Protocol

Female Fly Postmating Behaviors

Anne C. von Philipsborn,[1,4] Galit Shohat-Ophir,[2,5] and Carolina Rezaval[3,5]

[1]*Danish Research Institute of Translational Neuroscience (DANDRITE), Nordic-EMBL Partnership for Molecular Medicine, Aarhus University, 8000 Aarhus, Denmark;* [2]*The Mina and Everard Goodman Faculty of Life Sciences, The Leslie and Susan Gonda Multidisciplinary Brain Research Center, the Nanotechnology Institute, Bar-Ilan University, Ramat Gan 5290002, Israel;* [3]*School of Biosciences, University of Birmingham, Birmingham B15 2TT, United Kingdom*

Upon copulation, females undergo a switch-like change in their behavior and physiology, known as "postmating responses." These strong behavioral and physiological changes are triggered by the transfer of male seminal proteins during copulation. Postmating response is associated with strong reduction in receptivity, indicated by the females kicking their legs toward the suitor and curving their abdomen downward to hide their genitalia from them and extruding their ovipositor at the tip of the abdomen, which physically prevents copulation. The transfer of male-specific pheromones, such as 11-*cis*-vaccenyl-acetate, during copulation further reduces female attractiveness. In addition, mated females exhibit increased ovulation, egg-laying behavior, enhanced feeding behavior, and changes in food preference. However, females increase their rate of remating when they are in social groups or in the presence of food. This protocol describes methods for measuring female postmating behaviors, such as oviposition, female sexual receptivity, and mating plug ejection.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Drosophila apple juice agar plates <R>
Drosophila flies
Ethanol (70%)
Fly food (standard)

For example, Fly food recipes (2021). Prepare fly food in vials with regular and sloped surfaces (see Step 32).

RBS T230 (4%, Sigma-Aldrich) or Triton X-100 (10%) (see Step 38)

[4]Present address: Department of Medicine, Faculty of Science, University of Fribourg, 1700 Fribourg, Switzerland
[5]Correspondence: galit.ophir@biu.ac.il; c.rezaval@bham.ac.uk

From the *Drosophila* Neurobiology collection, edited by Bing Zhang, Ellie Heckscher, Alex C. Keene, and Scott Waddell.

Advanced Online Article. Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot108108

Equipment

Behavior boxes/behavioral room for experimenting

Rear and test the flies in an incubator with a light–dark cycle, set to 25°C, with 50%–60% humidity, and perform all behavioral experiments in an incubator, a behavior box, or a dedicated room with light, temperature, and humidity control. This allows for a quiet and controlled environment and reduces variability between experiments. For more detail, see Protocol: ***Single-Pair Courtship and Competition Assays in*** **Drosophila** *(von Philipsborn et al. 2023).*

Blocks (96-well, 2-mL, flat-bottom; QIAGEN 19585)

Fill each well with 0.5 mL of food, cool, and cover with a polymerase chain reaction (PCR) foil. Before use, punch air holes for each well and cut the foil along the walls separating the 12 rows with a scalpel or utility knife to allow each well to be filled individually.

CO_2 pad

Courtship chambers

Fabricate these by three-dimensional printing or acrylic laser cutting. For more detail, see Protocol: ***Single-Pair Courtship and Competition Assays in*** **Drosophila** *(von Philipsborn et al. 2023). The size and shape of the courtship chamber influences courtship initiation: The bigger the arena, the more time the male will take to find the female. Some researchers use round chambers 19 mm in diameter × 4 mm high (Cheriyamkunnel et al. 2021; Nojima et al. 2021), and others use chambers 10 mm in diameter (Demir and Dickson 2005; Shohat-Ophir et al. 2012). Detailed schemes for laser-cut chambers are presented in Supplemental Figures S1–S6 of Protocol:* ***Single-Pair Courtship and Competition Assays in*** **Drosophila** *(von Philipsborn et al. 2023). For scoring behavioral steps in detail or for automated tracking, it is advantageous to restrict vertical movement to prevent flies from walking on vertical chamber walls where they appear in side view from above. This can be achieved by designing chambers with sloped walls (Simon and Dickinson 2010; Nojima et al. 2021). For decreased climbing on walls, coat the walls with siliconizing agents such as the fluoropolymer resin polytetrafluoroethylene (PTFE-30).*

Dissection forceps (e.g., Dumont #5 Fine Forceps with tip dimensions of 0.05-mm × 0.01 mm; Fine Science Tools 11254-20)

Fly aspirator (foot pump)

Use the fly aspirator for selecting flies and transferring flies to the behavioral setups.

Paintbrush (fine) or feather (see Step 2)

Petri dishes (35-mm-diameter; 10-mm-tall)

Stereomicroscope

Stereomicroscope with UV filters or UV transilluminator (see Step 36)

Vials (glass or plastic; e.g., 80-mm × 25-mm plastic vials [Scientific Laboratory Supplies VIA6270], 80-mm × 25-mm plastic vials [VWR 734-2262P], or 25-mm × 28-mm polypropylene *Drosophila* breeding tubes [Semadeni 10405])

Use vials for rearing flies and for experimenting.

Video recording equipment

Use standard consumer camcorders or webcam equipment for recording behavior. For recording over longer time spans (e.g., when observing remating), it can be useful if the camera has a time-lapse option. For tracking behavior, use a FLIR Firefly camera with an infrared pass filter or a comparable camera, and record at a rate of least 30 frames per second to capture quick maneuvers. Place the courtship arenas in a rig to fix the position of the arenas. Backlight the arenas from below with an 880-nm near-infrared LED panel. To prevent buildup of heat, cool the backlight with H_2O or with constant air circulation and a heat sink. For details, see for example Kabra et al. (2013).

METHOD

Perform all behavioral experiments in a behavior box or a dedicated room with temperature and humidity control. Carry out all steps in the following protocol at room temperature unless otherwise noted.

Collection and Rearing of Flies

Temperature and humidity have a strong effect on mating success in flies (Miwa et al. 2018). In addition, social conditions during development modify fly behavior in adulthood (Bentzur et al. 2021). Therefore, keep rearing and testing conditions constant.

See Troubleshooting.

1. Rear the experimental flies under controlled humidity and temperature conditions (typically, 25°C with 50%–60% relative humidity) on a 12-h light–dark cycle.
2. Collect male and female flies 0–6 h after eclosion (newly emerged sexually naive [virgin] males and virgin females) on a fly pad with light CO_2 anesthesia using a fine paintbrush or a feather. Avoid exposing the flies for long periods (>2 min) to CO_2.
3. Using a fly aspirator (foot pump) to fill and remove flies, house flies in vials or 96-well blocks, keeping males and females separate before the defined start of the experiment. Place one fly per well in the blocks.
   - House virgin females in groups of five to 10 flies in vials with food.
   - House males singly or keep them in groups of two in vials with food.

   Some research groups place the males in bigger groups before testing them for behavior (e.g., in groups of 10). For housing many flies in isolation, use 96-well 2-mL flat-bottom blocks.
4. Age virgin males 4–7 d and virgin females 3–7 d under controlled temperature and humidity conditions until the experiment.

   Housing conditions and the age of the flies can vary depending on the aim of the experiment. We typically age males 5–7 d and females 3–5 d.

Testing and Data Analysis

A number of different female behaviors can be assessed: ovulation (Steps 5–7), egg laying (Steps 8–15), female fertility (Steps 16–23), postmating receptivity (Steps 24–33), and mating plug ejection (Steps 34–37).

See Troubleshooting.

Ovulation

5. Allow male and female flies to copulate in a regular food vial. Take females that have copulated with wild-type males at various time points (Chen et al. 1988) using a fly aspirator (foot pump) and place them in a food vial.

   See Troubleshooting.
6. Force the eggs out by dissecting the abdomen with dissection forceps under a stereomicroscope 2–3 h later or by cooling the female for 1–2 min on ice 2 h later (Bono et al. 2011; Laturney and Billeter 2016).

   Eggs are easily identified in the female abdomen once the outer cuticle is opened and the ovaries are exposed.
7. Count the number of mature eggs in the reproductive tract to quantify ovulation (Heifetz et al. 2000).

   See Troubleshooting.

Egg Laying

This can be done in food vials (Steps 8–11) or on apple agar plates (Steps 12–15).

Egg laying in food vials

8. Place single virgin females (3–7 d old) in food vials with three to five 5- to 7-d-old males.
9. Remove the males immediately after copulation using a fly aspirator (foot pump).

   See Troubleshooting.
10. Place mated females in food vials (one female per vial). Transfer them to fresh vials with fresh medium every 24 h.

If the chosen food recipe includes grains of dry yeast sprinkled on the food surface, omit this ingredient to make egg counting easier.

Egg laying is affected by social cues; therefore, we recommend placing females individually (Duménil et al. 2016).

See Troubleshooting.

11. Manually count the number of eggs laid in each vial under a stereomicroscope every 24 h for 10 d (Chapman et al. 2003; Wang et al. 2020).

    See Troubleshooting.

Egg laying on apple agar plates

Other laboratories measure egg laying using apple agar plates.

12. Transfer individual females (3–7 d old) to 35-mm Petri dishes 10 mm in height coated with apple agar.
13. Add three to five 5- to 7-d-old wild-type males and incubate them with the females for 2 h, allowing them to mate.

    See Troubleshooting.

14. After 2 h, remove males and keep the mated female in the plate for 24 h.

    See Troubleshooting.

15. Count the eggs (Mezzera et al. 2020).

    See Troubleshooting.

Female Fertility

16. Collect females 0–6 h (newly emerged sexually naive [virgin] females) after eclosion and store them in groups of three to five.
17. Age flies for 3–5 d.
18. Introduce flies individually into food vials containing three 5- to 7-d-old wild-type virgin males using a fly aspirator (foot pump).
19. Allow flies to mate.

    See Troubleshooting.

20. Remove males immediately after mating using a fly aspirator (foot pump).
21. Place mated females individually in food vials and transfer them to a vial with fresh medium.
22. Score vials for the presence of larval progeny after 10 d. Discount vials containing a dead female.
23. Calculate female fertility as the proportion of females that produce viable progeny (Vimal et al. 2018).

    See Troubleshooting.

Postmating Receptivity

This can be performed with (Steps 30 and 31) or without (Steps 24–29) food. Scoring can be done manually (Steps 28–31) or in an automated way (Steps 32 and 33).

Remating without food

24. Pair a 3- to 5-d-old virgin female with three 5- to 7-d-old wild-type males until she copulates in a food vial.

25. Immediately after mating, move females to food vials. Keep females in isolation or in groups of two to three mated females.

26. After 24 or 48 h, transfer one mated female into the courtship chamber using a fly aspirator. Transfer one new virgin 5- to 7-d-old wild-type male into the courtship chamber using a fly aspirator. Allow flies to mate.

    Avoid males with damaged wings. If the chamber has a removable slide separating the male from the female, keep the male and the female separated before the defined start of the experiment and allow them to acclimate to the chamber for 2–5 min before the start of the video recording.

    See Troubleshooting.

27. Start video and record at high resolution for 1 h at 25°C with >50% humidity. When the experiment is over, clean the chamber as described in Step 38.

    To quantify subtle female behaviors, make a high-resolution movie (e.g., one must zoom in to detect ovipositor extrusion). Depending on the size of the chamber, two chambers can be recorded at the same time to get enough resolution.

28. Calculate the percentage of females achieving copulation within 1 h by observing the recorded video (Chapman et al. 1994; Yapici et al. 2008; Yang et al. 2009; Rezaval et al. 2012, 2014).

    See Troubleshooting.

29. Analyze female rejection behaviors during courtship, including kicking, full ovipositor extrusion, wing flicking, and decamping (Aranha and Vasconcelos 2018). Measure these parameters during a 3-min observation period starting from courtship initiation. For example, measure ovipositor extrusion as the number of ovipositor extrusions performed by the female per minute during the observation period of courtship.

Remating on food (manual scoring)

In wild-type females, most remating events take place 1–5 h after the first mating when females are placed on food.

30. Place individual mated females with a new virgin 5- to 7-d-old wild-type male in a food vial.

    See Troubleshooting.

31. Observe the flies with the naked eye and note copulations.

    See Troubleshooting.

Remating on food (time-lapse imaging)

32. Place females with new males after their first mating in vials filled with food forming a sloped surface to allow flies to mate.

    See Troubleshooting.

33. Using video recording equipment, image by time lapse every 30–300 sec for up to several days to capture the full dynamics of remating (Fig. 1).

    See Troubleshooting.

Mating Plug Ejection

Females eject the mating plug together with unstored sperm within 2–5 h after the end of mating. The timing of this behavior is neuronally controlled and modulated by internal and external stimuli. As the mating plug contains high amounts of an autofluorescent male-derived protein, it can easily be identified under UV illumination (Lee et al. 2015; Laturney and Billeter 2016).

34. Pair virgin females with males in a food vial until they copulate.

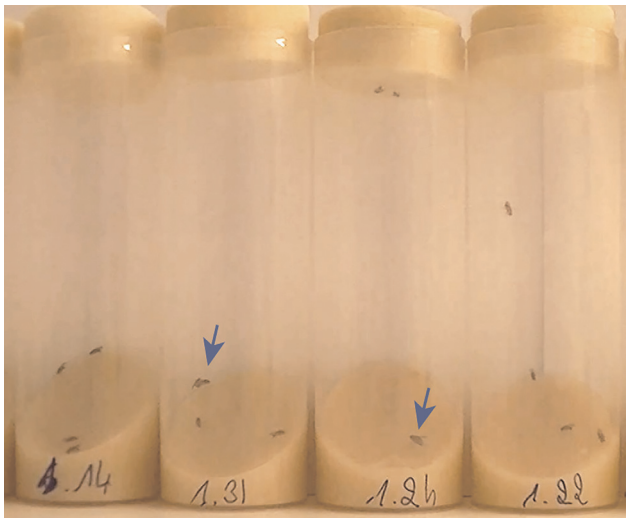

FIGURE 1. Postmating receptivity assay. Mated females are placed with three males in a food vial with a sloped surface. In this still image from a time-lapse video, the blue arrows indicate remating females. Vials with no mating events are easily spotted as they contain four separate flies.

35. Immediately after copulation, transfer females into courtship chambers or other small containers such as Petri dishes.
36. Video record ejection behavior (Lee et al. 2015) at sufficient resolution such that ejection is visible. Distinguish the mating plug from fecal matter using one of the two following options.
    - Distinguish the mating plug from fecal matter post hoc by inspection under UV illumination.
    - Inspect the chambers at regular intervals under a stereomicroscope with UV filters (Laturney and Billeter 2016).
37. Record the time between the end of copulation and mating plug ejection.

Cleaning Courtship Chambers

Wash and clean courtship chambers between experiments to remove fly scents.

38. Rinse the arenas with H_2O several times and then place them in a container filled with 4% RBS or 10% Triton X-100, wash actively, and let them soak for 30 min or 1 h. Rinse the arenas with hot H_2O several times (at least two or three times). Spray the arenas with 70% ethanol and let them dry overnight.

    Do not use ethanol to clean the arenas, as it can damage acrylic material, and remnants of ethanol vapor inebriate flies.

TROUBLESHOOTING

Problem (Steps 5, 9, 13, 19, 26, 30, and 32): Flies do not mate; there is low efficiency of preparing mated females.

Solution: Observe the interaction of the sexes to determine whether the failure to mate is likely to come from lack of male courtship or lack of female receptivity. Focus the troubleshooting on the sex that is most likely affected.

- Make sure that all flies are normally sized and did not originate from overpopulated cultures that give rise to small flies. Verify that wings are not damaged, as this may affect the male's ability to sing.
- Make sure that the females are not older than 7 d.
- In the case of transgenic flies, those with light eye colors are usually less successful in courtship; possible solutions to this issue are discussed in Protocol: **Measurement of Courtship Behavior in *Drosophila melanogaster*** (Ejima and Griffith 2007).
- Genetic background can affect courtship performance of males as well as female receptivity. To control for genetic background, follow the instructions outlined in the previous CSHL protocol on courtship measurement (see Protocol: **Measurement of Courtship Behavior in *Drosophila melanogaster*** [Ejima and Griffith 2007]). Compare flies from similar genetic backgrounds (backcross all genotypes to the appropriate genetic background).

Problem (Steps 10 and 14): Mated wild-type females do not lay eggs.
Solution: This problem has multiple potential solutions. Consider the following.

- Check the quality of food. Nutrition affects fly development, reproduction, and survival (Corrales-Carvajal et al. 2016; Gorter et al. 2016; Leitão-Gonçalves et al. 2017; Grangeteau et al. 2018). Variations in recipes could lead to behavioral differences. Make sure that the food is not too dry. Keep the vials hydrated. Sturdiness of the food is also a factor; ensure that the food is soft enough.
- Anesthesia by CO_2 can damage fly behavior. Reduce fly collection time as possible and allow recovery time. Refrain from using CO_2 after the initial collection.
- Make sure that flies are not contaminated with bacteria, fungi, or mites (see Troubleshooting for Steps 1–37).

Problem (Steps 1–37): Crosses are unhealthy and produce few and/or small progeny.
Solution: This problem has multiple potential solutions. Consider the following.

- Keep *Drosophila* cultures hydrated. If the fly food is too dry, females will not lay eggs. Make sure to add H_2O drops into the vials every ~2–3 d.
- Make sure that the *Drosophila* culture is mite-free. Follow this procedure to avoid this threat: http://www.flyfacility.gen.cam.ac.uk/Flylab/mites.
- Keep culture vials free of fungal and bacterial contamination.
- Check the quality of food. Nutrition affects fly development, reproduction, and survival (Corrales-Carvajal et al. 2016; Gorter et al. 2016; Leitão-Gonçalves et al. 2017; Grangeteau et al. 2018). Variations in recipes could lead to behavioral differences. Add food (in particular, fresh yeast) into vials or behavioral chambers to increase the likelihood of laying eggs (Grosjean et al. 2011; Gorter et al. 2016).

Problem (Steps 7, 11, 15, 23, 28, 31, and 33): Results are not reproducible or robust.
Solution: This problem has multiple potential solutions. Consider the following.

- Make sure to compare all genotypes at the same time.
- Make sure to test the flies at the same time of the day. Train the flies with daily cycles of 12 h.
- Use a power test to calculate the number of flies required for an experiment. Repeat at least three times on different days.

RECIPE

Drosophila *Apple Juice-Agar Plates*

MATERIALS

Reagents

Agar
Apple juice
Nipagin M (20% in absolute ethanol)
Sucrose

Equipment

Flasks, conical
Jug, plastic, 2-L
Magnetic stirrer and stirring rod
Oven, microwave
Petri dishes, 50- or 90-mm

This recipe will make 65 50-mm plates or 40 90-mm plates.

METHOD

1. In a conical flask, mix 18 g of agar in 600 mL of H_2O.
2. In a separate flask, mix 20 g of sucrose in 200 mL of apple juice.
3. Place both flasks in a microwave oven on a medium setting (e.g., 7 on a scale of 0–10) for 30 min.
4. When both the agar and sucrose are dissolved, pour the apple juice into the plastic jug with a magnetic stirring rod.
5. Carefully (to avoid bubbles) add the agar solution to the jug. Place the mixture on a magnetic stirrer. Allow the mixture to cool to at least 60°C.
6. Add 20 mL of 20% Nipagin M. Stir for a few minutes.
7. Pour into plates.
8. Allow the medium to solidify. Store the plates upside down at 4°C.

ACKNOWLEDGMENTS

We thank Dana Galili, Assa Bentzur, and Julia Ryvkin for insightful comments on this text. Work in our laboratory was supported by the Lundbeckfonden under grant number DANDRITE R248-2016-2518 to A.C.v.P., the Israel Science Foundation Grant 174/19 to G.S.-O., and BBSRC (BB/S009299/1), Wellcome Trust (214062/Z/18/Z), and Royal Society Research (RGS\R2\180272) grants to C.R.

REFERENCES

Aranha MM, Vasconcelos ML. 2018. Deciphering *Drosophila* female innate behaviors. *Curr Opin Neurobiol* **52:** 139–148. doi:10.1016/j.conb.2018.06.005

Bentzur A, Ben-Shaanan S, Benichou JIC, Costi E, Levi M, Ilany A, Shohat-Ophir G. 2021. Early life experience shapes male behavior and social networks in *Drosophila*. *Curr Biol* **31:** 486–501.e3. doi:10.1016/j.cub.2020.10.060

Bono JM, Matzkin LM, Kelleher ES, Markow TA. 2011. Postmating transcriptional changes in reproductive tracts of con- and heterospecifically mated *Drosophila mojavensis* females. *Proc Natl Acad Sci* **108:** 7878–7883. doi:10.1073/pnas.1100388108

Chapman T, Trevitt S, Partridge L. 1994. Remating and male-derived nutrients in *Drosophila melanogaster*. *J Evol Biol* **7:** 51–69. doi:10.1046/j.1420-9101.1994.7010051.x

Chapman T, Bangham J, Vinti G, Seifried B, Lung O, Wolfner MF, Smith HK, Partridge L. 2003. The sex peptide of *Drosophila melanogaster*: female post-mating responses analyzed by using RNA interference. *Proc Natl Acad Sci* **100:** 9923–9928. doi:10.1073/pnas.1631635100

Chen PS, Stumm-Zollinger E, Aigaki T, Balmer J, Bienz M, Böhlen P. 1988. A male accessory gland peptide that regulates reproductive behavior of female *D. melanogaster*. *Cell* **54:** 291–298. doi:10.1016/0092-8674(88)90192-4

Cheriyamkunnel SJ, Rose S, Jacob PF, Blackburn LA, Glasgow S, Moorse J, Winstanley M, Moynihan PJ, Waddell S, Rezaval C. 2021. A neuronal mechanism controlling the choice between feeding and sexual behaviors in *Drosophila*. *Curr Biol* **31:** 4231–4245.e4. doi:10.1016/j.cub.2021.07.029

Corrales-Carvajal VM, Faisal AA, Ribeiro C. 2016. Internal states drive nutrient homeostasis by modulating exploration-exploitation trade-off. *eLife* **5:** e19920. doi:10.7554/eLife.19920

Demir E, Dickson BJ. 2005. *fruitless* splicing specifies male courtship behavior in *Drosophila*. *Cell* **121:** 785–794. doi:10.1016/j.cell.2005.04.027

Duménil C, Woud D, Pinto F, Alkema JT, Jansen I, Van Der Geest AM, Roessingh S, Billeter J-C. 2016. Pheromonal cues deposited by mated females convey social information about egg-laying sites in *Drosophila melanogaster*. *J Chem Ecol* **42:** 259–269. doi:10.1007/s10886-016-0681-3

Ejima A, Griffith LC. 2007. Measurement of courtship behavior in *Drosophila melanogaster*. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot4847

Fly food recipes. 2021. http://bdsc.indiana.edu/information/recipes/index.html [Accessed August 12, 2022].

Gorter JA, Jagadeesh S, Gahr C, Boonekamp JJ, Levine JD, Billeter J-C. 2016. The nutritional and hedonic value of food modulate sexual receptivity in *Drosophila melanogaster* females. *Sci Rep* **6:** 19441. doi:10.1038/srep19441

Grangeteau C, Yahou F, Everaerts C, Dupont S, Farine J-P, Beney L, Ferveur J-F. 2018. Yeast quality in juvenile diet affects *Drosophila melanogaster* adult life traits. *Sci Rep* **8:** 13070. doi:10.1038/s41598-018-31561-9

Grosjean Y, Rytz R, Farine J-P, Abuin L, Cortot J, Jefferis GSXE, Benton R. 2011. An olfactory receptor for food-derived odours promotes male courtship in *Drosophila*. *Nature* **478:** 236–240. doi:10.1038/nature10428

Heifetz Y, Lung O, Frongillo EA, Wolfner MF. 2000. The *Drosophila* seminal fluid protein Acp26Aa stimulates release of oocytes by the ovary. *Curr Biol* **10:** 99–102. doi:10.1016/S0960-9822(00)00288-8

Kabra M, Robie AA, Rivera-Alba M, Branson S, Branson K. 2013. JAABA: interactive machine learning for automatic annotation of animal behavior. *Nat Methods* **10:** 64–67. doi:10.1038/nmeth.2281

Laturney M, Billeter J-C. 2016. *Drosophila melanogaster* females restore their attractiveness after mating by removing male anti-aphrodisiac pheromones. *Nat Commun* **7:** 12322. doi:10.1038/ncomms12322

Lee K-M, Daubnerová I, Isaac RE, Zhang C, Choi S, Chung J, Kim Y-J. 2015. A neuronal pathway that controls sperm ejection and storage in female *Drosophila*. *Curr Biol* **25:** 790–797. doi:10.1016/j.cub.2015.01.050

Leitão-Gonçalves R, Carvalho-Santos Z, Francisco AP, Fioreze GT, Anjos M, Baltazar C, Elias AP, Itskov PM, Piper MDW, Ribeiro C. 2017. Commensal bacteria and essential amino acids control food choice behavior and reproduction. *PLoS Biol* **15:** e2000862. doi:10.1371/journal.pbio.2000862

Mezzera C, Brotas M, Gaspar M, Pavlou HJ, Goodwin SF, Vasconcelos ML. 2020. Ovipositor extrusion promotes the transition from courtship to copulation and signals female acceptance in *Drosophila melanogaster*. *Curr Biol* **30:** 3736–3748.e5. doi:10.1016/j.cub.2020.06.071

Miwa Y, Koganezawa M, Yamamoto D. 2018. Antennae sense heat stress to inhibit mating and promote escaping in *Drosophila* females. *J Neurogenet* **32:** 353–363. doi:10.1080/01677063.2018.1513507

Nojima T, Rings A, Allen AM, Otto N, Verschut TA, Billeter J-C, Neville MC, Goodwin SF. 2021. A sex-specific switch between visual and olfactory inputs underlies adaptive sex differences in behavior. *Curr Biol* **31:** 1175–1191.e6. doi:10.1016/j.cub.2020.12.047

Rezaval C, Pavlou HJ, Dornan AJ, Chan Y-B, Kravitz EA, Goodwin SF. 2012. Neural circuitry underlying *Drosophila* female postmating behavioral responses. *Curr Biol* **22:** 1155–1165. doi:10.1016/j.cub.2012.04.062

Rezaval C, Nojima T, Neville MC, Lin AC, Goodwin SF. 2014. Sexually dimorphic octopaminergic neurons modulate female postmating behaviors in *Drosophila*. *Curr Biol* **24:** 725–730. doi:10.1016/j.cub.2013.12.051

Shohat-Ophir G, Kaun KR, Azanchi R, Mohammed H, Heberlein U. 2012. Sexual deprivation increases ethanol intake in *Drosophila*. *Science* **335:** 1351–1355. doi:10.1126/science.1215932

Simon JC, Dickinson MH. 2010. A new chamber for studying the behavior of *Drosophila*. *PLoS One* **5:** e8793. doi:10.1371/journal.pone.0008793

Vimal D, Kumar S, Pandey A, Sharma D, Saini S, Gupta S, Ravi Ram K, Chowdhuri DK. 2018. Mlh1 is required for female fertility in *Drosophila melanogaster*: an outcome of effects on meiotic crossing over, ovarian follicles and egg activation. *Eur J Cell Biol* **97:** 75–89. doi:10.1016/j.ejcb.2017.12.002

von Philipsborn AC, Shohat-Ophir G, Rezaval C. 2023. Single-pair courtship and competition assays in *Drosophila*. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot108105

Wang F, Wang K, Forknall N, Patrick C, Yang T, Parekh R, Bock D, Dickson BJ. 2020. Neural circuitry linking mating and egg laying in *Drosophila* females. *Nature* **579:** 101–105. doi:10.1038/s41586-020-2055-9

Yang C-H, Rumpf S, Xiang Y, Gordon MD, Song W, Jan LY, Jan Y-N. 2009. Control of the postmating behavioral switch in *Drosophila* females by internal sensory neurons. *Neuron* **61:** 519–526. doi:10.1016/j.neuron.2008.12.021

Yapici N, Kim Y-J, Ribeiro C, Dickson BJ. 2008. A receptor that mediates the post-mating switch in *Drosophila* reproductive behaviour. *Nature* **451:** 33–37. doi:10.1038/nature06483

Female Fly Postmating Behaviors

Anne C. von Philipsborn, Galit Shohat-Ophir and Carolina Rezaval

Cold Spring Harb Protoc; doi: 10.1101/pdb.prot108108; published online February 13, 2023