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Measuring Crop Motility and Food Passaging in Drosophila

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1 TITLE:
2 **Measuring Crop Motility and Food Passaging in *Drosophila***
3
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20 **KEYWORDS:**
21 crop, gastrointestinal, *Drosophila*, spectrophotometry, contraction, gut
22
23 **SUMMARY:**
24 The goal of this protocol is to measure crop contraction and quantify food distribution in the
25 *Drosophila* gut.
26
27 **ABSTRACT:**
28 Most animals use the gastrointestinal (GI) tract to digest food. The movement of the ingested
29 food in the GI tract is essential for nutrient absorption. Disordered GI motility and gastric
30 emptying cause multiple diseases and symptoms. As a powerful genetic model organism,
31 *Drosophila* can be used in GI motility research. The *Drosophila* crop is an organ that contracts
32 and moves food into the midgut for further digestion, functionally similar to a mammalian
33 stomach. Presented is a protocol to study *Drosophila* crop motility using simple measurement
34 tools. A method for counting crop contractions to evaluate crop motility and a method for
35 detecting the distribution of food dyed blue between the crop and gut using a
36 spectrophotometer to investigate the effect of the crop on food passaging is described. The
37 method was used to detect the difference in crop motility between control and *nprl2* mutant
38 flies. This protocol is both cost-efficient and highly sensitive to crop motility.
39
40 **INTRODUCTION:**
41 Most animals have a digestive tube called the gastrointestinal (GI) tract to absorb energy and
42 nutrients from the environment. The human GI tract is composed of four parts: the esophagus,
43 stomach, small intestine, and large intestine (colon). Food passage from the stomach to the
44 intestine is essential for nutrient absorption. Some effectors, such as aging, toxic drugs, and
45 infection, cause disordered GI tract motility and gastric emptying, which is related to some

diseases and their symptoms such as dyspepsia, gastroesophageal reflux disease, and constipation[1].

The fruit fly (*Drosophila melanogaster*) is a widely used model animal in biomedical research due to its easy genetic manipulation. Importantly, about 77% of genes associated with human disease have a homolog in *Drosophila*[2]. Research using *Drosophila* has made enormous advances in our understanding of many disease mechanisms. As a powerful genetic model organism, *Drosophila* is widely used in GI tract research[3]. *Drosophila* has a simpler digestive tract, which is divided into three discrete domains: foregut, midgut, and hindgut[4]. The crop, a part of the foregut, is a bag-like structure that serves as a site for ingested food storage. The midgut is a long tube and functions as the site for food digestion and nutrient absorption through the epithelial layer, which consists of absorptive enterocytes (ECs) and secretory enteroendocrine (EE) cells[5]. Interestingly, the stomach function in *Drosophila* is divided into two parts: the crop functions as food storage and the copper cell region (CCR) is a highly acidic region with a pH < 3[6]. In *Drosophila*, the ingested food is initially moved to the crop and subsequently pumped into the midgut[7]. Thus, the crop plays a critical role in food passaging. Enveloped by visceral muscles and consisting of a complex array of valves and sphincters, the crop keeps contracting and moving food into the midgut for further digestion.

This protocol allows for the detection of food movement from the crop to the midgut in *Drosophila*. Crop contraction is evaluated by counting crop contraction frequency. In addition, the effect of the crop on food passaging is investigated by detecting the food distribution between crop and gut. Furthermore, the food distribution can be used to reflect immediate food movement or basic food status using different feeding periods. Taken together, this protocol provides methods to rapidly evaluate crop motility and food passaging in *Drosophila*.

PROTOCOL:

1. Maintaining and preparing experimental flies

1.1. Maintain flies in vials containing 10 mL of freshly made food (1% agar, 2.4% brewer's yeast, 3% sucrose, 5% cornmeal) in an incubator at 25 °C with 60% humidity. Set the light cycle of the incubator to 12 light:12 dark.

1.2. To ensure that a large number of the desired genotype flies ecloses simultaneously, culture young flies (1−3 days old) in standard food with dry yeast on the surface for 3 days. Transfer the adults to a new food vial with standard food including wet yeast, for 2 days to allow egg laying. Leave the eggs in the incubator to develop and transfer adult flies to a new vial to collect more eggs.

1.3. Collect the eclosed male or female flies each day and culture them in new vials with standard food at the maintenance condition to the desired age.

NOTE: To get more same-age flies, multiple vials of the desired genotype may be set up simultaneously. The vials for adult fly culture should be changed every 3−5 days.

92 **2. Counting crop contractions**
93
94 2.1. Anesthetize the flies with CO_2 and take one fly into a dissecting plate well containing 200
95 µL of 1x phosphate buffered saline (PBS, pH = 7.4) composed of 136.89 mM NaCl, 2.67 mM
96 KCl, 8.1 mM Na_2HPO_4, and 1.76 mM KH_2PO_4.
97
98 2.2. Grasp the fly at its thorax using one pair of tweezers, smoothly open the thorax using
99 another pair of tweezers, and then pull the end in opposite directions to open the abdomen.
100 Take the crop and the gut out from the body carefully.
101
102 2.3. Wait for the fly to wake up and then visualize the crop and count the number of times it
103 contracts in 1 min.
104
105 NOTE: Only a complete wave on the crop lobes is counted as one contraction.
106
107 2.4. Repeat step 2.3 for 5x between 30 s intervals.
108
109 2.5. Calculate the average number of crop contractions per minute.
110
111 NOTE: During the contraction counting, the fly should be alive, and the gut should be intact
112 and attached at its anterior and posterior ends after dissection.
113
114 **3. Preparing dyed food**
115
116 3.1. Weigh and dissolve the blue dye (**Table of Materials**) in PBS at a concentration of 20%
117 (w/v).
118
119 3.2. Add the 20% blue dye into the boiled liquid maintenance food (step 1.1) with a 1:40
120 dilution to a final concentration of 0.5% (w/v) during the food cooling process.
121
122 NOTE: The blue dye is added before the food cooling down and mixed well with stirring. It is
123 optional to dissolve the blue dye in PBS; distilled water is also suitable.
124
125 **4. Feeding flies with dyed food**
126
127 4.1. Transfer groups of same-aged flies to the vials with starvation food (1% agar in distilled
128 water) for 4 h to ensure food intake.
129
130 4.2. Transfer the flies to new vials with food dyed blue and culture the flies for the desired
131 time.
132
133 NOTE: The feeding time is a critical factor and depends on the research purpose. Short feeding,
134 within the time of food passing through, can be used to evaluate the speed of food motility
135 from crop to gut. At the maintenance conditions, the food passes through in about 2 h.
136 However, the time of passing through might be related to culture conditions. Long feeding, up
137 to a few days, can be used to evaluate persistent food distribution status between crop and
138 gut.

139
140 **5. Dissecting flies and collecting dye samples in crop and gut**
141
142 5.1. Anesthetize the flies with CO_2 and take one fly into a dissecting plate well containing 200
143 µL of 1x PBS.
144
145 5.2. Grasp the fly at its thorax using one pair of tweezers and take the head off the body using
146 another pair of tweezers. Move the remaining body to a new well containing 200 µL of 1x PBS.
147
148 5.3. Wash the body 2x by gently shaking it in 200 µL of 1x PBS using a pair of tweezers to clean
149 the dye attached to the fly body.
150
151 5.4. Gently and smoothly open the abdomen using two pairs of tweezers and carefully
152 separate the whole gut from the body.
153
154 5.5. Carefully take off the crop from the whole gut and put it in a tube with 100 µL of 1x PBS.
155
156 5.6. Lastly, put the whole gut without crop (hereafter referred to as gut) in another tube with
157 100 µL of 1x PBS.
158
159 5.7. Grind the crop and gut respectively in tubes using pipette tips to make the dye dissolve in
160 the PBS.
161
162 5.8. Repeat steps 5.1−5.7 until enough crops and guts are collected for the experiment
163 designed.
164
165 NOTE: The crop and gut should be fully homogenized, and all dye should be dissolved in the
166 buffer. For research purposes, one or multiple crops or guts can be collected in one tube.
167
168 **6. Calculating dye amounts in crop and gut**
169
170 6.1. Centrifuge the sample tubes at the highest speed for 1 min and transfer 90 µL of
171 supernatant to the wells of a 96 well plate.
172
173 6.2. Make a series of blue dye dilutions at concentrations from 1×10^{-7} g/mL to 1×10^{-4} g/mL
174 as standards.
175
176 6.3. Add a series of 90 µL standards to the wells of the 96 well plate.
177
178 6.4. Measure the absorbance of the samples and standards at 630 nm with a plate
179 spectrophotometer.
180
181 6.5. To create a standard curve, plot a line graph of absorbance vs. concentration for each of
182 the standards. Then draw a line of best fit through the points to get the equation used to
183 calculate the dye concentration in the samples.
184
185 6.6. Calculate the amount of dye by multiplying the sample concentration by 0.1 mL.

186
187 **REPRESENTATIVE RESULTS:**
188 These methods to count crop contraction rate and detect dyed food distribution can be used
189 to evaluate crop function on food motility. The crop contraction reflects the frequency of
190 pushing food into the gut. The distribution of dye in the fly after a short feeding period
191 indicates immediate food passaging from crop to midgut.
192
193 Target of rapamycin complex 1 (TORC1) is a master regulator that mediates nutrient and cell
194 metabolism. TORC1 inhibition extends lifespan in many organisms, including *Drosophila*. As
195 an inhibitor of TORC1, the nprl2 mutant fly displays hyperactivation of TORC1 and GI digestion
196 defects[8,9]. The crop size in a *nprl2* mutant is normal at 3 days old and enlarged at 15 days old,
197 compared with its genetic background control (*yw*)[8]. To evaluate the crop motility assay, 3-
198 day-old *nprl2* mutant flies and *yw* controls were used. Each crop was counted five times and
199 the average value was used (**Supplemental Table 1**). The numbers of the crop contraction
200 during the five repeats were similar, which suggests that PBS might not affect the crop
201 physiology in short feedings and is suitable for crop contraction counting. The *nprl2* mutation
202 significantly decreased the rate of crop contraction (**Figure 1**).
203
204 Similar to the mammalian stomach, the *Drosophila* crop keeps contracting to move the food
205 into the gut. To further confirm the function of Nprl2 on the crop, food movement was
206 detected. The flies were fed with dyed food for 30 min and dissected immediately to detect
207 the amount of dye in the crop and the gut using spectrophotometry. As shown in **Figure 2A**,
208 the blue dye amounts in the crops of control and *nprl2* mutants were similar, consistent with
209 the previously reported comparable crop size[8]. The *nprl2* mutants had less dye in the gut,
210 which may be associated with the decreased contraction rate (**Figure 2B**).
211
212 **FIGURE AND TABLE LEGENDS:**
213 **Figure 1: Crop contraction difference between control and *nprl2* mutant males.** The 3-day-
214 old male flies were dissected, and the crop contraction rate was counted. The crop
215 contraction frequency of each fly is displayed as a data point. Error bars represent SD from
216 the indicated data point. ***, P < 0.001.
217
218 **Figure 2: Food distribution difference between control and *nprl2* mutant males after a short**
219 **feeding time.** The 3-day-old male flies were fed with food containing 0.5% blue dye for 30
220 min and then dissected immediately to detect the dye amount. (**A**) The dye amount in the
221 crop. (**B**) The dye amount in the gut without crop. **, P < 0.01; NS, not significant.
222
223 **Supplemental Table 1: The original data of crop contraction used in Figure 1.**
224
225 **DISCUSSION:**
226 In *Drosophila* ingested food moves from the crop to the gut for digestion. During this process,
227 the nutrients are absorbed, and the waste is expelled out of the body as feces. Thus,
228 comparing food ingestion together with feces ejection can be used to roughly assess the
229 speed of food movement in the body. The method of capillary feeder (CAFE) is widely used to
230 measure food ingestion[10,11]. The method of feces number counting can be used to estimate

231 the amount of feces creation[12]. However, the food movement in *Drosophila* body is under the
232 control of many factors, including crop motility. Crop function cannot be easily evaluated
233 using CAFE and feces counting methods. This protocol can quantitatively evaluate crop
234 motility and food passaging from crop to gut in *Drosophila*.
235
236 Crop motility is essential for food passaging and *Drosophila* survival. Some gene mutations or
237 virus infections that affect crop function result in decreased lifespan[7,13,14]. This protocol can
238 be used to screen and evaluate the mutants and drugs that affect crop motility. The crop
239 contraction counting is used to detect crop motility frequency and the spectrophotometry
240 measuring food distribution in crop and gut is used to predict crop motility efficiency. These
241 two methods are easy to perform and highly sensitive. Furthermore, the spectrophotometry
242 method can be modified to detect food usage in *Drosophila*. For example, food ingestion
243 within a short time can be evaluated by detecting the dye amount in the whole gut. The
244 continuous food distribution status between crop and gut can be assessed by detecting the
245 dye amounts in the flies fed with dye food for few days.
246
247 There are a few technical considerations in this protocol. For the crop contraction counting
248 method, it is essential to dissect and take out the crop carefully in PBS buffer. Saline solution
249 is not the physiological environment of the crop. The crop must remain connected to the body,
250 and the fly must be alive and awake; otherwise, the crop loses the ability to contract. It is
251 suggested to count crop contraction in intact flies as well[13]. For the spectrophotometry
252 method, the separation of the crops from the dissected GI and their transfer into a 96 well
253 plate after food dye feeding should be done carefully and quickly. The dye is used to indicate
254 the amount of food in the crop and gut. During the dissection process, the crop and gut should
255 be in contact. If dye leaks into the dissection media, the sample cannot be used. With practice,
256 a skilled technician can finish dissection and crop separation within 30 s.
257
258 Previously, these methods were used to evaluate the crop motility in 15-day-old *nprl2* mutant
259 flies that had enlarged crops[8]. In this case, the crop contraction and food distribution in 3-
260 day-old *nprl2* mutants with normal crop size were quantified. Consistent with the decreased
261 crop contraction rate, the *nprl2* mutants displayed less food dye in the gut. These results
262 suggest that the *nprl2* mutants have some defects in crop motility even at a young age. The
263 *yw* background was used as a wild type control because it is the genetic background of the
264 *nprl2* mutant. For other experiments, strains like *Canton S* and w^{118}, might be used as controls.
265 Other groups use a different dissecting solution (123 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 8
266 mM $MgCl_2$, 35.5 mM sucrose, pH = 7.1) for detecting crop contraction[13-15]. The crop
267 contraction rate found in the control flies in this study is lower than that reported by Solari et
268 al.[15], but higher than Chtarbanova et al.[14] and Peller et al.[13]. This difference may be caused by
269 the different genetic backgrounds or dissection media.
270
271 In all, blue dye spectrophotometry together with crop contraction can be efficiently used to
272 evaluate crop motility. The protocol presented here helps to make *Drosophila* a good model
273 for GI tract physiology study.
274
275 **ACKNOWLEDGMENTS:**
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279
280 **DISCLOSURES:**
281 The authors have nothing to disclose.
282
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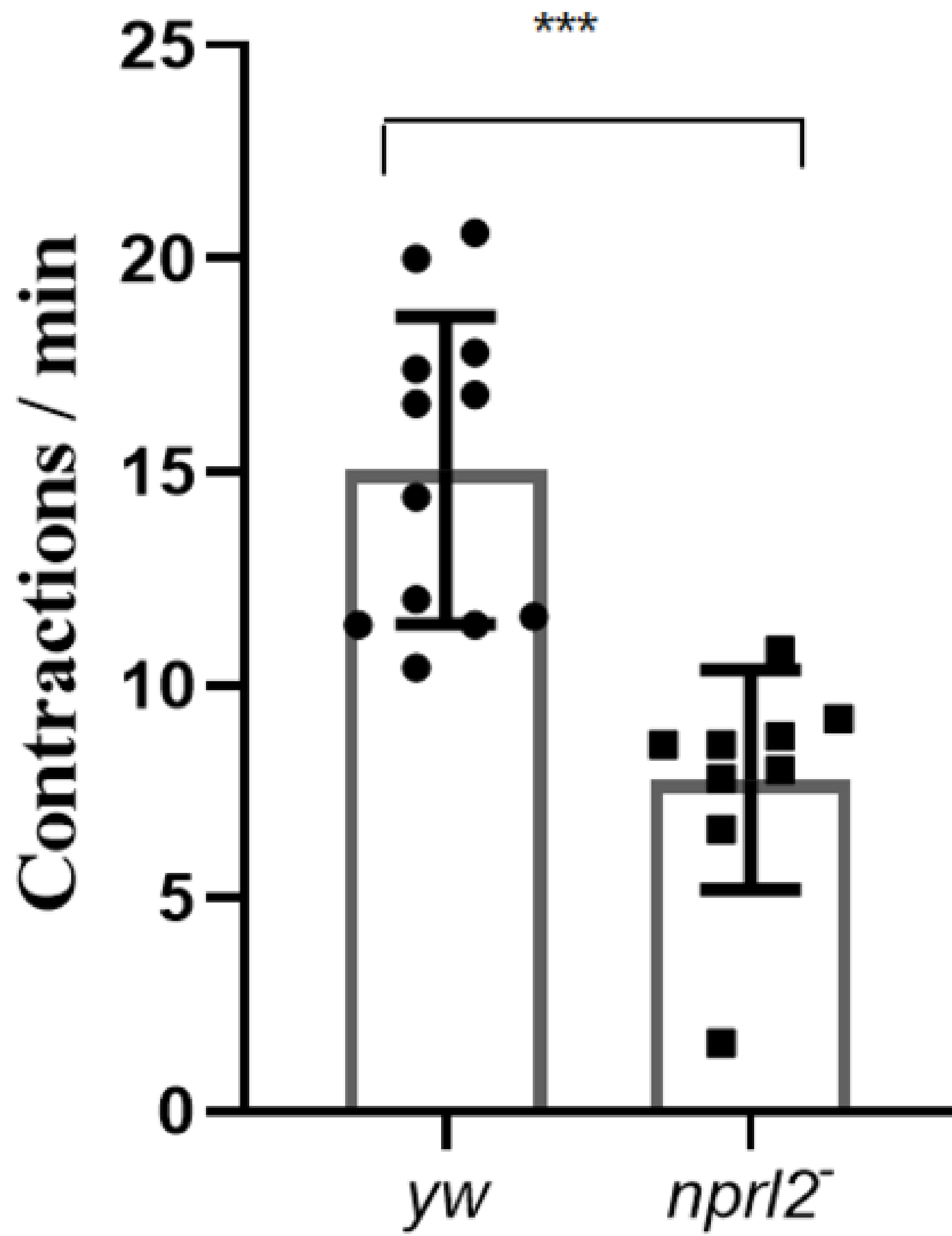

Figure 2

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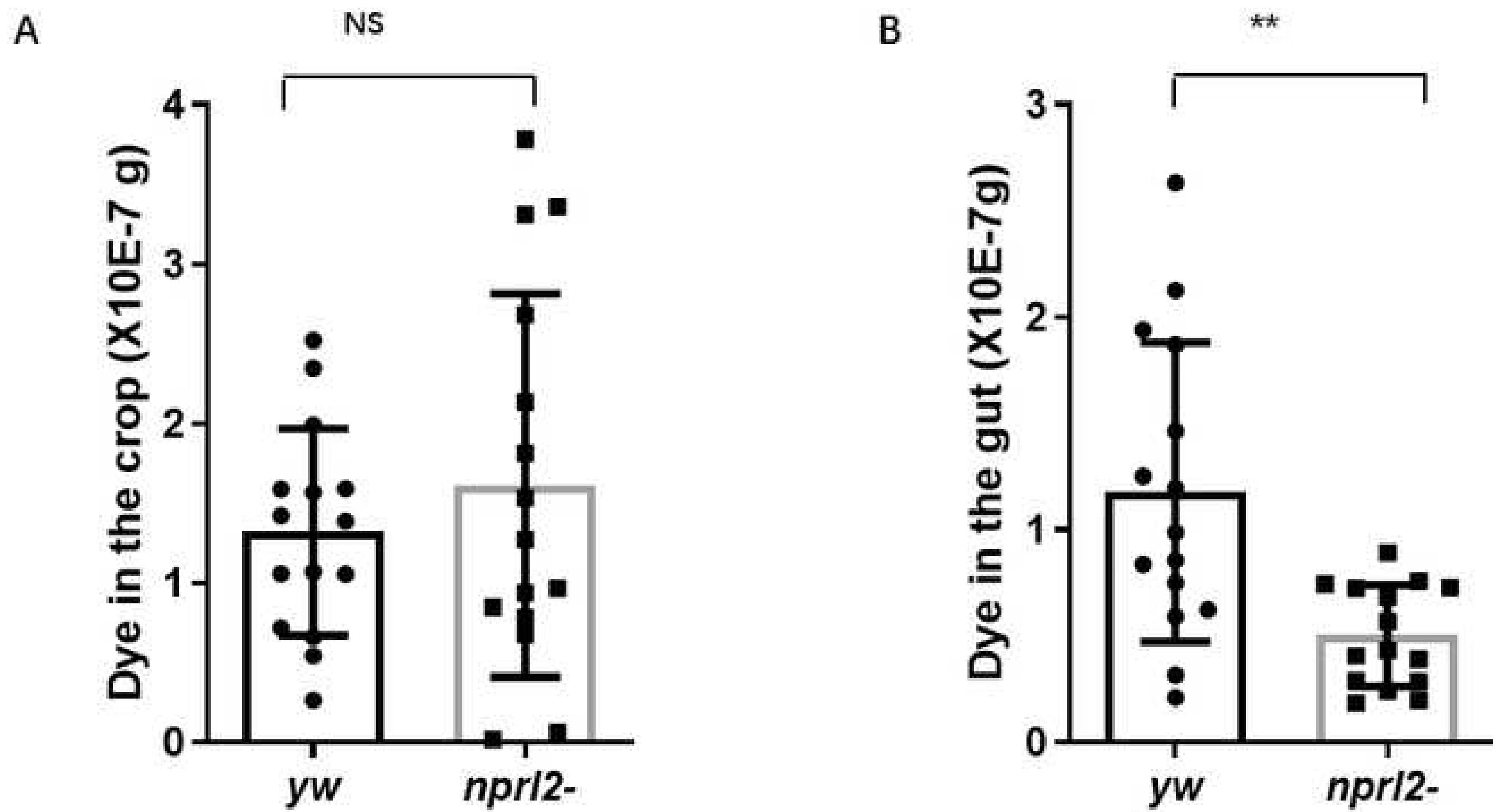

 Click here to access/download;Table of Materials;61181_Table_of_Materials.xlsx

Name of Material/Equipment	Company	Catalog Number	Comments/Description
96-well plate	Thermo fisher	269620	
Brillant Blue FCF	Solarbio	E8500	also called FD&C Blue No. 1
Centrifuge	Thermo fisher	Heraeus Pico 17	
Spectrophotometer	Spectra Max	cMax plus	
Tweezers	Dumont	11252-30	

 Click here to access/download;Rebuttal Letter;rebuttal.docx

This response is in reference to the manuscript “Measurement of crop motility and food passaging in Drosophila”. We thank the editor and reviewers for their careful consideration of our work. We apologize for not being sufficiently clear description and some language errors in previous protocol.

As detailed below, we have addressed the reviewers’ concerns by making recommended editorial changes to the text. In our response, reviewers’ comments are in *italics* while our response to the reviewer’s questions are in **bold type.**

In response to reviewer’s requests and concerns we have added the original data of crop contraction as supplemental table 1 to the manuscript. The revised parts in the manuscript are marked as yellow.

Editorial comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

2. Please submit each figure as a vector image file to ensure high resolution throughout production: (.psd, ai, .eps., .svg).

3. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Please sort the Materials Table alphabetically by the name of the material.

4. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

5. 1.2: Culture as in step 1.1?

Yes. We have revised it in step 1.3.

6. 3.2: What boiled liquid food? Same as 1.1?

Yes. We have revised it in step 3.2.

7. 4.1: What is starvation food? Just 1% agar?

Yes. 1% agar in distilled water. We have revised it in step 4.1.

8. 5.2: Remove heads how?

Grasp the fly at its thorax using one pair of tweezers, take off the head from the body using another pair of tweezers. We have revised it in step 5.2.

9. 5.7: Homogenize how? If using a machine, what are the settings?

Grind the crop and gut respectively in tubes using pipet tips to make the dye dissolved in the PBS. We have revised it in step 5.7.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

In this JoVE article by Jiadong et.al, authors have developed a protocol to analyze GI tract in Drosophila melanogaster. GI tract analysis is one of the techniques to evaluate feeding or metabolism related conditions in flies. Drosophila GI tract is composed of crop and gut. Crop is an organ similar to stomach in mammals. Crop stores food for short time and pump it to gut for further digestion. In this article, they describe a method for counting crop contractions as well as measuring food volume inside the crop. Similarly, they

have designed a protocol to measure food distribution throughout the gut for short and long period. They measured crop movement and food distribution in nprl2 mutants flies along with control flies as a model experiment.

Major Concerns:
Crop contraction measurement or quantification of food ingestion in Drosophila melanogaster is not a novel experiment. However, they tried to systematically document the protocol for the future reference. This protocol seems okay but some of the issues are pointed below:
1. In the beginning they described method to prepare experimental flies. They mentioned about different forms of yeast used (dry or wet), which I don't think essential. Otherwise, just mention it optional. Further, authors should mention the condition of the fly culture such as temperature, humidity and light/dark cycle and so on.
Providing supplemental yeast can greatly increase the eggs laying, which helps to collect more flies at same age. We changed it optional at the note part. In the revised version, we presented the condition of the fly culture in step 1.1.

2. 1.2 section, when did you exactly separate the males and females? Did you recommend using virgin flies? Is there any difference between virgin and mated? Furthermore, authors use yw as control. I think it would be good if authors provide several different controls here such as yw, w, and CantonS.
The male or female flies should be collected each day. Using male or female flies is depended on the experiment design. In the example, we use the *yw* as control because the *nprl2* is mutant in *yw* background. In fact, *w118*, *Canton S* or other wild-type flies can be used as control, which depends on the experiment design. We revised it in step 1.3 and the discussion.

3. Because opening the cuticle will alter the environment of the crop and potentially dilute circulating factors that could affect crop motility, dissecting solutions should contain similar constituents of the fly body. pH of the dissecting solution should be more accurate. Other group reported that dissecting solutions (128 Mm NaCl, 36 mM sucrose, 4 mM MgCl2, 2 mM KCl, 1.8 mM CaCl2, and % mM HEPES; PH-7) (CR Peller et. al., 2013) is used previously. I wonder if there is any different result.
We agree with the reviewer. Here we use the 1X PBS for dissection. The PBS contains NaCl 136.89 mM, KCl 2.67 mM, Na_2HPO_4 8.1 mM, KH_2PO_4 1.76 mM, and the PH is 7.4. In the PBS solution, the crop contraction rate didn't have great difference between the first counting and the fifth counting (supplemental data). We revised the step 2.1 and the discussion.

4. The procedure of fly dissection should be clearly mentioned (immobilization, dissection and observation). The main objective of this experiment is to count crop contractions. For that flies should be immobilized to some solid base. Otherwise it is hard to count exact number of crop movements for such a long time. 2.2 is not well described for orientation of flies to dissect and its following steps. Did you manually count the number of crop contraction? I highly recommend to record it with video.

We tried both immobilized the fly to the solid base and freely opened, which is easily dissect and time saving. We found that there was no significant difference between these two methods because we only count the complete wave of the crop lobes as contraction, which is easy to identify. We manually count the number of crop contraction rather than using video. We will provide the example of crop contraction in the video file.

5. What is the size of dissecting plate? Is 200 µL 1x PBS enough to dissect flies?

Yes. We used 12-well Spot Plates for dissection. In each well 200 µL 1x PBS is enough to dissect one fly.

6. In 2.3, they mentioned to count contractions for 1 min, 5 times and 30 sec intervals. Authors should provide the crop contraction for 5-10 min and justify whether the method is proper. In other words, is it consistent for 10 min?

We added the original contraction counting as the supplemental data. The data suggest that the crop contraction rate didn't change a lot in the PBS solution.

7. In 3.1, I did not get why they are using PBS to dissolve blue dye? I think distilled water should be okay.

Yes. Use the distilled water is also okay. We added this information in the notes of step 3.

8. In 4.2, Is there any concerns required for 2 hr feeding time depending on zeighber time (ZT; circadian time)? I suspect there should be different feeding and physiology depending on the ZT. This makes lots of trouble to audience if they don't provide the exact timing.

We agree with the reviewer. The culture condition can affect the time of food passing through. We found that at our maintenance condition, it need about 2 hours from feeding the flies with dye food to getting the waste with dye. Feeding flies within the time of food passing through can be used to detect the food movement capability from crop to gut. We have revised the note of step 4.

9. In 5.7, they mentioned tips to homogenize the crop or gut tissues. Are you talking about a grinder (homogenizer) or pipet tips? Make clear.

We use pipet tips to grind the crop and gut. We have revised it in step 5.7.

10. In 5.8, how many crops they used for their model experiment? There should be some standard number so that experimental error is minimum and data is consistent.

The number of flies used in each tube depends on the experiment designed. In the example, we wanted to test the variation and the difference between the individuals. So we used one crop in each tube. This assay can also use multiple crops or guts in one tube. We have revised the note of step 5.

11. In Fig 1. crop contractions per minute of control is less than that of control mentioned in Solari et. al., 2017. This can be due to different dissecting solution they used. Can you compare the different results between yours and other methods?

Our control flies had less contraction rate than mentioned in Solari et. al., 2017 and more contraction rate than mentioned in *Peller et. al., 2009*. This difference may be caused by the different genetic background or dissection media. We have revised it in the discussion.

12. Line 248, "make sure no dye leaking during this process". This should be more explanation in detail.

We revised the sentence as "The dye is used to reflect food amount in crop and gut. During the dissection process, the crop and gut should be contact. If there were dye leaking into the dissection media, the sample cannot be used any more".

13. 6.1 typo: tubes

We have corrected it.

14. Line 62, provide original reference here.

We have corrected it.

Reviewer #2:

Manuscript Summary:

This manuscript describes a method for studying GI function in the model organism Drosophila melanogaster by measuring both the contraction rate of the crop and the movement of food from the drop into the midgut. Given the growing popularity of the Drosophila gut as a subject of study, the publication of the protocol is both timely and relevant.

Major Concerns:

1. In step 2.2 of the procedure, more detail is required. Is the gut completely removed from the body or left attached at its anterior and posterior ends? Do you have to open the thorax in addition to the abdomen in order to avoid damaging the crop? How do you grab the thorax with forceps without damaging the crop?
Yes. The gut should still attach at its anterior and posterior ends after dissection. To get out the crop, we need to open the thorax. We have revised it in step 2.2. Grasp the fly at its thorax using one pair of tweezers, smoothly open the thorax using another pair of tweezers, and then pull the end in opposite directions to open the abdomen.

2. In our experience, the two lobes of the crop and the crop duct do not always contract at the same time or even at the same rate. In step 2.3 of the procedure, does one count any crop contractions or just contractions at one given point on the crop? If the latter, which area of the crop should be counted?
We count the contraction of crop lobes. We have revised it in step 2.3. Only the complete wave on the crop lobes was counted as contraction.

3. Some mention should be made in the discussion that counting crop contractions in a semi-intact preparation could miss the acute influence of hemolymph factors on contraction rate. As shown in Peller at al 2009, it is possible to combine contraction measurements with dyed food in order to visualize crop contractions in the intact fly.
We agree with the reviewer. We added it in discussion.

Minor Concerns:
1. On lines 82 and 89, "hatched" should be changed to "eclosed."
We have corrected it.

2. Line 222, "wildly" should be changed to "widely."
We have corrected it.

Reviewer #3:
Manuscript Summary:
The MS describe a simple methods for measurement of crop motility and food passaging in Drosophila as model insect by combined two well known protocols of crop contraction and dyed food estimation of food consumption

Minor Concerns:
Some information must be added to the protocol
1/ LINE 55 give reference
We have added the reference.

2/ Precise temperature, relative humidity and photoperiod for flies rearing
We have added it in step 1.1.

3/ Are insects starved and water satiated?
Yes. The flies were starved and water satiated for 4 hours on starvation food (1% agar in distilled water) before dye food feeding. We have revised step 4.1.

4/Are the flies anesthetized before dissection?
Yes. We have revised it in the step 2.1 and 5.1.

5/ Are contraction measured by eye under stereoscopic microscope or video-microscopy is used?
It is measured by eye under stereoscopic microscope. We will provide the example of crop contraction in the video file.

6/ Is there any time of acclimatization of the preparation of the saline before counts contraction?
No. We dissected the crop in PBS and counted the crop contraction directly.

7/ How authors labeled the muscle contraction (small twitch, wall or a complete wave?), is point chosen on the crop lobes to Labelle contraction?
Only the complete wave of the on the crop lobes was counted as muscle contraction. We have revised it in step 2.3.

8/ For feeding assay precise that the head must be taken off from the body to prevent eye pigment from interfering with absorbance spectrum of the dye.
We have revised it in step 5.2. Grasp the fly at its thorax using one pair of tweezers, take off the head from the body using another pair of tweezers.

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Genotype	First	Second	Third	Fourth	Fifth	Average
yw	12	11	17	17	15	14.4
yw	16	16	18	17	16	16.6
yw	20	19	21	21	19	20
yw	19	19	25	21	19	20.6
yw	17	19	19	15	17	17.4
yw	15	16	16	18	19	16.8
yw	18	17	17	19	18	17.8
yw	10	12	12	11	12	11.4
yw	9	10	10	11	12	10.4
yw	10	11	12	12	12	11.4
yw	12	12	12	12	12	12
yw	13	12	11	11	11	11.6
nplr2	10	8	8	7	7	8
nplr2	9	9	10	9	9	9.2
nplr2	1	1	3	1	2	1.6
nplr2	9	10	12	12	11	10.8
nplr2	8	9	10	9	8	8.8
nplr2	8	8	9	8	10	8.6
nplr2	10	9	9	8	7	8.6
nplr2	8	8	8	7	8	7.8
nplr2	7	7	7	5	7	6.6