In Vitro Culture of the Dechorionated Eggs in the Silkworm, *Bombyx mori* Linné: Development of the Embryos Recovered from the Culture Media

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Dechorionated insect eggs or even isolated embryos can develop into advanced stages under *in vitro* culture (Takami, 1958; Ohtsuki and Kitazawa, 1974; Koch, 1964; Counce, 1966; Krause and Krause, 1972; Lenoir-Rousseaux and Lender, 1971; Shankland *et al.*, 1982). However there has been no report that eggs cultured *in vitro* can complete embryonic and subsequent post-embryonic development. We report here that we have obtained adult moths from silkworm embryos cultured *in vitro* at a comparatively high frequency.

Eggs of the silkworm, *Bombyx mori*, (bivoltine hybrid race, Daizo×N124) were chilled to terminate diapause. Then at the elongated germ band stage before appendage formation, the eggs were mechanically dechorionated in Grace's insect tissue culture medium (pH 6.1) (Grace, 1962), with a care of avoiding injury to the serosal membrane. The dechorionated eggs were then cultured in hanging drops at 25°C basically according to the method of Takami *et al.* (1966).

In this *in vitro* system, develoment proceeded faster than in intact eggs with most of the embryos beginning to swallow the serosa under the yolk on the 6th day, one day earlier than the control. When they were left in the medium, they survived more than 2 weeks. To obtain normal development to a feeding first instar larva, the following protocol was devised: About 12 hours after they had finished swallowing serosa, the embryos were rinsed twice in the sterilized distilled water, then transferred to a petri dish in which they were placed on a fine gauze patch with a small amount of glass wool underneath. A few drops of water were added to the glass wool to keep moderate moisture of 70–80% relative humidity. Twenty-four hours later, they were fed mulberry leaves.

As shown in Table 1, 109 well developed embryos out of 136 eggs cultured *in vitro* were recovered, and 89% began to feed on the mulberry leaves. A few individuals died without entering 2nd instar. However, most of the remainder grew well and metamorphosed into pupae and finally into moths. The duration of growth and the final size attained were similar to the controls (Table 1). Moreover, the adults mated normally, and laid almost the same number of fertilized eggs as normel females do (Table 1). These eggs were diapause eggs as expected from this bivoltine race being cultured *in vitro* at 25°C.

Table 1 Development and characteristics of the individuals derived from eggs cultured *in vitro*.

		Cultured	Control
No. of eggs cultured *in vitro*		136	—
No. of embryos recovered from the culture media*		109	—
No. of individuals which began to eat diet		97	31
No. of individuals ecdysed into the 5th instar		78	31
No. of individuals ecdysed into a pupa		73	31
No. of individuals ecdysed into a moth		66	31
Developmental duration (day) (from feeding to adult emergence)		31.4±2.3 (n=66)	30.0±1.2 (n=31)
Cocoon weight (g)	Male	1.55±0.13 (n=25)	1.71±0.07 (n=12)
	Female	2.08±0.25 (n=46)	2.28±0.13 (n=19)
Pupal body weight (g)	Male	1.27±0.14 (n=25)	1.38±0.05 (n=12)
	Female	1.80±0.23 (n=46)	1.91±0.11 (n=19)
No. of eggs laid		739±62 (n=40)	777±30 (n=18)

* The embryos which completed serosa swallowing and dorsal closure were recovered from the culture media.

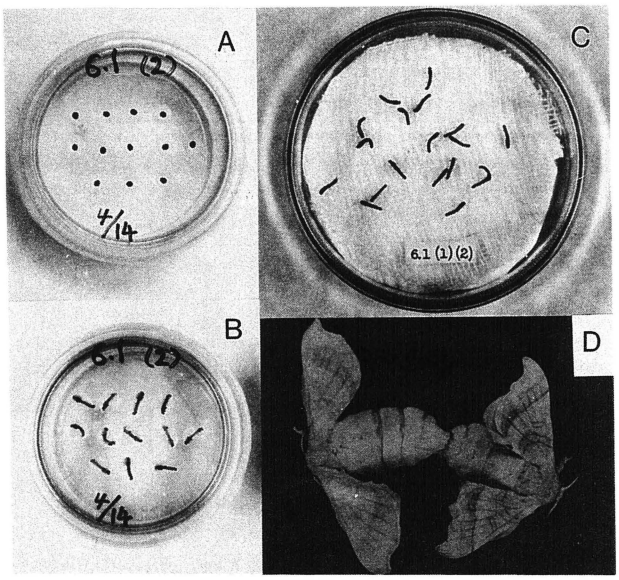

Fig. 1 Pictures showing the completion of embryogenesis *in vitro* and subsequent post-embryonic development. A. Just after setting up the hanging drop culture. B. Six days after of culture. The embryos have finished swallowing serosa and yolk. C. The embryos rescued from the culture media onto a fine gauze patch in a petri dish. D. Mating moths developed from the cultured embryos.

The embryos rescued from the culture media appeared to be fairly pale until the first larval ecdysis. Thereafter they were similar in colour to the controls. Morphology of the resultant 5th instar larvae, pupae and adults was more carefully examined under the dissecting microscope. There are no noticeable defects in their external and internal characteristics, except for one difference in their cuticlar markings. Namely the semilunar larval markings on the 2nd abdominal segment proved to be split fore and back at the middle part of the markings. Although there were slight variations, such abnormal pigmentation pattern was observed in all individuals developed from *in vitro* cultured embryos, but never in the controls. The disruption of the pattern formation was apparently caused by *in vitro* culturing.

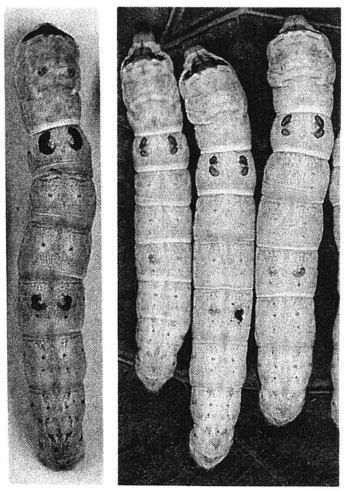

Fig. 2 Pictures of the 5th instar larvae developed from the cultured embryos. The control larva was shown in the left. Note that the markings were split fore and back at the center of the segment.

Why does the marking separation occur at the central (therefore spiracle) level of the segment? Dorsal closure seemed not to be concerned with, because it occurred on the 2nd thoracic segment, but not on the 2nd abdominal segment. Possibly this separation results from incomplete fusion of embryonic segmentation boundaries. In *Drosophila melanogaster* one of the first steps in segmentation of the embryo is the formation of parasegments which comprise the posterior half of one segment and the anterior half of the next segment (Martinez-Arias and Laurence, 1985; Scott and Carroll, 1987). Although it is uncertain whether the marking separation observed in this experiment is related to parasegment formation, it must be a clue for better understanding of the embryonic segmentation in a lepidopteran insect *Bombyx mori*.

This completion of embryogenesis *in vitro* and subsequent larval, pupal and adult development is the first case reported in insects. We believe that such culture system will be extremely usefull for embryonic and endocrinological studies, especially for the study on the mutual relationship between embryonic and post-embryonic developments.

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