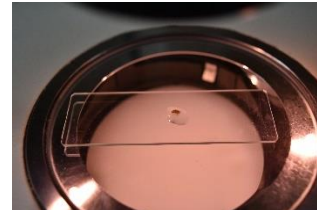


Protocol for the spotted wing drosophila (SWD) dissection, isolating spermatheca, and defining SWD mating status

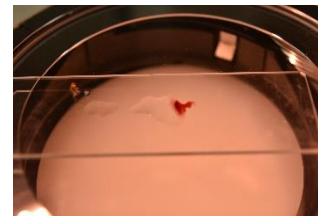
1. Place a drop of water on a slide.



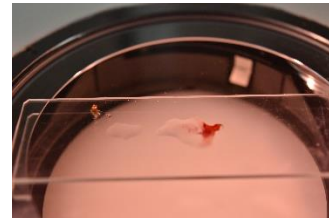
2. Select a female fly and place it in the center of the slide.



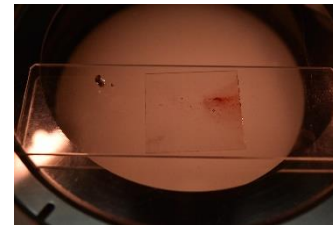
3. Using a pair of tweezers (or tweezers and a pin) pull the ovipositor out and separate two spermathecae from the rest of the tissues.
4. Remove all fly tissues except spermathecae from the slide.
5. Place one small drop of aceto-orcein (dye)* right next to the water drop and let it mix.



6. While looking through the oculars and using a pin help the stain to mix with water around spermathecae.



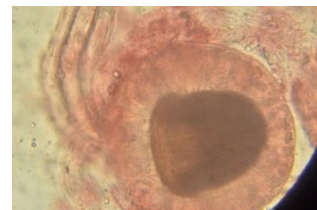
Cover the slide with a cover slip.



7. Bring the slide under a microscope and observe the intact spermathecae under 10x, 25x, and 40x.
8. Look at the content of spermathecae and surrounding tissues. Note if the spermathecae look “empty” or “full” (solid dark color), and whether the surrounding tissues look uniform.



Spermatheca looks “empty” (non-mated fly), 40x

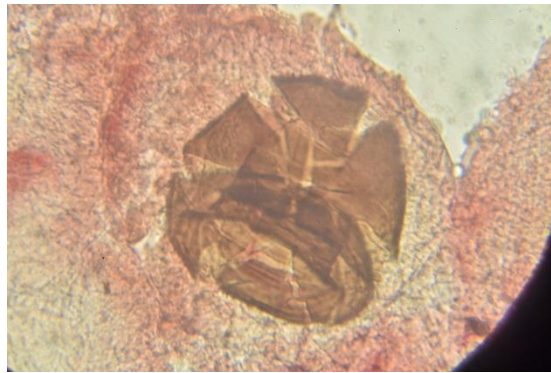


Spermatheca looks “full” (mated fly), 40x

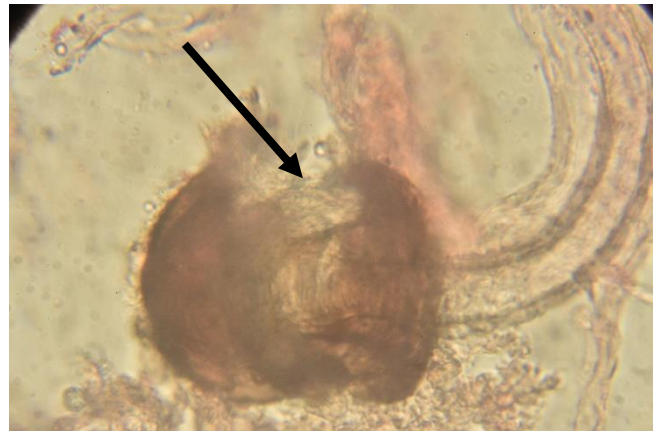
9. If the spermathecae look “full” look for thread-like sperm mass packed tight inside of the spermathecae (it looks like it is wrapped around the center).



10. Take a picture at 25x and 40x.
11. Crush spermathecae. To crush spermathecae, remember what part of the slide contains it. Place the slide on the table. Hold a pencil with the pencil-top eraser upside down approximately 5mm above the target part of the slide. Gently release the pencil.
12. Look at the spermathecae again under 10x, 25x, and 40x.
13. If the fly has not mated, the crushed spermathecae will not release sperm.



14. If the fly has mated, the sperm mass will be visible between broken parts of the spermatheca and beyond.



* Aceto-Orcein Solution (2%) for staining spermathecae can be purchased at Fisher Scientific (<https://www.fishersci.com/shop/products/aceto-orcein-solution-2-2/p-4416713>); 30mL is enough.
Manufacturer: AquaPhoenix Scientific, 9 Barnhart Drive, Hanover, PA 17331.