15. The Control of Growth and Development in *Bombyx mori*. XXV

Induction of Supernumerary Larval Ecdysis and Non-Diapause Eggs by Injection of the Juvenile Hormone

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Recently the effect of the synthetic juvenile hormone on morphogenesis of insects has attracted attention of many investigators (Akai and Kobayashi, 1971; Kenneth and Gilbert, 1970; Ohtaki, Kiguchi, Akai and Mori, 1972; Srivastava and Gilbert, 1969). In *Bombyx mori*, Akai, Kiguchi and Mori (1973) produced dauer larvae or extra ecdysed larvae by injecting the synthetic juvenile hormone (JH) into the 5th instar larvae. Morohoshi, Ishida and Fugo (1972) succeeded in the induction of non-diapause egg batches following injection of the JH into "diapause larvae" during the 5th instar. This paper reports further data of the related subjects with some remarks on voltinism.

Materials and methods. The race used was a tetramolting and bivoltine hybrid between Japanese No. 106 and Daizo. Eggs of this race were incubated at 17°C in the dark (LD series) or at 25°C in the light (HL series). The former produced only non-diapause egg batches while only diapause egg batches from the latter. The JH of 1 γ to 50 γ dissolved in olive oil were injected into 30 larvae at each assay 0.004 ml per animal every day during the 4th or 5th instar. Larvae received injection of olive oil provided control material. The JH used consisted of two types of methylene-dioxyphenyl derivatives (M.D.D. No. 1 and No. 2: see Report XVII).

Results. I. Induction of supernumerary larval ecdysis. (1) *Effect of the JH (30 γ) on the subsequent larval development in the 5th larval instar.* The injection of the JH in the 0-, 24-, 48-hr-old 5th instar larvae lengthened the subsequent larval development as compared with the control specimens. Especially, the 48-hr-old 5th instar larvae which received the JH of 30 γ remained as dauer larvae without spinning. Larvae received injection in the 72-, 96-, 120-hr-old 5th instar somewhat lengthened the subsequent larval development, and all of the larvae received injection in the 72- and the 96-hr-

old 5th instar had one more extra molting, though they died in the 6th larval stage without eating mulberry leaves owing to their intermediate form. (2) *Effect of the JH on the subsequent larval development in the 4th larval instar.* The production of extra ecdysed larvae increased more in larvae received injection in the 4th instar than in those received injection in the 5th instar. The extra ecdysed larvae derived from the JH-injection in the 4th instar pupated and emerged through the 6th larval instar, whereas those received the JH-injection in the 5th instar died in the 6th larval instar without eating mulberry leaves. The numbers of the extra ecdysed larvae derived from the JH-injection of 5 γ , 10 γ , 30 γ and 50 γ were about 5 to 70% of the treated ones. As a rule, the more the dose is administered, the higher becomes the frequency of extra ecdysed larvae. The extra ecdysed larvae from the 4th instar injection group somewhat quickly entered into the 4th molting as compared with the controls. These larvae showed lighter coloration of larval markings, though they recovered from the lighter coloration after the 5th ecdysis.

II. Effect of the JH on voltinism. In Report XVIII was reported that when the larvae destined to produce diapause eggs of the HL series were injected the JH (M. D. D. No. 1) of 10 γ in the 5th instar, about 50% of them transformed into non-diapause egg batches. Larval and pupal duration was prolonged, and cocoon and cocoon shell weight increased than those of the controls. In the previous experiment no change of voltinism was observed in injection of the M. D. D. No. 2, while some non-diapause egg batches were produced following injection of the M. D. D. No. 2 into the 5th instar larvae of the HL series in the present experiment.

In order to produce diapause egg batches from "non-diapause insects" by prolongation of the 5th larval duration, the injection of the JH of 10 γ in the 4th or 5th instar larvae of the LD series was carried out four times, but resulted in no change of voltinism in the experimental series as in the controls. When the JH of 1 γ was injected into the 4th instar larvae developed from the eggs which were incubated at 19°C in the dark, however, they produced a few diapause egg batches (3/193) in addition to a great majority of non-diapause egg batches in comparison with non-diapause egg control batches. The individuals received the JH-injection of 10 γ showed longer larval and pupal duration, more weight in cocoon and cocoon shell, as seen in the HL experimental series (Report XVIII). No change of voltinism occurred in the LD and HL series by the injection of ecdysterone at the 5th larval and the pupal stages.

Discussion. The extra ecdysed larvae derived from the injection of the JH in the 5th instar larvae died during the 6th instar without eating mulberry leaves. When the JH-injection was made in the 4th instar larvae, the resultant extra ecdysed larvae ate mulberry leaves, pupated and emerged through the 6th instar. In the 5th instar the extra ecdysed larvae was produced by the injection at the later half stage, while in the 4th instar injection series, they appeared in the 0-hr-old 4th instar larvae. Lighter coloration of larval markings was described by Kiguchi (1972) in larvae received injection in the later stage of the 4th larval instar, while it appeared in larvae injected in the earliest stage.

Morohoshi, Ishida and Fugo (1972) reported that the injection of the synthetic juvenile hormone induced the production of non-diapause egg batches. The present experiments showed the similar results. It seems then that when the function of the CA in the final instar larvae is extremely enhanced by injection of the JH, the change of voltinism occurs from diapause to non-diapause direction. This seems to be due to a rejuvenation of ovaries. From the results of our transplanting or extirpating experiments (Report XVI), the CA hormone in the final larval or pupal stage seems to be related to the determination of voltinism, although its function is weaker than that of the SG during the pupal stage. When voltinism is unstable as in bivoltine strains, however, voltinism seems to be much controlled by quantity of the CA hormone. The SG hormone is considered as a diapause-producing one and the CA hormone as a non-diapause-producing. The following conditional relations are particularly noted in the determination of voltinism:

(1) *Relationship between the duration of the final larval instar and voltinism.* The duration of the final larval instar in multivoltine strains is shorter than in univoltine strains, while in bivoltine strains it is intermediate (Report I). "The non-diapause larvae" which developed from low temperature incubation in bivoltine strains display shorter development in the final larval instar than "the diapause larvae" which developed from high temperature incubation (Report XXII). (2) *Relationship between the duration of the final larval instar and the activity of the CA.* The duration of the final larval instar is under control of the inactivity of the CA (Report XXI and XXII). The weaker the activity of the CA, the shorter becomes the duration of the final larval instar. (3) *Relationship between the inactivity of the CA in the final instar larvae and voltinism.* From the above mentioned features, it appears that the weaker the activity of the CA in the final instar larvae, the greater becomes the percentage

of non-diapause egg batches. (4) *Relationship between the activity of the SG during the pupal stage and voltinism*. The stronger the activity of the SG during the pupal stage, the greater becomes the percentage of diapause egg batches (Fukuda, 1951; Hasegawa, 1952; Morohoshi *et al.* Report X, XVI and XXII).

In the light of the above findings, it seems very probable that voltinism can be determined by both the activity of the CA and that of the SG in the pupal stage as follows:

	Activity of the CA	Activity of the SG
Non-diapause	Weaker	Weaker
Diapause	Stronger	Stronger

Generally, non-diapause egg batches in tetramolting strains usually occur in groups of shorter development or lighter cocoon of the same batches. In tri- or pentamolting strains, however, an abnormal voltinism is sometimes observed (see Fig. 1). For example, the trimolters which appeared from multivoltine Cambodge (c), contrary to the trimolters which appeared from uni- or bivoltine strains, showed a higher percentage (46.87) of diapause egg batches than that (16.46) of the tetramolters of the same batches. The pentamolters of the 5 m C showed a higher percentage (39.08) of non-diapause egg batches than that (27.48) of the tetramolters which segregated from the same batches.

Fig. 1. Relationship between the duration of larval development and voltinism.

In tetramolting strains or the trimolters which segregated from uni- or bivoltine and tetramolting strains, the shorter the larval and pupal development, the more occurs the production of non-diapause egg batches (see II in Fig. 1). When the larval development is accelerated until a critical limit (c_1) like the trimolters which segregated from multivoltine Cambodge, however, the trimolters produce more diapause egg batches than the tetramolters of the same batches. On the other hand, when the larval development is delayed until a critical limit (c_2) like the pentamolting (5 m C), the pentamolters produce more non-diapause egg batches than the tetramolters which segregated from the same batches (see III in Fig. 1). Such relational features can not be understood only by considering the activity of the

SG. The activity of the CA during development is important for the understanding. The activity of the CA in the final instar larvae must be stronger in the trimolters (c) and weaker in the pentamolters (5 m C). Since the trimolters segregating from multivoltine Cam-bodge are too short to develop, the activity of the CA of the trimolters in the final larval instar may be stronger.

The CA hormone inhibited the incorporation of ^{14}C -leucine into pupal ovary proteins, while the SG hormone promoted the incorporation of ^{14}C -glucose into pupal ovary glycogens and lipids (refer to Report VII, XI and XII). The radioactivity of ^{14}C -leucine per mg ovary weight in pupal ovary proteins decreased gradually with pupal age and the radioactivity of ^{14}C -glucose per mg ovary weight in pupal ovary glycogens and lipids showed a gradual increase with pupal age (see Fig. 2). The degree of the incorporation of ^{14}C -leucine into pupal ovary proteins was higher in the LD series (non-diapause) than in the HL (diapause), and also in the allatectomized pupae than in the control ones. On the other hand, the degree of the incorporation of ^{14}C -glucose into pupal ovary glycogens and lipids was higher in the HL series than in the LD, and also in the SG-transplantation pupae than in the control ones. It seems therefore that diapausing eggs contain less protein (N) and more glycogen and lipid (C) in any pupal period than non-diapausing eggs (see Fig. 2).

Fig. 2. Diagram illustrating the rate of incorporation of radio-active substances per mg dried ovary weight during pupal stage. Ordinate: the rate of ^{14}C -leucine or -glucose incorporated into pupal ovary proteins or glycogens and lipids. Abscissa: days in pupal stage. D: "diapause pupae", ND: "non-diapause pupae", C: glycogen and lipid, N: protein.

In mass-analysis experiments in silkworm eggs, Bito (1936) reported that non-diapause and diapause eggs which were produced from a bivoltine race under different conditions during incubation, showed percentages of dried egg weight as follows: 58.29:53.38 in protein, 8.51:10.63 in glycogen and 22.01:26.64 in lipid. When the inherent activity of the CA is stronger since embryonic development

Fig. 3. Two diagrams showing relative quantity of CA and SG during life cycle in various strains. Br: brain, CA: corpus allatum, SG: subesophageal ganglion, N: protein, C: glycogen and lipid, 1,2,3,4,5,6: instar.

Lm^e , Lm : sex-linked early and late maturing genes. M^3 , $+M$, M^5 : major genes controlling tri-, tetra- and pentamolting characteristics. V^1 , $+V$, V^3 : major genes controlling uni-, bi- and multivoltine characteristics.

like trimolting, non-diapause egg batches are usually produced more in trimolting insects (M^3) than in tetramolting ($+M$), and more in tetramolting insects than in pentamolting ($5MA$) (M^5) (see Fig. 3). And also when the brain strongly stimulates the CA throughout nerve commissures in the earlier larval stage, the secretion of the CA becomes weaker in the later larval stage, resulting in the formation of more non-diapause egg batches. For example, the insects which had the Lm^e gene produce more non-diapause egg batches than the insects which had the Lm gene (see Fig. 3).

References

- Akai, H., and Kobayashi, M. (1971): Appl. Ent. Zool., **6**, 138-139.
 Akai, H., Kiguchi, K., and Mori, K. (1973): Bull. Sericul. Exp. Sta., **25**, 287-305.
 Bito, S. (1936): Rep. Ibaraki Sericult. Sta., **7**, 1-16.
 Fukuda, S. (1951): Proc. Japan Acad., **27**, 672-677.
 Hasegawa, K. (1952): Jour. Fac. Agri. Tottori Univ., **1**, 83-124.
 Kenneth, J. J., and Gilbert, L. I. (1970): J. Morph., **131**, 301-314.
 Kiguchi, K. (1972): J. Sericult. Sci. Japan, **41**, 407-412.
 Morohoshi, S., and etc. (1971-1974): Series of Proc. Japan Acad., I (1969), VII (1971), X (1971), XI (1972), XII (1972), XVI (1972), XVII (1972), XVIII (1972), XXI (1974), XII (1974).
 Ohtaki, T., Kiguchi, K., Akai, H., and Mori, K. (1972): Appl. Ent. Zool., **7**, 161-167.
 Srivastava, U. S., and Gilbert, L. I. (1969): J. Insect Physiol., **15**, 177-189.