

Comparison of Preservation Techniques for Silkworm (*Bombyx mori* L.) DNA Based on Polymerase Chain Reaction (PCR) Products

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ABSTRACT

*There are many ways to preserve insect specimens that will protect its DNA from degradation for the period of time from collection to use in a molecular genetic study. However, techniques vary among groups of insects, suggesting that the determination of preservation should be done before working at the molecular level with large number of individuals from a particular group. This work compares four preservation techniques for the Thai native silkworm, *Bombyx mori* L. (UB1XNangnoi Srisaket 1): (1) killed and stored at -20°C, (2) killed and stored at -80°C, (3) killed and stored in 70% ethanol, and (4) rapid hot-air drying at 60°C. All specimens were stored for six months. DNA of each specimen was extracted by two different methods: (1) freezing in liquid nitrogen and transferring to lysis buffer for grinding and (2) grinding directly in lysis buffer. The integrity of DNA from those were determined and compared to live specimens using amplification of COI-COII DNA as a target region and genetic mapping with RAPDs. The method using 70% ethanol was found to be most practical and could prevent degradation of silkworm DNA for at least six months.*

Key words: Silkworm, *Bombyx mori* L., Preservation, PCR

INTRODUCTION

Since the polymerase chain reaction (PCR) procedure was discovered, the study of insect molecular genetics has developed and increased dramatically (Brower and DeSalle, 1994). Molecular systematics is one area of insect molecular genetics that has been revolutionized by the implementation of PCR. Molecular techniques provide an understanding of relationships among insects in terms of their evolution and genetic diversity. Techniques such as PCR-RFLP, RAPDs, microsatellites, AFLPs, and DNA sequences have allowed comparison of nucleotide sequences and fragments produced from the DNA of many kinds of insects (Hoy, 1994). In the past decade, there have been many such studies on the systematics of insects focusing on both higher and lower taxonomic levels such as Campbell et al., (1994), Downton and Austin (1994), Tuda et al., (1995), Langor and Sperling (1997), Hwang et al., (1999), Aoki et al., (2001).