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Virgin Collection and haplodiploid Crossing Methods in *Nasonia* (Parasitoid Wasp)

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INTRODUCTION

This protocol covers most procedures used by the *Nasonia* geneticist, including virgin collection. *Nasonia* are easily sexed as pupae, so collecting virgins is easily done with a window of several days. The female-specific ovipositor is visible at even the earliest pupal stages as a longitudinal ridge on the ventral posterior abdomen. To prolong generations, yellow pupae can be stored at 4-8C for up to two months and adults for two to three weeks. Haplo-diploid crossing methods are introduced in the example of making a new *Nasonia vitripennis* mutant homozygous, using an aunt-nephew mating approach. The protocol can be extended to inbreeding strains. Inbreeding depression is generally not a problem in *Nasonia* unless the mutation itself is female sterile. Mother-son mating and sibmating are also reasonable approaches to inbreeding.

MATERIALS

REAGENTS

12mm culture vials and cotton plugs

Fly hosts

Honey water (40% honey, 60% sterile H₂O)

EQUIPMENT

Dissecting microscope

CO₂ diffuser

Dissecting needle bent at 45 degrees 2cm from tip ("hockey stick")

Index card (fold long edges to 90 degrees, 1cm from edge, to form a tray)

25C Incubator

21C Incubator

4C refrigerator

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PROCEDURE

A. VIRGIN COLLECTION

1. Start with the offspring of a mated female. *Nasonia* pupae can be collected between days 10 and 13 if cultured at 25C, days 14-20 if cultured at 21C, and usually after day 27 if cultured at 18C, varying with the strain's development time.
2. Open fly hosts at midline using thumbnail or bent dissecting needle. Pour wasp pupae onto index card under dissecting scope.
3. Sex pupae and separate using bent-angle dissecting needle. Females will have a longitudinal ovipositor ridge at the ventral posterior of the abdomen (Fig XX). The ovipositor ridge interrupts the transverse abdominal segments. Males will have uninterrupted abdominal segments. Male *Nasonia vitripennis* also have noticeably smaller wing pads (less obvious in other species).
4. Put virgin pupae in 12mm culture vials and plug with cotton. Aim for 10 females per vial to reduce risks from sorting mistakes. Females should emerge in 14 days from original hosting at 25C or 21 days at 21C.

B. GENETIC CROSSES FOR MUTANT ISOLATION

1. Document phenotype of male mutant. (If the mutant appears in a female, host female as virgin for 2 days, then mate to a male of a wildtype strain and host).
2. Identify wildtype stock to use for mating. If new mutant is *Wolbachia*-free, use an uninfected strain such as the genome strain AsymCX. Otherwise, or if in doubt, use an infected strain such as LabII.
3. If adult virgin females are not available, place a drop of honey water on the side of the tube containing the mutant using a fine brush, allow to feed for 1 hour, and refrigerate until adult virgin females are available (See Virgin Collection above).
4. Place male mutant in culture vial with 2-5 virgin wildtype females and drop of honey water. Observe copulation and wait 3 hours for sperm incorporation before hosting, or else allow to mate overnight.
5. Host mated females with 1 fly host per female at 25C.
6. 10 days later, collect three sets of ten F1 virgin females. The first set will be virgin-hosted to produce F2 males. The second set will be aunt-nephew mated to the F2 males. The third set will be virgin hosted to produce males to mate to the F2 females. Back up the rest of the yellow pupae at 4C. All F1 females will be heterozygous.
7. Allow F1 virgin females to eclose. Check to see if mutation is dominant.
8. Host first set of virgin females individually, with two hosts each, and incubate at 25C. Give honey water as above to the second and third set of virgins and allow to feed for 1 hour. Then store at 4C.
9. Allow F2 males to emerge (Day 15). Place males on CO2 diffuser under dissecting microscope and check that phenotype was inherited. Some mutant phenotypes may be unable to escape the host; crack open the hosts to check..

10. Sort 2-4 F2 mutant males and then mate them to one of the tubes of emerged F1 heterozygous females which was stored at 4C. Add new drop of honey water and allow mating to proceed overnight.
11. Host mated females individually at 25C. Also host remaining tube of virgin F1 females.
12. 10 days after hosting, collect 20 F2 virgins. These will be a mix of heterozygotes and homozygotes. Back up the rest at 4C.
13. Host F2 virgins individually with two hosts at 25C. Label each vial to keep track of the females.
14. 48 hours later, transfer virgin females to new tubes containing a drop of honey water. Add in a mutant male from the virgin setting and allow the mating to proceed overnight in the 21C incubator.
15. After mating, add two fly hosts and incubate the cross at 21C.
16. 15 days later, F3 males will emerge. Sort through male populations using CO2 diffuser, making sure to crack open the hosts. Identify homozygous mothers.
17. Collect 20 virgins from F2 homozygous mothers. Also collect 10 virgins from heterozygous mothers. Back up the rest at 4C.
18. Repeat steps 12-15 to confirm that the strain is homozygous. Continue maintaining strain as in Protocol 1.

TROUBLESHOOTING

Problem

No female pupae seen.

Solution

Female parent may not have mated, or asymmetric *Wolbachia* infections caused cytoplasmic incompatibility. Unmated (virgin) *Nasonia* females will produce all-male broods. Recently emerged females (< 3 hr) from stocks may not have mated. Collect virgin females from 4C backup and resume cross. Check *Wolbachia* infection status of stocks. In general, infected males can only successfully fertilize eggs that have the same *Wolbachia* types. Virgins from an infected lab strain such as LabII can be used if male infection status is unknown.

Problem

homozygous females do not lay eggs or do not mate.

Solution

The mutation may be female-sterile. Maintain in heterozygous fashion (Steps 12-16 above) or discard.

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