Environmental Factors Compensating for Chemical Conditions during Larval Development of the Silkworm *Bombyx mori*.

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The nutritional condition for the 1st instar larvae of silkworm *Bombyx mori* was found to be critical for the regulation of the entire length of larval development. This regulation could also be achieved by some physical environmental factors experienced during the embryonic stage. The treatment of embryos with low temperature was effective in promoting the delayed larval development under the poor nutritional condition. The interrelationship between these chemical and physical environmental factors may suggest a compensatory mechanism to achieve the proper larval development in this insect.

Recently, the poor nutritional diet for the silkworm *Bombyx mori* has been shown to prolong the 1st instar period for more than 5 days and to produce the extra 5th larval ecdysis.\(^1,3\) However, the development of insects could be influenced by many environmental factors, some of which possibly facilitate and others interfere with the prolongation effects of the poor diet on larval development.

The present communication concerns the physical environmental factors which, supplemented at a given stage of a life cycle, could compensate for the chemical (nutritional) deficiency and facilitate the larval development of the affected insects.

**MATERIALS AND METHODS**

The experiments were carried out with nondiapaused hybridized larvae of the cross races between *Gunko* and *Manri*, bivoltine races of the silkworm *Bombyx mori*. The composition of the artificial diet (MO) which did not contain any mulberry leaf powder or its extract was according to our previous report.\(^3\) The Takeda commercial food (MLP), which contained a high proportion of mulberry leaf powder, was used as the control diet. The eggs were incubated at DD-18°C or DD-15°C (DD=constant dark). They were sterilized by the usual alcohol-formaline method before hatching. The larvae were reared with autoclaved food under the aseptic conditions of DL 8:16–25°C using a special facilities of gnotobiotron.

**RESULTS AND DISCUSSION**

Under the control diet condition one generation of silkworm usually takes 56 days. This includes 18 days for embryonic period at DD-18°C, 23 days for larval
Fig. 1 Length of larval period from hatching (i) to mounting stage to spin cocoon (i).
A: reared with MLP commercial prepared food ( ), B: reared with artificial MO diet ( ), C: reared with MLP commercial prepared food only for first instar of larval development.

Fig. 2 Homeostatic regulation between embryonic stage and larval stage.
A: OL-18°C (DD-18°C) for embryonic stage and MLP commercial prepared food ( ) for larvae; B: OL-18°C for embryonic stage and MO diet ( ) for larvae; C: OL-15°C for embryonic stage and MO diet for larvae, 16 L:25°C, (DL 8:16), (i) hatching, (i) mounting stage to spin cocoon.
Number in the square indicates days and total days in the parentheses.

advanced the entire larval development. This indicates that certain chemicals, contained in the MLP diet, may be involved in the developmental facilitation during the larval stage. Our preliminary data suggest that the chloroform extract from the MLP is effective in advancing the development of the larvae fed on MO diet.

In addition to the nutritional (chemical) effects, Morohoshi et al. suggested that there might be some humoral effects which affect the length of larval growth periods by three successive regulatory mechanisms: (1) the physical factors such as length of day time and the temperature first affecting the physiological state of the embryo, (2) subsequently, a regulatory effect on the state of humoral interaction between juvenile hormone and ecdysone, and (3) finally, the humoral interaction regulates the length of larval growth periods. In fact, the treatment period at DL 8:16-25°C and 15 days for pupal period at DL 8:16-25°C. The maintenance of the larvae on MO artificial diet significantly prolonged the larval periods from 23 days to 34 days (Figs. 1A, B). This effect, however, was completely removed by introducing the control diet during the 1st larval instar (Fig. 1C). This indicates that the diet condition of the 1st larval instar period is very critical for the regulation of subsequent periods of larval development.

The prolongation effect of MO diet on larval periods was also diminished by exposure of the embryonic periods to low temperature (Fig. 2B and C). This is likely to be due to some kind of homeostatic regulatory mechanism which prolonged the periods of embryonic stage and at the same time shortened the larval developmental periods.

It has been hypothesized that physiological state of the later development of animals was already predetermined by the environmental conditions, which has been given at the earliest developmental stages. In fact, the nutritional (chemical) condition for the 1st instar larvae was previously shown to influence the physiological state of matured larvae. The proper MLP diet, given at the 1st instar period, clearly

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of silkworm embryo with low temperature caused the prolongation of the embryonic periods and, more importantly, reduced the length of the larval stage, during which the larvae were constantly maintained on the MO diet. This may indicate that the larval growth depends not only on the chemical factors originated in the mulberry leaves, but on some flexible regulatory mechanism which might have been built up within the larval body by certain physical conditions experienced during the preceding embryonic development. The latter mechanism could be activated to enhance the larval development utilizing the special biosynthetic precursors derived from the contents of the MO diet.

Thus it may be speculated that the physical and chemical environmental factors which, when rendered available at a critical stage of the insect life cycle, will produce a compensatory homeostatic balance with regard to the proper length of the larval stage. The more detailed analysis of the compensatory mechanisms may require: (1) the extraction from the mulberry leaves of physiologically active substances which will facilitate the larval growth, and (2) the biochemical characterization of those substances actually synthesized by the MO-treated larvae which had been pre-exposed to the special physical conditions, such as low temperature throughout the embryonic development.

REFERENCES

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<和文抄録>

カイコの幼虫発育の化学的条件を代償する環境因子

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昆虫の幼虫発育期間の長短は、食べものの質と量によって大きく影響を受ける。いま、群光・万里というカイコ2化性品種の交雑種を18℃・暗において飼育し、孵化した幼虫をノートバイオトロンの中
で無菌的に、DL 8:16、25°C のもとで、桑粉末の混入した人工飼料を用いて飼育すると、最初の個体がマユをつくりやすまで 23 日を要する。しかし、桑粉末を全く混入しないと、34 日を必要とすることがわかった。しかるに、ふ化後の 1 令期である約 5 日間のみを桑粉末の混入した人工飼料、2 令から全く桑粉末の混入しない人工飼料を用いても、最初のマユつくりが同時に 24 日に短縮され、全幼虫期間の長さが、わずか最初の 1 令期の栄養条件によって支配されていることが判明した。

また、全令に桑粉末の混入しない人工飼料を用いても、その胚期が 15°C・暗という低温環境を経験しておれば、胚期が約 2 週間延長するかわりに、その幼虫期間はホメオスタティックに 24 日に短縮されることも判明した。

以上の事実は、幼虫発育が単に桑粉末中の化学物質に依存しているという点だけではなく、物理的環境因子による、胚期に受けた生理的変動によって、桑粉末の混入しない人工飼料も発育を促進する化学物質として利用できるようにする応応性に富んだ調節メカニズムにも依存することを示している。このような相互依存性のあるホメオスタティックなメカニズムを解明するためには、蚕からの発育促進物質の抽出・単離、また胚を低温におくことによって、その幼虫体に発現するともわれる桑粉末の混入しない人工飼料に由来する有効成分の特徴が明らかにされる必要がある。