SHORT NOTE

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Hatching ability of *Bombyx mori* (Lepidoptera: Bombycidae) eggs micro-injected with different concentrations of DNA at selected embryonic stages

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***Bombyx mori*, microinjection, DNA concentration, embryonic stage, hatching ability**

Abstract. The hatching ability of *Bombyx mori* eggs subjected to microinjection of about 30 nl plasmid DNA (10 µg/ml) at 1–30 h of embryonic development and eggs injected at 8 h with DNA concentrations (0.5 to 500 µg/ml), averaged to about 22%. In respect to hatching ability, the optimum stage for microinjection is the preblastoderm (8–10 h old eggs) and the DNA concentration up to 10 µg/ml.

Introduction

The technique of microinjection has been used widely to produce transgenic individuals (Rubin & Spradling, 1982; McMahon et al., 1985; Miller et al., 1987). In eggs with soft chorion, DNA may be injected directly into the cytoplasm (McMahon et al., 1985), whilst eggs with a hard chorion need to be dechorionated (Rubin & Spradling, 1982) or drilled manually before microinjection (Chorrout et al., 1986). Dechorionation was successful in the eggs with sufficient perivitelline space (Rubin & Spradling, 1982) but, in those with a rigid and non removable chorion, dechorionation resulted in mortality of all the eggs (Ninaki et al., 1985; Morris et al., 1989).

Eggs of *Bombyx mori* (Lepidoptera: Bombycidae) have hard and non-removable chorion. Using an automatic injector, Ninaki et al. (1985) microinjected into *B. mori* eggs, and determined the hatching ability. Tamura et al. (1990) improved the efficiency of injection and studied the transient expression of the injected gene. Coulon-Bublex et al. (1993) and Joy & Gopinathan (1994) used manual microinjection and ascertained transient expression of the injected genes. However none of these studies, examined the dependence of egg hatching ability on the amount of injected DNA and this is examined in the current study.

Material and methods

Freshly emerged silk moths (Pure Mysore race) were allowed to mate and to lay eggs on a transparent cellophane paper. Eggs were stored at 10°C untill the time of injection. The surface of the eggs were sterilised with gentamycin (300 units/ml commercial grade) in order to prevent infection in the microinjected eggs during incubation. Eggs were microinjected following a simple manual method described by Shamila & Mathavan (1996). Each egg received about 30 nl solution of supercoiled plasmid DNA (pBm FRT). Control eggs were injected with TE buffer (10 mM Tris- HCl pH 8.0 and 1mM EDTA). Eggs collected 1, 2, 3, 6, 8, 10, 12, 15, 20, 25, and 30 h after egg laying were microinjected with DNA concentration of 10 µg/ml. In another set of experiments preblastoderm stage eggs (8 h old) were injected with DNA concentrations of 0.5, 1.0, 1.5, 2.5, 5, 10, 75, 125, 250, and 500 µg/ml. The microinjected eggs were kept at 10°C for a few minutes prior to incubation at 27°C.

Results and discussion

Larvae emerged successfully from some microinjected eggs, whilst the rest died. The death of fully developed larvae that attained head pigmentation was recorded as "eggs dead after head pigmentation".

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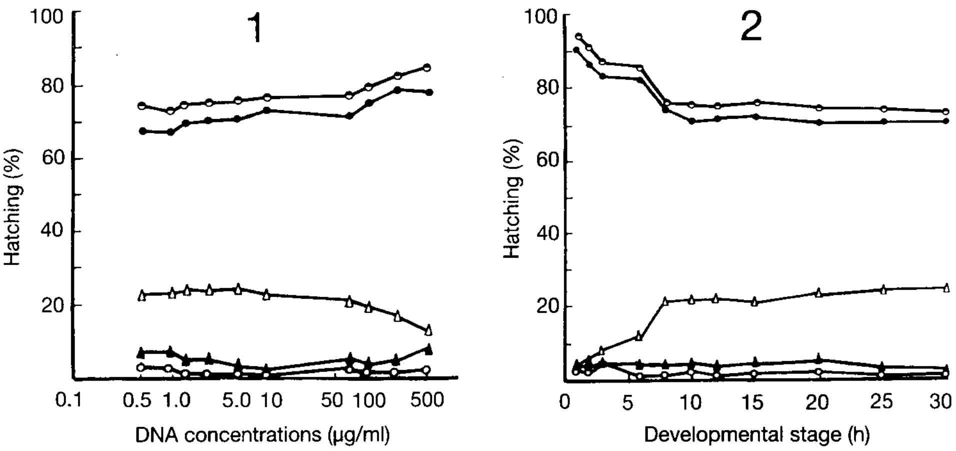

Figs 1–2: Hatching ability of *B. mori* eggs. 1 – microinjection with different concentrations of vector DNA at 8 h after oviposition; 2 – microinjection during different hours of embryonic development with a DNA concentration of 10 μg/ml. ○—○ – percentage of undeveloped eggs; ▲—▲ – percentage of eggs dead after head pigmentation; △—△ – percentage of eggs hatched; ●—● – percentage of eggs dead before head pigmentation; ◓—◓ – percentage of eggs dead due to microinjection (cumulative full symbols).

Death of embryos prior to head pigmentation is referred to as "eggs dead before head pigmentation". Dead eggs that did not show any sign of embryonic development and remained uniformly yellow were termed as undeveloped eggs. Of the 27,167 eggs microinjected at different developmental stages with the DNA concentration 10 μg/ml, 16.84% emerged successfully, 2% were undeveloped, 3.62% died after and 77.53% before head pigmentation. Minimum hatching ability of 3.27% was recorded in eggs subjected to microinjection 1 h after oviposition. Hatching ability increased with advancing stage of embryonic development upto 24.29% in eggs injected at 30 h (Fig. 1). Hatching ability of control injected eggs at 6, 8, 15 and 30 h was 12.90, 22.98, 24.32 and 26.78%, respectively. Hence there was no significant difference in the survival of control injected or plasmid injected eggs.

Microinjection of exogenous DNA during preblastoderm stage of the insect egg is a means of gene transfer (Handler & O'Brochta, 1991). Injection of DNA close to the site of pole cell is critical for the incorporation of DNA. In addition, the timing of injection is also important if the DNA is to be taken up by the developing germ line cells (Crampton & Egglestone, 1994). Insects with slower embryonic development may have a longer perblastoderm period which is suitable for microinjection. In *B. mori*, the eggs attain the blastoderm stage around 12 h after oviposition (Miya, 1984). Tamura et al. (1990) showed maximum expression of CAT gene in the eggs injected 8 h after oviposition. Similarly, Coulon-Bublex et al. (1993) showed a transient expression of β-galactosidase injected at 3–9 h old eggs. All these observations clearly suggest that *B. mori* eggs 8–10 h old are optimal for DNA injection.

Our results are consistent with reports of other authors; Kanda & Tamura (1991) recorded 20% hatching ability of eggs injected with 3 nl DNA (200 μg/ml) just after oviposition, Joy & Gopinathan (1994) registered 3–5% hatching ability in eggs supplied with DNA (200–400 μg/ml) at 2 to 2.5 h, Ninaki et al. (1985) observed 17.95% hatching ability with 5–10 nl DNA (75 μg/ml) injected at 6–7 h, Nikolaev et al. (1993) obtained 6% viable hatchlings with 1–10 nl DNA (8–150 μg/ml) injected at 8–12 h after oviposition.

A total of 26,806 eggs were microinjected with different concentrations of DNA; 21.05% hatched successfully, 4.93% died after and 72.18% before head pigmentation and 1.8% remained undeveloped. Hatching ability gradually decreased from 23.11% to 13.60% when the amount of injected DNA increased from 0.5 to 500 μg/ml (Fig. 2). The percentage of hatching ability declined when the DNA concentration increased above 10 μg/ml.

In cytoplasmic injection, considerable quantities of DNA must be injected to ensure that a few copies of DNA establish contact with chromosomal DNA (Chorrout et al., 1986). However, higher DNA concentrations might be lethal to the developing embryo. For example, in the migratory locust *Locusta migratoria* 50% of the embryos survived after receiving a DNA concentration of 200 μg/ml, but an increase more than 300 μg/ml resulted in mortality of all the eggs (Mathi et al., 1991). In the case of *B. mori* DNA concentration upto 10 μg/ml is the optimum for the survival injected eggs. Further increase in the concentration of DNA reduced the viability of the eggs.

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