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Strain Maintenance of *Nasonia vitripennis* (Parasitoid Wasp)

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Abstract

This protocol describes standard rearing of *N. vitripennis* strains on *Sarcophaga bullata* hosts. By using incubators at different temperatures, *Nasonia*'s development rate can be adjusted to conform to the investigator's schedule. *Nasonia* will produce diapause larvae when reared at 18C with a short-day light cycle (Saunders 1965); these can be archived at 4C for over a year. Diapausing *Nasonia* may lose their *Wolbachia* infections (Perrot-Minnot et al. 1996). *N. longicornis*, *N. giraulti* and *N. oneida* can be reared under similar conditions, with the caveat that *N. giraulti* and *N. oneida* lay all-diapause broods more frequently and are therefore best reared at 25C.

MATERIALS

REAGENTS

Sarcophaga bullata pupae (see Protocol 2 – fly rearing) or other calliphorid fly pupae (stored at 4C)

25mm diameter Drosophila vials and plugs (cotton, foam, rayon etc.)

12mm diameter plastic vials and cotton plugs

honey water (40% honey : 60 % H₂O aliquots in 1.5mL tubes)

EQUIPMENT

Incubators: 25C and 21C (constant light), 18C (8h light : 16h dark)

Refrigerator: 4C (preferably two for redundancy).

Small paintbrushes (number 2 or similar)

Rubber or foam pad

METHOD

I. 25C and 21C strain maintenance

1. Transfer 20 mated females and 4 or fewer males to new 25mm Drosophila vial. If transferring from same sized vial, place the new vial upside-down over the old vial. Mated females will climb up into the new vial. Otherwise, use a brush.
2. Cover vial opening with two fingers and knock vial bottom against rubber pad. This will make most *Nasonia* fall to the bottom.
3. Add 20 *Sarcophaga* hosts (or fill the vial 1/4 full if hosts are small).

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4. Plug with foam or cotton plugs and incubate new vial in 25C or 21C incubator. Used foam plugs can be autoclaved and reused.
5. Backup emerged cultures in 4C refrigerator. Mated females will survive and remain fertile for approximately 1–3 weeks.
6. Adults will emerge approximately 14 days later (25C) or 21 days later (21C). *Nasonia* females will mate immediately upon emergence.

II. 18C strain maintenance

1. Follow the same procedure as for 25C maintenance, except incubate at 18C using a 8h light : 16h dark cycle. *Nasonia* reared at 18C will emerge in approximately one month and live for several weeks.
2. If no *Nasonia* have emerged after six weeks, or after all emerged *Nasonia* have died, check the vial for diapause larvae. Crack open all hosts at the midline using thumbnail or a probe. Diapause larvae are as large or larger than the average *Nasonia* and are distinguished by prominent white fat cells visible through the cuticle.
3. Transfer hosts containing diapause larvae to a new 12mm plastic vial, plug with a cotton ball, label, and store in a 4C refrigerator for 6–24 months. Store in box with air holes, such as empty pipet tip box. Record strain and deposit time in a diapause log. If possible, split tubes between two 4C refrigerators to reduce the impact of equipment failure.

III. Recovery of diapause larvae

1. Remove tubes of diapausing larvae from 4C refrigerator. Strains are most likely to recover after refrigeration for 6–18 months.
2. Remove cotton plug. Take new cotton plug and gently moisten with one or two drops of distilled water (do not saturate or mold will grow). Cover all tubes with a sheet of aluminum foil to reduce loss of humidity.
3. Incubate recovering diapause larvae at 25C until wasps pupate, eclose and mate (about 14 days). If greater than 50% males emerge, separate approximately 20 females into a new vial using a paintbrush, add 2–4 males, paint a dot of honey water on the wall of the tube, and allow mating to occur for 4h or overnight.
4. For long-term strain maintenance, pull 2–3 full 12mm vials from diapause at 18–20 months. After rebuilding the population for a generation or two at 25C, return the strain to 18C culture to produce more diapause larvae.
5. Remove from 18C culture only when several batches of diapause larvae have been collected and stored (generally >6 vials worth).

TROUBLESHOOTING

Problem: low sex ratio (mostly males)

Solution: Crowding by multiple males may prevent some females from mating, who then produce all male broods and repeat the cycle. Remove as many females as possible using paintbrush, or, if necessary, knock out with CO₂ and sort. Separate up to 20 females into a new vial, add 2–4 males, paint a dot of honey water on the wall of the tube, and allow mating to occur for 4h or overnight. All-male broods means no female mated. Recover strains from a culture backed up at 4C.

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