Protocol

Establishing a *Culex* Colony from Field-Collected Eggs

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Culex larvae are well adapted to growing and developing in containers, and therefore collecting and rearing field-collected *Culex* to adulthood in the laboratory is relatively straightforward. What is substantially more challenging is simulating natural conditions that encourage *Culex* adults to mate, blood feed, and reproduce in laboratory settings. In our experience, this is the most difficult hurdle to overcome when establishing new laboratory colonies. Here, we detail how to collect *Culex* eggs from the field and establish a colony in the laboratory. Successfully establishing a new colony of *Culex* mosquitoes in the laboratory will allow researchers to evaluate physiologically, behaviorally, and ecologically relevant aspects of their biology and better understand and manage these important disease vectors.

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 material 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

Bleach solution (3%–5%)

Blood source

Large amounts (~500 mL) of sodium-citrate treated chicken's blood can be purchased from biological suppliers and divided into 6-, 9-, or 15-mL aliquots. These aliquots can be frozen for up to 16 mo at −70°C and thawed when needed.

Distilled H_2O

Reverse osmosis (RO) H_2O can also be used.

DNA extraction kit

In our experience, the DNeasy (QIAGEN) and Phire tissue-direct PCR kits (Thermo Fisher Scientific) work well.

Dry fish food (e.g., Tetramin tropical fish flakes pulverized using a coffee grinder)

Grass clippings (freshly cut; one handful of Kentucky bluegrass, crabgrass, or other locally available grasses; see Step 26)

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From the Mosquitoes collection, edited by Laura B. Duvall and Benjamin J. Matthews.

Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot108079

Gravid H_2O for Mosquito Larvae Collection <R>

Most protocols to generate gravid H_2O entail placing organic material (usually grass clippings or straw) in H_2O and allowing it to ferment at high temperatures such as outside during summer (Zulueta 1950; Gjullin et al. 1965; Gubler 1971; Kramer and Mulla 1979). We have also had success in allowing the H_2O to ferment in environmental chambers set to 27°C. Yeast increases carbon dioxide production and facilitates the breakdown of organic material, and milk protein increases the amino acid content of the H_2O and supports bacterial growth (Burkett-Cadena and Mullen 2008).

Honey (optional, see Step 20)
PCR master mix (e.g., DreamTaq, Fisher Scientific)
Primers

See Crabtree et al. (1995), Smith and Fonseca (2004), and Bahnck and Fonseca (2006).

Raisins (optional, see Step 20; organic)
Soap (optional, see Step 33; e.g., Dawn or Palmolive dish soap)
Sucrose solution (10%) made from table sugar or laboratory-grade sucrose (e.g., Domino granulated sugar; Fisher Scientific)

Equipment

Black paper or tape (see Step 19)
Black tubs (plastic, 20.5-in × 15-in × 5-in)

Research shows that large containers (≥35-L) are more effective at collecting mosquitoes than small containers (≤6-L; Allan and Kline 2004; Popko and Walton 2016).

Compound light microscope (optional, see Step 21)
Cotton
Dissecting microscope (100×–200× magnification with backlighting)
Environmental chamber

A room where lights are on a photoperiod timer and temperature and/or humidity are controlled can also be used. To stimulate mosquito growth and reproduction, we recommend setting the chamber/room to long-day conditions (>14 h light/d) and temperatures of 24°C–27°C. Culex *mosquitoes will develop at temperatures as low as 15°C, but development will be slower. If you want to induce diapause/overwintering dormancy,* Culex *mosquitoes should be exposed to photoperiods <12 h light/d and temperatures <21°C. Both reproductively active and diapausing* Culex *mosquitoes prefer humid conditions (relative humidity >70%). This can be achieved by using a humidifier and/or placing moist sponges on top of the mosquito cages.*

Forceps (optional, see Step 21)
Large syringe/turkey baster (e.g., Oxo good grips turkey baster)
Mesh cages (30 cm^3 or larger recommended; e.g., BugDorm-1 Insect Rearing Cage)
Paper towels or filter paper
Plastic deli cups with lids (e.g., diameter of 11.4 cm; height of 4.4 cm; holding volume of 250–500 mL; Fabri-Kal Corp)
Plastic shoe-box-sized containers (e.g., Sterlite, 28-cm × 16.7-cm × 6.9-cm)
Plastic transfer pipette (e.g., ~3-mL; Fischer Scientific)
Plastic utensils (e.g., spoon or spatula)
Sponges
Thermocycler

METHOD

Field Collections

Many species of Culex *mosquitoes are container breeders and prefer to lay their egg rafts in nutrient-rich H_2O. Therefore, in the summer, it is relatively easy to collect* Culex *eggs in black tubs containing gravid H_2O.*

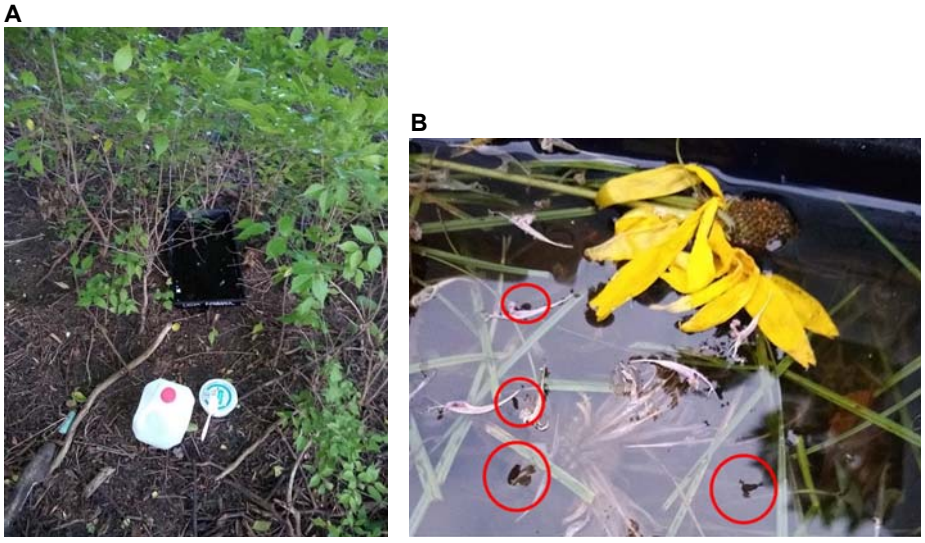

FIGURE 1. (*A*) Placement of a container of gravid H_2O used to collect *Culex* egg rafts. (*B*) Close-up of *Culex* egg rafts in a pan (egg rafts circled in red).

1. Place a black tub containing 4–11 L (1–3 gallons) of gravid H_2O along the edge of forested or semiforested areas. For best results, place one to three tubs at least 12 m apart within a site and select three to 10 sites (>1.5-km-apart) within a study locale.

   In our experience, this setup allows researchers to collect the highest number of Culex *egg rafts, which will allow for a great degree of genetic diversity and improve your chances of establishing a viable colony. Placing the tubs near vegetation allows researchers to easily access to the traps while ensuring that the traps are close to mosquitoes' resting habitat (Fig. 1A).*

2. Monitor the tubs daily for the presence of eggs, as egg rafts hatch between 24 and 72 h after being laid, depending on temperature (Miller et al. 2015).

3. Remove egg rafts (Fig. 1B) from the gravid traps using plastic utensils.

   White spoons are especially useful, as they provide a strong contrast with the black egg rafts.

   Although mosquito larvae can also be collected from gravid traps using large syringes or turkey basters, researchers will likely find it helpful to collect individual egg rafts as each raft is laid by a single female, meaning that only one to three of the larvae emerging from that egg raft need to be morphologically and/or genetically identified. Moreover, collecting unique egg rafts that belong to a single mosquito species will allow researchers to easily ascertain the effective population size and genetic diversity of their initial colony.

4. Place eggs into plastic deli-cup-sized containers lined with dry paper towels or filter paper.

   As a small amount of the gravid H_2O will also be transferred with the egg rafts, using dry paper towels ensures that the eggs will not be too moist and will adequately stick to the paper material until they are transported to the laboratory.

5. If mosquito larvae are present in the traps, remove them by using a plastic transfer pipette or large syringe/turkey baster and place them into plastic deli cups (250- to 500-mL) and seal with leak-proof lids.

6. After an adequate number of mosquito eggs/larvae have been collected (~500–1000 egg rafts or 2500–150,000 *Culex* larvae), dump the gravid H_2O from the tubs. Bring the mosquitoes and tubs back to the laboratory.

   The last step ensures that researchers are not creating additional breeding habitats for container-breeding mosquitoes that transmit disease.

Species Separation

After Culex *egg rafts or putative* Culex *larvae are collected, the next step to establishing a robust colony is to identify and separate your desired species from other mosquito species.*

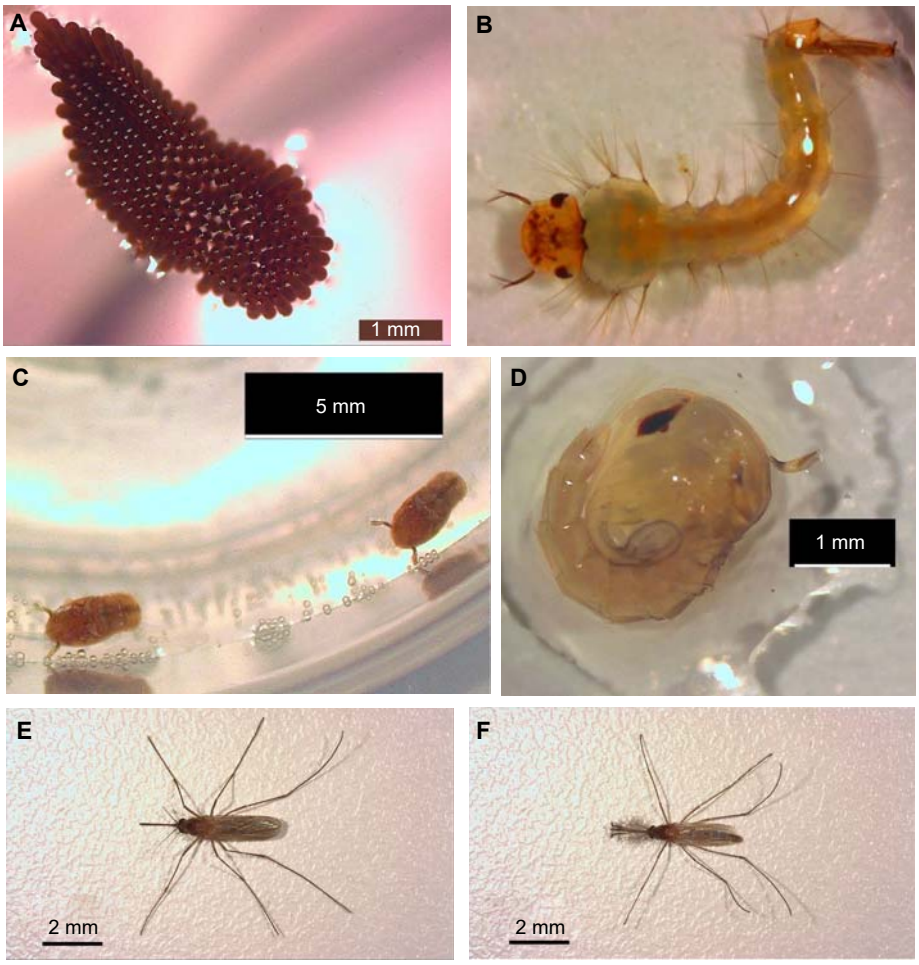

FIGURE 2. Life stages of laboratory-reared *Culex pipiens*. (*A*) A representative egg raft under a microscope. (*B*) A single larva of *Culex pipiens*. (*C*) Dorsal view of two pupae. (*D*) Lateral view of one pupa. (*E*) Nondiapausing female. (*F*) Adult male.

7. If egg rafts were collected, place a single raft into an individual plastic deli cup with ~100 mL distilled H_2O (Fig. 2A).
8. Place the containers with egg rafts in an environmental chamber set to long-day conditions (>13 h light/d) and a high temperature (>24°C).

   These conditions will prevent the mosquitoes from entering their overwintering dormancy upon reaching adulthood (Eldridge 1968; Spielman and Wong 1973).
9. Monitor the containers daily, and upon seeing first-instar larvae, add a small amount of dry fish food (~25 mg) to the container.

   Waiting until the larvae emerge is helpful, as some egg rafts may never hatch, and it is easier to see the larvae in clean H_2O. Additionally, waiting to add the food until larvae are present limits bacterial growth and promotes greater larval survival.
10. Provide the larvae with more food according to the feeding schedule in Protocol: **Rearing and Maintaining a *Culex* Colony in the Laboratory** (Meuti et al. 2023a).

Identifying *Culex* Larvae

Morphological Identification

Although Culex *larvae can be identified as early as the first instar (Dodge 1966; Haeger and O'Meara 1983; Jackson and Paulson 2006), in our experience, larger third- and fourth-instar larvae allow researchers to see diagnostic differences between species more clearly.*

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11. If *Culex* larvae from individual egg rafts were maintained separately, morphologically identify one to three larvae (Fig. 2B) by using a dissecting microscope and a published key.

    Darsie and Ward (2005) as well as Gaffigan (2021) provide excellent keys for identifying Culex *larvae.*

12. However, if egg rafts were maintained together and/or if putative *Culex* larvae were collected, morphologically identify each larva with a key.

PCR-Based Identification

Although determining the species of Culex *larvae is much easier than identifying pupae and adults (Michener 1947; Harrington and Poulson 2008), researchers will likely benefit from confirming morphological identifications with PCR-based assays to ensure that they have the desired species, especially if researchers are relatively inexperienced in identifying mosquitoes visually. Moreover, some PCR-based assays allow researchers to distinguish between hybrids and/or different subspecies, particularly within the* Cx. pipiens *complex. For example, Crabtree et al. (1995) provide a protocol and primers to distinguish* Cx. pipiens*,* Cx. restuans*, and* Cx. salinarius*; Smith and Fonseca (2004) describe how to distinguish* Cx. pipiens *from* Cx. quinquefasciatus *and their hybrids; and Bahnck and Fonseca (2006) provide instructions to estimate the proportion of introgression between autogenous* Cx. pipiens molestus *and anautogenous* Cx. pipiens pipiens.

13. Isolate genomic DNA (gDNA) from one to five larvae by using a DNA extraction kit.

    One single larva contains enough gDNA for subsequent PCR assays. However, researchers may elect to isolate RNA from a group of larvae, either originating from the same egg raft or from multiple egg rafts, to confirm morphological identifications.

14. Mix the extracted DNA with PCR master mix and species-specific primers to amplify the genomic fragments of interest in a thermocycler, per manufacturer instructions.

15. After PCR amplification, separate the DNA fragments using gel electrophoresis (see protocols in Smith and Fonseca 2004 and Bahnck and Fonseca 2006 for details).

16. Determine the species identity based on the presence/absence and sizes of various PCR products.

Rearing the Identified Larvae to Adulthood

17. Pool larvae belonging to the desired species by placing them in plastic shoe-box-sized containers.

    This strategy is used because makes it more likely that the larvae will survive to adulthood. Moreover, pooling larvae from multiple egg rafts into larger containers helps to retain the genetic diversity of the population that could otherwise be lost if bacterial growth in one or several small containers of larvae derived from a unique egg raft were to excessively grow, causing all or most of the larvae to die.

18. Estimate the number of mosquito larvae in each container and add an appropriate amount of larval food to the containers, by using the guidelines in Protocol: **Rearing and Maintaining a *Culex* Colony in the Laboratory** (Meuti et al. 2023a).

19. Upon pupation (Fig. 2C,D), with a plastic transfer pipette, carefully move pupae from their larval containers into an open plastic deli cup half-filled with H_2O derived from the larval pan, which is then placed into a mesh cage where the adults can emerge (Fig. 2E,F).

    We recommend placing pupae derived from field-collected eggs or larvae into large cages (>30 cm^3; cages 62 cm^3 are ideal) and placing black paper or tape along the bottom third of the exterior of the cage. This serves as a marker that allows males of Culex *to form swarms within the cage and may increase mating success.*

20. Provide emerging adults in cages with sugar sources that simulate natural sources, such as honey-soaked sponges and/or raisins, in addition to a 10% sucrose solution.

    Offering a variety of sucrose sources should facilitate the health of both male and female mosquitoes and ensure that they successfully mate in the confined conditions of a cage.

21. (Optional) To ensure that mating takes place, use forceps to dissect the spermathecae from a subset of females under a compound light microscope and examine them for the presence of sperm according to Bellamy and Reeves (1963).

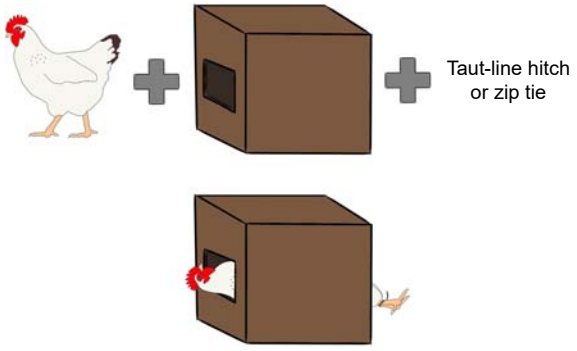

FIGURE 3. Cartoon diagram showing how to restrain a chicken for blood feeding. Supplies that are needed include a small cardboard box (L × W × H of ~25.4-cm × 20-cm × 20-cm) with holes cut on either side, a live rooster or hen, and either plastic ties or a rope tied in a taut line hitch. First place the tie/hitch around the chicken's legs. Then carefully and gently place the head of the chicken through one end of the box and the feet through the other. The chicken should be oriented on its back or side. Once the chicken is safely restrained, its feet can be placed inside the cloth sleeve of a mosquito cage. The sleeve should be secured with an additional zip tie to prevent mosquitoes from escaping.

Blood Feeding Field-Collected Mosquitoes

22. Allow females to age for 2–5 d. Then, to encourage the females to take a blood meal, remove all sucrose solutions for 24–48 h before blood feeding (3–7 d after adult emergence).
23. During this period, provide a H_2O source (e.g., via wet sponge or cotton) to ensure that males and females do not become dehydrated and die.

    However, as dehydrated female mosquitoes are more likely to bite (Hagan et al. 2018), it might be helpful to remove the H_2O source 2–4 h before blood feeding.

24. Blood feed female mosquitoes (see Introduction: **Points to Consider When Establishing and Rearing *Culex* Mosquitoes in the Laboratory** [Meuti et al. 2023b] for options or Protocol: **Rearing and Maintaining a *Culex* Colony in the Laboratory** [Meuti et al. 2023a] for specifics).

    As most Culex *mosquitoes are crepuscular, offering a blood meal at dawn or dusk will likely stimulate blood feeding. Additionally, we recommend offering the first blood meal 3 d after emergence. Whenever possible, we also recommend offering a live vertebrate host (Galun 1967). Blood meal analyses are common for a wide range of* Culex *in the United States and Canada, and you should investigate the host preferences of your target species in advance. To establish laboratory colonies of* Cx. pipiens*, we have worked with the Institutional Animal Care and Use Committee and Poultry Sciences at The Ohio State University to develop a protocol to restrain a live hen or rooster in a small cardboard box (Fig. 3).*

25. (Optional) If field-collected *Culex* females do not feed well, offer a blood meal every day or two until mosquitoes blood feed.

Collecting Eggs from Blood-Fed Females

26. Two days after blood feeding, create an infusion (oviposition H_2O) of freshly cut grass clippings and 500 mL of distilled H_2O. Age the infusion by setting it for ~48 h at room temperature or in an incubator/growth chamber set to ~25°C.
27. Four days after blood feeding, place ~200 mL of oviposition H_2O in a plastic deli cup inside the mosquito cage.
28. Monitor oviposition H_2O daily for the presence of egg rafts. Transfer egg rafts to clean plastic deli cups with distilled H_2O.

    Clean H_2O makes it easier to count the larvae.

29. Record the number of egg rafts that were collected as an accurate measure of the genetic diversity of the newly established laboratory strain.

Establish the Colony

30. After the larvae have hatched from the egg rafts, count approximately 200 of the first-generation larvae (F_1) and place them in shoe-box-sized plastic containers with ~450 mL distilled H_2O.

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31. Follow the procedures in Protocol: **Rearing and Maintaining a *Culex* Colony in the Laboratory** (Meuti et al. 2023a) to rear the larvae to adulthood. Place F_1 pupae inside new cages, and blood feed females as previously described in Step 24.
32. Continue rearing and blood feeding to establish a colony, allowing each new generation of mosquitoes to emerge within a new cage.

Wash Mosquito Containers and Cages

33. After any containers are no longer needed for rearing, wash them with a 3%–5% bleach solution, scrubbing the mosquito pan thoroughly and vigorously.

    Alternatively, soap can be used to clean mesh cages where adult mosquitoes are housed. Soap should be used sparingly, and the containers should be rinsed several times to remove all residue.

34. Rinse the containers at least three times with H_2O, as bleach residue will kill mosquito larvae and pupae.

DISCUSSION

As previously mentioned, establishing a laboratory colony of *Culex* mosquitoes from field-collected individuals is quite difficult. Some species of *Culex* are better suited to laboratory rearing than others, likely because of species-specific differences in stenogamy (the ability to mate in confined spaces) and the likelihood that mosquitoes will blood feed. While *Cx. pipiens* and *Cx. quinquefasciatus* are stenogamous and will mate within laboratory cages (Laven 1951; Sebastian and de Meillon 1967; Kim et al. 2018), *Cx. restuans* will not (Eldridge et al. 1972). To facilitate mating, it may be helpful to allow field-collected mosquitoes to emerge in large cages (>60-cm^3). Furthermore, wrapping the bottom third of the exterior of the cage with black paper or tape and/or lining the half of the bottom of the cage with black paper and the other half with white paper may also facilitate mating by providing markers allowing *Culex* males to know where to swarm within the cage. Sebastian and de Meillon (1967) report that for *Cx. pipiens fatigans* ratios of two males to one female facilitate the greatest mating success in the laboratory and that females typically mate 3–4 d after adult emergence within the laboratory. If mating is a concern, researchers can dissect the spermathecae of female *Culex* to examine them for the presence of sperm (Bellamy and Reeves 1963; Kim et al. 2018).

The other major hurdle to establishing a laboratory population of *Culex* is inducing females to blood feed. We discuss this in Introduction: **Points to Consider When Establishing and Rearing *Culex* Mosquitoes in the Laboratory** (Meuti et al. 2023b), as well as in Protocol: **Rearing and Maintaining a *Culex* Colony in the Laboratory** (Meuti et al. 2023a). Notably, even if researchers are unable to establish a breeding population of *Culex* mosquitoes, following the protocol that we have detailed here will allow scientists to collect *Culex* from the field and rear at least one generation in the laboratory. In doing so, researchers can gather important biological information about different populations and species of *Culex* mosquitoes. This information includes the developmental rates and responsiveness of *Culex* mosquitoes to various temperatures, photoperiodic conditions, and food sources, as well as their mating behavior under controlled conditions.

RECIPE

Gravid H_2O for Mosquito Larvae Collection

Add 17.3 g of milk protein powder, 3.35 g of baker's yeast, and a large handful of moldy or freshly cut grass clippings to 11.35 L (3 gallons) of tap H_2O. Allow the mixture to ferment for 3 d outside in the summertime or at least 4 d at 27°C.

ACKNOWLEDGMENTS

We thank Robert Aldridge, Josh Benoit, David Denlinger, Leeanne Garrett, Woodbridge Foster, Joe Rinehart, Rebecca Robich, David Shetlar, and Cheolho Sim for helping us to identify locations and trapping techniques to collect mosquitoes and for assistance in developing these protocols. We also thank members of the Meuti Laboratory, past and present, for their diligence in rearing mosquitoes. M.E.M. and M.W. were supported by a National Science Foundation grant (IOS 1944324), and M.E.M. and A.S. were supported by a National Institutes of Health grant (R21-AI144266).

REFERENCES

Allan SA, Kline D. 2004. Evaluation of various attributes of gravid female traps for collection of *Culex* in Florida. *J Vector Ecol* **29:** 285–294.

Bahnck CM, Fonseca DM. 2006. Rapid assay to identify the two genetic forms of *Culex* (*Culex*) *pipiens L.* (Diptera: Culicidae) and hybrid populations. *Am J Trop Med Hyg* **75:** 251–255. doi:10.4269/ajtmh.2006.75.2.0750251

Bellamy RE, Reeves WC. 1963. The winter biology of *Culex tarsalis* (Diptera: Culicidae) in Kern County, California. *Ann Entomol Soc Am* **56:** 314–323. doi:10.1093/aesa/56.3.314

Burkett-Cadena ND, Mullen GR. 2008. Comparison of infusions of commercially available garden products for collection of container-breeding mosquitoes. *J Am Mosq Control Assoc* **24:** 236–243. doi:10.2987/5597.1

Crabtree MB, Savage HM, Miller BR. 1995. Development of a species-diagnostic polymerase chain reaction assay for the identification of *Culex* vectors of St. Louis encephalitis virus based on interspecies sequence variation in ribosomal DNA spacers. *Am J Trop Med Hyg* **53:** 105–109. doi:10.4269/ajtmh.1995.53.105

Darsie RF, Ward RA Jr. 2005. *Identification and geographical distribution of the mosquitoes of North America, north of Mexico*. American Mosquito Control Association, Mount Laurel, NJ.

Dodge HR. 1966. Studies on mosquito larvae: II. The first-stage larvae of North American Culicidae and of world Anophelinae. *Can Entomol* **98:** 337–393. doi:10.4039/Ent98337-4

Eldridge BF. 1968. The effect of temperature and photoperiod on blood-feeding and ovarian development in mosquitoes of the *Culex pipiens* complex. *Am J Trop Med Hyg* **17:** 133–140. doi:10.4269/ajtmh.1968.17.133

Eldridge BF, Bailey CL, Johnson MD. 1972. A preliminary study of the seasonal geographic distribution and overwintering of *Culex restuans* Theobald and *Culex salinarius* Coquillet (Diptera: Culicidae). *J Med Entomol* **9:** 233–238. doi:10.1093/jmedent/9.3.233

Gaffigan TV. 2021. Identification key to the genera of mosquito larvae for the world. The Walter Reed Biosystematics Unit, retrieved from: http://www.wrbu.org/keys/WW_GN_L/Mosq_Gen_Worldwide_L.html.

Galun R. 1967. Feeding stimuli and artificial feeding. *Bull World Health Organ* **36:** 590–593.

Gjullin CM, Johnson JO, Jr FWP. 1965. The effect of odors released by various waters on the oviposition sites selected by two species of *Culex*. *Mosq News* **25:** 268–271.

Gubler DJ. 1971. Studies on the comparative oviposition behavior of *Aedes (Stegomyia) albopictus* and *Aedes (stegomyia) polynesiensis* Marks. *J Med Entomol* **8:** 675–682. doi:10.1093/jmedent/8.6.675

Haeger JS, O'Meara GF. 1983. Separation of first-instar larvae of four Florida *Culex* (*Culex*). *Mosq News* **43:** 76–77.

Hagan RW, Didion EM, Rosselot AE, Holmes CJ, Siler SC, Rosendale AJ, Hendershot JM, Elliot KSB, Jennings EC, Nine GA, et al. 2018. Dehydration prompts increased activity and blood feeding by mosquitoes. *Sci Rep* **8:** 6804. doi:10.1038/s41598-018-24893-z

Harrington LC, Poulson RL. 2008. Considerations for accurate identification of adult *Culex restuans* (Diptera: Culicidae) in field studies. *J Med Entomol* **45:** 1–8. doi:10.1093/jmedent/45.1.1

Jackson BT, Paulson SL. 2006. Seasonal abundance of *Culex restuans* and *Culex pipiens* in southwestern Virginia through ovitrapping. *J Am Mosq Control Assoc* **22:** 206–212.

Kim S, Trocke S, Sim C. 2018. Comparative studies of stenogamous behaviour in the mosquito *Culex pipiens* complex. *Med Vet Entomol* **32:** 427–435. doi:10.1111/mve.12309

Kramer WL, Mulla MS. 1979. Oviposition attractants and repellents of mosquitoes: oviposition responses of *Culex* mosquitoes to organic infusions. *Environ Entomol* **8:** 1111–1117. doi:10.1093/ee/8.6.1111

Laven H. 1951. Crossing experiments with *Culex* strains. *Evolution* **5:** 370–375. doi:10.1111/j.1558-5646.1951.tb02795.x

Michener CD. 1947. Mosquitoes of a limited area in Southern Mississippi. *Am Midl Nat* **37:** 325. doi:10.2307/2421659

Miller N, Mettelman RC, Baker SC, Harbison JE. 2015. Use of Centers for Disease Control and Prevention gravid trap in catch basins: proof-of-concept trials. *J Am Mosq Control Assoc* **31:** 196–199. doi:10.2987/14-6470R

Meuti ME, Siperstein A, Wolkoff M. 2023a. Rearing and maintaining a *Culex* colony in the laboratory. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot108080

Meuti ME, Siperstein A, Wolkoff M. 2023b. Points to consider when establishing and rearing *Culex* mosquitoes in the laboratory. *Cold Spring Harb Protoc* doi:10.1101/pdb.top107823

Popko DA, Walton WE. 2016. Large-volume gravid traps enhance collection of *Culex* vectors. *J Am Mosq Control Assoc* **32:** 91–102. doi:10.2987/moco-32-02-91-102.1

Sebastian A, de Meillon B. 1967. Experiments on the mating of *Culex pipiens fatigans* in the laboratory. *Bull World Health Organ* **36:** 47.

Smith JL, Fonseca DM. 2004. Rapid assays for identification of members of the *Culex* (*Culex*) *pipiens* complex, their hybrids, and other sibling species (Diptera: Culicidae). *Am J Trop Med Hyg* **70:** 339–345. doi:10.4269/ajtmh.2004.70.339

Spielman A, Wong J. 1973. Studies on autogeny in natural populations of *Culex pipiens*. 3. Midsummer preparation for hibernation in anautogenous populations. *J Med Entomol* **10:** 319–324. doi:10.1093/jmedent/10.4.319

Zulueta JD. 1950. Comparative oviposition experiments with caged mosquitoes. *Am J Epidemiol* **52:** 133–142. doi:10.1093/oxfordjournals.aje.a119413

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Cold Spring Harb Protoc; doi: 10.1101/pdb.prot108079 originally published online February 22, 2023