub> Hyb. yellow X hyb. yellow; produced 86 yellow, 34 white cocoons.

F<sub>2</sub> Hyb. yellow X hyb. yellow; produced 42 yellow, 7 white cocoons.

F<sub>2</sub> ♂ Hyb. white X ♀ hyb. yellow; produced 40 gold-yellow, 26 white cocoons.

F<sub>2</sub> ♂ Hyb. yellow X ♀ hyb. white; produced 36 white, 29 gold-yellow cocoons.

Thus within a group of the same race crosses first one color and then the other proved dominant or neither was dominant. But in all cases the inheritance was strictly alternative, never particulate or blended.

Using other races of yellow cocooners and other races of white cocooners various similarly conflicting results were obtained. For example, in Italian Yellow X Chinese White, yellow was dominant; in Italian Yellow X Japanese White, yellow was dominant; in Turkish and French Yellow X Bagdad White, white was dominant; in Istrian Yellow X Chinese White, yellow was dominant; in Istrian Yellow X Japanese White, yellow was dominant; in Persian Yellow X Chinese White, yellow was dominant; in Persian Yellow X Bagdad White, white was dominant; in Italian Yellow X Italian White, approximately half of the offspring of generation F<sub>1</sub> were white and half of them yellow.

In crossing yellow with green, first generation results were: in Istrian Yellow X Japanese Green, offspring were all yellow; in Persian Yellow X Japanese Green, a majority of the young were yellow, a minority green (numbers too small to be of significance as to proportions); in Italian Yellow X Japanese Green, majority of the offspring were yellow, minority green (numbers were too small to be of significance as to proportions).

In crossing white and green, first generation results are: in Bagdad White X Japanese Green, all offspring are *yellow*.

In crossing yellow and salmon, as in Persian lemon Yellow X Italian Salmon, the offspring represented all shades from pale salmon to golden yellow; in Istrian golden Yellow X Italian Salmon, the offspring were salmon.

In crossing white and salmon as in Bagdad White X Italian Salmon, salmon is usually dominant (numerous cases of the dominance of white, however, see Strain and Individual Idiosyncrasies) but the cocoons are not of single salmon tint characteristic of the Italian Salmon race but are of all shades from very pale or whitish salmon to very yellowish salmon or indeed definitely yellow even golden. But in second generation lots produced by intermating hybrids the white color usually appears again as a Mendelian recessive distinct from the pale to yellow salmon shades constituting a Mendelian dominant. In some white and salmon crosses as Italian White X Italian Salmon, white was dominant. For example of the breaking of salmon into all shades of pale salmon to golden-yellow:

F<sub>1</sub> ♂ Bagdad White X ♀ Italian Salmon; produced all salmon cocoons.

F<sub>2</sub> Hyb. salmon X hyb. salmon; produced 28 pale to yellow salmon and 7 white cocoons.

F<sub>2</sub> Hyb. salmon X hyb. salmon; produced 30 pale to yellow salmon and 15 white cocoons.

F<sub>1</sub> ♂ Italian Salmon X Bagdad White; produced all yellowish salmon to strong yellow cocoons.

F<sub>2</sub> Hyb. yellow salmon X hyb. yellow salmon; produced 23 pale to yellow salmon and 19 white cocoons.

F<sub>2</sub> Hyb. yellow salmon X hyb. yellow salmon; produced 50 salmon to yellow and 16 white cocoons.

The behavior of cocoon color crosses is evidently so erratic (at least is *apparently* so erratic) that it may more appropriately be discussed in the section on "Strain and Individual Idiosyncrasies" rather

than in this section which is concerned primarily with recording Mendelian behavior. Cocoon colors often follow Mendelian proportions but are not rigorously related as dominant and recessive to each other; and are not even rigorously alternative.

It should be noted in this connection that whereas I have found the larval color pattern characteristics to behave for the most part in very satisfying Mendelian manner, being rigidly alternative in inheritance and following in their transmission with close approximation the Mendelian proportions, I have found the cocoon colors to be much less consistent in behavior.

Toyama on the other hand found both larval and cocoon characters to be equally consistent and Mendelian in behavior.

#### LARVAL PATTERN AND COCOON COLORS IN THE SAME MATINGS.

It is of interest to note the results in matings combining crosses of opposed larval patterns and opposed cocoon colors at the same time.

In the first place the occurrence of typical Mendelian two-pair combinations may be noted; as in crossings of Bagdad white larva, white cocoon, with Italian Salmon, tiger-banded larva, salmon yellow cocoon, where in first generation all the larvæ are tiger-banded and all the cocoons salmon, *or white* (see reference to this in section on Strain and Individual Idiosyncrasies), with the second generation lots from intermated hybrids breaking into 3 to 1 of tiger-banded to white larvæ and inside of each of these into 3 to 1 salmon to white (*or white to salmon*) cocoon lots.

But I want particularly to call attention to the fact that in these crossings of combined opposed larval and cocoon characteristics we are dealing with characters of different life stages of the animals and that we can often note the interesting fact of the offspring following the paternal parent in a characteristic of one life-stage and the maternal parent in a characteristic of another life-stage. For example the following is a type of the inheritance behavior of the larval and cocoon characteristics in scores (hundreds indeed) of lots: ♂ Italian Salmon, tiger-banded larva, salmon yellow cocoon, X ♀ Bagdad, white larva, white cocoon; produced all tiger-banded larvæ and all white cocoons.

Such examples only serve to bring out in still stronger relief the fact that the inheritance behavior is a function of the character not of the influence of the parent.

In numerous other cases we find the inheritance in both larval and cocoon characteristics agreeing in following a single one of the

parents, as where all the young of a cross-mating between a tiger-banded larva, salmon cocoon race, as Italian Salmon, and a white larva white cocoon race as Chinese White, being tiger-banded larvæ spinning salmon-colored cocoons. But these cases of coincidence in both larval and cocoon characters being those of either the father or the mother are really only coincidences in the possession by the one parent of the two dominant members of a double pair of allelomorphs. It is still the dominance of the character and not of the parent that determines the condition of the offspring as concerns the appearance or lying latent of the character in question.

It is unfortunate that none of the adult characters has yet been found to be of the alternative Mendelian inheritance type, so that a comparison of the transmission of characters in all three stages, larval, pupal and imaginal, might be made. The adult variations in wing-pattern and in the color and adhesiveness of the eggs are fluctuating and not alternative in character.

#### PREPOTENCY OF SEX AND VIGOR.

It was desired to determine whether the dominance of a character in heredity could be weakened or destroyed by weakening or lessening the vigor of the parent representing the character, or whether in general any prepotency in heredity was due to vigor or sex.

Experiments were begun, therefore, in 1904 by rearing certain individuals under conditions of short food and others of full food and making matings between these starvelings and full-fed vigorous individuals. The individuals used for experiment were selected so as to represent two races offering a Mendelian pair of allelomorphs both as to larval and cocoon characteristics. Examples of these 1904 matings and their results are as follows:

A male starveling of race Italian Salmon, zebra larva, salmon cocoon, was mated with a full-fed, vigorous female of race Chinese White, unpatterned white larva, white cocoon. The young were all zebra larvæ.

A male starveling of Italian Salmon race, zebra larva, salmon cocoon, was mated with a full-fed, vigorous female of race Chinese White, unpatterned white larva, white cocoon. The young were 70 zebra larvæ and 75 unpatterned white larvæ; all the cocoons were salmon.

A full-fed, vigorous male of Italian Salmon race, zebra larva, salmon cocoon, was mated with a starveling female of Japanese White

race, patterned white larva, white cocoon. Two-thirds of the young were blended zebra and patterned larvæ, and one-third were patterned white larvæ.

In the first case the starveling male was undoubtedly a homozygote; in the second case a heterozygote. In the first case the weak condition of the starveling male did not affect or modify in any degree the characteristic dominance of the zebra over the white larval type. In the third case there seems to be an interesting varying from the Mendelian or alternative type of inheritance to an unmistakable and perfect blending in the eggs of two-thirds of the progeny.

In 1905 another set of experiments along this line of testing the prepotency of vigor and sex was made. The following examples of these matings and their results may be referred to:

A male starveling, Italian Salmon, zebra larva, salmon cocoon, mated with a vigorous female, Bagdad race, white larva, white cocoon. Offspring all zebra larvæ; cocoons showing many shades of color from greenish white through salmon and dull yellow to golden yellow.

A male starveling, Italian Salmon, zebra larva, salmon cocoon, mated with vigorous female, Bagdad, white larva, white cocoon. Offspring were all zebra larvæ.

A male starveling, Italian Salmon, zebra larva, salmon cocoon, mated with vigorous female, Bagdad, white larva, white cocoon. Offspring were 133 zebra larvæ and 129 white larvæ. Cocoons all salmon.

A vigorous male, Italian Salmon, zebra larva, salmon cocoon, mated with female starveling, Bagdad, white larva, white cocoon. Offspring all zebra larvæ; cocoons all white.

A male, vigorous, Italian Salmon, zebra larva, salmon cocoon, mated with female starveling, Bagdad, white larva, white cocoon. Offspring all zebra larvæ; cocoons of several shades from yellowish salmon to strong golden yellow.

A male starveling, Bagdad, white larva, white cocoon, mated with female, vigorous, Italian Salmon, zebra larva, salmon cocoon. Offspring all zebra larvæ; cocoons 78 white and 71 salmon.

A male, vigorous, Italian Salmon, zebra larva, salmon cocoon, mated with female starveling, Bagdad, white larva, white cocoon. Offspring all zebra larvæ; cocoons 63 white and 65 salmon to yellowish salmon.

A male, vigorous, Italian Salmon, zebra larva, salmon cocoon, mated with female starveling, Bagdad, white larva, white cocoon. Offspring all zebra larvæ, cocoons  $\frac{1}{3}$  salmon and  $\frac{2}{3}$  white.

From these matings and results it seems obvious that what determines the behavior in inheritance of a character, that is, what determines its prepotency or lack of prepotency, its dominance or recessiveness, is something fully apart from (a) sex of the parent and (b) physical vigor of the parent. In all the above matings the larval character zebra striping is regularly dominant in all lots, whether the parent representing the zebra larval characteristic be male or female, vigorous or weak bodied.

## STRAIN AND INDIVIDUAL IDIOSYNCRASIES.

In numerous conversations with Luther Burbank the distinguished plant-breeder of Santa Rosa, California, I have heard a certain phrase fall often from his lips. Many years of close observation and of extraordinarily wide experimentation in inheritance have deeply impressed on Burbank the actuality of "individual idiosyncrasy" in the matters of heredity. And I use this term as expressing what I believe actually to exist in the case of the silkworms. Coupled with it I use also the phrase "strain idiosyncrasy" to indicate a varying inheritance behavior of certain characteristics according to races or strains of long breeding.

These phrases are not used to obscure explanation or to relegate the matter to hopeless confusion—there is of course regularity at the bottom somewhere—but are used because no generalization or law of inheritance so far formulated seems to offer an expression or explanation sufficiently defining the actual phenomena or order of inheritance as exhibited by the silkworms (and by other animals).

As examples of the condition described as "individual idiosyncrasy," we may take the following:

♂ Bagdad pure race, white larva, white cocoon X ♀ Italian Salmon, pure race, tiger-banded larva, salmon cocoon; produced 135 tiger-band, 129 white larvæ, and all salmon cocoons.

♂ Italian Salmon pure race, tiger-banded larva, salmon cocoon, X ♀ Bagdad pure race, white larva, white cocoon; produced all tiger-band larvæ, and all white cocoons.

♂ Bagdad pure race, white larva, white cocoon, X ♀ Italian Salmon, pure race, tiger-banded larva, salmon cocoon; produced all tiger-band larvæ and 78 white cocoons and 71 salmon.

Now the differences in the larval inheritance in these three first cross rearings are explicable on the basis of the Italian Salmon parent having been a homozygote (as regards the larval characteristic) in two cases and a heterozygote in one. But the differences in cocoon character inheritance are not to be so explained.

In the  $F_2$  generations from intermated hybrids of these rearings the larvæ in all cases (except white X white) segregated according to parental characters and did so in Mendelian proportions; the cocoons also segregated according to the parental characters and also did so in most cases with some approximation to Mendelian proportions.

Now to illustrate "strain idiosyncrasy."



Mating Istrian, golden-yellow cocoon, with Chinese White, pure white cocoon race, produced all golden-yellow cocoons; also mating Istrian with Japanese White, pure white cocoon race, produced all golden-yellow cocoons; but mating Istrian with Bagdad pure white cocoon race produced (in some instances) all white cocoons. In fact although the cocoon character of most white cocoon races is recessive in matings with the yellow, green or salmon colors of other races, the white cocoon character of the Bagdad race is dominant in most crossed race matings.

The importance of this matter of a difference in inheritance behavior of the same characteristic in different strains and in different individuals of the same strain leads me to offer in some detail an account of the data obtained from several series of rearings. These data will reveal also certain irregularities in the inheritance behavior which make it difficult or impossible for me to accept Toyama's sweeping conclusions as to the rigorous alternative and Mendelian or in any way thoroughly consistent behavior of the silkworm cocoon colors. In fact my whole work disposes me to be very chary of accepting too quickly the fascinating generalizations concerning the simplicity or rigorous regularity of inheritance behavior. There is no doubt in the world that the Mendelian discoveries and conclusions are a great step forward in our understanding of inheritance phenomena. That they are as widely or as rigorously applicable as some Mendelian disciples assume I doubt very much.

Data of a series of crossings between Bagdad, pure race, bluish-white larva, white cocoon, and Istrian, pure race, clayey-white larva, golden-yellow cocoon.

F<sub>1</sub> ♂ Istrian X ♀ Bagdad; produced all golden-yellow cocoons.

F<sub>2</sub> Hyb. yellow X hyb. yellow; produced 64 yellow, 24 white cocoons.

F<sub>2</sub> Hyb. yellow X hyb. yellow; produced 61 yellow, 28 white cocoons.

F<sub>1</sub> ♂ Bagdad X ♀ Istrian; produced all white cocoons.

F<sub>2</sub> Hyb. white X hyb. white; produced 77 white, 17 yellow cocoons.

F<sub>2</sub> Hyb. white X hyb. white; produced 62 white, 15 yellow cocoons.

F<sub>1</sub> ♂ Bagdad X ♀ Istrian; produced 31 white, 21 yellow cocoons.

F<sub>2</sub> Hyb. white X hyb. white; produced 57 white, 31 yellow cocoons.

- F<sub>2</sub> Hyb. white X hyb. white; produced 51 white, 18 yellow cocoons.
- F<sub>2</sub> Hyb. yellow X hyb. yellow; produced 86 yellow, 34 white cocoons.
- F<sub>2</sub> Hyb. yellow X hyb. yellow; produced 42 yellow, 7 white cocoons.
- F<sub>2</sub> ♂ Hyb. white X ♀ hyb. yellow; produced 40 yellow, 26 white cocoons.
- F<sub>2</sub> ♂ Hyb. yellow X ♀ hyb. white; produced 29 yellow, 36 white cocoons.
- F<sub>1</sub> ♂ Bagdad X ♀ Istrian; produced 10 white, 9 yellow cocoons.
- F<sub>2</sub> Hyb. yellow X hyb. yellow; produced 26 yellow, 11 white cocoons.
- F<sub>2</sub> ♂ Hyb. yellow X ♀ hyb. white; produced 56 yellow, 54 white cocoons.
- F<sub>2</sub> ♂ Hyb. white X ♀ hyb. yellow; produced 45 yellow, 67 white cocoons.
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| <p>F<sub>1</sub> ♂ Bagdad X ♀ Istrian;<br/>produced all white cocoons.</p> <p style="text-align: center;">⚧<br/>↓</p> <p>F<sub>2</sub> ♂ Hyb. white<br/>produced 66 yellow, 41 white cocoons.</p> <p>F<sub>1</sub> ♂ Bagdad X ♀ Istrian;<br/>produced 31 white, 21 yellow cocoons.</p> <p style="text-align: center;">⚧<br/>↓</p> <p>F<sub>2</sub> Hyb. white<br/>26 yellow cocoons.</p> <p style="text-align: center;">⚧<br/>↓</p> <p>F<sub>2</sub> Hyb. yellow<br/>13 white cocoons.</p> <p>F<sub>2</sub> ♀ Hyb. yellow<br/>low, 48 white cocoons.</p> <p>F<sub>2</sub> ♀ Hyb. white<br/>low, 52 white cocoons.</p> <p>F<sub>2</sub> Hyb. white<br/>33 yellow cocoons.</p> | <p>♂ Istrian X ♀ Bagdad; produced<br/>all yellow cocoons.</p> <p style="text-align: center;">⚧<br/>↓</p> <p>X ♀ hyb. yellow;</p> <p>♂ Bagdad X ♀ Istrian; produced<br/>10 white, 9 yellow cocoons.</p> <p style="text-align: center;">⚧<br/>↓</p> <p>X hyb. white; produced 85 white,</p> <p>X hyb. yellow; produced 71 yellow,</p> <p>X ♂ hyb. white; produced 72 yellow,</p> <p>X ♂ hyb. yellow; produced 73 yellow,</p> <p>X hyb. white; produced 86 white,</p> |
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In the above series there is a striking combination of alternative inheritance in Mendelian manner with marked individual idiosyncrasies. At first glance these idiosyncrasies seem to depend on sex-dominancy but an inspection of the  $F_2$  generations will show that sex is not the determinant of dominancy.

Data of a series of crossings between Italian Salmon, \*zebra larva, pinkish yellow (salmon) cocoon race and Bagdad, white larva, white cocoon race.

$F_1$  ♂ Ital. Sal. X ♀ Bagdad; producing all zebra larva, and cocoons varying from dirty white through salmon, pale straw yellow to golden yellow.

$F_1$  ♂ Bagdad X ♀ Ital. Sal; producing 133 zebra, and 129 white larvæ; all salmon cocoons.

$F_2$  ♂ Hyb. zebra larva, salmon cocoon, X ♀ hyb. white larva, salmon cocoon; producing 35 zebra larvæ spinning 17 salmon to yellow and 3 white cocoons, and 26 white larvæ, spinning 11 pale salmon to yellow and 4 white cocoons.

$F_2$  ♂ Hyb. white larva, salmon cocoon, X ♀ zebra larva, salmon cocoon; produced 30 zebra larvæ spinning all salmon to yellow cocoons, and 33 white larvæ spinning 18 salmon to yellow cocoons.

$F_2$  ♂ Hyb. white larva, salmon cocoon, X ♀ hyb. zebra larva, salmon cocoon; produced 48 zebra larvæ spinning 13 salmon to yellow and 9 white cocoons, and 36 white larvæ spinning 17 pale salmon to yellow and 6 white cocoons.

$F_2$  Hyb. white larva, salmon cocoon, X hyb. white larva, salmon cocoon; produced all white larvæ, spinning 25 salmon to yellow and 7 white cocoons.

$F_1$  ♂ Ital. Sal. X ♀ Bagdad; produced all zebra larvæ and all white cocoons.

$F_2$  Hyb. zebra larva, white cocoon, X hyb. zebra larva, white cocoon; produced 46 zebra larvæ spinning 27 white and pale salmon cocoons, and 15 white larvæ spinning 10 white and 2 pale salmon cocoons.

$F_2$  Hyb. zebra larva, white cocoon, X hyb. zebra larva, white cocoon; produced 40 zebra larvæ spinning 12 white and 5 salmon cocoons and 7 white larvæ spinning 2 white and 1 salmon cocoons.

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\* Italian Salmon race has two discontinuous types of larvæ, viz., Zebra and White, but where the race name is used without qualification I refer always to Zebra type.

F<sub>1</sub> ♂ Ital. Sal. X ♀ Bagdad; produced all zebra larvæ and all yellowish salmon to golden yellow cocoons.

F<sub>2</sub> Hyb. zebra larva, salmon-yellow cocoon X hyb. zebra larva, salmon-yellow cocoon; produced zebra larvæ spinning 19 white and 19 salmon to yellow cocoons, and white larvæ spinning 4 salmon to yellow cocoons.

F<sub>2</sub> Hyb. zebra larva, salmon-yellow cocoon X hyb. zebra larva, salmon-yellow cocoon; produced 68 zebra larvæ spinning 14 white and 38 salmon to yellow cocoons, and 26 white larvæ spinning 2 white and 12 salmon to yellow cocoons.

F<sub>1</sub> ♂ Bagdad X ♀ Ital. Sal.; produced all zebra larvæ and 78 white and 71 salmon cocoons.

F<sub>2</sub> Hyb. zebra larva, white cocoon X hyb. zebra larva, white cocoon; produced 96 zebra larvæ spinning 73 white and 23 salmon cocoons and 26 white larvæ spinning 4 white and 1 salmon cocoons.

F<sub>2</sub> Hyb. zebra larva, white cocoon X hyb. zebra larva, white cocoon; produced 108 zebra larvæ spinning 58 white and 20 very pale salmon to light yellow-salmon cocoons, and 40 white larvæ spinning 22 white and 8 pale salmon cocoons.

F<sub>2</sub> Hyb. zebra larva, white cocoon X hyb. zebra larva, white cocoon; produced 125 zebra larvæ spinning 64 white and 16 salmon cocoons, and white larvæ spinning 15 white and 72 salmon cocoons.

F<sub>2</sub> Hyb. zebra larva, salmon cocoon X hyb. zebra larva, salmon cocoon; produced 105 zebra larvæ spinning 9 white and 55 pale to yellow salmon cocoons, and 67 white larvæ spinning 17 white and 50 pale to yellow salmon cocoons.

F<sub>2</sub> ♂ Hyb. zebra larva, white cocoon X ♀ hyb. zebra larva, yellow cocoon; produced 97 zebra larvæ spinning 25 white and 24 salmon cocoons, and 20 white larvæ spinning 7 white and 9 salmon cocoons.

F<sub>2</sub> ♂ Hyb. zebra larva, white cocoon X ♀ hyb. zebra larva, yellow cocoon; produced zebra larvæ spinning 60 white and 45 salmon cocoons, and 35 white larvæ spinning 14 white and 17 salmon cocoons.

F<sub>2</sub> ♂ Hyb. zebra larva, yellow cocoon X ♀ hyb. zebra larva, white cocoon; produced 140 zebra larvæ spinning 40 white and 63 salmon cocoons, and 68 white larvæ spinning 21 white and 28 salmon cocoons.

F<sub>1</sub> ♂ Ital. Sal. X ♀ Bagdad; producing all zebra larvæ and 63 white and 65 salmon to yellow (20 really yellow) cocoons.

F<sub>2</sub> Hyb. zebra larva, yellow cocoon X hyb. zebra larva, yellow cocoon; produced zebra larvæ spinning 21 white and 81 salmon to yellow cocoons, and 47 white larvæ spinning 8 white and 20 salmon to yellow-salmon cocoons.

F<sub>2</sub> Hyb. zebra larva, yellow cocoon X hyb. zebra larva, yellow cocoon; produced 45 zebra larvæ spinning 6 white and 12 salmon to yellow cocoons, and 18 white larvæ spinning 3 white and 15 salmon to yellow cocoons.

F<sub>2</sub> Hyb. zebra larva, white cocoon X hyb. zebra larva, white cocoon; produced 66 zebra larvæ spinning 54 white cocoons and 17 white larvæ spinning 4 white and 1 pale salmon cocoons.

F<sub>2</sub> Hyb. zebra larva, white cocoon X hyb. zebra larva, white cocoon; produced 10 zebra larvæ spinning 3 white and 1 salmon cocoons, and 3 white larvæ spinning 1 white cocoon.

F<sub>1</sub> ♂ Ital. Sal. X ♀ Bagdad; produced all zebra larvæ and 11 white and 5 yellow-salmon cocoons.

F<sub>2</sub> Hyb. zebra larva, white cocoon X hyb. zebra larva, white cocoon; produced 158 zebra larvæ spinning 83 white and 35 pale salmon cocoons and 43 white larvæ spinning 26 white and 6 pale salmon cocoons.

F <sub>1</sub> ♂ Bagdad X ♀ Ital. Sal.;	♂ Ital. Sal. X ♀ Bagdad;
produced all zebra larvæ and 78 white and 71 salmon cocoons.	produced all zebra larvæ and 63 white and 65 salmon cocoons.



F<sub>2</sub> Hyb. zebra larva, white cocoon X hyb. zebra larva, white cocoon; produced 88 zebra larvæ spinning 43 white and 10 pale salmon cocoons, and 16 white larvæ spinning 10 white and 5 pale salmon cocoons.

F<sub>2</sub> Hyb. zebra larva, white cocoon X hyb. zebra larva, white cocoon; produced 98 zebra larvæ spinning 68 white and 21 pale salmon cocoons, and 39 white larvæ spinning 19 white and 10 pale salmon cocoons.

- F<sub>2</sub> Hyb. zebra larva, white cocoon X hyb. zebra larva, white cocoon; produced 112 zebra larvæ spinning 77 white and 19 pale salmon cocoons, and 51 white larvæ spinning 13 white and 4 pale salmon cocoons.
- F<sub>2</sub> Hyb. zebra larva, salmon cocoon X hyb. zebra larva, salmon cocoon; produced 85 zebra larvæ spinning 14 white and 38 salmon to yellow cocoons, and 26 white larvæ spinning 3 white and 21 salmon cocoons.
- F<sub>2</sub> ♂ Hyb. zebra larva, yellow cocoon X ♀ hyb. zebra larva, white cocoon; produced 99 zebra larvæ spinning 34 white and 28 pale salmon to salmon cocoons, and 37 white larvæ spinning 14 white and 19 salmon cocoons.
- F<sub>2</sub> ♀ Hyb. zebra larva, white cocoon X ♂ hyb. zebra larva, yellow cocoon; produced 227 zebra larvæ spinning 63 white and 68 pale to yellow salmon cocoons, and 47 white larvæ spinning 20 white and 27 pale to yellow salmon cocoons.

A few examples from other Bagdad X Italian Sal. series may be given to emphasize the actuality of individual idiosyncrasies in these crossings.

- F<sub>1</sub> ♂ Ital. Sal. X ♀ Bagdad; produced 50% zebra and 50% white larvæ, and all white cocoons.
- F<sub>2</sub> Hyb. zebra larva, white cocoon X hyb. zebra larva, white cocoon; produced 43 zebra and 11 white larvæ and 38 white and 9 salmon to golden yellow cocoons.
- F<sub>2</sub> ♂ Hyb. white larva, white cocoon X ♀ hyb. zebra larva, white cocoon; produced 61 zebra and 59 white larvæ, and 67 white and 20 salmon cocoons.
- F<sub>1</sub> ♂ Bagdad X ♀ Ital. Sal.; produced all zebra larvæ and all white cocoons.
- F<sub>2</sub> Hyb. X hyb.; produced 68 zebra and 42 white larvæ and 44 white and 19 salmon to golden cocoons.
- F<sub>2</sub> Hyb. X hyb.; produced 114 zebra and 26 white larvæ and 36 white and 9 salmon cocoons.
- F<sub>1</sub> ♂ Bagdad X ♀ Ital. Sal.; produced 58 zebra and 73 white larvæ, and all white cocoons.

- F<sub>2</sub> Hyb. zebra larva, white cocoon X hyb. zebra larva, white cocoon; produced 37 zebra and 14 white larvæ, and 23 white and 2 salmon cocoons.
- F<sub>1</sub> ♂ Bagdad X ♀ Ital. Sal.; produced 50% zebra and 50% white larvæ and all salmon cocoons.
- F<sub>2</sub> Hyb. zebra larva, salmon cocoon X hyb. zebra larva, salmon cocoon; produced 75 zebra and 16 white larvæ and 16 white and 45 pale salmon to golden yellow cocoons.
- F<sub>2</sub> ♂ Hyb. zebra larva, salmon cocoon X ♀ hyb. white larva, salmon cocoon; produced 52 zebra and 42 white larvæ, and 21 white and 59 salmon to golden cocoons.
- F<sub>2</sub> ♂ Hyb. white larva, salmon cocoon X ♀ hyb. zebra larva, salmon cocoon; produced 63 zebra and 78 white larvæ, and 21 white and 57 salmon to golden cocoons.
- F<sub>1</sub> ♂ Bagdad X ♀ Ital. Sal.; produced 50% zebra and 50% white larvæ and all salmon to yellow cocoons.
- F<sub>2</sub> ♂ Hyb. zebra larva, salmon cocoon X ♀ hyb. white larva, salmon cocoon; produced 43 zebra and 40 white larvæ, and 22 white and 39 salmon to golden cocoons.
- F<sub>2</sub> Hyb. zebra larva, salmon cocoon X hyb. zebra larva, salmon cocoon; produced 123 zebra and 44 white larvæ and 27 white and 109 salmon to golden cocoons.
- F<sub>2</sub> Hyb. zebra larva, salmon cocoon X hyb. zebra larva, salmon cocoon; produced 122 zebra and 15 white larvæ and 33 white and 91 salmon to golden cocoons.
- F<sub>2</sub> ♂ Hyb. zebra larva, salmon cocoon X ♀ hyb. white larva, salmon cocoon; produced 83 zebra and 66 white larvæ, and 19 white and 99 salmon to golden cocoons.
- F<sub>1</sub> ♂ Bagdad X ♀ Ital. Sal.; produced 60 zebra and 45 white larvæ and all salmon to yellow cocoons.
- F<sub>2</sub> Hyb. zebra larva, salmon cocoon X hyb. zebra larva, golden cocoon; produced 85 zebra and 22 white larvæ and 6 white and 47 salmon to golden cocoons.
- F<sub>1</sub> ♂ Ital. Sal. (white larva) X ♀ Bagdad; produced all white larvæ and 9 white and 15 salmon yellow cocoons.
- F<sub>2</sub> Hyb. white larva, salmon cocoon X hyb. white larva, salmon cocoon; produced all white larvæ, and 5 white and 16 salmon to yellow cocoons.

- F<sub>2</sub> Hyb. white larva, salmon cocoon X hyb. white larva, salmon cocoon; produced all white larvæ and 10 white and 22 salmon to golden-yellow cocoons.
- F<sub>1</sub> ♂ Bagdad X ♀ Ital. Sal.; produced all zebra larvæ and 19 white and 35 salmon to yellow cocoons.
- F<sub>2</sub> Hyb. zebra larva, salmon cocoon X hyb. zebra larva, salmon cocoon; produced 73 zebra and 28 white larvæ and 47 white and 38 salmon to golden-yellow cocoons.
- F<sub>2</sub> ♂ Hyb. zebra larva, salmon cocoon X ♀ hyb. zebra larva, white cocoon; produced 156 zebra and 39 white larvæ and 59 white and 61 salmon to yellow cocoons.
- F<sub>2</sub> Hyb. zebra larva, salmon cocoon X hyb. zebra larva, salmon cocoon; produced 123 zebra and 55 white larvæ and 17 white and 99 salmon to golden cocoons.
- F<sub>2</sub> Hyb. zebra larva, salmon cocoon X hyb. zebra larva, salmon cocoon; produced 50 zebra and 15 white larvæ and 17 white and 44 salmon to golden cocoons.
- F<sub>2</sub> Hyb. zebra larva, salmon cocoon X hyb. zebra larva, salmon cocoon; produced 60 zebra and 19 white larvæ and 15 white and 45 salmon to golden cocoons.
- F<sub>2</sub> Hyb. zebra larva, salmon cocoon X hyb. zebra larva, salmon cocoon; produced 34 zebra and 17 white larvæ and 19 white and 26 salmon to golden cocoons.

In these series are to be noted the regularly Mendelian behavior of the larval patterns (in many of the lots the numbers were either so reduced by disease or by the necessities of space, food and time of care-takers as to obscure the Mendelian proportions), the marked individual idiosyncrasies (reversal of dominance, splitting of colors equally in first crosses, impure recessive behavior in second (hybrid) generations, etc., etc.) in the cocoon color inheritance, the constant tendency for the salmon color to break into a series of graduating colors ranging from the very pale salmon through to strong (golden) yellow, and the influence of white toward making the salmon extremely pale, i. e., to produce a blending in inheritance rather than a sharp segregation. But the cocoon color does not always behave irregularly. In many cases it behaves in almost exact Mendelian manner, and this is true whether in F<sub>1</sub> the dominant color is salmon yellow or is white. In F<sub>2</sub> lots the splitting will then be respectively 3 salmon-yellow to 1 white or 3 white to 1 salmon-yellow, which



very regularity only emphasizes more the reality of individual idiosyncrasies in such cases of reversed dominance.

While white cocoon color is in most race crossings recessive in character, it is, as already pointed out, not so in all crossings of white and colored cocoon races. For example Bagdad race (white cocoon) seems to be an especial vigorous or potent race in race crossings, the white cocoon color being frequently dominant. Examples of this have already been given. In addition Italian White (white cocoon) crossed with Galbin Italiano (salmon-yellow cocoon) gives young all spinning white cocoons and in  $F_2$  lots the two colors segregate in Mendelian proportions. In these crossings we have examples (asked for by Bateson in his summing up of the "progress of genetics since the rediscovery of Mendel's papers," p. 389, *Progressus Rei Botanicae*, vol. I, 1907) of two whites producing a color. That is the color is carried germinally through an all white  $F_1$  to appear in  $F_2$ .

In some matings with this same Galbin Italiano salmon-yellow cocoon race a reversal of the above described condition occurred. For example Galbin Italiano crossed with Bagdad (in many crossings a prepotent race and almost always stronger than Japanese White) showed in  $F_1$  a dominance of the salmon-yellow cocoon color which in  $F_2$  lots split in Mendelian proportions.

Despite the inconsistencies in dominant-recessive relation between the cocoon color exhibited in the foregoing data the faithfulness to the alternative character of the inheritance (except in the matter of the break-down of salmon into all the shades from very pale salmon to strong golden yellow) and the adherence or approximation to Mendelian numerical proportions are striking. But these two features have also their marked exceptions in other series of crossings.

In mating Bagdad white cocoon race with Japanese green cocoon race white, greenish white, green, greenish yellow and yellow cocoons are got in the first generation.

In mating Bagdad white cocoon race with Persian lemon yellow cocoon race, green and strong yellow cocoons are got in the first generation. In mating Italian Salmon, pale pinkish yellow or salmon cocoon race, with Istrian, strong golden yellow cocoon race, cocoons of all gradations from salmon to golden are got in the first generation, and also in the second generation whatever two cocoon shades be mated together. In mating Italian Salmon and Chinese White, in first, and especially in later generations, there is a strong tendency for all sharp distinction between white and salmon to break down and cocoons

are got representing a continuous series of gradations from white up to well-marked salmon, the whitish and pale salmon shades being most abundant. But not always! As for example:

F<sub>1</sub> Italian Salmon X Chinese White; produced 60 zebra and 60 white larvæ and cocoons ranging from very pale to strong salmon.

F<sub>2</sub> Hyb. X hyb.; produced very pale salmon cocoons.

F<sub>2</sub> Hyb. X hyb.; produced 31 creamy to salmon cocoons.

F<sub>2</sub> Hyb. X hyb.; produced 38 whitish to salmon cocoons.

F<sub>2</sub> Hyb. X hyb.; produced 31 salmon cocoons.

F<sub>2</sub> Hyb. X hyb.; produced all salmon to strong yellowish salmon cocoons.

And repeated groups of F<sub>2</sub> generations varied among themselves although the parents of all the members of each group were brothers and sisters (i. e., all from a single F<sub>1</sub> lot). But in the large majority of lots the break-down was complete and the cocoons ran continually from white to salmon, with the modal shade a very pale salmon.

*Conclusions.*—Not to prolong unduly this discussion an end may be made of the presenting of data. The evidence could be piled high by introducing the details of other series of rearings, but this seems to me unnecessary.

It seems plain to me that the inheritance of the cocoon color character is not a consistent one. The characteristic may behave in strictly alternative and nearly exact Mendelian manner. Or it may be inconsistent as to dominance within the same races; that is of a pair of allelomorphs one may be dominant in one cross mating and the other dominant in a second cross mating between the same races. While in a third cross mating between the same pure races neither cocoon color may be dominant but half or another proportion of the offspring may be of one color and the rest of the other color. Or the color characters may not behave as a strictly alternative character but may blend or break down in transmission.

These variants or deviations from a strictly alternative Mendelian character may appear within the same race crossings and even within a single group of F<sub>2</sub> and F<sub>3</sub> generations, all derived from a common parental or grand-parental crossing, or these deviations may be characteristic of crossings between different races or strains possessing similar cocoon color. In the first place the deviations or inconsistencies in inheritance behavior may be attributed to "individual idiosyncrasies"; in the second to "strain idiosyncrasies."

The condition is different in the case of the larval characters. Here the inheritance behavior is consistent, is rigid. It can be prophesied. It follows the Mendelian principles of alternative inheritance with great fidelity.

What is the reason for this difference between the inheritance behavior of the larval characters and that of the cocoon characters? What is the significance of this difference?

In the five thousand years, or less during which the mulberry silkworm has been the subject of man's ameliorating attention the principal aim of all the manipulation by the various processes involved in artificial selection has been the modification of the cocoon characteristics. The attempt has been to produce more silk, better silk, silk of one color, silk of another color. As regards larval and imaginal characters, much less attention and manipulation have been given. Docile, disease-resistant and hearty-feeding larvæ, prolific and sedentary moths have been encouraged by selection. But larval patterns, diverse and distinct though they appear to us today, have not been the product of the breeder's work except as they may be correlated with valuable cocoon characters and thus preserved by the way. The diversity in larval pattern is a natural diversity; the differences have appeared and have persisted according to natural processes.

Not so with the cocoon characters. Or at least only in so far as natural variation has coincided with the breeder's wishes. The cocoon colors have originated as fluctuating variations fostered, accumulated, and fixed by careful, rigorous selection. Or if any of them have appeared as discontinuous variations or sports they have been given from the start all the advantage of the breeder's selective attention.

But the larval patterns have had to make their way alone. How have they come to exist then? As fluctuating variations fostered and fixed by selection? No; for neither artificial selection (except in rare possible cases of coincidence with a desirable cocoon variation), nor natural selection have played any part in their history in the last 4000 or 5000 years. Then they have probably arisen as discontinuous variations or sports, or as mutations, if the mutationists will admit them to their charmed circle. But in order to persist, these discontinuous larval variations or sports must have been endowed with a certain potency or prepotency, which prevented them from being lost or extinguished by interbreeding. If these discontinuous variations, sports, or mutations, have arisen, as seems probable from the analogy with other discontinuous variations, in small numbers, then the per-

sistence and final definite establishment of these larval characteristics must have been due to a potency in inheritance at least equivalent to that shown by such discontinuous variations as De Vries's mutations.

There is an important significance then, to my mind, in this difference of conditions between the cocoon characteristics and the larval characteristics of the silkworm. On the one hand we have different characteristics appearing originally, in most cases at least, as slight fluctuating or Darwinian variations, selected, fostered and fixed by the careful attention and manipulation of the breeder and by these means finally elevated to a condition apparently stable and of value equivalent to that of the usual differences in natural races or species. On the other hand we have, in the larval characteristics, a series of differences or variations which are strictly natural in their establishment. This establishment however cannot have come about by selection; not by natural selection, because during the many generations in the course of which this establishment has been brought about, the silkworm has not been exposed to natural selection; not by artificial selection, probably, because the characteristics are of no interest to the breeders. The establishment has come about, then, through natural methods, probably by the appearance of sudden discontinuous variations or mutations, which have been sufficiently potent in inheritance to have maintained themselves.

Despite this difference in the method of establishment the two sets of characteristics appear now on their faces to be of equivalent character and worth. But an experimental study of them by a protracted series of matings, pure and cross, shows that they are not of equivalent worth. The larval characteristics, established by Nature, are unbreakable, behave consistently and rigorously in inheritance through all possible manipulation. The cocoon characteristics, established artificially, break down under manipulation, are inconsistent in their inheritance behavior and reveal an instability which distinguishes them clearly and importantly from the larval characteristics.

And yet there are important and suggestive points of likeness. The cocoon characteristics as they stand today are discontinuous in their nature and show a strong tendency to become fixed, stable and consistent in inheritance, this stability and consistency being exactly of the type shown by the larval characteristics. In many crossings the cocoon characteristics are inherited in purely alternative manner and with close approximation to Mendelian proportions. In other crossings, using the same characteristics in different strains or races, or,

perhaps, indeed, within the same strains, the Mendelian behavior is lost, and even the discontinuous or alternative nature of the characteristics breaks down.

These different conditions displayed by the inheritance of larval and cocoon characters are to my mind extremely suggestive. They seem to me to indicate pretty clearly strong differences between naturally established and artificially established characters; they seem to indicate the difficulty of explaining fixed strain, race and species differences on the basis of selection of fluctuating variations; they seem to point toward explanation of such differences on the basis of discontinuous variations or mutations; but they seem, finally, to indicate an essential likeness, at bottom, between characteristics established by the selection of fluctuation variations and characteristics established by the appearance, full-fledged, of potent discontinuous variations. The differences established by the selection of fluctuating variations seem to require a long period of time to get upon that safe ground of independence which is attained almost at once by the difference established by discontinuous variations or mutations. And yet the fact seems plain that in a long time both kinds of differences will come to rest upon and be possessed of the same inheritance behavior and potency.

## DOUBLE MATING.

In connection with the question of prepotency of strain or race in cross mating, experiments have been begun in double mating, that is in pairing a female of one race with two (or more) males representing two different races. The silkworm is polygamous, both males and females usually mating more than once before egg-laying begins. Or this repeated mating may continue after egg-laying has begun.

In any consideration of the results of such repeated mating the unusual way in which the eggs of insects (at least of the silkworm moth and hosts of others) are fertilized must be remembered. This way is, simply, that the male fertilizing cells, the spermatozoa, are received by the female at mating into a special sac or receptacle, the spermatheca (there may be several spermathecae, as in flies) in which the spermatozoa remain alive and active. This spermatheca, a diverticulum of the oviduct, is situated near its external opening, the vagina. As the unfertilized eggs of the moth pass slowly down from the ovarial tubes into the oviduct they lack only fertilization to be entirely ready for development. They have already their full supply of yolk, they are already enclosed in their protecting envelopes (vitelline membrane and outer, firmer chorion). But these envelopes do not *completely* enclose the egg-mass; there is, at one pole of the egg, one or more small openings, the micropyle, through which the spermatozoa, issuing from the duct of the spermatheca as the eggs pass, enter the eggs. As soon as a single spermatozoan has entered, a jelly-like substance closes the micropyle and prevents polyfertilization.

Thus when the silkworm moth first mates she receives in her spermatheca, and holds there, a considerable number of spermatozoa representing the heritable characters of the male involved. When she couples again she receives another lot of spermatozoa, and if the second coupling is with a male of different race from the first these spermatozoa represent a new set of characters. What is going to be the result of this double mating as exhibited in the offspring?

In 1905 a female of Japanese White race (white patterned larva, white constricted cocoon) was mated with a male of the same race and allowed to lay some eggs and was then mated again, this time with a male of Italian Salmon (from a zebra larva) and allowed to lay another lot of eggs. All the larvæ (1906 rearings) from both sets of eggs

were of Japanese White race type, as were also all the cocoons spun by these larvæ.

In 1906 several double matings were made but in a different way. The female was not allowed to lay eggs after the first mating but was immediately, after the first mating, remated with a male of different race, then allowed to lay all of her eggs, and the offspring got in 1907 from these double matings all reared through to maturity, and their characters, larval and pupal, noted and tabulated. The matings and results were as follows:

(No. 111.) Female Bagdad (white larva, white cocoon) was mated with a male Bagdad and then with a male Istrian (buffy larva, golden yellow cocoon). Result, all the young were of Istrian larval type and of Bagdad cocoon type. Too much stress cannot be laid upon the larval type because the Bagdad and Istrian larvæ are much alike, although the noticeable clayey or buffy tinge of the Istrian larvæ is really a fairly distinguishing character.

(No. 112.) Female Bagdad mated with male Bagdad and then with male Istrian. Result, eggs all sterile; no hatches.

(No. 113.) Female of Italian Salmon (white larva, pink yellow cocoon) mated with male of same race and then with male Bagdad (white larva, white cocoon.) Result, all white, i. e., Bagdad cocoons.

(No. 236.) Female of Japanese Green race (white larva, green cocoon) mated with male Bagdad (white larva, white cocoon) for 1½ hrs., then with male Istrian (clayey-white larva, golden-yellow cocoon) for a longer time. Result, all golden-yellow, i. e., Istrian cocoons.

(No. 238.) Female of Bagdad race (white larva, white cocoon) mated with male Istrian (clayey-white larva, golden-yellow cocoon) for 1½ hrs. and then with male Japanese Green (white larva, green cocoon) for a longer time. Result, cocoons all golden yellow, i. e., Istrian.

(No. 239.) Female of Bagdad race (white larva, white cocoon) mated with male Japanese Green for 1½ hrs., then with male Istrian (clayey-white larva, golden-yellow cocoon) for a longer time. Result, all cocoons golden-yellow, i. e., Istrian.

These few experiments (the subject is being followed up more extensively this year) show that in such double matings one strain is potent over another. With two kinds of spermatozoa in the spermatheca, fertilization of the eggs does not occur according to the laws of probability, but the spermatozoa of one strain are successful in the race

or struggle to fertilize, or in some other way control the development of the egg. And the race that is potent in these mixed matings may be the one possessing those characters which are dominant in the Mendelian sense in cross matings. That is the yellow cocoon color represented by the Istrian race in several double matings where the Istrian male is either the first or second in mating, where his coupling time is either the shorter or the longer, is dominant in each case over the white cocoon color represented by the Bagdad female or male and over the green cocoon color represented by the female or other male (Japanese Green) involved in the double mating. (See lots numbered 236, 238, and 239.)

But in lots III and II3 we have a potency on the part of the Bagdad race, represented in one case by the female and one of the males, in the other by only one of the males, which does not correspond to any dominancy on the part of the character, i. e., white cocoon color, which reveals this potency. In both these double matings, Italian Salmon being the other race involved, the offspring all spun white cocoons. But in simple cross matings of pinkish-salmon cocoon-color with white cocoon-color, white is usually the recessive character. Hence dominancy of character does not explain the results obtained in lots III and II2.

But confirmatory matings are necessary before accepting the results of lots III and II2 as something regularly to be expected under similar conditions of mating. As I have already said the work is being more extensively carried on, and will be reported on in the future.



## FLUCTUATING VARIATIONS AND THEIR INHERITANCE.

While such characters as larval pattern and cocoon color seem to be essentially discontinuous in their appearance and alternative in inheritance, certain other silkworm characters are distinctly fluctuating or continuous in variation and non-alternative in inheritance. Such characters are amount and quality of silk thread composing the cocoon, shape of the cocoon, wing-pattern of the adults, wing-venation, certain larval markings subsidiary to the whole condition of color pattern, degree of adhesiveness of the eggs, polyvoltinism, etc.

A good deal of laborious work was done in the first three years of the six over which our experimental rearing has extended, in connection with these continuous or fluctuating variations. But little space, however, need be given to stating the results of the work.

Coutagne has already shown the strictly fluctuating character of the differences in "*richesse de soie*," which may be taken as including the quantity and quality of the silk. His series of rearings from matings based on a careful selection as regards the character extend over ten years and show clearly the variational and inheritance behavior of the characteristic. It is strictly continuous, fluctuating, and non-alternative.

For a knowledge of the behavior in variation and inheritance of the characteristic, shape of cocoon, Coutagne and Toyama's work is sufficient. They both show it to be fluctuating as to variation and non-alternative as to inheritance.

Toyama has worked also on the character polyvoltinism, or, better expressed, the brood character of the silkworm, whether of annual generation, or of two or more generations a year, expressed by silk-growers as univoltine, divoltine, multivoltine. He finds it to be a fluctuating character, to be maintained in one condition only by rigorous selection. "Thus," he writes, "when we crossed a multivoltine with univoltine breed, the eggs laid by the moth were either pure maternal or pure paternal, very rarely a mixture of both parents. Those forms raised from the first cross do not remain true to the parents in subsequent generations. Even when we selected multivoltine parents for five generations, we failed to get any constant multivoltine breed." Miss McCracken has carried on and still is maintaining in our labora-

tory an elaborate study of the inheritance behavior of this character and will report on her work in another year.

My own observations and experimental rearings on these various fluctuating characteristics touch especially the following: degree of adhesiveness of the eggs; subsidiary larval markings within the so-called "white" and "patterned" types (which behave as a whole in discontinuous and alternative fashion); wing pattern, and finally wing venation. I shall discuss these characteristics briefly in the order in which they have just been named.

#### INHERITANCE OF EGG CHARACTER.

The eggs of different silkworm races show differences apparently constant in size, color and shape. But none of these differences has seemed to me quite marked enough to be used in my studies; at any rate no attempt has so far been made to study the inheritance behavior of any of these characters.

But the character of adhesiveness (or lack of adhesiveness) is so conspicuous and so readily and certainly determinable that it has been made the subject of some experimental breeding. The one race in my possession whose eggs are regularly (this regularity is not absolute) non-adhesive is the Bagdad race, a strong white larva and white cocoon race much used in the laboratory. Females of this race simply drop the "non-sticky" eggs loosely in the mating boxes (small oblong boxes made by folding and pinning square sheets of strong paper in which the male and female to be mated are confined and in which the female deposits her eggs). These loose eggs are like so many little spherical seeds, yellowish at first but soon changing to lead-gray. The eggs of all the other races I have are strongly stuck to the paper of the boxes in a single layer with the eggs close together. Among the races depositing adhesive eggs there is practically no female which fails to fasten its eggs. Of course it would be quite possible for a female of such a race to show the teratological condition of absence of cement glands and such a one could of course not fasten her eggs. But in all our rearings I do not recall a single case of the oviposition of loose eggs by a female of an "adhesive egg" race. But the contrary is not true. That is the females of the Bagdad race, the one non-adhesive egg race that I have reared, show a certain degree of variation in regard to this characteristic. This variation comprises the deposition of eggs actually adhesive, that is fastened to the paper, but only weakly so, that is, they may be displaced by gentle rubbing (it requires

vigorous rubbing to remove eggs of the adhesive egg races). Or in rarer cases the eggs may be fairly firmly fastened. In other cases some eggs may be firmly fastened and some weakly fastened. In others some may be weakly fastened and some loose and the proportion of loose to fastened may be slight to large. But the females showing these variations in the egg character are very few compared with those showing the normal loose eggs condition. Matings were made pure and crossed on the basis of these variations in the egg laying, and the results, although the work has been only fairly begun, already show unmistakably the general character of the inheritance behavior of the characteristic.

This egg character or rather imaginal character of egg-laying is not a Mendelian or alternative character in inheritance. The non-adhesive condition exhibited by the Bagdad race however it may have originated, either as sport or as selected fluctuating variation, shows a plain tendency to change (back?) to the adhesive condition. From those few pure Bagdad matings (out of many pure Bagdad matings made) in which the female laid adhesive eggs, young were obtained which on being mated together produced some adhesive eggs in almost every case, and in most of these cases all the eggs laid were adhesive. From crossed race matings in which the female was a Bagdad laying adhesive eggs, young were obtained which, mated together, produced almost exclusively adhesive eggs. It seems from this plain that the adhesive egg character is very unstable, succumbing quickly in crossed matings to the character adhesiveness, and tending even in pure matings to throw partially or even completely (reversions?) the character adhesiveness.

But when there are mated together hybrids produced by crossing Bagdad with a non-adhesive egg race the young of these hybrids usually lay non-adhesive eggs. That is, this is true in practically all cases where the hybrids have for parents a Bagdad moth and a moth of any one of six other different races used in the matings. But where the parents were a Bagdad moth and a moth of a certain single adhesive egg race, viz., Italian Salmon, the hybrids deposited sometimes non-adhesive, sometimes adhesive eggs.

This character is one exhibited only by the females, of course, but capable of being transmitted through the males. Males of races laying adhesive eggs when mated with Bagdad females (laying non-adhesive eggs) may produce young tending to lay adhesive eggs. In other cases the young from Bagdad males crossed with non-adhesive race

females tend to lay non-adhesive eggs. This is very clear proof of the transmission through the males of this female characteristic as the females of races regularly laying adhesive eggs never tend to sport to non-adhesiveness.

The subject is being further studied and a report will be made later.

#### SUBSIDIARY LARVAL MARKINGS.

In larvæ of the white type the body is not wholly unmarked but certain markings known in our laboratory under the names of "eyebrows," or "eye-spots," and "anterior and posterior lunules" occur in very faint to fairly strong condition. Lunules occur as a single pair on the dorsum of each of the 2nd and 5th abdominal segments, and the "eyebrows" are markings on the dorsum of the mesothoracic segment. The posterior lunules (on the 5th abdominal segment) correspond externally to the situation of the developing internal reproductive organs (ovaries or testes) and are more elaborate in make-up than the anterior lunules. The "eyebrows" can also be quite elaborate in make-up and when well developed are really of the nature of eye-spots with a colored center, which may be red, yellow or pink, surrounded by purple or blackish lines (see Plates I and II).

All of these markings appear in the so-called "patterned" type of larva (Japanese White race type) and also vary in their degree of conspicuousness, that is, development.

On the basis of these variations in color and degree of development of these larval markings, selection among individuals was repeatedly made, matings instituted on a basis of this selection, and rearings made and all individuals examined and tabulated. The work was laborious and extensive. It was carried on chiefly by Mrs. Bell-Smith.

Her results show clearly the thoroughly continuous and fluctuating character of the variations and the non-alternative character of their inheritance.

#### WING-PATTERN.

A variation of distinctly fluctuating and continuous character is the wing-pattern of the adult moths. A good deal of attention and time were paid to the variations in wing-pattern through several years, with the result that the purely fluctuating character of the variation and its corresponding non-alternative behavior in inheritance seem certainly established, and hence make any use of it in cross matings of

only subsidiary interest. It is a variation or character strongly subject to Galton's law of regression and does not seem to be capable of any considerable modification or degree of fixation by even a most careful and persistent personal selection.

The pattern consists of the presence, in more or less well-marked condition, of a number of dark curving lines or bars crossing the white or creamy wings from anterior to posterior margin. These lines may be broad and strongly blackish, or narrower and only smoky, or very narrow and faint, or nearly invisible. All gradations from almost total absence of this pattern, when the wing may be called white (W), up to the most marked and elaborate condition of the pattern, when the wing may be called strongly patterned (S. P.) are to be noted. (See figures 1 to 3, Plate II.) For convenience I have established four arbitrary categories or pattern classes, which I call respectively White (W.), Barely Patterned (B. P.), Medium Patterned (M. P.), Strongly Patterned (S. P.). As examples of the manner of inheritance of this variation a short series of lots from the 1906 rearings (from the total series of nearly 300 in which pattern differences were tabulated) may be referred to. The small number of moths representing each lot is due to the fact that not all the cocoons were allowed to give up their moths and that from many that did the moths were allowed to make their condition of pattern undecipherable (by much beating of wings in the small mating boxes) before they were examined for tabulation as to wing-pattern. The records however show plainly the fluctuating and continuous character of the variation, even if the numerical representations of the different pattern types are not capable of being construed as indicating the actual proportions in any whole lot.

(Lot M. 22.) S. P. male mated with W. female. Result, 12 M. P., 5 S. P. and 6 melanic. (Note; male parent was a medium melanic.)

(Lot M. 44.) S. P. male mated with W. female. Result, 7 W., 20 B. P., 11 M. P.

(Lot M. 13.) S. P. male mated with B. P. female. Result, 17 B. P., 5 M. P.

(Lot M. 12.) M. P. male (cream color) mated with M. P. female. Result, 4 W., 13 B. P., 12 M. P., 1 S. P.

(Lot M. 41.) B. P. male mated with S. P. female. Result, 1 W., 3 B. P., 5 M. P., 15 S. P.

(Lot M. 29.) S. P. male mated with B. P. female. Result, 4 B. P., 13 M. P., 3 S. P.

- (Lot M. 49.) W. male mated with M. P. female. Result, 1 W., 10 B. P., 11 M. P., 3 S. P.
- (Lot M. 42.) M. P. male mated with M. P. female. Result, 18 W., 10 B. P., 11 M. P.
- (Lot M. 118.) S. P. male mated with M. P. female. Result, 5 W., 11 B. P., 6 M. P.
- (Lot M. 58.) M. P. male mated with W. female. Result, 5 W., 9 B. P., 10 M. P., 1 S. P.
- (Lot M. 38.) S. P. male mated with W. female. Result, 4 W., 7 B. P., 10 M. P., 5 S. P. (one of these a melanic).
- (Lot M. 45.) S. P. male mated with snowy white female. Result, 1 W., 1 B. P., 4 M. P., 1 S. P.
- (Lot M. 27.) S. P. male mated with a pure W. female. Result, 3 W., 1 B. P., 1 M. P.
- (Lot M. 52.) S. P. male mated with W. female. Result, 6 B. P., 4 M. P.
- (Lot M. 51.) S. P. male mated with W. female. Result, 2 W., 7 B. P., 3 M. P., 2 S. P.
- (Lot M. 78.) S. P. male mated with S. P. female. Result, 7 B. P., 5 M. P., 1 S. P. and 1 melanic.
- (Lot M. 113.) S. P. male mated with S. P. female. Result, 2 B. P., 1 M. P., 2 S. P.

The S. P. pattern is more common among males than females, but is not confined to either sex.

From these data, the fluctuating and continuous nature of the variation is apparent, and it is equally apparent that there is no alternative character in its inheritance. Rigorous selection would probably be able to produce parents which would throw a larger proportion of S. P. young, and other parents a larger proportion of W. young, but in neither case would this selection probably produce a fixed race. There would simply be produced a condition capable of being maintained as long as vigorous selection was practised, but only so long. This is the probability indicated by my experiments in attempting to foster the extremes of the pattern variation through several generations by selective matings.

#### WING VENATION.

The variations in the wing venation of a series of silkworm moths constituting a lot of experimental material were studied with a view to seeing whether there are indications of structural degeneration in this

functionally degenerate organ. The material consists of the wings of 52\* individuals derived from larvæ which had been subjected to various conditions of feeding as follows:

(Lot 399, sub. 1.) Moths from larvæ fed optimum amount of food during entire experimental history.

(Lot 399, sub. 2.) Moths from larvæ given short rations during a single (the immediate) generation.

(Lot 399, sub. 3.) Moths from larvæ given short rations for one year, optimum for the following (the immediate) generation.

(Lot 399, sub. 4.) Moths from larvæ given short rations during past two generations.

It was thought that upon seriation of the data there might be found some correlation between the variations and the conditions of feeding within each sub-lot. It was realized, however, upon seriation of the data, that while there are certain unique and suggestive variations in certain sub-lots, the series is numerically too short to justify any correlation of variations with conditions of nutrition. Therefore in the following tabulation of results, the variations are seriated for the 52 individuals as a whole, the interest centering in the degenerating structural condition of the venation in this organ which is functionally degenerate through disuse.

Many of the 104 wings exhibit numerous variations from the typical venation (Fig. 1) of the species. These variations may be classified in three groups as follows:

1. Variation by addition of spurs or of short veins to the typical venation.

2. Variation by loss of certain veins in full or in part.

3. Variation by loss of veins proper, i. e., the absence of chitinization combined with the persistence of tracheæ which are disposed:

(a) in such a position as to take the place of veins belonging to the typical venation of today;

(b) along ancient lines of development, as where the extinct base of media is preserved intact in the discal cell.

#### **1. Variations by addition.**

The variations classified under this heading are very few numerically and very insignificant in kind, adding but a fractional amount to the total extent of the wing's venation.

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\* In the 52 pairs of wings some were broken in certain areas so that every study does not include the entire 52 pairs.

Specifically, the variations by addition consist of:

(a) the presence of spurs in unexpected places; such as two short cephalic spurs between the forkings of  $R_2$  and  $R_{3+4}$  in a right wing (specimen Sub. 4, H); two longer spurs or short branches running from  $R_3$  near its distal end to the wing's costal margin in 1 right wing (specimen Sub. 1, O); a spur running proximad from  $R_2$  shortly beyond its forking in 1 left wing (specimen Sub. 4, L); a spur originating from the middle of  $R_4$  and running proximad in 1 right wing (specimen Sub. 2, I); a spur originating from the 2nd anal vein and directed toward the inner margin in 1 left wing (specimen Sub. 3, C);

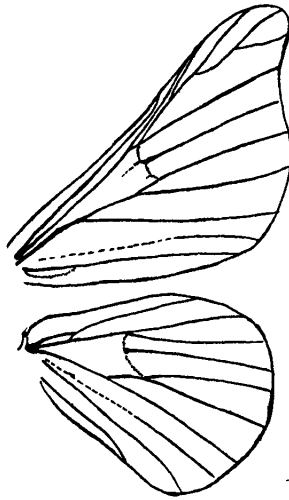


Fig. 1. Venation of the silk-worm moth, *Bombyx mori*.

(b) very short additional cross-veins, as where there is a cross-vein connecting  $R_3$  with  $R_4$  near their distal tips in 1 left wing (specimen Sub. 1, C); or a short cross-vein running cephalad from  $R_4$  to the costal margin in 1 right wing (specimen Sub. 2, I).

## 2. Variation by loss of certain veins entirely or in part.

The variations are numerous and striking in kind and are represented by many variants. The veins involved include members of the radial, medial and anal series in the fore-wings and the medial, cubital and anal series in the hind-wings.

The variation in the radial series consists of a "continuous" variation on the part of  $R_3$ . This summary included 46 left and 43 right



wings which were perfect and available for study.  $R_3$  is present and normal in 27 of the 46 left wings and in 22 of the 43 right wings.  $R_3$  is *entirely* absent as a separate branch in 29 of the 46 left wings and in 21 of the 43 right wings;  $R_3$  is present in part of its length in three wings as follows: (a) as a very short branch originating typically but ending freely in cell  $R_2$   $\frac{3}{4}$  of its length from the costal margin, in 1 left wing (specimen Sub. 4, I); (b) as in above under (a) but twice as long in 1 left wing (specimen Sub. 1, B); (c) in three sections, a basal, a terminal, and a middle section lying freely in cell  $R_2$  in 1 right wing (specimen Sub. 1, R).

The variations in the anal series of the fore wings affect the first and third anal veins. In the case of the first anal vein, the variations in 95 wings may be summarized under the following four classes: (a) 4 right and 5 left wings in which there is *not a trace* of the vein; not even a fold, furrow or surviving trachea; (b) 39 right and 33 left wings in which there are faint traces of a thickening or a faintly defined vein distally and not extending for as much as  $\frac{1}{2}$  the total length of the vein; (c) 5 right and 2 left wings in which as much as the distal half of the vein is present as a vein, fold, furrow, thickening or trachea or any combination of these; (d) 1 right and 3 left wings in which the distal  $\frac{1}{2}$ ,  $\frac{2}{3}$  or  $\frac{3}{4}$  of the vein is present as a vein. In no case is the vein found present in its entire length.

The third anal vein is also represented by all stages between and including total absence on the one hand and presence entirely on the other: (a) in 4 right and 2 left wings the vein is absent; (b) in 32 right and 35 left wings the vein is represented in part of its length by a fold, furrow, thickening or surviving trachea or combinations of these; (c) in 4 right and 3 left wings the vein is almost complete; (d) in 7 right and 5 left wings the vein is present in its entirety.

The other variation by loss in the fore wing consists of the absence of the cephalic, caudal, or middle third of the medial cross vein.

In the hind wing the variation by loss of parts concerns the medial, cubital and anal series of veins.

The variation by loss in the medial series of the hind wing consists of an incomplete condition of  $M_2$ : (a) in 1 left wing  $M_2$  is only  $\frac{3}{4}$  its normal length, stopping short of the outer margin (specimen, Sub. 1, N); (b) in 1 left wing the chitinization of the base of  $M_2$  is incomplete (specimen Sub. 3, B).

The variation by loss in the cubital series of the hind wing consists

of a single case in which  $Cu_1$  is but  $\frac{3}{4}$  its normal length, ending freely short of the outer margin (specimen Sub. 4, 1 left).

The variation by loss in the anal series of the hind wing consists of slight variations in length and character of the first anal vein which, in the typical venation, is incomplete proximally. In 18 right and 20 left wings the distal half of the vein is perfect or normal. This distal portion is in some wings either longer or shorter than  $\frac{1}{2}$  the total length of the vein from base of wing to outer margin; in 4 right and 5 left wings, the vein is longer than the normal, while in 14 right and 10 left it is shorter than normal. In 9 right and 8 left wings there is no true chitinization but some part of the vein's distal portion is represented by a thickening, fold or furrow. In 2 right and 2 left wings the vein is  $\frac{3}{4}$  its normal length and is continued proximad to the wing's base as a distinct fold. In one pair of wings the vein lies freely in the cell  $Cu_2$ , ending short of both proximal and distal margins of the wing.

The medial cross-vein of the hind wings varies by loss of parts as does its homologue in the fore wings.

The fact that variations by addition are of slight importance (found in only 7 wings and in no case contributing any considerable addition to the venation's total extent) as contrasted with the variations by subtraction or loss of venation in this functionally degenerate organ is suggestive. It would seem to indicate that the variations in this useless organ are characteristically of the nature of a breaking down or degeneration of structures. It is interesting in this connection to compare the conditions in these useless silkworm wings with those found in the highly specialized and useful wings of the honey bees\*, in which addition of veins and cells was clearly characteristic of the variation in their venation.

Finally we reach the third group of variations in venation, namely, variation by loss of the chitinization of the veins combined with the substitution of persisting tracheæ where the veins should be. This variation occurs in the 1st and 3rd anal veins and in the discal cell of the fore wings and in  $M_2$ , the medial cross vein, the 2nd anal vein, and the discal cell of the hind wings.

In the fore wing, there are tracheæ in the discal cell in 5 right and 7 left wings, the 1st anal vein is represented by a trachea only in 1 right wing; the 3rd anal vein is represented in part of its length by a trachea

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\* See Kellogg and Bell, *Studies of Variation in Insects*, Proc. Wash. Acad. Vol. VI, p. 212.

in 20 right and 22 left wings, while in 13 right and 17 left wings the 3rd anal vein is represented by a trachea only.

In the hind wing the medial vein is represented wholly or in part by tracheæ in 4 right wings; in 1 right and 1 left wing the vein  $M_2$  is in part of its length a trachea only; in 1 right wing the 2nd anal vein is a trachea only in its distal portion; there are tracheæ in the discal cell in 1 right and 4 left wings.

In one case (the left hind wing of specimen Sub. 3, I) the tracheæ in the discal cell show an arrangement which might be interpreted as throwing light on the ancient type of venation in the discal cell before it

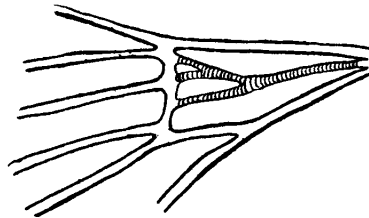


Fig. 2. Diagram showing relation of tracheal trunks to the radial and median veins in the silk-worm moth, *Bombyx mori*.

became a single cell. A single longitudinal trachea arises from the base of the wing and forks at about the center of the cell into two branches ( $M_{1+2}$  and  $M_3$ ). The cephalic branch forks again within the discal cell, separating  $M_1$  from  $M_2$ , while the caudal branch meets and fuses with the medial cross vein until  $M_3$  again turns longitudinally and continues as  $M_3$  distally to the outer margin. (Fig. 2.) It is only within the discal cell that the medial series is represented by tracheæ, the veins of the series being well chitinized outside the discal cell.

To sum up the variations in venation found in these functionally degenerate wings of the silkworm, we find very little variation by addition and no variations in the direction of specialization for a strengthening of the wing skeleton. We find a very large amount of variation by absence of certain veins or by loss of the parts of veins, in some cases the loss being total, in some cases an imperfection in the chitinization and in many cases the survival of tracheæ as substitutes for the missing veins.

This loss of parts of the disused supporting skeleton of the wing is, of course, exactly what we should expect to find in the light of that degeneration of function which has become characteristic of silk-

worm moths. The frequent persistence of tracheæ as the only traces of the last venation suggests that possibly the degeneration of the venation starts with a giving up of the structural features acquired latest in the race's and the individual's development (namely the chitinization which occurs about the tracheæ as trails) and, proceeding backward through time, repeats the story of the structure's birth—by what might be termed a reversed recapitulation of ancestral stages.

## MISCELLANEOUS.

### DOUBLE COCOONING.

Through all the years of our rearing and in lots representing most of the different races studied the appearance of occasional double cocoons was recorded. By double cocoon is meant a cocoon which is made by the joint labors of two larvæ, the one cocoon enclosing the two pupæ of these larvæ. (See Plate II.) In a few cases triple cocoons, produced by three larvæ working together, occurred. This double cocooning habit is of course a familiar one to silkworm growers and there is even a silkworm race aboriginal to the Riu Kiu Islands described by Sasaki (Bull. Coll. of Agric., Tokyo Imper. Univer., vol. 6, page 33, 1904) in which almost all the cocoons are double. They are large and variable in shape and usually enclose more than two pupæ, not rarely even seven or eight.

Coutagne (Recherches Experimentales sur l'Hérédité chez les Vers a Soie, 1902, p. 62 ff) questions whether an increase or decrease in number of double cocoons in a race is really hereditary, i. e., whether it is an acquired racial character, but inclines to hold it to be a purely ontogenetic character depending upon the amount of space available to the spinning worms.

But Duseigneur (Monog. du Cocon de Soie, 1875, p. 104) declares that the proportion of double cocoons is in some degree a fairly fixed characteristic of a race. Certain races come up to 30 per cent., in this proportion, while certain others do not get beyond 3 or 4 per cent.

Lambert (Revue de Viticulture, 1895, pp. 447) reports on a special Chinese race in which in 8 years he was able to reduce the percentage of double cocoons from 15 per cent. to 3 per cent.

Maillot and Lambert (Traité sur le Ver a Soie, 1906, pp. 342, ff) in giving the characteristics of many silkworm races regularly give the percentage of double cocoons, this percentage varying from 2 to 15. Also in their discussion of the effects and results of crossing they quote cases where the proportion of doubles in hybrid races is less than in either parent race. For example in a hybrid race produced by crossing two Chinese parent races the percentage of double cocoons is 1 in place of 2 per centum or 6 per centum characteristic respectively of the parent races. In other cases the proportion of double cocoons in hybrid races is the same as in one of parent races while in others the propor-

tion equals the sum of the parent races, while in still others it is midway between the percentage of the parent races.

All of these data would seem to indicate unmistakably that double cocooning is a heritable condition and not a purely ontogenetic one. They would also indicate that this condition can be fostered or modified by selection and thus made into a racial character.

My own work on double cocooning resolves itself practically into an attempt to foster (or to test) this habit by selection. Experiments were begun in 1902 by mating together a male and female, both of which had issued from the same double cocoon. All the eggs of this mating hatched prematurely except two, the larvæ from which were reared and spun single cocoons. The moths issuing from both these cocoons were both females and were mated one with a male from a double cocoon and one with a male from a single cocoon.

From the general 1903 rearings I collected 9 double cocoons (6 yellows, 3 whites). They varied considerably in shape, the extremes being, respectively, round, elliptical and elongate spindle shaped. In no case was a mixed yellow and white double cocoon found. With the moths from these 9 double cocoons together with some moths from single cocoons, 14 pure and cross matings (on a basis of cocooning habit) were made so as to bring together male moths from double with female moths from double, male moths from single with females from double, and males from double with females from single. Also in mating males and females from double cocoons together care was taken to cross the colors, i. e., a moth from a yellow double would be mated with a moth from a white double. Also pure matings were made in this color respect, thus yellow double with yellow double and white double with white double.

The results of the rearings of the various lots of eggs derived from these matings were as follows:

From 5 lots of eggs with both parents from double cocoons only two double cocoons were obtained, three of these matings producing no double cocoons at all. (These lots were greatly cut down so that comparatively few larvæ were allowed to spin up, but there was plainly no inherited tendency to produce doubles.)

From the matings in which one parent was from a double and the other from a single, 9 matings altogether, only 2 double cocoons were obtained, a single double cocoon appearing in each of two of the lots. (These lots also were very small.)

From a mating made between a moth from a double and a moth

from a single cocoon directly descended from the 1902 mating of doubles, no doubles were obtained.

From a large lot of eggs obtained by allowing three males from doubles to mate miscellaneously with three females from singles, one double and 31 single cocoons were obtained. (This lot was greatly cut down by disease.)

In no cases was a mixed double (i. e., yellow and white) produced.

No matings on a basis of double cocooning condition were made in 1904, but in the 1905 general rearings several double cocoons appeared and from the moths obtained from them 16 matings were made as follows: 11 of double with double (in one case the parents were from the same triple cocoon and in two other cases from the same double); and 5 of double with single. The proportion of doubles to singles produced in 1906 from the 11 1905 matings of double with double was one double to 16 singles, or a little more than 6 per cent. From the mating of the two moths obtained from the same triple cocoon only single cocoons were obtained. From the two matings in each of which both parents were obtained from the same double cocoon, in one case 12 double and 109 single cocoons were got, and in the other no doubles and 57 singles.

In 1907 a few more rearings were made from the eggs produced by the mating in 1906 of moths from double cocoons. In certain of these cases the parents were the offspring of moths which had issued in 1905 from double cocoons. Five of these 1907 rearings represented a second generation of individuals selected on a basis of double cocooning. In three of the cases of these five rearings each pair of parents issued from the same double. And in one of these three cases the grandparents had issued from the same triple cocoon. The results of these three rearings were as follows:

From two parent moths from a same double and grandparents from the same triple, two double cocoons and 68 singles were obtained.

From a second pair of parents, both from the same double, the grandparents each from a double, one double and 48 single cocoons were obtained.

From another pair of parents both from the same double and grandparents each from a double, 3 doubles and 73 singles were obtained.

In all the rearings (1904-5-6-7) the larvæ were *crowded* at spinning time if there were many larvæ in the lot.

The results seem to be plainly that (a) double cocooning is not

purely heritable and cannot be increased by selection or hybridization, and (b) that therefore it is an ontogenetic character but one not produced by crowding.

This conclusion seems quite opposed to that indicated by the statements of Duseigneur, Lambert and others. But nevertheless they are the only conclusions that can be derived from my data.

If the data did not include records of the second generation produced from matings made to test the possibility of the double cocooning habit as a Mendelian recessive, it might be assumed that this habit is of such a recessive character, not appearing in the first generation because of the dominance of the lack of the habit. But the data for the second generation although covering but few cases are unmistakable and definite so far as they go and show clearly that there is no basis for interpreting double cocooning behavior as a recessive character of alternative inheritance. The percentages of doubles appearing in the three second generation rearings are just about the percentages which might be expected to appear in any rearing, and are far too low to correspond to the expected percentage of a Mendelian recessive.

Although the single fact that in all the hundreds of rearings made in the laboratory in the last five years the spinning larvæ had been crowded in practically every case would indicate that the mere condition of crowding is not sufficient stimulus to determine double cocooning, it may be interesting to record the results of a few special experiments tried on individual worms to test the effect of crowding. Several times pairs of larvæ which had begun to spin double cocoons were separated and only very rarely in such cases was double cocooning given up. That is to say, such would-be double cocooners after being separated, in some cases 15 inches apart, would find their way together again and rebegin the double cocoon. In one case one of two larvæ which had begun a double cocoon together was removed and another larva ready to spin was substituted for it. The result was a desertion by both of the double cocoon already started and the spinning of a single cocoon by each. At another time 14 larvæ ready to spin were arranged in couples and each couple put into a space which compelled constant crowding of the two. Yet not one double cocoon was produced. All of the larvæ spun singles. Three larvæ ready to spin were introduced one into each of three nets already begun by three other larvæ. The result was six single cocoons, the introduced larvæ deserting the already begun net in each case.

Miss McCracken has paid some special attention to the question



of whether the double cocoon may not be the result of the labors of a single one of the two larvæ enclosed, the other being an individual which for some reason is not able to spin a cocoon, and hence attempts to become a room-mate with a normal spinning larva. She found in a number of cases that by separating two larvæ becoming enclosed in a common cocoon only one of the pair made a cocoon, the other spinning threads aimlessly or forming only a "carpet" and then pupating unprotected by a cocoon. In one or more cases the two larvæ becoming enclosed in a common cocoon showed by their attempts to spin single cocoons after being separated (one always succeeding) that they were spinners of differently colored silk. In no case however have we noted a double cocoon composed of two colors of threads. Miss McCracken's observations and suggestions should be followed up.

A little attention has been paid to note whether, in association of larvæ in spinning of double cocoons, sex cuts any figure. Double cocoons were often found to be produced by two females together or by a male and a female working together, but we have no recorded case of two males issuing from the same double cocoon. However, our records touching this point cover too short a series to be at all conclusive.

#### APPEARANCE AND BEHAVIOR OF "SPORTS."

In the seven years of our silkworm rearing there have appeared in various lots individuals showing sport characters of several kinds.

In 1903 various cocoonless pupæ were noted, the larvæ of these having spun no silk at all, or only a random "carpet," or they outlined cocoons only to neglect and leave them. Eight such cases were noted in 1903. Other larvæ spun only very thin, semi-transparent cocoons. In 1904 and 1905 other thin or skeleton cocooners were noted. Also larvæ that spun up after the third moulting (instead of the fourth as normally). Certain cocoons of extraordinary shape were also noted. Certain larvæ with caudal horn wholly wanting and others with this horn very short and small were observed. (Pl. I, fig. 12.) A few larvæ with curiously distorted body appeared (Pl. I, fig. 8). Also larvæ showing sport characters of coloration and pattern (Pl. I, fig. 9). The *moricaud* or all-dark color-pattern of the larva was found to be a frequent sport occurring in several races. (This has been the subject of breeding and inheritance testing by Coutagne, Toyama, Miss McCracken and myself and is referred to in the part of this paper devoted to a consideration of the alternative or Mendelian characteristics of the silkworm).

Moths appeared with sport wing patterns; also strongly melanic forms; also flying moths; also moths with rudimentary wings.

A great deal of work has been done in mating these sports and freaks, making rearings and following up the appearances for several generations, but for the most part only results of little value were got. In the matter of the *moricaud* or melanic larvæ more important data were obtained, especially by Miss McCracken. Some of the notes and results of the work with the other sports may be briefly referred to as follows:

*Cocoonless and skeleton cocoon pupæ.*—Rearings were made in 1905 from matings of sport individuals appearing in the general lots of 1904, and in 1906 from the 1905 moths produced from the 1904 matings. If there were no reappearance of the cocoonless character in the first hybrid generation (from a cocoonless and cocooning mating) it would not necessarily indicate the non-heritable character of the cocoonless habit but might show it to be a strictly recessive Mendelian character. The 1906 rearings from inbred hybrids should however reveal the recessive character again.

From seven rearings in 1905 from 1904 matings in each of which one or both parents were cocoonless, and seven rearings in 1906 from inbred matings from the 1905 generation the data show no transmission of the cocoonless character. It is ontogenetic.

*Miscellaneous larval coloration sports.*—From strongly pinkish, bluish and "black-face" larvæ descendants were obtained (the sports being crossbred with normal larvæ of their same race) without obtaining in either first or second generation (inbred hybrids) any reappearance of the sporting shades of color.

An interesting coloration sport which I have called "clouded head" (Pl. III, fig. 10) was noted in a lot of Bagdad race worms in 1906. Nearly one-half of a single lot of larvæ (a lot being the worms derived from all the eggs laid by a single female) showed in greater or less degree a "clouded head," a coloration of the dorsum of the thorax much like that of the familiar *moricaud* larval sport, but with the color pattern strictly limited to the dorsum of the thoracic segments. Four pure matings (i. e., "clouded head" with "clouded head") from this lot were made and the 1907 rearings from these were as follows:

(No. 230) More than 50 per cent. of the larvæ with clouded heads.

(No. 337) Eighty-nine clouded heads, forty-nine normals.

(No. 370) Twenty-two clouded heads, one hundred and fourteen normals.

(No. 415) One hundred and forty-four larvæ *all* with clouded heads.

Matings were made in 1907 from this material and will be reared this spring (1908).

*Congenitally "hornless" larvæ.*—Various matings were made in several years (inbred matings from first generation hybrids also mated for second generation rearings) of moths derived from larvæ born without the caudal horn or with it in greatly reduced condition (Pl. I, fig. 12). The results of all these matings show that the character is not heritable. That is, does not behave as a Mendelian or alternative character, nor can it be fostered and fixed by selection.

*Experimentally "dehorned" larvæ.*—The horn seems to be a useless structure. It is not an organ of defense, neither secreting an imitating or a mal-odorous fluid nor can it pierce or wound in any way an enemy. Besides, for nearly 5,000 years the silkworm has had no enemy except disease germs to defend itself against. This fact of the apparent present uselessness of the horn and the fact that it not infrequently appears in rudimentary condition or is even wholly wanting suggested the experimental mutilation of silkworm by removing this degenerating structure. Would such mutilations or removal of a structure already tending congenitally to degeneration or loss be more likely to be inherited as an "acquired character" than other mutilations such as have been brought about by experiment or custom and have shown no signs of being handed down to the young.

Considerable work was done during three successive years in testing this. In no case was there any indication of the transmission by inheritance of the mutilation. So this case may join the many others all of which (almost without question) have been repetitions of the same evidence of negation.

*Sport wing pattern of moths.*—In the seven years of rearings several well-marked sport variants of the wing pattern have appeared. Various matings to test the behavior in inheritance of these sports were made. For example, in 1905 two matings were made of a sport wing pattern with a normal wing pattern. In the first or hybrid generation there was no reappearance of the variant pattern. In rearings (1907) from inbreds from this hybrid (1906) generation there was also no reappearance of the sport pattern.

These pattern sports are various in character, some of them being asymmetries, some extreme emphasis of the normal faint patterning, some the appearance of large conspicuous well delimited black

blotches, etc. In no case has one of these sports yet shown any potency in heredity.

Melanic moths, not black, but with wings and body strongly smoky not infrequently appear (Pl. II, fig. 4). In various lots in various races these melanic or "darky" moths have been noted. And much work has been done in testing the inheritance behavior of this melanism. The general result is like that for all the other sporting characters (except the *moricaud* larval pattern) so far noted and studied, namely, it has no potency in heredity and does not behave as an alternative or Mendelian character. It shows a certain tendency in pure matings (that is smoky male mated with smoky female) to reproduce itself and careful selection could in time probably produce broods in which melanism would be the rule. The occurrence of melanic individuals is much more abundant among males than among females. No special evidence has yet been adduced to show that this melanism is not congenital, but is caused by special conditions surrounding the ontogeny. As all the individuals of any one lot of silkworms (by lot being meant all the worms derived from the eggs laid by a single female) are reared under as nearly identical conditions as possible, the occurrence of two or four or a dozen melanic moths in such a lot of two or three hundred individuals is evidence for the distinctly congenital nature of the variation. However, in some experiments which included the rearing of silkworms in an atmosphere of high humidity maintained during the whole larval life, the moths produced by these larvæ practically all showed a marked melanic tendency, although the character of the smoky coloration was somewhat different from that which often appears as a sport and which has given in my laboratory the name "darky" moths to the individuals showing the variation.

The studies into the nature and character of behavior in inheritance of this sporting melanism are being continued. (Eighteen matings were made on this basis in 1906 and most of the lots reared through to maturity in 1907, and a new set of matings made for the 1908 rearing season. The rearings made in earlier years from matings made on a basis of this character were unfortunately not well followed up).

*Flying moths and moths with rudimentary wings.*—The occasional appearance of male moths exhibiting a considerable power of flight (the silkworm moth although retaining its wings, probably in full size, has lost the power of flight, its wing-vibrations being no longer strong enough to carry its body), and the rarer appearance of moths with greatly reduced or rudimentary wings led to a number of matings to

test the inheritance possibilities of these variations. They were found to possess no special potency in transmission.

#### FERTILITY AS AFFECTED BY AGE OF THE GERM CELLS.

The theories of possible species differentiation on a basis of some sort of genetic or reproductive selection (Pearson) or reproductive divergence (Vernon) assume that without actual topographic isolation gradual differentiation within a species can come about through discriminate breeding or differences in fertility dependent on the association of sexual attraction or antipathy or actual degree of fertility with some other structural or physiological character in the individuals. It has been often suggested that such a relation may exist between age of the germ cells and degree of fertility. I have made a few observations in this connection.

When the silkworm moth issues from the cocoon it is sexually mature. Mating can take place and often does within a half hour after emergence and the results of this union are fertile eggs. The moths live usually for about three or four days after emergence, at the most but six or seven, so that the age of the moth, and accordingly of the germ cells in functionally active condition, should be reckoned by hours. Matings between moths of exactly known but differing ages were made. For example, males not over four hours old were mated with females as old as fifty-two hours, and with others not over four hours old. Males fifty-six hours old were mated with females just issued and with other females much older. And so on. The eggs from the matings were counted and after the development of the eggs had proceeded for some months the eggs were again counted to the end of determining how many were developing and how many were not.

The results of the experiments show that eggs from parents in which the male is old did not develop as well as eggs from other parents. That is, the extreme age of the female (egg cells) seems to make no difference in regard to the developing power of the fertilized eggs. But the age of the male (sperm cells) does seem to affect the fertility of the eggs. Very old males (sperm cells) seem to be less potent than younger ones.

## ECONOMIC ASPECTS OF STUDIES IN SILK- WORM INHERITANCE.

In their recent comprehensive treatise (*Traité sur le Ver a Soie du Murier et sur le Murier*, 1906) on commercial silkworm rearing, Maillot and Lambert of the principal government experimental silk culture station of France (at Montpellier) discuss the effects and advantages of the crossing of silkworm races and of individuals of the same race reared in separated localities. Their statements are based on the experience of long years of rearing, observation and selection.

First, they find that crossing, even between moths of closely allied races, produces individuals "more vigorous, more productive, more fecund."

Then they utter certain generalizations concerning the results to be expected from certain crossings. For example: "if one mate a male moth of a race that lays adherent eggs with a female moth of a race laying non-adherent eggs there will be more chances that the eggs produced by the hybrid young will be non-adherent; but in the reciprocal crossing [i. e. male of non-adherent eggs with female with adherent] the contrary will most often occur."

Also "if one crosses a race with large, cylindrical, yellow cocoons and worms large and of slow growth, with a race with small, oval, white cocoons and worms smaller and of rapid development, one will have in the first generation both yellow and white cocoons, of each type, sometimes in numbers almost equal, sometimes many more of one type than of the other; the worms will differ from worm to worm: some will be of the type of the male race, large and long lived; others will be of the type of the female race, small and short lived; others yet will show the characters of both races. Thus in a crossing of worms with white skin with worms of black skin one will find sometimes individuals which have half of the body with the skin black the other half with the skin white. The separating line being the median longitudinal one.

"In the crossings between races of differently colored cocoons the most advantageous one, that which offers the best guarantee in the matter of the homogenousness of the cocoons produced, both as to quality and quantity, will be a crossing of a male of yellow cocoon with a female of white cocoon. One can affirm nothing with certainty concerning the inheritance of the tendency which is shown

by the worms of certain races, as the races of Japan and several of China, to combine two or more in the same cocoon, that is to make what are called double cocoons; it seems, however, that in this respect the hybrids tend more often to follow the female than the male.

“Finally if one mates hybrids among themselves one will find in the worms and cocoons a diversity of size, form and color much greater than would be found in the direct descendants of the parents and this great diversity in the cocoons depreciates them much in the eyes of the spinners.

“The principal advantages of these crossings is the production of worms very vigorous and very robust which resist the disease of flaccidity better than do the native races of yellow or white cocoons and which give at the same time a tolerable harvest in places in which the European races produce rarely a harvest worth gathering; besides they are very much more precocious and form their cocoons sooner.

“But aside from these advantages these crossings are disadvantageous by producing worms and cocoons very often dissimilar, sometimes following more one race, sometimes more the other, and if one intermates the hybrids one obtains products of a still greater diversity. It is wise therefore to confine oneself to rearing worms issuing directly from an original crossing and of repeating this crossing each year. But for this it is necessary of course to make rearings each year of the two pure races of which one proposes to make the crossings. This is, of course, a disadvantage and a complication.”

These are practically all of the generalizations touching the “effects of crossing” which the authors of this modern authoritative treatise on silkworm culture permit themselves to express. Without doubt they might, from the large experience and the long series of rearings carried on by their station, utter many more. But, and this is the point to which I wish to call attention, of how curiously indefinite and unsatisfactory character are such generalizations compared with those which can be expressed after even so few years of experimental breeding as those of Toyama and myself in the light of the modern scientific study of heredity.

The knowledge of the definite Mendelian character of the inheritance of certain characteristics and the knowledge that certain other characteristics are not inherited according to Mendelian principles but must be fostered and maintained by strict personal selection, can be a potent help to the commercial silk grower in his attempts to produce new races especially fit for his particular need and use.

Whereas without a knowledge of the Mendelian behavior of certain characteristics it might take many generations of rearing the products of various crossings and selections—Maillot and Lambert record that it required 70 generations to establish a certain particular race—with this tested knowledge of the behavior in inheritance of specific characters it would be quite possible to fix certain characters in from three to five or six generations.

Experimental breeding with Mendelian principles in mind will enable the professional silk grower to determine speedily the simple or compound nature of the characteristics of the eggs, larvæ, and cocoons; will enable him to analyze the compound characters into their component simple ones; will permit him to establish combinations, even very elaborate ones, comparatively rapidly (at least of such characteristics, as show alternative inheritance, that is are Mendelian in behavior), and will save him much waste of time in purely empirical work.

His first aim in crossing and selecting will not be the establishment of the desired combination by long-continued miscellaneous trials, but will be the determination of the actual status, as regards behavior in inheritance, of the characteristics he desires to combine and fix. He will determine for each of these characteristics (and two or three generations will tell him) their inheritance habit. Are they unit characters? Are they strictly alternative in inheritance? Or do they combine in the hybrids in particulate (mosaic) manner, or as true blends? Or finally are they so strictly of the nature of simple fluctuations of varying degree or extent about a modal characteristic that they tend strongly to drop back towards this modal type or condition so that only the strictest and most continuous sort of personal selection can maintain them?

Among the characters and conditions of eggs, larvæ and cocoons forming, in various combinations and degrees of emphasis, the diagnostic marks of the present silkworm races, characteristics showing all these types or modes of inheritance are included. Color of silk, an important character, behaves usually as a unit character, alternative in inheritance, following, in some degree, the Mendelian principles. Certain colors are then recessive towards others, as white to yellow; salmon to yellow, etc. The relative status of potency (dominancy or recessiveness) can be definitely determined for any two colors, and the silk breeder thus have a knowledge of enormous usefulness in his work of crossing and selecting. Richness in silk (i. e. proportion of



quantity of silk to total weight of cocoon and enclosed pupa) is purely a fluctuating characteristic, capable of a certain amount of amelioration by persistent, rigid, personal selection. Double cocooning is a characteristic, from the evidence of my data, not heritable but ontogenetic, although from the statements of Duseigneur and Lambert it would seem to be heritable; it is a characteristic needing more study to determine its actual behavior or status in inheritance.

But it is, as said in the introductory paragraphs of this paper, not my intention to consider at present in any detail the economic aspects of our present knowledge of the status in inheritance of the characters. I hope to be able in a future paper to offer some discussion of this subject.

## GENERAL DISCUSSION.

I shall undertake no real general discussion of the problems of inheritance: not even of those particular ones upon which this silkworm work may have some bearing. The few points to which I shall here briefly call the reader's attention will be chiefly simply by way of indicating or drawing certain comparisons with the conclusions of Toyama (Bull. Coll. Agric., Tokyo Imp. Univ. v. 7, pp. 259-391, 1906) based on his similar work with silkworms, and with those of Davenport (Paper No. 7 of the Carnegie Station for Experimental Evolution, 1906) based on his work with poultry.

Toyama finds the larval variations of color-pattern and the cocoon differences of color to follow Mendel's law, and to behave with equal consistency and regularity. I do not. By the use of many repetition or check lots I find the larval characters to exhibit a great fidelity to Mendelian principles in their mode of inheritance, but with the cocoon colors I find exceptions so numerous, so various, and so pronounced as to lead me to lay great stress on the potency or influence of individual and strain idiosyncrasies. My position in this matter has been already definitely set out in this paper in the sub-section "*Conclusions*" of the section "Strain and Individual Idiosyncrasies" (p. 33).

I have stated there what seems to me to be the probable significance of the facts of this marked difference in the consistency of the inheritance behavior of these two sets of characters. This significance is, in a word, that the regularity and consistency of the behavior of the larval characters result from their natural origin and fixation as contrasted with the more artificial or man-controlled origin and fixation of the cocoon characters, and that the evidence suggests the mutational origin of the stable larval differences as contrasted with the origin of the cocoon characters through the selection of fluctuating variations.

However, this significance may not come to my readers with any of the force with which it comes to me. If not I still wish to direct their attention to the definite character, at least, of the differences in consistency and regularity of the inheritance behavior of the two sets of characteristics, and the inevitable conclusion that the heredity of the silkworm is not to be expressed by any single fascinating sweeping generalization as to its regularity. The longer the series of check lots, the greater the opportunity the silkworm is given to reveal inconsistencies in its heredity (that is, inconsistencies from our favorite point

of view today, i. e. the Mendelian point of view), the more numerous and various and pronounced and confusing (or illuminating if we are simply searching for truth and not the truth of a single hypothesis) these inconsistencies become.

On the other hand it is also a point not to be overlooked that these inconsistencies are only put into the conspicuous position they occupy by the strong and suggestive tendency through all the silkworm heredity towards Mendelian behavior. And it may very well be that some more thorough-going student and more subtle interpreter than I of inheritance phenomena will be able to analyze many of the phenomena which seem to me to be inconsistencies and exceptions to the Mendelian principles in such a way as to reveal the possibility if not actuality of their basic consistency with these principles. Professor Bateson has exhibited so much ingenuity in analysis of the various apparently un-conformable cases of inheritance presented to him that a student less well grounded and less gifted can not venture to be too certain in the interpretation of his data. By the addition of the hypothesis of determiners and cryptomeres to a keen analysis of the data offered him, Bateson has most plausibly brought into line with Mendelism numerous at first sight non-Mendelian cases. Very well. He has now on hand for treatment apparently un-conformable new data and interpretations from both Davenport and myself.

This reference to Davenport's results and conclusions leads me directly to say that on the whole my results with the silkworm and my interpretations of and conclusions from these results are very much like those of his, derived from his extended work with poultry. With Davenport, I find dominance and recessiveness often incomplete; prepotency as truly important as dominance; the theory of gametic purity not borne out with any rigorousness by the data of crossings. Differing from him, I find reciprocal crosses (on basis of sex) not exhibiting important or consistent differences in inheritance; where such differences in reciprocal cross results occur they can more readily be ranked in the category of "individual idiosyncrasies" than in the category of sex influence. I find no special evidence to favor Conklin and Guyer's contention for a larger influence in inheritance on the part of the female because of the larger mass of cytoplasm in the female germ cells. Indeed Miss McCracken finds in her intensive study of the inheritance in silkworms of larval melanism and imaginal polyvoltinism that if either sex shows any prepotency it is the male sex.

I find much more inheritance difference on a basis of strain or race

differences than Davenport seems to, although he finds some. These are my differences due to "strain idiosyncrasies."

The significance of my data as regards the pressing question of the chief influences in species change seems to me to be that of pointing toward the sudden appearance of definite discontinuous fixed differences either of the nature of new unit characters or of new combinations of old unit characters, endowed from the start with taxonomic stability, behaving in heredity as consistent alternative characteristics along Mendelian lines. In other words it seems to me that my data indicate the reality of mutations as real species differentiating characters. The visible differences between hereditary strains of organisms based on the accumulation of fluctuating variations by some method of selection may be even larger in appearance than the mutational differences and yet lack the stability and hence fundamental reality of these latter differences. Apparently, however, by some means they may come to acquire the inheritance behavior and stability of the mutational differences. At least the cocoon differences in silk worms which are the result of selection methods seem to be tending strongly toward the acquirement of the same type of inheritance, viz., alternative Mendelian inheritance, as that of the larval characteristics. If this condition can be really attained then the differences will be as real and species-distinguishing as those which arise as mutations.

But I concede readily that my conclusions are not so inevitable from my data as my expression of them would seem to indicate. And I wish to leave with my readers no wrong impression of an overestimate on my part either of the value of the data themselves or of the worth of the few generalizing conclusions expressed in this paper. I offer the data as facts as nearly as I can see and describe them, contributing toward our gradually growing knowledge of inheritance phenomena.

## SUMMARY OF RESULTS AND CONCLUSIONS.

Silkworms exhibit some characteristics which are alternative in inheritance and which follow in their transmission exactly or with more or less approximation Mendelian proportions. But some of these characteristics are not very stable in their alternative and Mendelian behavior. Other characteristics still are not discontinuous or alternative in character or inheritance but are of the nature of fluctuating variations and are strongly obedient to Galton's law of regression.

Larval color-pattern differences are consistently and rigorously alternative and Mendelian in inheritance; cocoon colors tend to be alternative and Mendelian in behavior but are inconsistent as to dominance and recessiveness and numerical proportions, and may even break down and blend, or one color be otherwise influenced or modified by the presence, in a mating, of another.

Larval pattern and cocoon color characters do not except as coincidences follow the same parent in dominance. In cross matings combining opposed larval and cocoon characters dominance in larval pattern may be with the paternal type, in the cocoon color with the maternal, or *vice versa*, or both dominances may rest with the paternal or with the maternal type. Dominance is a function of the characteristic not of the parental influence. Dominance is also not a function of sex or of bodily vigor.

While in larval color-pattern characters the inheritance behavior is rigorously alternative and Mendelian, dominance always being consistent in relation to a given color-pattern as related to another, this is not true of cocoon colors. With these characteristics differences peculiar to strain (or race) and individual are marked. Strain and individual idiosyncrasies are real and important and thus sweeping generalizations concerning the inheritance behavior of the cocoon colors tending to class them unqualifiedly in the Mendelian category cannot be made. The tendency is for them to behave in Mendelian manner, but it is a tendency subject to numerous, marked and various inconsistencies and irregularities.

In double matings, i. e. mating of one female with more than one male, these males representing different types of larval and cocoon characters, interesting modifications and interactions of influence are to be noted. The reality of strain potency over character potency is made manifest in these double matings.

Quantity and quality of silk, subsidiary larval markings, wing-pattern and wing-venation variations, and degree of adhesiveness of eggs are all fluctuating, non-alternative characters.

Double cocooning is a phenomenon determined by ontogenetic circumstances. Crowding is not the causal circumstance.

Of various sport appearances of larval, cocoon, and imaginal characters only one, namely, larval melanism or moricaudness, is of pre-potent or dominant nature when crossed with the normal condition. All other sport characteristics including various larval color and structural abnormalities, active flight of moths, absence or rudimentary condition of wings, unusual color patterns, including melanism, of moths, are extinguished in cross-matings.

Fertility is not affected by the age of the egg cells but seems to be unfavorably affected by the age of the spermatozoa. Old spermatozoa seem less potent than younger ones.

A scientific study of inheritance in silkworms can be of service to commercial silk culture.

PLATE I.

(LARVÆ, NAT. SIZE.)

- Fig. 1. Italian Salmon race, white type, in last instar.
- Fig. 2. Italian Salmon race, tiger-banded or zebra type, in last instar.
- Fig. 3. Galbin Italiano race, in 4th instar.
- Fig. 4. Japanese White race, in 4th instar.
- Fig. 5. Mosaic of tiger-banded and pattern types, last instar.
- Fig. 6. Clayey-yellow or "muddy" type, last instar.
- Fig. 7. Chinese White race, in 4th instar.
- Fig. 8. Sport or abnormality, in 4th instar.
- Fig. 9. Sport pattern, spot marking on segments, in 4th instar.
- Fig. 10. Japanese White race, in 3rd instar.
- Fig. 11. Front view of head and thoracic segments, showing variations in thoracic markings ("eyebrows").
- Fig. 12. Posterior segments of three specimens, showing "hornless," "tuberclcd" and "fully horned" conditions.





PLATE II.

(MOTHS AND DOUBLE COCOONS.)

- Fig. 1. Moth of white wing pattern ( $\times 1\frac{1}{2}$ ).
- Fig. 2. Moth of medium patterned wings ( $\times 1\frac{1}{2}$ ).
- Fig. 3. Moth of strongly patterned wings ( $\times 1\frac{1}{2}$ ).
- Fig. 4. Sport wing pattern and melanism of wings and body ( $\times 1\frac{1}{2}$ ).
- Figs. 5 to 8. Types of double cocoons (nat. size).

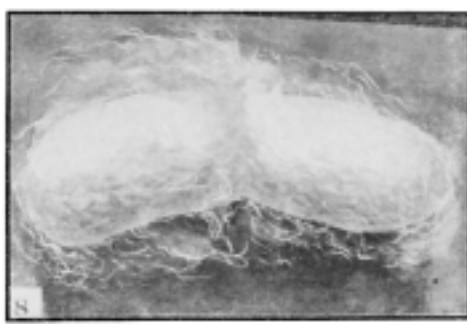
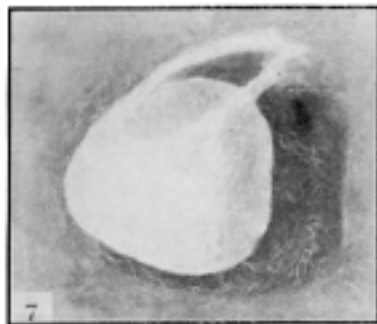
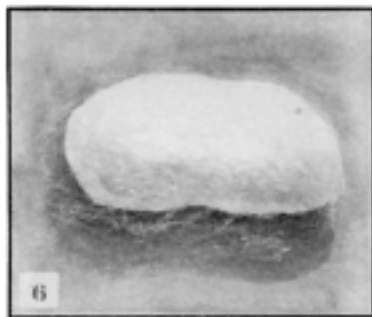
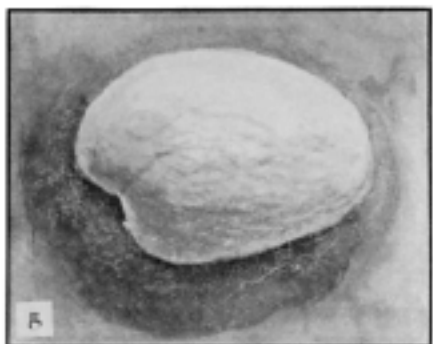


PLATE III.

(LARVÆ, NAT. SIZE.)

- Fig. 1. Italian Salmon race, white type, last instar.
- Fig. 2. Italian Salmon race, tiger-banded or zebra type, last instar.
- Fig. 3. Japanese White race, last instar.
- Fig. 4. Bagdad race, moricaud type, last instar.
- Fig. 5. Chinese White race, last instar.
- Fig. 6. Italian Yellow race, last instar.
- Fig. 7. Japanese White race, last instar.
- Fig. 8. Bagdad race, moricaud type, last instar.
- Fig. 9. Mosaic of zebra and moricaud types, last instar.
- Fig. 10. Clouded head type, last instar.



PLATE IV.  
(COCOONS, NAT. SIZE.)

- Fig. 1. Chinese White race.
- Fig. 2. Japanese White race.
- Fig. 3. Spherical shape.
- Fig. 4. Bagdad race, white.
- Fig. 5. Bagdad race, faintly greenish.
- Fig. 6. Bagdad race, more strongly greenish.
- Fig. 7. Japanese Green race.
- Fig. 8. Turkish and French Yellow race.
- Fig. 9. Istrian race.
- Fig. 10. Creamy yellow from a "break-down" lot.
- Fig. 11. Stronger yellow from a "break-down" lot.
- Fig. 12. Golden yellow from a "break-down" lot.
- Fig. 13. Italian Salmon race.
- Fig. 14. Italian Yellow race.
- Fig. 15. Yellow-salmon from a "break-down" lot.



## APPENDIX.

(Abstracts or summaries of papers already published by the author on various phases of silkworm biology.)

(with R. G. Bell) Notes on Insect Bionomics, in Jour. Exper. Zool., v. 1, pp 357-367, August, 1904.

*Food Conditions in Relation to Sex Differentiation.*—Various lots of silkworms were reared on reduced rations in the years 1901, 1902, and 1903, to test the alleged influence of nutrition on sex differentiation. It has been assumed by some authors that poor nutrition of developing organisms is an extrinsic influence tending to determine the sex of the organism to be male and good nutrition an influence tending to produce females. The most important part of the assumption is the idea that sex is subject to control by the environment of the organism—that sex is not inherently predetermined in the germ.

The data obtained test the possible influence of poor nutrition of the parents (and grandparents) in determining the sex character (if predetermined) of the germ cells, as well as of the possible immediate influence of nutrition in determining the sex of developing individuals.

No positive influence of the poor nutrition on sex determination of the silkworm is shown by the data presented.

*Forced Pupation.*—Experiments were made to determine how early in larval life the food supply could be cut off without stopping the metamorphosis (development) of the silkworm, whether such forced abbreviation of the food-taking period results in any unusual structural or physiological modification in the stages which follow the withdrawal of food, and whether the metamorphosis (in particular, pupation) is hastened when food is withdrawn in late larval life, an adaptation often assumed to be possessed by Lepidoptera. Such an adaptation would obviously be of real advantage, as it might often save individuals from death due to a sudden disappearance of the food supply, or to sudden accidental incapacity to gain access to the food supply.

The silkworm race experimented with in this regard spends normally about sixty days in the larval (feeding) stage, divided into five actively feeding intermoult periods of about ten days each, by four brief two-day moulting periods, during which no food is taken. On the eleventh or twelfth day (from 270 to 300 hours) after the fourth moult, the larva "spins up" and pupates.

Twenty healthy silkworms were selected at random from a large lot (several hundred) which had been reared in one tray, all the individuals, of course, under the same condition of food supply, temperature, humidity, light, etc. Of the twenty, one was fed as long as it would take food; the other nineteen were deprived of food variously from the time of the fourth moult, from one day after the fourth moult, from two days after, from three days after, and so on until individuals were obtained representing a withdrawal of food supply for a period of but a day before the normal time of giving up

eating to begin spinning, through periods of two days before, three days before, four, five and so on to twelve days before, the twelve-day period being the whole of the feeding period normally lasting from the fourth moulting up to spinning time.

From these results it may be said that silkworms may be cut off from a food supply nearly seven days before the normal limit of their feeding time and yet complete their development (spin, pupate and emerge as imago). These seven days represent a little more than half of the last intermoulting actively feeding period, or about one-ninth of the whole larval (feeding) life. The deprivation of food for from one to four days seems neither to hasten the metamorphosis nor to modify it appreciably, nor to result in the production of a moth of lessened size or lessened fertility. The larvæ deprived of food not more than four days before normal close of feeding time do not immediately spin and pupate, but wait restlessly for the normal time of pupation (approximately twelve days after the fourth moulting), and then normally spin and pupate. If deprived of food for more than four days and less than seven, the larvæ shorten their last intermoulting stage to about seven days, forming, however, a normal cocoon and transforming into a normal moth. If the larvæ are deprived of food eight days or more before their normal spinning-up time, they invariably die without forming a cocoon, and in only one case was pupation accomplished. A beginning at spinning is made by larvæ fed for more than two days after the fourth moulting, but no spinning at all is done by larvæ deprived of food from the day of fourth moulting or from the first or second day thereafter.

The twentieth larva of the lot was to be deprived of food 216 hours after the fourth moult, but it began spinning up in 200 hours (eight days) after, and pupated on the following day. Here is a normal variation of four days out of the usual twelve of the last feeding stage, just about as much shortening as the extreme that could be induced by actual deprivation of food.

*Loss of Weight During Pupal Life.*—A belief among commercial breeders of silkworms that there is a loss in weight of the cocoons (silk) accompanying pupal life is indicated by their recognized wish to make an early sale of the cocoon product. This loss is generally attributed to "evaporation from the cocoon." The question arose as to whether the loss in weight of the pupa-containing cocoon might be not a loss in weight of silk but an accompaniment of developmental changes in the pupa, a process in which stores of nourishment (in the larval body) are being converted into moth with chemical changes which might occasion some loss in weight. Therefore in four individuals the cocoon and pupa were weighed separately once each day from the time of pupation to time of emergence of the moth, while at the same time the daily weights of the naked chrysalids of three other lepidopterous species were determined to see if a loss of weight accompanied pupal aging in them as well as in the silkworm moth. From the data obtained it is apparent that the silken cocoon loses a very small amount, about 4 per cent., of its weight in the first day after its completion, and then loses no further weight; that the pupa loses weight slightly but persistently and steadily from day to day throughout its entire duration, the total loss amounting to about 14 per cent.; and that the pupæ of three other lepidopterous insects, namely, the tent caterpillar (*Chisiocampa sp.*), checkerspot butter-



fly (*Melitæa sp.*), and mourning-cloak butterfly (*Euvanessa antiopa*) also steadily lose weight from day to day, this loss being very considerable in two of these species, viz., about 35 per cent. in the case of one and 65 per cent. in the case of the other.

(with R. G. Bell) Variations Induced in Larval, Pupal and Imaginal Stages of *Bombyx mori* by Controlled Varying Food Supply, in *Science N. S. v. 18*, pp 741-748, December, 1904.

One of the races of the mulberry silkworm was made the subject of experiments directed toward a determination of the exact quantitative relation which quantity and quality of food bear to the development and variations of the individual insect, and to the maintenance or transmission of these variations to its progeny.

The change in quality of food consisted of a substitution of lettuce for mulberry. The lettuce-fed worms went through their moults, spinning up, pupation and issuance as adults successfully. They mated freely and laid eggs which developed normally. The young larvæ adopted the unusual diet very reluctantly, but in later life these same larvæ, "educated" to its use, ate lettuce with a relish which rivaled that displayed by the normal larva with its mulberry leaf.

The most striking variation induced by this lettuce regimen was that the time consumed by the metamorphosis was double the time appointed for that of the normal mulberry-fed larva—being three months as compared with six weeks for the latter. In the commercial world this fact would offset the advantage of the lettuce, as a cheaper food and as one available at all seasons, by demanding twice the labor that is required to rear to spinning time larvæ fed on mulberry. Thus it appears that the lettuce experiment can not be of economic value to sericulture unless it should prove that lettuce-made silk is worth the cost of double labor.

The other variations noted among the lettuce-fed "worms" have to do with the larva and cocoon. All of the lettuce-fed larvæ appeared to be unusually "thin skinned," the body wall being stretched and shiny. The larvæ were at all stages characteristically heavier than mulberry-fed larvæ, each of them weighing at spinning time as much as, and two of them weighing 400 mg. more than the heaviest of the mulberry-fed. The weights of the cocooned pupæ were somewhat above the average among the mulberry-fed, a fact due to the large pupa rather than to the amount of silk in the cocoon, as was demonstrated by weighing cocoon and pupa separately, whereupon it was found that the cocoon was, on the average, but one-half as heavy as that of the average among the mulberry-fed, in some cases falling as low as two-fifths of the mulberry cocoon's average weight, and in no case rising above three-fifths. The silk appears to be less strong and elastic than that of the mulberry-made cocoon.

In the mulberry-fed worms there exists a very definite and constant relation between amount of food and size as indicated by weight, the starveling individuals being consistently smaller than the well nourished, the lingering effects of this dwarfing being handed down even unto the third generation, although the progeny of the famine generation be fed the optimum amount of food; in case the diminished nourishment is imposed upon three or even two successive generations there is produced a diminutive, but still fertile, race of Lilliputian

silkworms whose moths, as regards wing expanse, might join the ranks of the micro-Lepidoptera almost unremarked.

In illustration may be quoted the typical or modal larval weights for each of the lots of 1903 at the time of readiness to spin, which marks the completion of the feeding and is, therefore, an advantageous point for a summary of the results of the three years' experimental feeding.

The history of the eight lots referred to may be gathered from an examination of the accompanying table, in which "O" means optimum amount of food and "S" means short rations. The column to the right indicates the relative rank of the various lots as judged by the modes of frequency polygons erected to include all the individual weights for each lot at spinning time.

Lot Number	HISTORY OF LOTS			Modal Rank 1903
	1901 Grandparents	1902 Parents	1903	
1	O	O	O	1
2	O	O	S	6
3	O	S	O	3
4	O	S	S	7
5	S	O	O	2
6	S	O	S	5
7	S	O	O	4
8	S	S	S	8

We find that control lot 1, consisting of normally fed individuals of normal ancestry, holds first rank in weight, as was to be expected. Second comes lot 5, whose grandparents experienced a famine but whose parents as well as themselves enjoyed years of plenty. Lots 2 and 3 have likewise had one ancestral generation on short rations, and the fact that they are lighter in weight than lot 5 illustrates a general rule which obtains throughout the entire company of experimental worms, namely, that the effects of famine grow less evident the further removed the individuals are from its occurrence in their ancestral history. Thus lot 5 is two generations removed from the famine of 1901, while lot 3 has had but one generation in which to recover its ancestral loss. Lot 2, which has had a total of but one famine year—the current year—nevertheless ranks below lot 7, which has had two famine years in its ancestry succeeded by plenty during the current year. Lot 2 also ranks below lot 6, a fact which appears strange, considering that lot 6 has suffered two generations of famine, including the current year, which is the only famine year experienced by lot 2. In explanation of this anomalous condition it is suggested that possibly the larvæ of lot 6 were better fitted for enduring the making the best of hard conditions than were the individuals of lot 2, the ancestors of the former lot having been selected two years ago on a food-scarcity basis. This suggestion gathers support from an inspection of the mortality notes, from which it appears that the number of

deaths—for which the famine was probably a contributing and not a primary cause—in each lot which is for the first time subjected to short rations is almost doubly greater than the number of deaths in lots which are descended from starved ancestors, whether these ancestral famines occurred in successive or alternate years. The figures indicate that a reduction of food is almost twice as destructive upon the first generation which is subjected to it as it is when visited on a second generation. Lot 4 follows lot 2 as the seventh in rank and its position is in accord with the rule above noted, its latest ancestral generation which enjoyed an optimum amount of food having been grand-parental, whereas the ancestors of all the other lots except lot 8 have had the optimum amount of food during 1902 or 1903. Lot 8 holds lowest rank, it and its ancestors having been subject to trying conditions throughout the entire three years, during some one or two of which all the other lots have enjoyed the best of food conditions. Thus it appears that a generation of famine leaves its impression upon at least the three generations which succeed it, yet the power of recovery through generous feeding exhibited by the progeny of individuals subjected to famine is so extensive (witness lot 5) that it appears probable that every trace left by the famine upon the race would eventually disappear. It is even conceivable that the ultimate result of the famine would be a strengthening of the race, the famine having acted the part of a selective agent, preserving only the strong.

That conditions of alimentation bear a directive relation to functional activity may be demonstrated by reference to the records of the physiological functions of moulting, spinning, pupating and emerging, of the individuals of the experimental lots.

An abnormal extension of the time needed for the metamorphosis follows upon a reduction of the food supply. The degree of extension depends with the utmost nicety upon the amount of food given the larvæ. For example, among the 1901 generation of silkworms, one control lot of twenty larvæ was given the optimum amount of food, a second lot of twenty larvæ one-half this amount, and a third lot of twenty larvæ one-quarter of the amount. To take the time of the fourth moulting as an illustration, the moulting was begun by the first lot, which led the way by two and a half days, at the end of which the second lot began to moult, while the third lot was twenty-four hours behind the second. All the individuals of the first lot had finished moulting on April 20, all of the second on April 24, while the moulting in the third lot continued until April 29.

As in the matter of weight, this retarding of the functions, by means of a reduced food supply, affects not only the immediate generation which is subjected to the famine, but the lingering effects of it may be traced in the progeny of the dwarfed individuals at least unto the third generation, even though two years of plenty follow the one year of famine. The conditions which obtain in each lot of individuals of the 1903 generation at spinning time are shown in the following table, which is based upon polygons erected to include all the individuals in each lot.

LOT NUMBER	HISTORY OF LOTS			RANK OF 1903 LOTS AS TO PROMPTNESS IN SPINNING			
	1901 Grandparents	1902 Parents	1903	Earliest Spinner	When Two-Thirds of Each Lot Were Spinning		Latest Spinner
					Date	In Order of Rank	
1	O	O	O	1	May 12	1	1
2	O	O	S	5	" 25	4	4
3	O	S	O	2	" 13	2	3
4	O	S	S	4	" 26	5	5
5	S	O	O	3	" 13	2	2
6	S	O	S	6	" 29	6	7
7	S	S	O	6	" 22	3	5
8	S	S	S	7	" 30	7	6

This period in the silkworm's life is particularly advantageous for consideration here because it marks the completion of the feeding, so that the individuals of under-fed ancestry have been given the best chance to recover, while those subject to altered food conditions have had the benefit of the alteration during the entire food-taking period of life.

In the table "O" means optimum amount of food and "S" means short rations. To the right of the history of the lots is a section showing the rank of the lots as to the extreme time limits of the spinning time (emphasized congenial differences again), with a safer criterion, as to their relative promptness, in the column between the extremes—a column of figures intended to show the relative promptness with which a two-thirds majority of the larvæ in each lot arrives at the spinning time, this proportion being taken to represent the typical condition for the lot. The order in which the lots are arranged in this column corresponds in a general way with that prevalent for the weights at spinning time, and the generalizations indulged in there may with few exceptions be applied here. The lots which were well fed during the 1903 generation are ahead of all of those given short rations in 1903, whatever ancestry they may have had. Lot 1 leads here as in the matter of weight. Lots 3 and 5 tie for second place, having held second and third places in weight. Lots 2 and 4 stand in the same relation to one another that they held as to weight. Contrary to the weight relation, lot 6 follows lot 2 at the spinning—a fact which illustrates again the general rule that two generations of famine are more disastrous than one, but does not lend support to the notion of natural selection on a food scarcity basis as previously suggested. Lot 8, which has had no relief from famine during the entire three years, brings up the rear at the spinning, as might be expected.

As to the life and death selection due to famine, it may be said, in addition to the previous discussion of mortality among the experimental silkworms, that while lots subjected to two years of famine (themselves in one year, their parents in the year before) were fertile in so far as number of young hatched is concerned, it was found to be exceedingly difficult to rear from them a 1903 generation. Indeed, at the time of the second moulting there were but nineteen

individuals (and tolerably vigorous larvæ they were) alive in the lot which had experienced two years of famine, although every individual of the 149 hatched was carefully preserved and royally fed—a fact which goes to prove that the equipment at birth of many of these larvæ was inadequate.

The fact that some larvæ of starved ancestry have exhibited a superiority over their fellows, in surviving and recovering from hard conditions, is testimony for the existence of individual variations which can not be defined anatomically, and yet which serve as “handles” for natural selective agents. Such variations might be called physiological variations, since it seems that the surviving larvæ must be those which are in best trim physiologically. These larvæ are able to make the most of the food offered to them. If competition were allowed, they would probably be the individuals which would cover the area most rapidly, securing whatever food there might be. But under our experimental conditions there was no competition allowed and yet certain precocious individuals made more grams of flesh and more yards of silk, than other larvæ furnished with the same amount of raw material under like conditions; that this was due to the possession by the former of certain congenital qualities of adaptability can scarcely be doubted.

As to the fertility of the variously fed lots; in so far as number of eggs produced is a measure of fertility, our records already demonstrate the fact that the better nourished are the more fertile. Furthermore, the economy in this matter practised by the starvelings is not merely numerical, quality as well as quantity of eggs being affected. In witness of this point may be recalled the story of the dying 1903 generation, produced from eggs of the starvelings of 1901 and 1902, which would seem to offer conclusive evidence that a famine suffered by the parents works its way into the germ cells so that most of their progeny have but a poor birthright.

**Regeneration in Larval Legs of Silkworms, in Jour. Exper. Zool., v. 1, pp 593-599, 10 figs., Dec., 1904.**

Experimenters in regeneration in insects have too often overlooked the fact that the imaginal (adult) legs of insects of complete metamorphosis are produced not by a direct transformation of the corresponding larval thoracic legs but from new centers called imaginal discs or histoblasts. These histoblasts are developed from an invagination of the larval cellular skin layer (hypoderm) and only in comparatively late larval life do the new developing imaginal legs lie within the larval ones. It follows from this that if a larval leg be cut off in early larval life the imaginal leg is in no way mutilated, and that if it appears of full size and normal character in the adult insect, this is not due to restorative regeneration but simply to its normal growth and development. If a leg be cut off in late larval life, the developing imaginal leg may or may not be at the same time mutilated. If mutilated, however, it will always be by a removal of much less of its extent than of the extent of the larval leg taken off. A cut which severs the larval leg near its base (for example, through the base of the femur), will not take off more than the tarsus or perhaps part of the tibia and tarsus of the imaginal leg, which, in its development, is beginning to extend into the larval one. Thus if the imaginal leg be found, when the imago issues, to lack a tarsus

but to possess a complete femur and tibia, this is no indication that there has been a partial regeneration; there may have been none whatever.

To make a definite test of the capacity of the silkworm to regenerate lost parts, legs, both thoracic and abdominal, were cut off of the larva at various ages and at various places between the tarsus and the body, and notice was taken of whether or not regeneration of these legs took place before pupation, and if so in what degree, and whether normally, *i. e.*, so as to produce an exact replica of the lost leg, or not.

The results of the experiments show, (a) that the larva of the silkworm moth, *Bombyx mori*, has the capacity of regenerating its thoracic and abdominal (prop-) legs from the stumps of these legs, but not from the body (trunk), *i. e.*, that each leg has the capacity to regenerate any distal part from any proximal part, but that the body can not produce a wholly new leg; (b) that this regeneration shows externally not after the first moulting after the mutilation but after the second moulting, and that the regenerative processes are completed with the appearance of the new parts after this second moulting succeeding the mutilation.

The small caudal horn, a pointed non-segmented, but movable, process projecting upward from the dorsal surface of the penultimate abdominal segment was cut off in many larvæ (silkworms) of various ages, and in no case was there the slightest regeneration. After the first moulting succeeding the mutilation the new skin always extended smoothly over the place where the horn had been, without any sign of scar.

The function of this horn, which occurs on some other lepidopterous larvæ, notable and characteristically on the larvæ of the Sphingid moths, is unknown. It has been explained by some entomologists as an ornament, by others as a "terrifying organ." It is not a sting nor in any way an effective weapon of defense, as even where long and conspicuous ( $\frac{1}{2}$  in. long) it is weak and easily bent. Nor does it secrete an acrid or ill-smelling fluid. Certainly in the silkworm it has had for many hundreds of generations no possible function as a weapon. It is interesting to note that this useless organ is not regenerated.

*Relation of Regeneration to Natural Selection.*—This suggests to us a consideration of the relation of regeneration, as we have observed it in the silkworm, to its causes, or at least to natural selection as an explaining cause. If the caudal horn is now a useless organ in the silkworm body its lack of capacity to regenerate (loss of capacity, if it ever had it) would seem to favor the theory of the natural selectionists concerning regeneration. At first glance, also, the retaining of the regenerative capacity of the legs, useful organs, may seem to favor this theory. But it must be borne in mind that the silkworm has been for approximately 5000 years a domesticated animal cared for under such conditions as to make the natural loss of legs almost an impossible occurrence.

Perfectly protected against such natural enemies as bite off legs, there has certainly been nothing of that sharp necessity, during all the life of countless successive generations of silkworms, which is supposed to be the basis for maintaining the advantageous capacity for regeneration. There has been a clear field for panmixia. But the regenerative capacity still exists in effective degree. The silkworm offers little aid and comfort to those who would explain regeneration wholly as a phenomenon fostered and maintained by natural selection on a basis of utility.

**Influence of the Primary Reproductive Organs on the Secondary Sexual Characters, in Jour. Exper. Zool., v. 1, pp 601-605, Dec., 1904.**

In this paper is recorded an account of the process of extirpating the developing ovaries and testes of various silkworm individuals in various larval stages. These individuals after pupation and issuance as adults were then examined to note if any change or lack of normal development had taken place in those structures showing secondary sexual differences, particularly the antennæ.

The extirpation of the developing reproductive organs, which lie just beneath the dorsal wall in the fifth abdominal segment, was accomplished by searing with a hot needle. The slight wounds soon closed, and most of the larvæ were reared to moths. In all cases the moths were dissected to be sure that the destroying of the ovaries or testes had been complete and to see whether any regeneration of these parts had taken place. No such regeneration occurred, and in a score of moths the ovary or testis of one or both sides was found to be wholly wanting.

There was no case of the absence or modification of the secondary sexual characters in any of the moths. All males had both antennæ of the usual male type, although the testis of one side or the other, or of both sides, was wholly wanting.

**Some Silkworm Moth Reflexes, in Biol. Bull., v. 12, pp 152-154, Feb., 1907.**

Silkworm moths, *Bombyx mori*, are sexually mature and eager to mate immediately on issuing from the pupal cocoon. They take no food (their mouth parts are atrophied), they do not fly, they are unresponsive to light; their whole behavior, in fact, is determined by their response to the mating and egg-laying instincts. We have thus an animal of considerable complexity of organization, belonging to a group of organisms well advanced in the animal scale, in a most simple state for experimentation.

The female moth, nearly immobile, protrudes a paired scent-organ from the hindmost abdominal segment, and the male, walking nervously about and fluttering its useless wings, soon finds the female by virtue of its chemotactic response to the emanating odor. Males find the females exclusively by this response, but orient themselves for copulation (after reaching the female) by contact. When two males accidentally come into contact in their moving about they try persistently to copulate.

A male with antennæ intact, but with eyes blackened, finds females immediately and with just as much precision as those with eyes unblackened. A male with antennæ off and eyes unblackened does not find females unless by accident in its aimless moving about. But if a male with antennæ off does come into contact, by chance, with a female it always (or nearly so) readily and immediately mates. The male is not excited before touching the female, but is immediately and strongly so after coming in contact with her. Males with antennæ on become strongly excited when a female is brought within several inches of them.

The protruded scent-glands of the female are withdrawn into the body immediately on her being touched by a male. If the scent-glands are cut off and put wholly apart from the female, males are as strongly attracted to these

isolated scent-glands as they are to unmutated females; on the contrary they are not at all attracted to the mutilated females. If the cut-out scent-glands are put by the side of and but a little apart from the female from which they are taken, the males always neglect the near-by live female and go directly to the scent-glands. Males attracted to the isolated scent-glands remain by them persistently trying to copulate with them, moving excitedly around and around them and over and over them with the external genitalia vainly trying to seize them.

The behavior of males with the antenna of only one side removed is striking. A male with left antenna off when within three or four inches of a female (with protruded scent-glands) becomes strongly excited and moves energetically around in repeated circles to the right, or rather in a flat spiral thus getting (usually) gradually nearer and nearer the female and finally coming into contact with her, when he is immediately controlled by the contact stimulus. A male with right antenna off circles or spirals to the left. It is a curious sight to see two males with right and left antenna off, respectively, circling violently about in opposite directions when he immobile female a few inches removed protrudes her scent-glands. This behavior is quite in accordance with Loeb's explanation of the forward movement of bilaterally symmetrical animals.

The results of all the experiments tried show how rigorously the male moths are controlled by the scent attraction (chemotropism) and how absolutely dependent mating (the one adult performance of the males) is on this reaction. If we can find specialized animals in a condition where all attractions and repulsions (stimuli) but one are eliminated we may readily perceive the rigorous control exercised by this remaining one. We are, unfortunately, in the general circumstances of animal life too much limited to the use of very simply organized animals for reaction and reflex experimentation. This tends to make it difficult to carry over to the behavior of complexly organized animals the physico-chemical interpretation which is steadily gaining ground as the key to the understanding of the springs and character of the behavior of the simplest organisms. But where the complex stimuli and reactions that determine the behavior of complexly organized forms can be isolated and studied the inevitableness of much of this behavior can be recognized.

*Reflexes of Moths Without Cephalic and Thoracic Ganglia.*—A number of experiments was made to determine the need, or absence of need, of the principal ganglia of the central nervous system in the performance of the two chief reflexes in the silkworm moth's life, *viz.*, mating and egg-laying.

Males mate with headless females, and the headless females, after mating, lay a few eggs which develop normally, that is become fertilized by the release of spermatozoa from the spermatheca in the female's body, are oviposited by the repeated extrusion and retraction of the ovipositor, and make the usual color changes (from yellow to cherry-red and then to lead-gray) incidental to normal development. But in no case did a headless female lay her full complement of eggs, in fact in no case were more than a score of eggs laid (the normal number is from 200 to 350). Headless females (and headless males) usually live as long as unmutated individuals, *i. e.*, from a week to two weeks.

Females with head and thorax cut off (and even part of the abdomen) can be mated with by males, and this fractional part of the female can fertilize



and oviposit a few eggs which begin normal development. In one case 10 eggs, of which 8 are now normally developing were oviposited by such an impregnated part of female abdomen, this abdominal relict remaining alive (!), *i. e.*, flexible and responsive to stimulus and capable of extruding the ovipositor and laying eggs, for forty hours.

Males with head removed can not find females, nor can they mate if placed in contact with them. When the head or head and prothorax of a male is cut off immediately after the male and female are *in copulo* the female, although uninjured, lays no eggs. If heads of both males and females *in copulo* are removed no eggs are laid although both moths remain alive usually as long as do unmutated individuals.

A silkworm moth can maintain itself right side up with antennæ off or with antennæ off and eyes blackened, but with head off one position seems indistinguishable from another to it, *i. e.*, it lies on one side or the other, on the venter or dorsum equally willingly. The organs of equilibrium are not on the antennæ, then, but are lost when the rest of the head is removed.

**Sex Differentiation in Larval Insects, in Biol. Bull., v. 12, pp 380-384, 8 figs., May, 1907.**

Dissections and sections of larvæ of *Bombyx mori* of various ages from just after hatching to the last instar show that the reproductive organs (ovaries or testes) are already in such an advanced stage of development that the distinction between male and female (testes and ovaries) can be recognized in larvæ from the time of the first moulting. Also that the just hatched larva has the reproductive organs already well developed. Careful scrutiny by a special student of oogenesis and spermatogenesis would probably enable him to determine the sex of the larva immediately on hatching.

The sex of the silkworm is then not to be tampered with by gorging or starving, and what is true of this lepidopteron is undoubtedly true of its cousins, the other moths and the butterflies. It is probably also true of other insects with complete metamorphosis. I recall dissections of various larvæ, notably of *Corydalid cornuta* (a neuropteran) and of *Holorusia rubiginosa* (a dipteran) in which the reproductive organs appear of two sizes in specimens of the same age: indeed in *Corydalid*, of two shapes. These organs need histologic examination. Some student should laboriously work through a long and representative series of insects and settle the question as to the time of sex differentiation. That is, find out whether it be true for all, as it is in the silkworm, that the time of sex differentiation is obviously before, or, at latest, at very little after the time of hatching. If it is true, the question of the influence of nutrition in sex determination will also be settled—for insects. And we need waste no more time in tedious feeding and tabulating.

**Artificial Parthenogenesis in the Silkworm, in Biol. Bull., v. 14, pp 15-22., December, 1907.**

In a clutch of unfertilized eggs oviposited by a virgin silkworm moth (*Bombyx mori*) almost always a small number of eggs begins development. This development extends to the formation of the embryonic envelopes and sometimes farther, and is clearly indicated to the observer by the change in color of the egg from yellow to cherry or through cherry to gray. Non-developing eggs

remain yellow and, after a while, collapse. Eggs which begin to develop either persist in spherical shape, which indicates persisting life, or collapse, which means death. The development of unfertilized eggs rarely proceeds, without artificial stimulus, beyond a very early embryonic stage. In fully 500 clutches or broods of unfertilized eggs (from confined females from isolated cocoons) under observation, not a single egg gave up its larva, although an average of about seven or eight per centum of the eggs began to develop.

Although this parthenogenetic development always ceases and the embryo dies before reaching hatching stage, much difference in vitality or duration of life of the egg (strictly, embryo) is noticeable. Some of the developing eggs collapse within a few days, some in a few weeks, while a few persist for several months. (The normal egg stage, *i. e.*, time from egg laying to hatching of larvæ in the silkworm univoltin races, is about nine months.) There is also to be noted a difference among races in the proportion of unfertilized eggs which begin to develop. Among a dozen races in our rearing rooms, one (a vigorous white-cocoon race called Bagdad) is strongly inclined to normal parthenogenesis, from twenty-five to seventy-five per centum, even in a few cases ninety-five per centum, of the eggs in unfertilized lots beginning to develop. The more usual proportion, however, *i. e.*, that shown by the other races, is, as already noted, less than ten per centum. So much for normal parthenogenesis in the species.

In 1885 Tichomiroff discovered that by bathing the unfertilized eggs with concentrated sulphuric acid, or by rubbing them gently, he could induce a considerably larger per centum than the normal to begin development. He repeated his experiments, confirming and extending his results, in 1902. By histologic examination of the eggs he learned that the artificially stimulated eggs which develop do so in a somewhat abnormal manner. Tichomiroff held the stimulus to development to be neither the action of specific ions, osmotic pressure nor catalysis. He believes that the eggs respond by segmentation to any appropriate excitation, "whatever the nature of this excitation."

Version, in 1899, used electricity as a stimulus, and found that the development thus initiated ceased at a point about corresponding with that reached by a fertilized egg on the third day after oviposition.

Quajat (1905) submitted unfertilized eggs to the action of oxygen, high temperatures, sulphuric acid, hydrochloric acid, carbon dioxide, and electricity. His account of the experiments indicates that he was able to stimulate development by several of these agents, but he gives no data to show the proportion of developing eggs in the various treated lots. No larva issued, but by an examination of the eggs he found that several embryos had practically completed their development and growth.

My own experiments include the treatment of something over a hundred lots of unfertilized eggs (a "lot" is all the eggs laid by a single female, averaging from 100 to 350 in number), and of several lots of fertilized eggs (to serve as controls to indicate possible injury to the eggs from the reagents used). The stimuli or agents used were dry air (obtained by drawing air through vessels of calcium chloride and then of concentrated sulphuric acid), high temperature, sunlight, friction, sulphuric acid, hydrochloric acid, glacial phosphoric acid, glacial acetic acid, absolute alcohol, potassium hydroxide, ammonia, and lime

water. The reagents were used in different dilutions and for varying lengths of time. The treatment was applied to eggs not more than twelve hours old; mostly to eggs but a few minutes to a few hours old. Five hundred or more lots of untreated, unfertilized eggs were observed in order to determine the extent of normal parthenogenetic development. The eggs of half a dozen silkworm races were used and all the eggs were preserved from time of laying until their death.

As it seemed to me that most of the favorable results obtained by Tichomirow and Quajat were obtained by treatments which had as common effect a dehydration (such as high temperature, friction, sulphuric acid, etc.) I attempted to test this first by using various dehydrating agents, especially a dry chamber in which the eggs could be submitted for from a minute or two to several hours to a nearly perfectly dry atmosphere. Friction, heat, sulphuric acid, phosphoric pentoxide and glacial phosphoric acid were also used as dehydrating agents. At the same time other treatment, not dehydrating, was used on other lots and gave results hardly less favorable than the dehydrating. The results at the end of this first course of treatment seemed to point to the hydrogen ions as the most likely development-inciting factor. Hence various agents agreeing in containing hydrogen ions though differing radically in other particulars were used. The results gave no encouragement to the hydrogen ion theory. In fact I have not been able to come to an opinion concerning the true *causa efficiens* in the matter. My results simply show to me that various stimuli, acid or alkaline, dehydrating or non-dehydrating, possessing or not possessing hydrogen ions, are able to increase materially the proportion of eggs that develop in lots of unfertilized eggs.

The data of the experiments are given in considerable detail.