5.3 Adult Male Testes Dissection

MR4 Staff

Introduction

Male reproductive organs can provide valuable data about population age and mating history (Mahmood and Reisen 1982; Huho et al. 2006). Testes size has been associated with male age in *An. stephensi* in which it was found that longer testes were seen in sexually mature individuals while shorter ones were found in sexually immature or older mosquitoes (Mahmood and Reisen 1982). Additional studies in *An. gambiae* have found that differences in the appearance of the male accessory glands (MAG) were related to the age of the mosquito (Huho et al. 2006). Mosquitoes that were greater than 4 days old typically had no visible clear area in their MAG and large sperm reservoirs while mosquitoes that were younger than 4 had a small, transparent area on the edge of their MAG and small sperm reservoirs. Adult male testes are also a good source of metaphase chromosomes (French et al. 1962). The dissection technique described below should successfully remove all the reproductive organs.

Materials

- Stereoscope
- Compound microscope
- Fine forceps
- Dissecting needles or minutien pin mounted on a long wooden stick
- Clean microscope slides and cover slips
- PBS solution (0.01M, pH 7.2)

Protocol

1. Aspirate males into a container and anesthetize by gently chilling them at -20ºC for 5-7 minutes.
2. Place a drop of PBS on a clean slide
3. Under the stereoscope, gently grasp the male by the thorax with a pair of forceps and place ventral side up with the abdomen resting in the PBS.
4. Take a fine tip needle or forceps and gently remove the claspers of the male by piercing them and gently pulling them away while holding the thorax with forceps (**Figure 5.3.1**).
5. Using the dissecting needles, gently remove the extraneous tissues isolating the male reproductive organs then cover with a clean cover slip.
6. Examine both the testes and MAG to determine their dimensions and presence or absence of a clear zone (**Figures 5.3.2-9**).

References

French WL, Baker RH, Kitzmiller JB (1962) Preparation of mosquito chromosomes. Mosq News L1 - 2927pdf 22:337-383

Huho BJ, Ng'habi KR, Killeen GF, Nkwengulila G, Knols BG, Ferguson HM (2006) A reliable morphological method to assess the age of male *Anopheles gambiae*. Malar J 5:62

Mahmood F, Reisen WK (1982) *Anopheles stephensi* (Diptera: Culicidae): changes in male mating competence and reproductive system morphology associated with aging and mating. J Med Entomol 19:573-588

Figure 5.3.1. Dissected male *Anopheles gambiae* mosquito. The MAGs are visible as the yellow sacs near the terminalia.

Figure 5.3.2. MAG of a mosquito that is <4 days old. Nnote the clear zone on the edge of the MAG.

Figure 5.3.3. MAG of a mosquito that is > 4 days old. Note the absence of a clear zone.

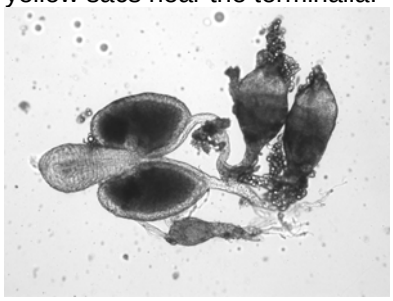

Figure 5.3.4. MAG and testes of a 1 day old *An. gambiae* mosquito.

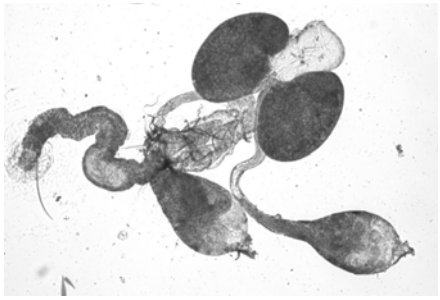

Figure 5.3.5. MAG and testes of a 5 day old *An. gambiae* mosquito.

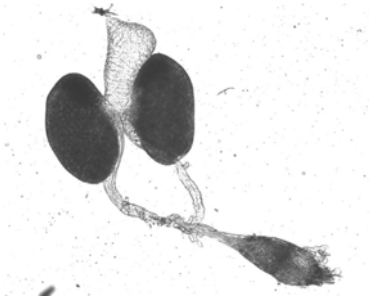

Figure 5.3.6. MAG and testes of a 7 day old *An. gambiae* mosquito.

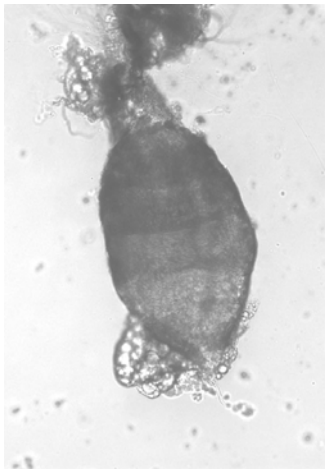

Figure 5.3.7. Testis of a 1 day old *An. gambiae* mosquito.

Figure 5.3.8. Testis of a 5 day old *An. gambiae* mosquito.

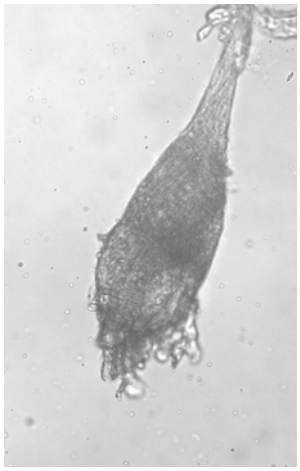

Figure 5.3.9. Testis of a 7 day old *An. gambiae* mosquito.