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# Distinguishing Mated and Unmated Mountain Pine Beetles in Alcohol-Preserved Specimens

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## ABSTRACT

*Alcohol-preserved female mountain pine beetles from both short-term and long-term storage were examined for evidence of insemination. The spermathecae of inseminated females were opaque, smooth, and rounded, while spermathecae of unmated females were translucent, grainy, and wrinkled. The analysis shows that mated and unmated segments of the mountain pine beetle population now can be distinguished in field collections.*

**KEYWORDS:** *Dendroctonus ponderosae*, insemination, stored specimens

McCambridge (1969) observed the need for distinguishing mated from nonmated MPB (mountain pine beetles; *Dendroctonus ponderosae* Hopkins [Coleoptera: Scolytidae]) because of their differing reactions to pheromones. He subsequently developed an accurate method to detect sperm in live females. This enhanced evaluation of pheromone experiments concerning MPB control and population surveys. McCambridge's technique is only useful for detecting sperm in live or recently killed specimens.

But there is great need for measuring proportions of mated and unmated females that have died during other types of behavioral studies involving field traps—for example, when studying the response of mountain pine beetles to lodgepole pine stands subjected to various silvicultural treatments (Schmitz and others 1980). To prevent their escape, beetles caught during such studies drown in water kept in the traps (Schmitz 1984) and are

subsequently transferred to vials containing 70 percent alcohol. Therefore, I initiated a study to determine if mated and unmated MPB could be distinguished among alcohol-preserved specimens.

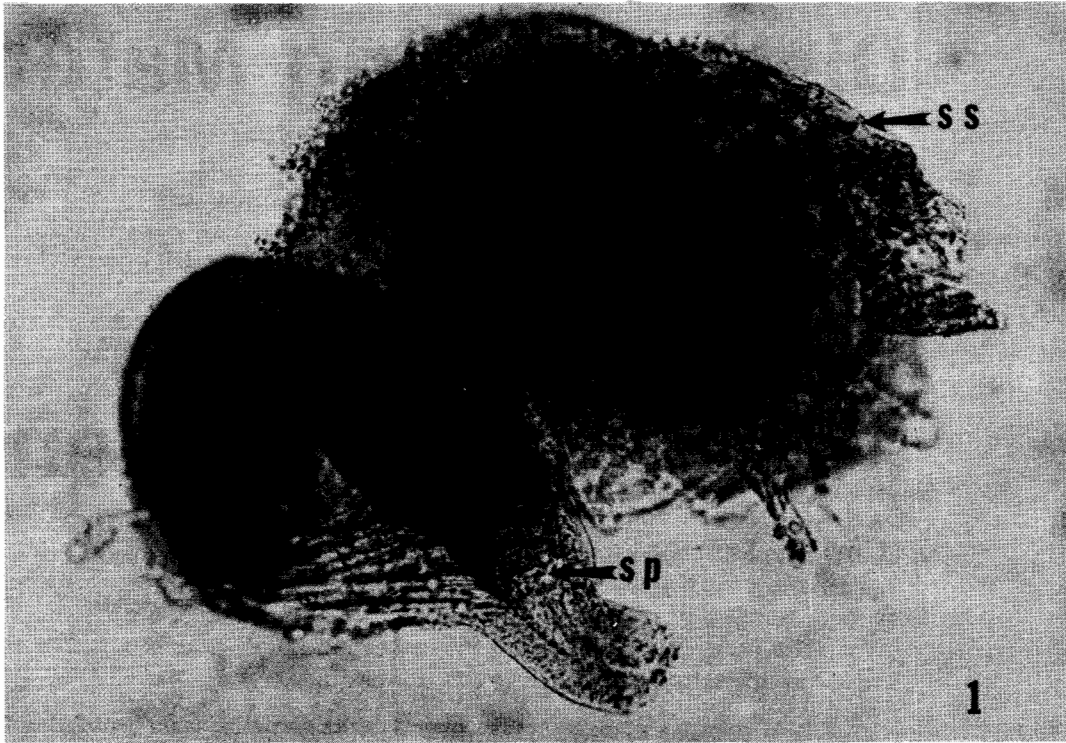
## MATERIALS AND METHODS

On October 9, 1980, several log sections were cut from a lodgepole pine naturally infested with MPB in northern Utah. The log sections were transported to Ogden, UT, and stored in a walk-in cooler at 40 °F until October 28, 1980. The logs then were moved to a laboratory and kept at room temperature (72 °F) until December, when the developing brood of the succeeding generation were force-reared to adults.

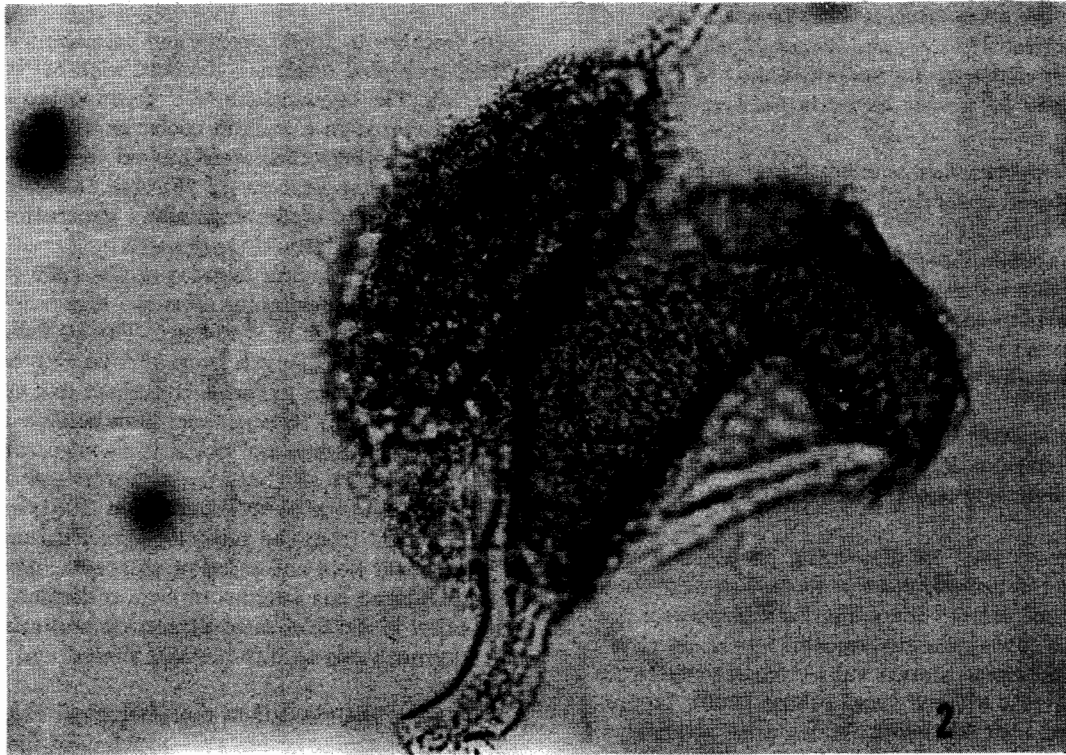
I removed egg-laying females of the 1980 generation from the logs immediately after the logs were moved to the laboratory from cold storage. The 50 females from galleries ranged in length from 15.2 to 53.3 cm (average of 30.5 cm). All egg galleries contained live eggs or young larvae, and 16 contained male adults. Because parthenogenesis does not occur in *Dendroctonus* (Gibson 1927; Reid 1962), all these females were mated. Mature unemerged females of the successive (1981) generation were removed from the same logs. In February 1982, a supplemental field collection of egg-laying females was made. I placed the females of each collection in 70 percent ethyl alcohol for later dissection and examination of the spermathecae to differentiate mated and unmated females.

Cerezke (1964) described morphological characteristics of the MPB reproductive systems. I used his description along with a modification of McCambridge's (1969) methods of detecting live inseminated females. The spermatheca was removed by holding the female under alcohol (except for six individuals dissected under water in 1983) and grasping the base of the most distal abdominal tergite with fine forceps. The tergite was then pulled outward while holding the rest of the beetle stationary.

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*Figure 1—Spermathecal sac (ss) and pump (sp) (length = 0.84 mm) from 70 percent alcohol-preserved mated female mountain pine beetle.*



*Figure 2—Spermathecal sac and pump from 70 percent alcohol-preserved unmated female mountain pine beetle.*

This removed the hindgut and female genitalia. The spermatheca was then easily identified by the sclerotized pump associated with it (fig. 1).

In November 1980, I dissected 50 alcohol-preserved mated females from the first collection in the above manner and, in January and February 1981, I dissected 50 spermathecae from the females of the unmated 1981 generation. Finally, in January 1983, six additional individuals were used for photographic documentation—three unemerged females from December 1980 and three egg-laying females from the February 1982 collection.

The six females analyzed in 1983 were dissected under water and required total abdominal dissection because the internal organs had become firm by remaining in 70 percent ethyl alcohol for an extended time.

I dissected all beetles under a dissecting stereomicroscope at 16 power and 40 power under reflected light and examined the spermathecae under 40 power, using diffuse reflected light with a dark background.

## RESULTS AND DISCUSSION

The spermathecae taken from mated females were opaque white, smooth, and quite rounded (fig. 1). This appearance contrasted strongly with the spermathecae removed from unmated females collected from pupal chambers in December 1980, which were translucent, grainy in appearance, and wrinkled (fig. 2).

The consistency of color and texture of the spermathecae (arising from protein fixation of the sperm by the alcohol) among all 50 mated females of variable ages suggests that an abundance of sperm is received during copulation. This probably accounts for the distinct differences in spermathecae between all mated and unmated females observed. The appearance of spermathecae from mated and unmated females remained consistent for analyses made in January 1983. Therefore, mated and unmated females can be diagnosed from alcohol-preserved specimens stored for long periods, although dissection is more difficult.

The beetles dissected in 1983 demonstrated that dissection under water was more efficient than under alcohol. This is because it was not necessary to replenish the water as it sometimes was with alcohol, and observations were unaffected by currents as occurs from evaporating alcohol. There were also no observable short-term changes of the spermathecae during dissection and analysis under water.

The results of spermathecal analysis from alcohol-preserved females demonstrate that it is an easy technique that can be used to accurately determine mated from nonmated MPB. These two segments of the mountain pine beetle population now can be distinguished in field collections.

## ACKNOWLEDGMENT

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