THE EFFECT OF MOISTURE UPON THE SILK OF
THE HYBRID PHILOSAMIA (ATTACUS) RICINI
BOISD. ♂ × PHILOSAMIA CYNTHIA
(DRURY) ♀

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Philosamia ricini and of *P. cynthia* from Ning-po were crossed in 1914 in order to study the method of inheritance of certain spots on the larva, the colour of the cocoons, and the arrangement of the long white hairs on the abdomen of the moth. The results of the preliminary work on cocoon colour are given in this paper, no reference being made to the still unfinished work on the larva and moth.

When the cocoon colour work was undertaken, it was hoped that the results would help to explain the lack of coincidence between the work of Kellogg and Toyama on the inheritance of colour in cocoons. The breeding, thus far, has thrown no light upon the method of inheritance, but, if the cocoons used by them, are as much affected by moisture as the hybrid here discussed, their results may have to be considerably modified.

Philosamia ricini is found wild in Assam, but has also been domesticated to a considerable extent in that province, and to a smaller degree, in other parts of India. A careful study of the life history has been made at Pusa, India. The form there cultivated is not pure, some larvae being spotted instead of spotless, and some of the cocoons being a pale fawn instead of a pure white. Every effort is, however, made to keep the broods as nearly as possible like the wild form, which has a spotless larva and a pure white cocoon. Owing to the uncertainty of the Pusa stock, the specimen used in this cross was chosen from a batch of white cocoons collected in the Assam forests.

In order that there should be no doubt about the stock, Mr. J. H. Watson and myself, mated males and females of this batch

and reared the offspring, to find that they produced spotless larvae and white cocoons. One seemed, therefore, justified in presuming that the parent *P. ricini* came from a stock which produced only white cocoons.

P. cynthia differs markedly from *P. ricini* in that it has, typically, a red-brown cocoon. Owing to some unknown cause, the intensity of colour varies considerably. I have not up to the present been able to rear this species in order to study the variations of intensity and the possible causes which may produce that variation. A related species, *P. canningi*, also has the cocoon colour varying from a rich red-brown to a pale fawn: this species has been reared, but only for one generation. Experiments were to be made on the second generation to test if moisture had any effect on the intensity of colour, but the moths unfortunately did not pair. It is important to notice that neither *P. cynthia* nor *P. canningi*, is known ever to have produced a white cocoon.

As the result of the crossing, the female, *P. cynthia* laid about 180 eggs, a large proportion of which were fertile. At the end of three weeks there were 149 larvae, which produced 135 cocoons and from them there emerged 111 moths, only three being cripples. The family was exceptionally healthy in spite of the exotic conditions under which it was grown. The larvae grew quickly and reached a length of $2\frac{1}{2}$ to $3\frac{1}{2}$ inches—as large as any grown at Pusa. There was very little disease.

These cocoons exhibited every variety of fawn and pale brown—none were white, none were the deep red-brown characteristic of *P. cynthia*. One was therefore, inclined to conclude that the brown colour acted as a weak dominant.

The cocoons (F_1 generation), were spun in the privet leaves on which the worms had been reared or on the nets which covered their cages. The nets were either black or white. The colour of the nets had no influence upon the intensity of the colour of the cocoons, as both darker and lighter cocoons occurred side by side on both black and white nets. One colour phenomenon, however, constantly occurred; when a cocoon was spun in leaf, it was always a darker colour on the side towards the leaf,

the leaf never being large enough to extend entirely around the cocoon.

The second generation of cocoons (F_2) varied much more than F_1 some being a dark red, the majority fawn, and a few so light in colour that one was inclined to describe them as white. These very light cocoons, however, were found, when opened, to have a fawn coloured lining, whereas those of *P. ricini* which I had reared myself, were white throughout. All the nets used in rearing this generation were black.

Mr. W. Bateson very kindly looked at the cocoons of the F_1 and F_2 generations, and, influenced by his own early work on cocoon colour, advised a very careful study of every possible environmental influence. As a result, hours were spent in watching the larvae spin. It was thus ascertained amongst other facts, that the first silk produced might be either brown or white. I was, however, never successful in collecting this first silk straight from the mouth of the larvae, but have seen sufficient on the glass lid of a cell, to have no doubt of the colour. The brown silk, even when thus seen in some quantity, was a fawn brown, never the deep red-brown which characterised so many completed cocoons.

Bateson had worked at *Eriogaster lanestris* and *Saturnia carpin*i and concluded that they spun a very light or white cocoon as a result of unhealthy conditions or "unnatural conditions such as disturbance at the time of spinning or removal from the food plant when the growth is nearly complete." That unhealthy conditions had nothing to do with the light cocoons produced in this hybrid is, I think, quite clear from the general condition of the larvae as described above. Mr. J. H. Watson, a well known breeder, inspected the larvae and stated that he had never seen any in better condition. Disturbance, in the case of this hybrid had no influence, as is demonstrated by the experiments presently to be described.

Whilst these general observations were in progress, a number of larvae were being reared, for special breeding purposes, in separate cells. In every case except one, these larvae produced deep brown cocoons. These cells, being filled with privet leaves,

were invariably damp, or even saturated with moisture. The brown of the cocoons was so striking, that a special cause was at once sought. Now, the only difference in the environment of these solitary larvae and those in the large cages was moisture: hence, there seemed a possibility that moisture might be a factor in cocoon colour. In all my experiments the larvae were reared exclusively on Privet (*Ligustrum vulgare*).

The forests in which the *Philosamias* live are very moist at certain seasons, but I have been unable to obtain any satisfactory information as to the degree of moisture of these forests, and as to whether the moist seasons coincide with the periods when these larvae spin their cocoons.

In order to test the effect of moisture, the following typical experiments were made.

A. Experiments on unfinished cocoons

1. A larva began to spin a cocoon in a dry room. At 10 p.m. the first threads were a pale brown. The larva was removed from the cage and placed in a dried box. The next morning, at 8 a.m., the cocoon was complete and a pale brown colour. This experiment was repeated several times and it was found that if the original silk were brown, the cocoon was brown, but not a deep brown. When subsequently exposed to moisture, cocoons made under these conditions, became a deep red-brown.

2. A larva began a cocoon of white silk at 9 a.m. At 10 p.m., the cocoon was white but incomplete; the cocoon was then removed to a dry box containing CaCl_2 . By 8 a.m., the following morning, a good cocoon was complete, the colour being a dirty white. The cocoon was left in the cell and the pupa died of desiccation.

3. A larva at 9 p.m. had completed a pure white platform. The larva and the platform were then placed in a damp cell with a dozen broken privet leaves. The following day at 10 a.m., the platform had become brown, but it was deserted by the larva, which had spun a complete new brown cocoon. On the evening of the same day, the leaves were removed from the box, the box dried and CaCl_2 introduced. After ten days the colour was unaltered, but the pupa had died of desiccation. This larva finally spun a dark cocoon although it was disturbed by me and as a result deserted its original cocoon.

4. A larva began to spin brown silk in a damp cell. The leaves which produced the moisture were at once removed, the cell dried, and CaCl_2 introduced. The cocoon was finished and was a pale brown colour. Three months later, when the cocoon was placed in a very damp box, it became much darker.

5. One white cocoon was spun in a damp cell and remained white. Unfortunately it was not possible to breed from the moth which emerged from this cocoon.

B. Experiments on finished cocoons

Cocoons containing larvae

1. On Jan. 26, at 10 p.m., four pure white, completed cocoons were found together on the net. One was placed in a damp cell, leaving a tiny piece of silk outside in the dry room. The following day at 9 a.m. the cocoon in the box had become brown, whilst the piece outside remained white. Of the three cocoons remaining on the net, two were still pure white, but the third had become brownish at the mouth of the cocoon. By 10 p.m. there was no change in the cocoons. The brown cocoon was then placed in a dry cell with CaCl_2 . When the cocoons were examined on January 28 no change had taken place in the colour. On February 10, on which date the cocoons contained pupae, the cocoons were still the same colour. During March and April, when the moths were being forced to emerge by warmth and damp, the three white cocoons became a pale brown.

2. A cocoon was white except at one end where it was a pale fawn. It was placed in a damp cell, with the fawn end hanging in the dry room. By the following morning all the cocoon was a dark brown except the excluded end.

3. A cocoon was fawn except a small attachment piece, which was brown; the whole cocoon was placed in a damp cell, and in fourteen days was a dark, dirty brown.

4. A medium red-brown cocoon was opened, when the interior was found to be a lighter colour than the exterior. This cocoon was placed in a damp cell, and after eleven days had become a deep brown throughout, the inside now being the same colour as the exterior. The original and subsequent coloration of this cocoon are an interesting demonstration of the influence of external conditions.

5. All the white cocoons of F_3 generation became various shades of fawn and red-brown when subjected to warmth and moisture.

The pieces of silk used in these observations, were taken, it will be noted, from, (a), fresh cocoons which contained the larva, (b), cocoons which contained the pupa, and (c), cocoons up to four months old. These experiments all suggest that moisture is a factor of considerable importance in the cocoon colour of this particular hybrid. Moisture, again, would explain the phenomenon already noted, that in all cases of cocoons spun in a fresh leaf, the side towards the leaf was always darker, for owing

to transpiration, this side would be more or less moist. It also probably explains why no white cocoons appeared in F_1 generation, as that family was reared in an unusually moist room.

It has been stated above, that the larvae have been seen to begin by spinning either pale brown or white silk, and that when the first silk was brown, the cocoon was pale brown, whether it was spun under moist or damp conditions. Up to the present only one case is known, in which a white cocoon was spun in a cell saturated with moisture. When the white or pale brown cocoons are subsequently placed in a very damp atmosphere, they became more deeply coloured, and thus approximated to or even attained, the colour characteristic of the species.

These then are the problems to be solved—is the original colour, whether it be white or pale brown, affected subsequently by (a), atmospheric moisture; (b), by this moisture plus oxidation; or (c), by an excretion in addition to these two?

If a larval excretion has any influence, there are two possible factors which have to be considered—moisture and a definite colouring matter. Or, the excretion may possibly contain a colourless substance which, indirectly, causes the silk to change from white to brown.

Experiment to test whether the colouration was due to oxidation plus water, or to the effect of water vapour alone

A piece of silk was taken from a pure white cocoon, fixed to the end of a glass rod and plunged into a flask from which all air had been excluded by prolonged boiling, and which therefore contained only water vapour. The piece of silk became brown instantaneously, the rapidity of the change being hastened by the temperature. Other pieces of silk from this same cocoon had been kept in boxes at 60°–65° F. and 75°–85° F. respectively, and all had become brown, the change taking place more quickly at the higher temperature. The degree of colouration varied considerably in both boxes. It appeared that temperature acted solely as an accelerating factor, as equally deep browns were produced at both lower and higher temperatures. No experiments were tried at a temperature below 42° F., as that is the minimum temperature at which the pupa remains healthy.

The above experiments make it quite clear that moisture, by itself, can change white silk to brown and consequently, atmos-

pheric conditions are of paramount importance in determining the ultimate colour of the cocoons. The experiments, however, throw no light upon the innate difference between the white silk of the hybrid which becomes brown in a moist atmosphere and the white silk of the parent *Philosamia ricini*, which remains white under all variations of atmospheric moisture.

When two cocoons are formed, one white and one brown, side by side on the net, during the same night, there is some difference in the condition of the two which is difficult to explain by the slight difference in atmospheric moisture which would occur in a small, closed room, between 10 p.m. and 8 a.m. There is here, doubtless, a difference in inheritance, such that the one possessed and the other did not, the power to produce brown silk at the particular temperature and degree of moisture, which occurred in that room and on that night. Nevertheless, all the white or light cocoons made thus, side by side with brown ones, became, even after four months, brown when exposed to a very moist atmosphere. It is a matter of regret that the conditions under which the experiments were made, did not admit of constant observations with a wet and dry bulb thermometer.

But, although atmospheric moisture can change white silk to brown, there remains the possibility, that cocoon colour is normally affected by an excretion.

The larvae, just before beginning to spin, pass a large evacuation which consists of the ordinary frass in a drop of greenish liquid. When dry, this liquid becomes a deeper green. Observations were made five or six times a day, to ascertain whether the cocoons were wet or even damp, as if soaked by an excretion, but such were never found, the cocoons were invariably dry. The colour of the cocoons was frequently irregular, thus the body of the cocoon might be a medium brown, but the peduncle, by which it was attached to the branch or wire, might be almost colourless. This condition suggested that an excretion might have reached to the end of the cocoon, but did not extend as far as the peduncle. The constant dryness of the cocoons, however, appears to negative this possibility. The irregularity of the colour was possibly due to the looser arrangement of the silk on

the cocoon, which would allow it to be more quickly affected than the very closely woven silk of the peduncle.

Moisture may possibly affect cocoon colour in a number of Lepidoptera, but, so far, the only record which I have been able to discover, concerns the English moth, *Plusia moneta*. This species has both white and yellow cocoons and several observers state that the white cocoons become yellow, when damped. I have been unable to obtain any cocoons in order to confirm these observations.

Each fibre of silk consists of a core of fibroin, surrounded by a layer of sericin and that in its turn by a mucous layer. The silk fibres occur in pairs. The fibroin is composed of fibrils embedded in a structureless matrix. In order to demonstrate the chemical difference which presumably exists between the brown and the white fibres, they were stained as shown in the following table. No attempt was made to ascertain the nature of the chemical difference.

Until more is known concerning the chemistry of silk, and its reactions to environmental influences, it is impossible to disentangle its heredity.

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CONCLUSIONS

1. The larvae of the hybrid moths may begin to spin with a white or a fawn silk.

2. In all but two cases, cited in the text (pp. 00), the white or fawn cocoons became various shades of red-brown when placed in a very moist atmosphere.

3. The above change can take place even when the silk is removed from the cocoon and is due to the effect of water vapour and not to atmospheric oxygen.

4. No evidence has been collected to indicate that the colouration is due to any excretion from the larva, and there is some negative evidence suggesting that excretion has no influence at all.

5. Further research is needed to ascertain the chemical differences between brown and white silk in those species which produce both kinds. Further breeding is needed to discover the causes to which these variations are due.

REAGENT	WHITE SILK	BROWN SILK
Picrocarmine.	1. Stains rapidly: the fibroin and sericin staining the same colour but the former of a deeper shade. 2. When treated with strong ammonia for a day the picrocarmine does not stain the silk at once. When the stain does act, the mucous layer appears broken and swollen.	1. Did not stain even after an immersion of 18 hours. 2. (a) When treated with strong ammonia for one hour, the fibre did not take the stain but the sericin became very granular in appearance. (b) When treated with strong ammonia for 24 hours the sericin became reddish and the fibroin a deeper yellow. Some fibres did not stain at all.
Water.	No effect.	Did not dissolve out the colour.
Alcohol.	Transverse striations appear in the mucous layer which is gradually dissolved.	Reduces the colour a little by the end of half an hour but never removes it all.
G. Mann's methyl blue eosin.	After one week the fibre became entirely red.	After one week the fibre becomes red.
Water solution of Giemsa's methylene blue	The mucous and fibroin became wine red, the sericin became blue.	All the fibre becomes blue.
Bordeaux red and Heidenhain's iron-alum haematoxylin.	Transverse sections of this preparation were examined. The mucus was of a very deep red, the sericin had not stained, the fibroin appeared as red fibrils in a grayish matrix.	The fibres become red.
Eosin.	The mucus and fibrils are red, the remainder is unstained.	The fibres stain red rapidly.

LITERATURE CITED

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