

Euro Biotechnology 2018: Standardization of products of biotechnological sericulture by parthenocloning and cryobanking of transgenic clonal silkworms- Valeriya Zabelina- National Agriculture and Food Research Organization

Valeriya Zabelina

Abstract

The technology of silkworm (*Bombyx mori*) rearing and silk harvesting from the cocoons was discovered in China about 5000 years ago. Sericulture spread to many other countries and a handful of other moth species became exploited in the silk industry but *B. mori* has remained the major producer of commercial silk. The economic importance of sericulture declined after the discovery of the man-made polymers in early forties of the last century. Nylon and other synthetic materials rapidly replaced silk in most applications and their cost has been decreasing, while the cost of silk has increased because of the gradual rise of the labor cost. However, in spite of the relatively high cost, the excellent silk properties and the tradition of silk dresses in some countries create sufficient demand for the maintenance of traditional sericulture and associated textile industry. Silk exploitation in medicine and cosmetics is of a lesser but nevertheless persisting commercial importance.

The profitability of sericulture can be enhanced with the aid of genetic engineering that can improve silk properties or employ silkworm as a bioreactor for the production of diverse commercially attractive proteins other than silk. This short review describes the principles of methods used to produce transgenic silkworms and to establish them as genetically stable lines. It must be mentioned that silkworms (and some other insects) can be used as bioreactors without interventions with their genotype. Instead, genes encoding desired proteins are introduced into microbial vectors that express them in the infected insects or in the infested insect cells grown *in vitro*. The technique was first published by Maeda et al. (1985) who demonstrated production of human α -interferon in *B. mori* infected with the genetically engineered nucleopolyhedrovirus (BmNPV). Parallel investigations in the alfalfa looper *Autographa lifornica* and associated AcMNPV virus led to the development of commercial baculovirus expression vector system employing the cell line Sf9 derived from the fall armyworm, *Spodoptera frugiperda* (Roth et al. 1993). This expression system is now

widely used for the production of diverse proteins in many laboratories. Exploitation of AcMNPV in *B. mori* has been unsuccessful for many years because the tested strains of *B. mori* resisted infection by this type of baculovirus (reviewed by Kato et al. 2010). Only the recent study by Wöltje et al. (2014) described AcMNPV expression in several tissues of certain *B. mori* strains. The authors also demonstrated baculovirus penetration into the silk glands. We can expect commercial exploitation of this finding because purification of desired proteins from the glands or from their silky secretion is easier than the extraction from haemolymph or tissue culture media. The baculovirus expression systems are suitable for the production of specific proteins in cultured cells, less so in living insects. Transgene insertion into the insect genome is preferable to the viral infections because it permits establishment of stable silkworm lines that can be further modified, for example by crosses with other genotypes. The production of strains with defined and inheritable genome modifications requires (a) intervention with the genome such as transgene insertion and (b) subsequent breeding of transgenic insects for stable transgene expression and eventually homozygosity in the affected locus. In the following text we show that the techniques of both steps have been sufficiently elaborated for research purposes, while commercial applications seem to be hindered by fear of a chancy escape of genetically engineered silkworms into the environment. The same concern applies to the use of genetically engineered baculoviruses and other insect pathogens. We believe that these fears are not justified because the risk of silkworm escape into the wild is small (*B. mori* cannot survive without human care) and pathogens can be selected for high virulence in *B. mori* and low in other Lepidoptera. The risks are outweighed by the perspective that the deployment of genetically engineered silkworms could secure jobs to millions of people in the developing countries. Previous investments (mulberry plantations, facilities for silkworm rearing, training of farmers) into the declining traditional sericulture would be redeemed.

Valeriya Zabelina
National Agriculture and Food Research Organization, Japan

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