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OXFORD INSECT TECHNOLOGIES

Mosquito rearing protocol.

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i Insectary conditions

It should be possible to control the temperature, humidity and light cycle in any room to be used as an insectary. The room should be maintained at **26°C** and **80%** humidity with a **12 hour** light cycle and constant air flow to help prevent temperature gradients. There should also be room for storage of larval trays and adult cages as well as a surface on which to work. Ideally there should be a water supply and a sink or bucket for disposing of water.

ii Eggs

An egg paper will usually contain between a few hundred and a few thousand eggs. We usually hatch one egg paper per tray regardless of how many eggs are present, unless there are very few (i.e. <100 per paper) in which case we submerge several papers per tray.

To hatch eggs:

- Egg papers are placed into about 100mls of water (no tet is added at this stage) in a small pot. The pot is then placed into a vacuum desiccator (example shown opposite) and a vacuum applied (we use a tap-water driven vacuum device, but any sort of vacuum apparatus is suitable). The eggs are left in the vacuum device for 30-60 minutes and the hatch rate is generally very high (80-95%).
- Fill a standard rearing tray just under half full (**1 litre**) with purified/de-ionised water or tetracycline water (tet water) as appropriate for the strain (see Appendix 2 for tet water protocol).
- Pour the larvae and the egg paper into the appropriate tray. We label our trays with the name of the line and with the date the eggs were submerged. If tet water has been used we also label trays with a piece of yellow tape to indicate that tet water is required (in case there are several members of staff maintaining the trays and changing the water).

iii Trays

Our trays are white plastic storage trays measuring 35 x 25 x 5 cm (14 x 10 x 2

inches); with a capacity of 3.5 litres (from Global Food Service http://www.globalfse.co.uk); product code ST2220. £2.19 (GB pounds) each (incl. VAT) + handling and shipping.

iv Larvae

To look after larvae:

- Each day, check the larval trays for;
  - The quality of the water; if an oily film or a cloudy scum appear, the water needs to be changed and replaced with new water or tet water (we do this by passing the larvae through a funnel with mesh across the narrowest part but any fine sieve would be sufficient). Tet water will change colour over time, and become darker yellow, this is normal. We usually filter our trays approximately once a week.
  - The number of larvae in the tray; if a tray is overcrowded the larvae will take longer to pupate and the adults will be smaller and weaker than in a less crowded tray. The ideal number of larvae in a tray is around **250**.

  - Food; on the first day after hatching, we feed the larvae with two drops of liquifry per tray. After this we feed with Tetramin ornamental fish flakes (also available from www.aquatics-warehouse.co.uk) which we crush almost to a powder before using. We use one pinch of food per tray (approximately 0.15 – 0.2g) per day.

Using these guidelines, at **26°C**, with **80%** humidity, we have pupae approximately one week after putting eggs into water to hatch. These pupae can be removed using a 2.5ml pastette (VWR international; www.vwr.com Cat. 6121681) They will require screening for the presence of fluorescence according to the marker used for each strain (see appendix 3). We cover our trays with mesh covers so that if any pupae are missed and adults emerge they are confined within the trays. Any adults that emerge can then be easily removed using a small vacuum or aspirator (CPC http://.cpc.farnell.com Cat. AV00714).

v Pupae

Larvae become pupae after 7-10 days at 26°C.

Once larvae pupate:

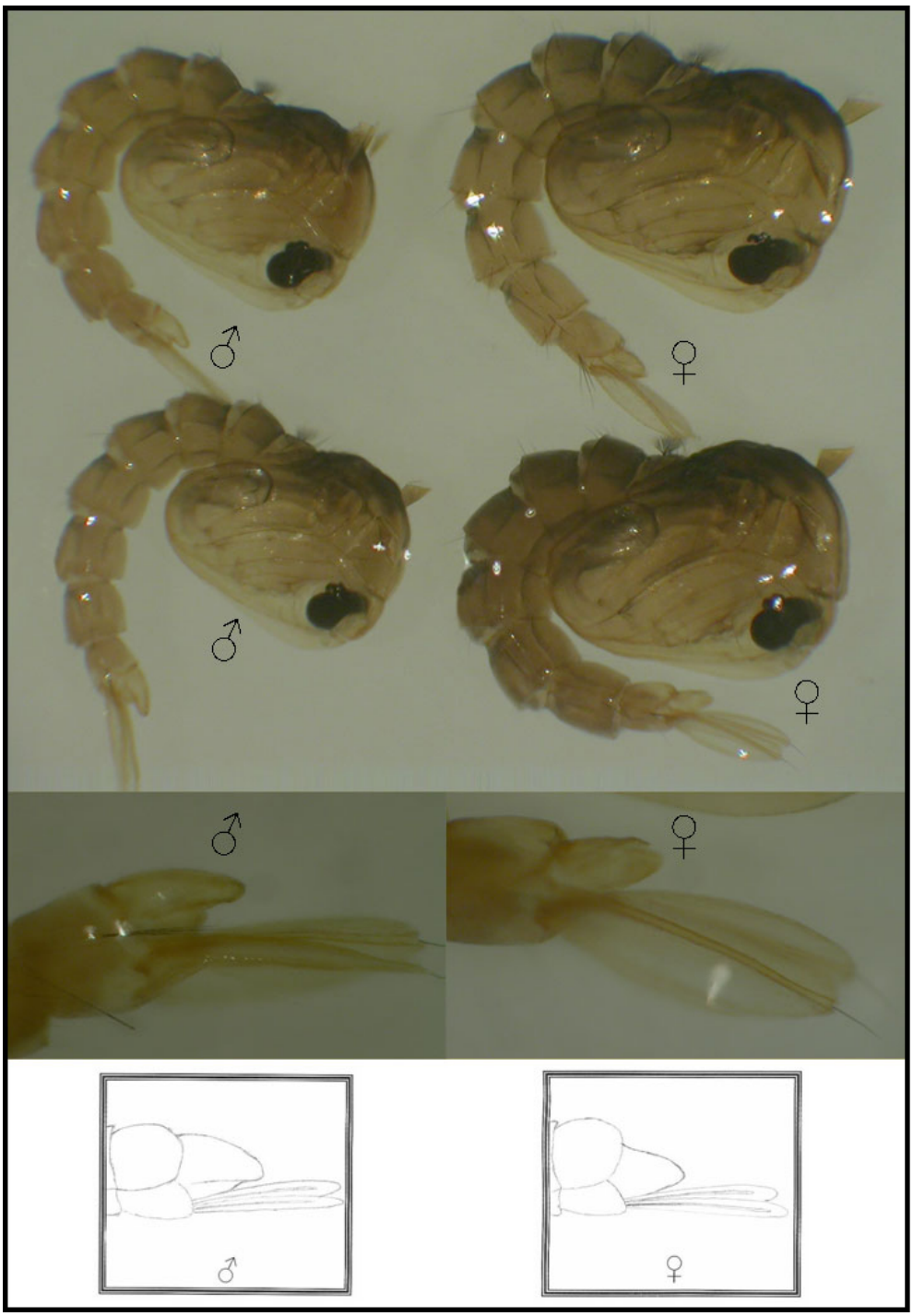
- Pupae must be removed from the larval trays within 48 hours, after which adults will emerge, so it is safest to remove pupae daily.

- Use a 2.5ml pastette to suck up the pupae in a small volume of water and remove them to an appropriate container such as a 100ml weigh boat

vi
Sexing pupae

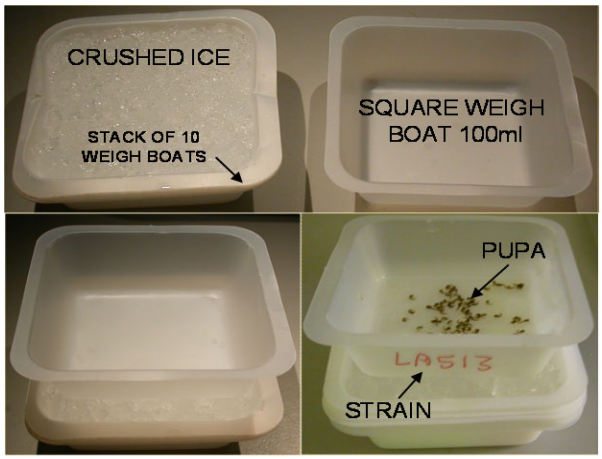
Sexing pupae:

Pupae can be reliably sexed by the structure on the end of the pupal abdominal segments just below the paddles. Males also tend to be much smaller then females.



vii Quality control and screening

Screening:



- Remove most of the water from the weigh boat using a pastette so that the pupae lie flat on the base of the weigh boat. Place the pupae weigh boat on top of another weigh boat containing ice. This will slow down the movement of the pupae so that it is possible to view them under a microscope. Pupae can survive on ice like this for several hours if necessary.
- For quality control, determine whether the fluorescent profile is consistent with the strain. If there is loss of fluorescence or the fluorescent profile changes significantly in any way please contact us immediately.

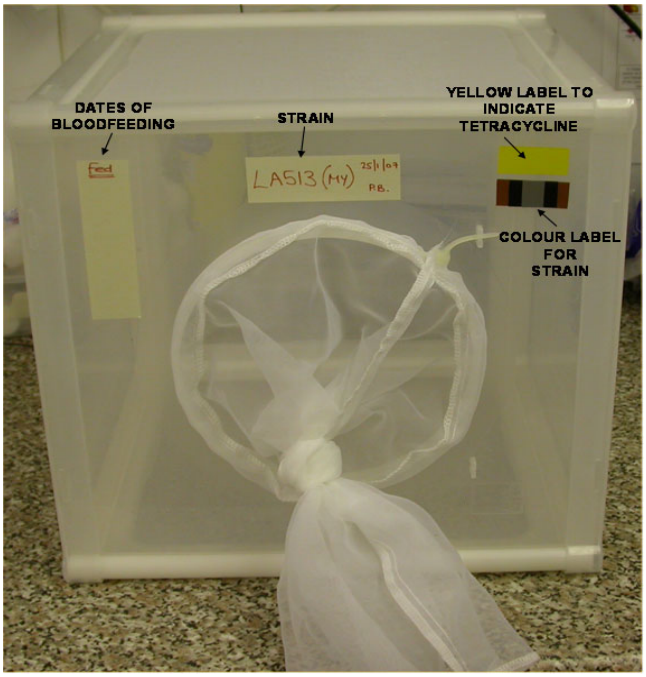
Please note that screening for fluorescence should be carried out in a darkened room. Ideally there should be no windows, or dark curtains that can shut out all light. The door should be closed and there should be no other light sources such as computer screens in the same room. Any additional light interference like this can lead to inaccuracies in screening especially for some lines reared on tetracycline.

viii Screening transgenic lines

Different transgenic lines can have different fluorescent markers and different fluorescent profiles. Therefore, please refer to the appropriate '**PROFILES AND QUALITY CONTROL**' document for detailed information on the fluorescence profile(s) of the particular transgenic line you are analysing.

ix
Cages

30cm x 30cm x 30cm mesh sided with a net sleeve which can be tied shut. (From www.megaview.com.tw).



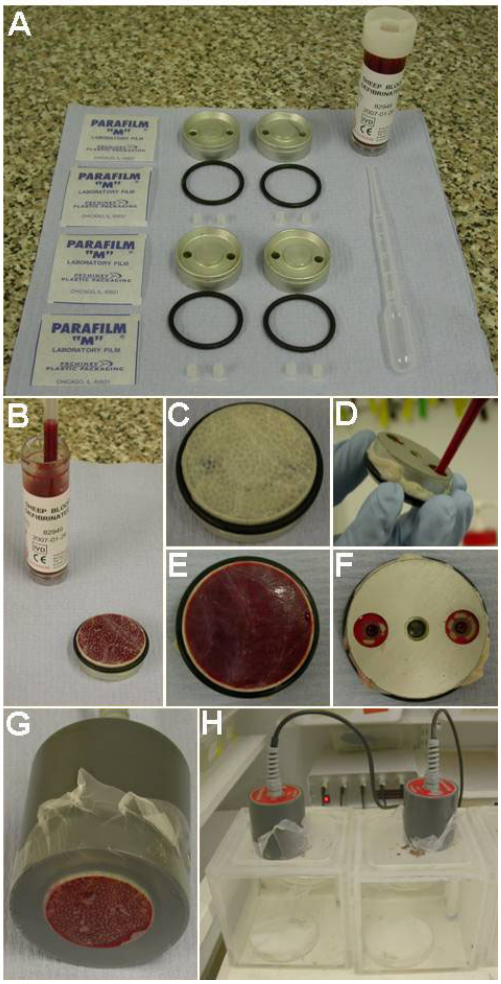
x
Adults

- Once pupae have been screened re-fill the weigh boat with water and place in an adult cage to emerge.
- Adult cages should be labelled with the strain of mosquito, the date that the cage was started and dates when the cage is blood fed.
- Adult mosquitoes should be provided with a source of sugar water. We drill a 10mm diameter hole in the lid of a 30ml bijou tube (VWR international, www.vwr.com Cat; 2150321) and place a Parotisrol dental cotton through this hole (Size 3, www.kentexp.com Cat. 30805), see picture opposite.
- Secure these sugar tubes in the cages using Blu-tack or similar.

xi Blood feeding

In order to get eggs from female mosquitoes they need to blood feed. We use the Hemotek blood feeding system: (details at hemotek@discoveryworkshops.co.uk). Other methods of blood feeding (such as animals or other membrane systems) may also be used.

- The general equipment for blood feeding is shown in picture A.
- Stretch the feeding membrane (we use animal intestine obtained from the butcher) over the feeding reservoir and secure with the rubber rings supplied (C). Fill 3ml reservoir with blood (we use de-fibrinated sheep blood (B) screened for pathogens, available in 25ml vials from TCS Biosciences www.tcsbiosciences.co.uk) using a 2.5ml pastette. Fill at a slight angle to avoid introducing air bubbles (D + E).
- Plug holes in reservoir with plastic plugs provided (F).
- Attach reservoir to Hemotek feeding unit, and stretch Parafilm over the whole feeding surface of the Hemotek feeder (G).
- Breathe on the Parafilm and touch it on a human arm a few times to pick up the odours.
- Place the feeder on top of an adult cage (H) and breathe into the cage, carbon dioxide stimulates the females to feed.



xii Obtaining Eggs

A female *Aedes* mosquito has a **three day** gonotrophic cycle so she will lay eggs three days after a blood meal.

To obtain eggs:

- Place some cotton wool in a Petri dish and soak with water.
- Rub a piece of filter paper (e.g. Whatman 3M) on a human arm to coat it with human odours. Place this piece of paper over the wet cotton wool and press it down to thoroughly soak it. The paper should be very wet, but with no water standing in the dish.
- Egg papers should be labelled with the strain of mosquito and the date, ideally by using a piece of tape attached to the filter paper. This prevents

cross contamination and mistakes.

- To obtain eggs quickly, the cage can be covered with a box or dark cloth; otherwise eggs will be laid during the usual night cycle of the insectary.
- There are many different methods to obtain eggs and the method that you are most familiar with is perfectly acceptable.

xiii Weekly Maintenance

- Adult cages should be fed twice a week; we feed Monday and Wednesday if we want eggs to be laid over the weekend.
- Sugar tubes should be replenished weekly.
- Once eggs are laid they should be kept damp for at least two days, after which the wet cotton wool should be removed from underneath the paper. The eggs should be allowed to dry and mature for four days before attempting to hatch them.

xiv Other maintenance

When a cage is full of mosquitoes (400-500 adults) then stop adding more pupae and remove all weigh boats before blood feeding a cage. This is to ensure that females lay only on the egg papers provided.

Appendix 1 Sugar Water

To make sugar water:

- Dissolve **100g** of sucrose in **1 litre** of de-ionised water and add **3ml/l** of penicillin/streptomycin (Sigma Aldrich, www.sigmaaldrich.com Cat. P0781).
- Filter using 0.2µm filter (VWR international, www.vwr.com Cat. 7345037) to remove un-dissolved sugar and other impurities.

Appendix 2 Tetracycline water

Protocol for making up Tetracycline water for larval trays (for RIDL only).

To make up aliquots of tetracycline stock solution:

- We make up stock solution at a concentration of **3mg/ml**
- Add **3g** of tetracycline powder to **1 litre** of purified/de-ionised water
- Mix until dissolved
- Aliquot into 50ml tubes and freeze

Tetracycline degrades in sunlight so keep the powder and stock solution in the dark.

To make up tetracycline water for mosquito larval trays:

- To make **5 litres** of tetracycline water (at a working concentration of **30µg/ml**), defrost **1 x 50ml** aliquot of tetracycline stock solution and add to **5 litres** of purified/de-ionised water
- We do not currently use tetracycline in the sugar solution for adults (but if you wanted to, use the same concentration as the water for larval trays).

Tetracycline hydrochloride

3g of tet powder makes 20 aliquots of concentrated tet water. Each of these makes 5 litres of tet water. So, **3g** of tet powder is required to make **100 litres** of water or **100 trays**.