CHAPTER I

INTRODUCTION

Honeybees are economical insects, especially *Apis mellifera* is a commercial species for beekeeping which have many subspecies. It is important for improvement of bee subspecies, strains, and breeding stocks for commercial. For these purposes, it is necessary to control mating. However, it is difficult in controlled mating because mating of honeybee occurs in the air. Instrumental insemination is an alternative for honeybee breeding in beekeeping industry and also enable to make mating that are not possible for natural mating (Ruttner, 1975; Harbo, 1986).

In Thailand, three of four species used in the experiments such as *Apis cerana*, *A. dor*sata, and *A. florea* are native species. Only one introduced species is *A. mellifera* (Wongsiri *et al.*, 2000). Natural mating does not occur among these four species because of behavioral and copulatory barriers that prevents copulation between queen and drone. Therefore, the use of instrumental insemination has a high potential to study heterospecific sperm transfer and storage in the spermatheca. Usually, semen of *A. mellifera* drone is collected from ejaculation by everted endophallus. In practice, it is necessary to use many drones for collecting sperm by this method in each time for inseminating a *A. mellifera* queen. However, large quantities of *A. mellifera* sperm are easily available, but it is not feasible in *A. cerana*, *A. dorsata* and *A. florea*. Because the number of drone per colony and the amount of spermatozoa per drone in *A. cerana*, *A. dorsata* and *A. florea* are less than in *A. mellifera* (10 -12 x 10^6 spermatozoa in *A. mellifera*, 1.2 x 10^6 spermatozoa in *A. cerana*, 1.59 x 10^6 spermatozoa in *A. dorsata*, and 0.43 x 10^6 spermatozoa in *A. florea* (Woyke, 1975; Rinderer *et al.*, 1985; Koeniger *et al.*, 1989, 1990).

Due to difficulties in collecting many drones and obtaining semen from everted endophallus of *A. cerana*, *A. florea* and *A. dor*sata, then the technique of collecting semen had to be modified for increasing the percent yield of sperm and the efficacy of

sperm collection. So, the technique of sperm collection from seminal vesicles and spermatozoa reconcentration by centrifugation will be conducted in this research. The success for this method of sperm collection depends on the diluent and period of sperm storage. Therefore, the first step for this research is to find an appropriated diluents for spermatozoa storage before using for instrumental insemination.

1.1. Objectives

1. To study and develop techniques of sperm collection for instrumental insemination.
2. To investigate the number of spermatozoa and its motility of hetero - and conspecific spermatozoa in the spermatheca of *Apis mellifera*.
3. To investigate reproductive incompatibility among honeybee species.

1.2. Anticipated benefits of this research

1. The technique of sperm collection from seminal vesicles and spermatozoa reconcentration by centrifugation has practical potential on research of cross insemination between species. Further, it can be applied to commercial instrumental insemination in *Apis cerana*.
2. This research will be the part of explanation for understanding a physiological differences in reproductive organs of queens between species and the sperm's contribution to the condition of storage and to the passage of spermatozoa from the oviduct into a spermatheca.