EXPERIMENTS UPON THE EFFECT OF COLD APPLIED TO CHRYSALIDS OF BUTTERFLIES.

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1. In May 1878, I placed many chrysalids of *Papilio ujox*, from eggs laid by form *walshii*, in the ice box. The youngest were aged but 10 to 15 minutes after pupation and were still soft; others were added at intervals up to the age of 24 hours (the chrysalis is hard at about 12 hours), and others yet at 2 days, 3 days and so on to 8 days after pupation. All were removed from the box on the same day. The exposure had been from 19 to 5 days, those chrysalids which were put in latest generally having had the shortest exposure. I wished to determine, if possible, whether, in order to effect any change, it was necessary that cold should be applied immediately after pupation, or if one or several days might intervene between pupation and icing. Inasmuch as no color begins to show itself in the pupa till a few hours, or at most a day or two, before the butterfly emerges, I thought it possible that cold applied shortly before that time might be quite as effective as if applied earlier, and particularly very soon after pupation. The result was that more than half the youngest and immature chrysalids died; one which had been exposed at 10 minutes after pupation, two at 1 hour, one at 2 hours, two at 3 hours. On the other hand, one exposed at 15 minutes, one at 2 hours, and one at 12 hours, produced butterflies. The temperature was from 32° to 34° F. [0° to 1° C.] most of the time, rising somewhat daily as the ice melted. The normal chrysalis period in this species is from 11 to 14 days, in case the butterfly emerges the same season, but now and then a single butterfly will emerge several weeks after pupation. The latter is an uncommon occurrence, however, as usually the chrysalids which do not give imagos within 14 days retain them till the following spring. The form which would naturally emerge the first season from these chrysalids is *marcellus*, but all the overwintering chrysalids would produce *telamonides or walshii*, which latter are the winter forms of the species. If then, from the chrysalids subjected to cold, the winter form should emerge the same season, it would be owing to the exposure to cold.

On the 14th day after taking the chrysalids from the ice, one *telamonides* emerged, from a chrysalis placed in the ice box 3 days after pupation, and kept there 16 days.
On the 19th day, emerged one *telamonides*, or a form between that and *walshii*, from a chrysalis put in the box 12 hours after pupation and kept there 11 days. On the 19th day emerged one *walshii*, from chrysalis 2 hours old, and on ice 11 days. All the rest emerged unchanged *marcellus*, but at periods prolonged in a surprising way:

One on 43d day, exposed at 15 minutes.
One on 46th day, exposed at 2 hours.
One on 53d day, exposed at 24 hours.
One on 62d day, exposed at 6 days.
One on 63d day, exposed at 4 days.
One on 66th day, exposed at 7 days.
One on 77th day, exposed at 4 days.
One on 81st day, exposed at 12 hours.
One on 91st day, exposed at 5 days.
One on 96th day, exposed at 19 hours.

Five chrysalids lived over until the spring of 1879, when all produced *telamonides*.

2. In June 1879, I obtained eggs of the form *marcellus*, and in due time had from them 104 chrysalids. I placed about one third, or 35, in the ice box, at from 12 to 24 hours after pupation, dividing them into three lots: 1st, 9 chrysalids, kept on ice 14 days; 2nd, 12 chrysalids, 20 days; 3rd, 11 chrysalids, 25 days. Temperature and conditions the same as in experiment No. 1.

Of the 69 chrysalids not exposed to cold, 34 gave butterflies at from 11 to 14 days after pupation, and one additional example emerged 11 Aug., or at least 22 days past the regular period. I reserved these chrysalids that I might be able to compare the butterflies from these with the butterflies from the same lot of eggs, but whose chrysalids were iced.

Of the chrysalids on ice, from lot No. 1, emerged 4 ♀ at 8½ to 9½ days after removal from the ice, and 5 are now living and will pass the winter. From lot No. 2, emerged 1 ♂ 5 ♀, at 8 to 9 days; another ♀ came out at 40 days, and 5 go over the winter. From lot No. 3, emerged 4 ♀, at 9 to 12 days; another ♀ at 54 days, and 6 were found to be dead.

In this experiment, I wished to see, as exactly as possible: 1st, in what points changes would occur; 2d, if there would be any change in the shape of the wings, as well as in markings and coloration,—that is, whether the shape might remain that of *marcellus*, while the markings might be of *telamonides* or *walshii*, or a summer form with winter markings—(in previous experiments I had not noted this point so carefully as I wished now to do); 3d, to ascertain more closely than I had yet done what length of exposure to cold was required to bring about a decided change, and what would be the effect of prolonging that period. After my experiments with *Phyciodes tharos*, as shown in Butterflies of N. A., v. 2, and which had resulted in a suffusion of color, I hoped that I might see a similar change in *Papilio ajae*, brought about either by the low temperature or prolonged exposure.

I obtained from these chrysalids 11 perfect butterflies, 1 ♂ 10 ♀. Some others emerged crippled, and these I rejected, as it was not possible to make out their markings satisfactorily. From lot No. 1, 14 days: 1 ♀ between *marcellus* and *telamonides*, 2 ♀ *marcellus*. These latter were pale colored, the light parts a dirty white, the submarginal lunules on hind wing only two in number and small, at anal angle one large and one small red spot, and the frontal hairs very short.
The black of the first, or intermediate, female was also pale, but the light parts were more green and less sordid; there were three large lunules, the anal red spot was double and connected as in telamonides, the frontal hairs were short as in marcellus. I find these the most salient points for comparing the several forms of P. ajax. In nature there is much difference in shape also between marcellus and telamonides, still more between marcellus and walshii, and the latter may be distinguished readily from telamonides by the white tips of the black tails. In telamonides the white edges both sides of the tail. Walshii is smaller and the anal spot is larger and edged with white on upper side, and the frontal hairs are long and brush-like and black. In marcellus they are very short and with much yellow; in telamonides they are of a length between the other two, black with yellow next the eyes.

From lot No. 2, 20 days, came: 1 ♀ marcellus, with single red spot; 1 ♀ between marcellus and telamonides, the colors pale, the lunules all obsolescent, two large red spots but not connected, frontal hairs of medium length, as in telamonides; 1 ♀ between marcellus and telamonides, color not pale, but black and green, three lunules, two large red spots, frontal hairs short; 1 ♀ telamonides, colors black and green, four lunules, a large double and connected red spot, frontal hairs medium; 2 ♀ telamonides, colors like last, three and four lunules, two large red spots, not connected, frontal hairs medium.

From lot No 3, 25 days: 1 ♀ telamonides, clear colors, four large lunules, one large and one small red spot, frontal hairs long; 1 ♀ telamonides, of medium color, four lunules, large double and connected red spot, frontal hairs long.

In general shape all are marcellus, the wings produced, the tails long.

By which it appears that those exposed 25 days were fully changed; of those exposed 20 days, three were fully, two partly changed, and one not at all. Of those exposed 14 days, one partly, two not at all.

The butterflies from this lot of 104 chrysalids, but which were not subjected to cold, were put in papers as they emerged. Taking 6 ♂ 6 ♀ from the papers, as they came to hand, I spread them and compared with the iced examples.

Of the 6 ♂, 4 have one red anal spot only, 2 have one large and one small; 4 have two green submarginal lunules on hind wing, 2 have three, and these latter have a fourth, which is obsolescent, at outer angle; all have short frontal hairs.

Of the 6 ♀, 5 have but one red spot, 1 has one large and one small spot; 5 have two lunules only, 1 has three; all have short frontal hairs.

Comparing 6 of the females from the iced chrysalids, being those in which a decided change occurred, with the 6 females not iced:—

1. All the former have the colors more intense, the black blacker, the light, green.

2. In 5 of the former the green lunules on hind wings are decidedly larger; 3 of the 6 have four distinct lunules, 1 has three, 1 has three, and a fourth, at outer angle, obsolescent.

Of the 6 not iced, none have four, 2 have two, and a third, being the lowest of the row, obsolescent, 3 have three, the lowest being very small, 1 has three and a fourth, at outer angle, obsolescent.
3. In all the former the subapical spot on fore wing and the stripe on same wing which crosses cell inside the common black band, are distinct and green; in all the latter these marks are either obscure or obsolete.

4. In 4 of the former there is a large double and connected red spot at anal angle, and in 1 of them it is edged on its upper side by white; 2 have one large and one small red spot. Of the latter, 5 have one spot only, and the 6th has one spot and a red dot.

5. The former have all the black portions of the wing of deeper color but of less extent—the bands being narrower; on the other hand, the green bands are wider as well as deeper and clearer colored. Measuring the width of the outermost common green band along middle of upper median interspace on fore wing in tenths of a millimetre, I find it as follows:—

- On iced chrysalids, 81, 66, 76, 76, 66, 66.
- On not iced, 56, 56, 51, 51, 46, 51.

Measuring the common black discal band along middle of lower median interspace on same wing:—

- On the not iced, 76, 71, 66, 63, 71, 76.

In other words, the natural examples are more melanic than the others.

I find no difference in length of tails or in the length and breadth of the wings. The cold has produced no effect in the shape of the wings.

Comparing 1 male from the iced chrysalids with the 6 males not iced:—

1. The former is bright colored; 2 of the latter are the same, 4 have the black pale, the light pale and sordid.

2. The former has four lunules; 3 of the latter have three, 3 have two only.

3. The former has a large double connected red anal spot, edged with white; 3 of the latter have but one red spot, 2 have one large and one small spot, 1 has one large red spot and a red dot.

4. The former has the subapical spot and stripe in cell clear green; 1 of the latter has the same, 5 have these obscure or obsolete.

Looking over all, of both sexes, in both lots, the iced and not iced, the largeness of the green submarginal lunules on fore wings in the iced examples is conspicuous as compared with all the others,—though this feature is included in the general widening of the green bands spoken of.

In all my experiments at any time made with _P. ajax_, if any change at all has been produced by cold, it is seen in the enlarging or doubling of the red anal spot, and in the increased number of clear green lunules on hind wings. Almost always the frontal hairs are lengthened, the color of the wings deepened. So also the extent of the black area is diminished. All these changes are in the direction of _telamoniodes_, or the winter form.

That the effect of cold is not simply to precipitate the emerging of the winter form, making the butterfly which would naturally leave its chrysalis in the succeeding spring to emerge in the season in which it fed as a caterpillar, is evident from the fact that the shape is always that of the summer form, while the markings are of the winter form. Those chrysalids which go over the winter, on the other hand, do not have the summer form, but the winter, and the markings agreeing thereto, just as in examples in nature.

On these the cold has produced no effect whatever. (To be continued on p. 15.)