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Project Page Link: <https://review.jove.com/account/file-uploader?src=21970723>

Title: Protocols for Efficient Rearing and Functional Analysis of the Endoparasitoid Wasp *Asobara japonica* and its Host *Drosophila melanogaster*

Authors and Affiliations:

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes, all done!**

If **Yes**, can you record movies/images using your own microscope camera? **Yes**

SCOPE shots: 2.2.2, 2.5.1, 3.1.2, 3.5.2, 3.6.1, 4.4.1, 4.4.2, 4.5.2, 4.7.4, 4.11.1, 4.11.2, 4.12.2

SCOPE shot 4.7.4 requires the scope kit.

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **Yes, on the second and third floors of the same building.**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 23

Number of Shots: 56 (11 SCOPE shots)

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Hayato Kondo:** Our research investigates the mechanisms by which parasitoid wasps manipulate their hosts, aiming to identify the key molecules critical for successful parasitism.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Takumi Kamiyama:** This protocol improves rearing efficiency and reproducibility by coordinating the parasitoid and host developmental timing and optimizing the injection protocol.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Anastasiia Staroverova:** This protocol allows researchers to investigate the molecular mechanisms underlying parasitoid-host interactions.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Yuko Shimada-Niwa:** Maintaining wasps and host flies in good condition and completing the infection assay within 15 minutes ensures reproducible results.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

1.5. **Hayato Kondo:** This protocol will facilitate comprehensive analyses of gene functions involved in parasitism and host manipulation.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

Note: The timestamps for SCOPE shots are added by the postshoot note integrator at the postshoot stage. Please contact Kathyayani Sridharan for any queries regarding SCOPE shots.

2. Laboratory Rearing of the Host *Drosophila melanogaster* and Endoparasitoid *Asobara japonica*

Demonstrator: Anastasiia Staroverova

- 2.1. To begin, transfer the adult Oregon-R, or OR (*O-R*)- strain flies maintained at 25 degrees Celsius under a 12-hour light-dark cycle to a new food vial containing standard cornmeal-yeast-agar medium [1]. Label the vial with the date of transfer and the strain name [2].
 - 2.1.1. WIDE: Talent transferring adult flies to a new food vial containing standard cornmeal-yeast-agar medium. *Videographer: Please obtain CU shot as well for this shot.*
 - 2.1.2. Talent labeling the food vial with the date and strain name OR.
- 2.2. Clear the adult flies from the vial, one day after the transfer, to prevent excessive number of eggs in the food [1]. Ensure that the offspring larvae are feeding on the surface of the food [2].
 - 2.2.1. Talent removing adult flies from the food vial.
 - 2.2.2. SCOPE: 2_2_2_20260701_Tsukuba. 00:11–00:19
- 2.3. On the fourth day after egg laying, transfer a spoonful of fly larvae to a new food vial, keeping them unexposed to parasitoids for the next generation and to control the host larval density [1].
 - 2.3.1. Talent transferring a spoonful of fly larvae to a new food vial.
- 2.4. For rearing the endoparasitoid wasp, transfer 10 to 20 adult wasps of Tokyo, or TK (*T-K*) strain to the new vial containing fly larvae [1] and label it with the transfer date and the wasp strain name [2].
 - 2.4.1. Talent transferring adult wasps into the vial containing fly larvae.
 - 2.4.2. Talent labeling the vial with the date and strain name.
- 2.5. Allow the adult wasps to oviposit in the fly larvae for 1 to 2 days [1], then remove the wasps to prevent superparasitism [2]. After 10 days, remove the eclosed adult flies that escaped parasitism [3]. Two weeks later, transfer the newly eclosed adult wasps to a

fresh food vial containing sterilized filter paper with 25 percent glucose and 50 millimolar Vitamin C solution [4].

2.5.1. SCOPE: 2_5_1_20260701_Tsukuba. 01:41–01:45; 01:59–02:03

2.5.2. Talent removing adult wasps from the vial.

2.5.3. Talent removing eclosed adult flies from the vial.

2.5.4. Talent transferring newly eclosed adult wasps to a new food vial.

3. Single-Oviposition Infection Assay

Demonstrator: Hayato Kondo

3.1. To synchronize the developmental stage of fly larvae, place adult flies in a 50-milliliter tube containing a grape-juice agar plate spread with fresh yeast paste to allow egg laying for 24 hours [1]. Transfer 20 to 30 newly hatched first-instar larvae from the grape-juice agar plate to a 12-milliliter vial containing 2 to 3 grams of fly food paste, allowing them to grow at 25 degrees Celsius for 3 days [2].

3.1.1. Talent placing adult flies into a 50-milliliter tube containing a grape-juice agar plate.

3.1.2. SCOPE: 3_1_2_20260701_Tsukuba. 00:03–00:09; 00:18–00:23

3.2. Prepare a new small vial containing an appropriate amount of fly food paste for rearing host larvae after the infection assay [2]. Prepare the infection assay arena in a 4-centimeter Petri dish containing a thin and evenly spread fly food paste layer covering approximately 1.5 to 2.0 centimeters in diameter [1]. **Note:** The VO is edited for inverted shots

3.2.2. Talent preparing a small vial containing fly food paste. **Note:** 3.2.2 was placed before 3.2.1

3.2.1. Talent preparing the infection assay arena in a 4-centimeter Petri dish.

3.3. Collect the larvae at the third-instar stage and transfer them to a dish containing deionized water for rinsing to remove residual food from their bodies [1]. Gently remove excess moisture from the larvae using lint-free paper wipes [2]. Transfer the larvae to the infection assay arena [3].

3.3.1. Talent transferring staged larvae into a dish containing deionized water.

3.3.2. Talent drying larvae with lint-free paper wipes.

3.3.3. Talent transferring larvae to the infection assay arena.

3.4. Gently place approximately 3 anesthetized adult wasps into the infection assay arena [1]. Close the lid and allow the wasps to recover on the fly food paste [2].

- 3.4.1. Talent placing female wasps into the infection assay arena.
- 3.4.2. Talent closing the Petri dish lid.
- 3.5. Observe the oviposition behavior of the wasps under a stereomicroscope [1]. Confirm infection for a standardized time of 15 minutes, when a wasp inserts its ovipositor into a moving fly larva, vibrates its abdomen, deposits an egg, and withdraws the ovipositor [2].
 - 3.5.1. Talent placing the Petri dish containing the infection arena under a stereomicroscope.
 - 3.5.2. SCOPE: 3_5_2_20260701_Tsukuba. 00:03–00:09
- 3.6. After infection, use forceps to transfer the infected fly larva to a new small vial containing fly food paste [1]. Label the vial with the date, sample name, infection time, and number of individuals [2]. Place the vial in a humid container and maintain it at 25 degrees Celsius [3].
 - 3.6.1. SCOPE: 3_6_1_20260701_Tsukuba. 00:10–00:13; 00:17–00:20
 - 3.6.2. Talent labeling the small vial with experimental information.
 - 3.6.3. Talent placing the vial into a humid container.

4. Generation of RNA interference (RNAi) wasps by dsRNA injection

Demonstrator: Takumi Kamiyama

- 4.1. Prepare double-stranded RNAs, or dsRNA (*D-S-RNA*) of 400 base pairs or larger using the T7 RiboMAX (*T-seven-Ribo-Max*) Express RNA interference system, as per manufacturer's instructions [1]. Store the dsRNA samples at minus 20 to minus 80 degrees Celsius until use [2].
 - 4.1.1. Talent setting up the T7 RiboMAX Express RNA interference system.
 - 4.1.2. Talent placing double-stranded RNA samples into cold storage.
- 4.2. Prepare a 5.5-centimeter Petri dish filled two-thirds with 2% agar to create a thick agar layer for injection [1]. Place a lint-free paper wipe moistened with water on the lid of a plastic dish [2].
 - 4.2.1. Talent preparing a 5.5-centimeter Petri dish containing agar.
 - 4.2.2. Talent placing a moistened lint-free paper wipe on a plastic dish lid.
- 4.3. At 7 days post-infection, add a drop of water to the vial wall [1] to help detach the host fly pupal cases [2]. Use forceps to collect host fly pupae from the vial [3]. Place them on the prepared lint-free paper wipe [4].
 - 4.3.1. Talent adding a drop of water to the vial wall.

- 4.3.2. Shot of the fly pupal case getting detached from the vial.
- 4.3.3. Talent collecting host fly pupae from the vial using forceps.
- 4.3.4. Talent placing pupae on the moistened lint-free paper wipe.
- 4.4. Use forceps to remove the host fly pupal case and expose the wasp body [1], confirming that the head, thorax, and abdomen are distinguishable, and that the wasp body is immobile [2].
 - 4.4.1. SCOPE: 4_4_1&2_20260701_Tsukuba. 00:16–00:20; 00:27–00:30; 00:32–00:34
 - 4.4.2. SCOPE: 4_4_1&2_20260701_Tsukuba. 00:40–00:44; 00:49–00:53
- 4.5. Place 20 to 30 retrieved wasp bodies on the prepared agar plate [1]. After aligning the body axes in the same orientation, arrange them with the dorsal side facing upward in 1 to 2 lines [2].
 - 4.5.1. Talent placing retrieved wasp bodies on the agar plate.
 - 4.5.2. SCOPE: 4_5_2_20260701_Tsukuba. 00:09–00:16; 00:51–00:53
- 4.6. Set up the injection apparatus and stereomicroscope according to the manufacturer's instructions [1]. Place the agar plate so that the wasp heads are oriented toward the injection needle tip [2]. Set the injection volume to 50 nanoliters per wasp and the injection speed to 50 nanoliters per second on the injector [3].
 - 4.6.1. Talent setting up the injection apparatus and stereomicroscope.
 - 4.6.2. Talent positioning the agar plate relative to the injection needle.
 - 4.6.3. Talent setting the injection volume to 50 nL/s.
- 4.7. Use PC-10 (*P-C-ten*) needle puller to prepare glass capillary needles [1]. Adjust the shape by modifying the heater temperature [2]. Place the glass capillary needle on the needle grinder at an angle of 30 to 35 degrees [3]. Grind the needle until the tip length reaches 0.04 millimeters [4].
 - 4.7.1. Talent preparing glass capillary needles with a needle puller.
 - 4.7.2. Talent adjusting the heater temperature on the needle puller.
 - 4.7.3. Talent positioning the glass capillary needle on the needle grinder.
 - 4.7.4. SCOPE: 474. 00:13–00:20

Videographer: Please film this shot using the scope kit.
- 4.8. Before injection, thaw the dsRNA sample [1] and prepare a concentration of 0.25 to 1 microgram per microliter [2]. Add blue dye for visualization [3-TXT]. Use a microloader pipette tip to fill the glass capillary with 4 microliters of the dsRNA solution, ensuring there are no air bubbles [4].
 - 4.8.1. Talent thawing the double-stranded RNA sample.

- 4.8.2. Talent preparing the required concentration of double-stranded RNA solution
- 4.8.3. Talent adding the blue dye to the dsRNA solution. **TXT: Blue Dye: Erioglaucine disodium salt, 2 % w/v**
- 4.8.4. Talent filling the glass capillary with the double-stranded RNA solution.
- 4.9. Use a 1-milliliter syringe and a needle to fill the remaining portion of the glass capillary with mineral oil without air bubbles [1]. Attach the glass capillary needle containing the dsRNA solution and mineral oil to the needle holder [2], then mount it onto the injection device [3].
 - 4.9.1. Talent filling the glass capillary with mineral oil using a syringe and needle.
 - 4.9.2. Talent attaching the glass capillary needle to the needle holder.
 - 4.9.3. Talent mounting the loaded glass capillary needle onto the injection device.
- 4.10. Adjust the position of the agar plate and glass capillary needle on the injector so that the needle is oriented at a 30-to-45-degree angle relative to the wasp bodies [1].
 - 4.10.1. Talent adjusting the agar plate and glass capillary needle alignment.
- 4.11. Insert the glass capillary needle into the slightly lateral side of the dorsal midline of the wasp body [1] and microinject the dsRNA solution, confirming successful injection via the blue dye inside the wasp body [2]. **Note: The timestamps are provided for the combined shots 4.11.1 and 4.11.2, as separating the 2 steps is difficult.**
 - 4.11.1. SCOPE: 4_11_1&2_20260701_Tsukuba 00:13–00:32
 - 4.11.2. ~~SCOPE: 4_11_1&2_20260701_Tsukuba Talent microinjecting the double-stranded RNA solution.~~ **Note: This shot is removed at the postshoot stage by the postshoot integrator, as the timestamp for this shot is included in 4.11.1.**
Videographer: Please zoom in to film the blue coloration appearing in the wasp body after successful injection.
- 4.12. After injection, place the agar plates in a humidity-controlled container, in an incubator for 1 week until adult wasps eclose [1]. Remove injured wasp bodies from the agar plate to prevent bacterial contamination [2].
 - 4.12.1. Talent placing the agar plates in an incubator.
 - 4.12.2. SCOPE: 4_12_2_20260701_Tsukuba. 00:16–00:20

Results

5. Results

- 5.1. The evaluation of the parasitism success after single-oviposition infection assay under different rearing conditions showed an 81.7% parasitism success rate with normal-yeast diet [1] and 35.0% when yeast content was reduced to 10 percent [2].
 - 5.1.1. LAB MEDIA: Figure 2C. *Video Editor: Please emphasize the peach color region in the second bar along with its X-axis label "+", the text 1X on top, and the legends "Wasp adult" and "A. japonica infected."*
 - 5.1.2. LAB MEDIA: Figure 2C. *Video Editor: Please emphasize the peach color region in the fourth bar along with its X-axis label "+", the text 0.1X on top, and the legends "Wasp adult" and "A. japonica infected."*
- 5.2. Similarly, while a yeast-poor diet delayed host eclosion by up to 9 days [1], it delayed the wasp eclosion by only 4–5 days [2].
 - 5.2.1. LAB MEDIA: Figure 2D. *Video Editor: Please emphasize the space between black and grey lines.*
 - 5.2.2. LAB MEDIA: Figure 2D. *Video Editor: Please emphasize the space between peach and orange lines.*
- 5.3. The RNA interference, or RNAi (*R-N-A-eye*), knockdown of the wasp venom gene, imaginal disc degradation factor 1, or IDDF-1 (*I-D-D-F-one*)—conducted to evaluate the efficiency of the optimized protocol—showed significantly decreased expression levels in eclosed adult wasps [1] when compared against the control [2].
 - 5.3.1. LAB MEDIA: Figure 3D. *Video Editor: Please emphasize the height of the small bar corresponding to X-axis label "IDDF-1 RNAi"*
 - 5.3.2. LAB MEDIA: Figure 3D. *Video Editor: Please emphasize the height of the large bar corresponding to X-axis label "Control (GFP RNAi)"*
- 5.4. Functional validation using a single-oviposition infection assay with these IDDF-1 knockdown wasps and host fly larvae expressing a wing-pouch apoptosis reporter revealed a significantly reduced apoptotic signal [1] compared to the control [2].
 - 5.4.1. LAB MEDIA: Figure 3E. *Video Editor: Please emphasize the low green fluorescence in the panel labelled "IDDF-1 RNAi" in the "GC3Ai" row.*
 - 5.4.2. LAB MEDIA: Figure 3E. *Video Editor: Please emphasize the increased green fluorescence in the panel labelled "Control (GFP RNAi)" in the "GC3Ai" row.*

Pronunciation Guide:

- *Drosophila melanogaster*
Pronunciation link: <https://www.merriam-webster.com/dictionary/Drosophila>
IPA: /drə'säfələ/
Phonetic Spelling: druh·sof·uh·luh
- *Asobara japonica*
Pronunciation link: No confirmed link found
IPA: /,ɑ:sos'ba:rə dʒə'pɑ:nɪkə/
Phonetic Spelling: ah·soh·baa·ruh juh·pon·ih·kuh
- *Endoparasitoid*
Pronunciation link: <https://www.merriam-webster.com/dictionary/endoparasite>
IPA: /,endoʊ'perə,sɪ,tɔɪd/
Phonetic Spelling: en·doh·pair·uh·sih·toyd
- *Cornmeal-yeast-agar*
Pronunciation link: <https://www.merriam-webster.com/dictionary/agar>
IPA: /'eɪ,gɑ:r/
Phonetic Spelling: ay·gahr
- *Oviposit*
Pronunciation link: <https://www.merriam-webster.com/dictionary/oviposit>
IPA: /,oʊvə'pɑ:zɪt/
Phonetic Spelling: oh·vuh·poz·it
- *Superparasitism*
Pronunciation link: No confirmed link found
IPA: /,su:pər'perə,sɪtɪzəm/
Phonetic Spelling: soo·per·pair·uh·sih·tih·zum
- *Eclosed*
Pronunciation link: <https://www.merriam-webster.com/dictionary/eclose>
IPA: /ɪ'kloʊz/
Phonetic Spelling: ih·kloh
- *Sterilized*
Pronunciation link: <https://www.merriam-webster.com/dictionary/sterilize>
IPA: /'sterə,ləɪz/
Phonetic Spelling: stair·uh·lyz
- *Millimolar*
Pronunciation link: <https://www.merriam-webster.com/dictionary/molar>
IPA: /,mɪlə'moʊlər/
Phonetic Spelling: mil·uh·moh·ler
- *Synchronization*
Pronunciation link: <https://www.merriam-webster.com/dictionary/synchronize>
IPA: /'sɪŋkrə,nəɪzɪʃən/
Phonetic Spelling: sing·kruh·ny·zay·shuhn

- First-instar
Pronunciation link: <https://www.merriam-webster.com/dictionary/instar>
IPA: /'ɪnˌstɑːr/
Phonetic Spelling: in·star
- Petri
Pronunciation link: <https://www.merriam-webster.com/dictionary/Petri%20dish>
IPA: /'piːtri/
Phonetic Spelling: pee·tree
- Deionized
Pronunciation link: <https://www.merriam-webster.com/dictionary/deionize>
IPA: /di'æɪˌnaɪzd/
Phonetic Spelling: dee·eye·uh·nyzd
- Anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>
IPA: /ə'nesθəˌtaɪzd/
Phonetic Spelling: uh·ness·thuh·tyzd
- Stereomicroscope
Pronunciation link: <https://www.merriam-webster.com/dictionary/stereomicroscope>
IPA: /ˌsteri.əʊˈmaɪkrəˌskoʊp/
Phonetic Spelling: stair·ee·oh·my·kruh·skohp
- Oviposition
Pronunciation link: <https://www.merriam-webster.com/dictionary/oviposition>
IPA: /ˌoʊvəpəˈzɪʃən/
Phonetic Spelling: oh·vuh·puh·zish·uhn
- Ovipositor
Pronunciation link: <https://www.merriam-webster.com/dictionary/ovipositor>
IPA: /ˌoʊvəˈpɑːzətər/
Phonetic Spelling: oh·vuh·poz·uh·ter
- RNA interference
Pronunciation link: <https://www.merriam-webster.com/dictionary/RNA>
IPA: /ˌɑːrˌenˈeɪˌɪntərˈfɪərəns/
Phonetic Spelling: ar·en·ay in·ter·feer·uhns
- dsRNA
Pronunciation link: No confirmed link found
IPA: /ˌdiːˌes ɑːr ɛnˈeɪ/
Phonetic Spelling: dee·ess·ar·en·ay
- RiboMAX
Pronunciation link: No confirmed link found
IPA: /ˈraɪboʊˌmæks/
Phonetic Spelling: rye·boh·maks
- Capillary
Pronunciation link: <https://www.merriam-webster.com/dictionary/capillary>

IPA: /'kæpə,leri/

Phonetic Spelling: kap·uh·lair·ee

- Microliter

Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>

IPA: /'maɪkroʊ,li:tər/

Phonetic Spelling: my·kroh·lee·ter

- Nanoliter

Pronunciation link: <https://www.merriam-webster.com/dictionary/nanoliter>

IPA: /'nænoʊ,li:tər/

Phonetic Spelling: nan·oh·lee·ter

- Microinject

Pronunciation link: <https://www.merriam-webster.com/dictionary/microinject>

IPA: /,maɪkroʊɪn'dʒekt/

Phonetic Spelling: my·kroh·in·jekt

- Erioglaucine

Pronunciation link: No confirmed link found

IPA: /ɪ,raɪoʊ'glɔːsiːn/

Phonetic Spelling: ih·rye·oh·glaw·seen

- Disodium

Pronunciation link: <https://www.merriam-webster.com/dictionary/disodium>

IPA: /dɑɪ'soʊdiəm/

Phonetic Spelling: dye·soh·dee·uhm