3D imaging reveals potential anticipatory responses to increasing intraspecific competition in the *Drosophila* male reproductive system

Authors: Juliano Morimoto[1], Renan Barcellos[2], Todd A. Schoborg[3], Liebert Parreiras Nogueira[4], Marcos Vinicius Colaço[5]

Authors' Affiliations:

[1]School of Biological Sciences, University of Aberdeen, Zoology Building, Tillydrone Ave, Aberdeen AB24 2TZ

[2]COPPE, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

[3]Department of Molecular Biology, University of Wyoming, 1000 E. University Ave, Laramie, WY 82071

[4]Department of Biomaterials, Institute of Clinical Dentistry, University of Oslo. Geitmyrsveien 71, 0455 Oslo, Norway

[5]Laboratory of Applied Physics to Biomedical Sciences, Physics Institute, State University of Rio de Janeiro, Rio de Janeiro, Brazil

*Correspondence: juliano.morimoto@abdn.ac.uk

Data accessibility

Raw data will be available as supplementary information upon acceptance of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

JM designed and conducted the experiment, analysed the data, wrote the first draft, revised the manuscript. RB performed the segmentation of the regions of interest, quantification and image processing. TAS assisted with identification of structures and editing of the manuscript. LPN performed the chemical staining of the samples, microCT scannings, data analysis, study design and writing and editing. MVC designed the micro-CT experiment, conducted data analysis and editing. All authors contributed to the revision of the manuscript and agreed on the final version submitted to the journal.

Abstract

Selection favour males that anticipate intraspecific competition levels and develop appropriate competitive traits. In holometabolous insects, larval density can act as an ecological cue of males' future intraspecific competition levels while also limiting access to food allocated to sexually selected traits (e.g., testes). To date, however, most studies have been conducted in larval densities that may have little or no relevance to species' ecology. Here, we manipulated *Drosophila melanogaster* larval density based on the natural history of the species and used 3D micro-CT imaging to show a strong modulation of testes volume based on larval density: males from high larval densities generally had larger testes. Conversely, males from high densities tended (albeit not statistically significantly) to have the smallest accessory glands and ejaculatory bulb. Overall, we used micro-CT to measure male reproductive morphology in an ecologically relevant design for which the findings broadly support predictions from sexual selection theory.

Keywords: Bateman gradient, sexual dimorphism, seminal fluid, x-ray microtomography

Introduction

Ecological cues in the environment modulate how individuals respond to perceived levels of intraspecific competition for mating opportunities, thereby shaping individuals' mating and reproductive success and, at the population level, the strength of sexual selection (Bretman et al., 2011; Johnson et al., 2017; Morimoto et al., 2016). In holometabolous insects, larval density is an important ecological factor shaping individual fitness and can be an important ecological cue of intraspecific competition levels which individuals – particularly males – are likely to encounter in the adult stage (Johnson et al., 2017). The implicit theoretical prediction is that as larval density increases, males should allocate more (of the fewer) resources per capita to traits related to either migration (e.g., longer wings) or (post-copulatory) reproductive success (e.g., better ejaculates), or both (Katsuki et al., 2013). Empirical work suggests that many insect species conform at least partly to this expectation [reviewed in (Than et al., 2020)].

In *Drosophila melanogaster*, larval density modulates adult morphological, physiological, and behavioural traits that affect individual fitness (Bath et al., 2018; Borash et al., 2000; Lushchak et al., 2019; Prasad et al., 2001; Shenoi et al., 2016). For instance, males from high larval densities have smaller body sizes and lower mating and reproductive success (Amitin and Pitnick, 2007; Morimoto et al., 2016; Morimoto et al., 2017) but have disproportionately higher ejaculate investment relative to body size in each mating opportunity (Wigby et al., 2015), potentially as a mitigatory (adaptive) response to the morphological constraints imposed by nutrient limitation and competition at the larval stage (Klepsatel et al., 2018). This effect appears to be above and beyond changes in sperm length or transcriptional levels of nine major seminal fluid proteins (Amitin and Pitnick, 2007; McGraw et al., 2007). Therefore, it is possible that male reproductive responses to larval intraspecific competition originate in changes in male's morphology (rather than physiology) that enables males to invest relatively more ejaculate per mating opportunity. Anatomical changes such as increased testes sizes are known responses to increased larval density [e.g., (Gage, 1995; Stockley and Seal, 2001)], but little is known about other aspects of the male reproductive system. Yet, non-sperm components of the ejaculate (e.g., seminal fluid proteins) which are produced in tissues other than testes can be as important for male fertility (Perry et al., 2013). Thus, a more holistic view of the morphology of the male reproductive system is needed to gain insights into the responses to larval density.

In this study, we used 3D micro-CT imaging to analyse the volume of *D. melanogaster* male' reproductive organs over a range of larval densities. Larval density range was chosen based on our previous ecological assessment of *D. melanogaster* larvae in a natural population (Morimoto and Pietras, 2020) and included densities ranging from low, natural range, and high larval densities relative to the densities observed in nature. This approach allowed us to apply our 3D micro-CT technique to an ecologically relevant experimental design for the species. Based on the literature, we predicted that male reproductive organs' volume – particularly accessory glands and testes – should be positively associated with larval density assuming that (1) relatively larger reproductive organs are correlated with higher ejaculate investment, (2) larval density is an ecological cue for the level of (post-mating) competition in the adult stage and (3) males respond adaptively to this ecological cue as to enhance their reproductive success. This prediction emerged from the rationale that, as larval density increased, the perceived level of post-copulatory competition would likewise increase, resulting in high demand for high-quality ejaculates that in turn, require larger reproductive organs to produce it (Parker, 2016). Our study applied 3D imaging to reveal the morphological changes in male reproductive organs in response to a naturally-occurring range of larval densities, highlighting the role of phenotypic expression in shaping individuals' responses to intraspecific competition.

Material and Methods

Fly stock and larval density manipulation

We used an outbred *D. melanogaster* population collected in September 2015 in Brittany (France) and kindly provided to us by Dr Herve Colinet (see (Henry et al., 2018)). Flies were maintained in large population cages (>1,000 individuals) with overlapping generations, at 20 ^{o}C and ca. 50 % humidity, with 12 h light: 12 h dark cycles. Fly stocks were maintained – and all experiments conducted – with a standard yeast-sucrose diet (Brewer's yeast MP Biomedicals 0290331205, commercial sucrose, agar Sigma-Aldrich A1296, Sigma-Aldrich, and 0.5 % Nipagin Sigma-Aldrich). Eggs were collected for 6 h using an oviposition device (Petri dish (90 mm) covered with a solution of commercial blackcurrant juice and 1 % agar and coated with a thin layer of yeast paste). Oviposition devices were incubated overnight until eggs hatched at 25 ^{o}C, after which, first instar larvae were counted and allocated to larval density treatments using a soft brush under a Leica M9i stereoscope. Larval density treatments were based on our survey of larval densities in a natural population of *D. melanogaster* (Morimoto and Pietras, 2020). We had 5 larval densities: 0.5, 5, 15, 30, and 50

larvae/g of diet in vials with between 3 g (highest density) to 6 g (lowest density) of diet. This larval density gradient corresponds to a range from low (i.e., 0.5 larvae/g, lower than the natural range), natural range (5 and 15 larvae/g), and high (30 and 50 larvae/g, higher than natural range) larval densities (Morimoto and Pietras, 2020). Vials were incubated at 25 ^{o}C with 12 h light: 12 h dark cycles until adult emergence. Within 8 hours of emergence, females were discarded, males were transferred to fresh vials with the standard diet and incubated for 5 days before being killed at -20 ^{o}C. This ensured that even in the unlikely case that males mated, males had enough time to replenish their reproductive organs prior to imaging. Males were transferred to an increasing gradient of ethanol (from 40 % to 100 %, 2h in each solution) for fixation before imaging.

Imaging and data analysis

Individual males were submerged in the iodine-ethanol (I2E) 1 % in 100 % ethanol (EtOH) solution and stored overnight, after which individuals were rinsed in 100 % EtOH three times for 10 min to wash off excess iodine. Males were then placed in heat-sealed pipette tips with 100 % EtOH and scanned using a SkyScan 2211 (Multiscale X-ray Nano-CT System, Bruker micro-CT, Kontich, Belgium) at Oral Research Laboratory, University of Oslo. Scanning parameters were as follows: 55 kV, 260 µA, 650 ms exposure time per projection and without the use of a physical filter. Males were scanned over 360° with rotation steps of 0.31°, resulting in 1162 projections. Each projection was averaged by 3 frames, leading to a total scan duration of about 40 min for each sample and a final voxel size of 1.40 µm. The reconstruction process for each sample was performed using the system-provided software NRecon (version 1.7.4.6). Image segmentation was done using Dragonfly software ORS (Object Research Systems Inc, Montreal, Canada, 2019; software available at http://www.theobjects.com/dragonfly). Each male reproductive organ was segmented individually employing semiautomatic processes and correcting for inconsistencies in the three axes to achieve optimal and accurate segmentation.

Statistical analyses

All statistical analyses were conducted in R (Team, 2010). We imaged 12 randomly selected males (0.5 larvae/g: N = 2, 5 larvae/g: N = 3, 15 larvae/g: N = 2, 30 larvae/g: N = *3*, 50 larvae/g: N = 2). Accessory glands and testes were measured individually. We compared the volumes (in μm^3) of each organ with a linear mixed model using the 'lme4' and 'lmerTest' packages (Bates et al., 2007; Kuznetsova et al., 2017) with models that included individual

ID or in the case of accessory glands and testes, side (left or right) nested within individual ID as a random effect and the fixed effect larval density (fitted as a factor with 5 levels) (Table S1). Post-hoc tests in linear mixed models were performed using the 'emmeans' package (Lenth and Lenth, 2018). In *Drosophila*, larval density influences male body size (Amitin and Pitnick, 2007). We therefore also included normalised abdominal volume as a fixed effect in all models to control the allometric relationship between larval density, body size, and reproductive organs, even though abdominal volume was not statistically different between larval densities (Density: $F_{4,21} = 1.959$, $p = 0.205$; Table S1). All data plots were made using the 'ggplot2' package (Wickham, 2016).

Results

We first identified the target organs for segmentation. Fig 1a shows an example of an image slice of a specimen used for segmentation and Fig 1b shows a 3D model reconstruction of the segmented abdomen highlighting the male reproductive system. A video with an example of the segmentation slice per slice is presented in the supplementary material (Video S1). This approach allowed to then test the effect of larval density on male reproductive organs. After controlling for abdominal volume, testes volume was statistically significantly influenced by larval density (Density: $F_{4, 19.3} = 13.278$, $p < 0.001$). For instance, males from the 30 larvae/g treatment had the testes with the highest volume while males from the 15 larvae/g treatment had the testes with the smallest volume. Males from the 5 larvae/g treatment had testes with intermediate volume which was not different from males from the lowest (0.5 larvae/g) or highest (50 larvae/g) larval densities (Table S1, Fig 2a). Accessory gland volume progressively decreased with increasing larval densities, although this effect was not statistically significant (Density: $F_{4,22} = 1.673$, $p = 0.191$). Nevertheless, this trend suggested that larger accessory glands (by volume) were observed in males that emerged from the lowest and natural larval densities of 0.5, 5 and 15 larvae/g, whereas relatively smaller accessory glands were observed from males that emerged from high larval densities of 30 and 50 larvae/g (Fig 2b, Table S1). Ejaculatory ducts volumes were marginally statistically influenced by larval density (*Ejaculatory duct:* $F_{4,8} = 3.714$, $p = 0.054$) while ejaculatory bulb displayed similar trend as that of accessory glands but likewise without reaching statistical significance (*Ejaculatory bulb:* $F_{4,8} = 2.446$, $p = 0.130$). The ejaculatory duct followed a similar (although less accentuated) trend to that observed for the testes (Table S1, Fig 2c). Conversely, the ejaculatory bulb followed a similar trend observed in the accessory glands, whereby males emerging from high larval densities of 30 and 50 larvae/g had smaller

ejaculatory bulbs by volume compared with males from other larval density treatments (Fig 2d).

Discussion

We manipulated *D. melanogaster* larval density, based on larval population levels observed in a natural population, and showed a tissue-specific response to larval density in adult males. Previous studies in *D. melanogaster* have found that larval density does not affect sperm size (Amitin and Pitnick, 2007) nor the transcription activity of seminal fluid proteins in the accessory gland (McGraw et al., 2007), suggesting that changes in ejaculate quality caused by larval density can be based upon anatomical differences that can affect overall ejaculate composition (e.g., total sperm number, total seminal fluid concentration). However, contrary to our expectations, reproductive organ volume was not always positively associated with larval density, suggesting that, if our assumptions are correct, responses to larval density are more nuanced than previously expected. For instance, while testes volume tended to increase with larval density, accessory glands and ejaculatory bulb volumes tended to decrease with increasing larval density. These differences could underpin a trade-off in ejaculate composition, whereby the ejaculate composition is sperm-biased for males from higher larval densities but balanced and seminal fluid-biased for males from natural range and larval densities, respectively (assuming reproductive organ size or volume reflects ejaculate composition (Gage, 1995; Lemaitre et al., 2011; Ramm et al., 2015; Stockley and Seal, 2001; Stockley et al., 1997)). Whether or not the ratio of ejaculate components is dependent upon larval density remains to be investigated. The specific functions of the male ejaculatory bulb and duct have not yet been fully uncovered and thus it is challenging to relate our findings to functional aspects of these organs. Nonetheless, both ejaculatory duct and tract produce important components of the male ejaculate (Heinstra and Thörig 1982; Lung et al. 2001; Bretman et al. 2010) and respond dynamically to mating (and potentially to ecological cues) (Cohen and Wolfner, 2018)

Drosophila males are known to modulate ejaculate composition and expenditure based on ecological cues of intraspecific competition in adults [e.g., (Bretman et al. 2010; Hopkins et al. 2019)] in ways that can favour male's exploitation of rivals' ejaculate (particularly of costly seminal fluids) under competitive scenarios (Hodgson and Hosken, 2006; Sirot et al., 2011). This is in agreement with sexual selection theory which predicts that male ejaculate

expenditure should increase with the risk of post-copulatory sexual selection, but decrease with increasing intensity of post-copulatory sexual selection (Parker and Pizzari, 2010; Parker et al., 2013). Thus, if males exploit rivals' seminal fluid, then it is possible that males from high-density environments (where competition is more intense but there is more opportunity for exploitation) (i) have lower cost *per* ejaculate due to the lower production of seminal fluid proteins and (ii) have higher sperm production to enhance efficiency of ejaculate exploitation. Our results suggest testes volume are higher (and accessory glands volume lower) for males from high larval density, which corroborates the idea that under high intraspecific competition levels, male ejaculate should be sperm-biased to enhance ejaculate exploitation. This contradicts the findings that males from high larval density invest relatively more *per* mating opportunity (Wigby et al., 2015). Our data do not allow us to investigate the causes of this contradiction, but it is worth mentioning that the larval densities used in (Wigby et al., 2015) are at least four times higher than the highest density used in this study and that of observed a natural population (Morimoto and Pietras, 2020). Such a high larval density could result in higher non-adaptive responses to ejaculate investment per mating opportunity as a response to extreme intraspecific competition levels. Nevertheless, our results are broadly consistent with the wider holometabolous insect literature (Than et al., 2020). For example, males from high larval density developed larger testes in the yellow dung-fly *Scatophaga stercoraria* (Stockley and Seal, 2001) and the moth *Plodia interpuctella* (Gage, 1995).

Conclusion

Our results show that larval density leads to a tissue-specific effect on male reproductive organs, which agrees with predictions from sexual selection theory and contributes to our understanding of the anticipatory morphological responses across larval intraspecific competition levels. Future studies across species using micro-CT will reveal whether the responses found in *Drosophila* are consistent across insects, and can help elucidate why some groups [e.g., Coleoptera (Gay et al., 2009)] appear to differ in their response to larval density compared to others (e.g., Lepidoptera and Diptera) (Than et al., 2020).

Data accessibility

Raw data will be available as supplementary information upon acceptance of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We would like to thank Dr Stuart Wigby for his useful comments in the early version of this manuscript.

References

Amitin, E. G. and Pitnick, S. (2007). Influence of developmental environment on male□and female□mediated sperm precedence in Drosophila melanogaster. *J. Evol. Biol.* **20**, 381–391.

Bates, D., Sarkar, D., Bates, M. D. and Matrix, L. (2007). The lme4 package. *R Packag. version* **2**, 74.

Bath, E., Morimoto, J. and Wigby, S. (2018). The developmental environment modulates mating-induced aggression and fighting success in adult female Drosophila. *Funct. Ecol.* **32**,.

Borash, Teotónio, Rose and Mueller (2000). Density-dependent natural selection in Drosophila: correlations between feeding rate, development time and viability. *J. Evol. Biol.* **13**, 181–187.

Bretman, A., Lawniczak, M. K. N., Boone, J. and Chapman, T. (2010a). A mating plug protein reduces early female remating in Drosophila melanogaster. *J. Insect Physiol.* **56**, 107–113.

Bretman, A., Fricke, C., Hetherington, P., Stone, R. and Chapman, T. (2010b). Exposure to rivals and plastic responses to sperm competition in Drosophila melanogaster. *Behav. Ecol.* **21**, 317–321.

Bretman, A., Gage, M. J. G. and Chapman, T. (2011). Quick-change artists: male plastic behavioural responses to rivals. *Trends Ecol. Evol.* **26**, 467–473.

Cohen, A. B. and Wolfner, M. F. (2018). Dynamic changes in ejaculatory bulb size during Drosophila melanogaster aging and mating. *J. Insect Physiol.* **107**, 152–156.

Gage, M. (1995). Continuous variation in reproductive strategy as an adaptive response to population density in the moth Plodia interpunctella. *Proc. R. Soc. B Biol. Sci.* **261**, 25–30.

Gay, L., Hosken, D. J., Vasudev, R., Tregenza, T. and Eady, P. E. (2009). Sperm competition and maternal effects differentially influence testis and sperm size in Callosobruchus maculatus. *J. Evol. Biol.* **22**, 1143–1150.

Heinstra, P. W. H. and Thörig, G. E. W. (1982). Multiple function of pteridines in Drosophila: the fluorescence of the ejaculatory bulb in Drosophila melanogaster. *J. Insect Physiol.* **28**, 847–855.

Henry, Y., Renault, D. and Colinet, H. (2018). Hormesis-like effect of mild larval crowding on thermotolerance in Drosophila flies. *J. Exp. Biol.* **221**, jeb169342.

Hodgson, D. J. and Hosken, D. J. (2006). Sperm competition promotes the exploitation of rival ejaculates. *J. Theor. Biol.* **243**, 230–234.

Hopkins, B. R., Sepil, I., Thézénas, M.-L., Craig, J. F., Miller, T., Charles, P. D., Fischer, R., Kessler, B. M., Bretman, A., Pizzari, T., et al. (2019). Divergent allocation of sperm and the seminal proteome along a competition gradient in Drosophila melanogaster *Proc. Natl. Acad. Sci.* **116**, 17925 LP – 17933.

Johnson, T. L., Symonds, M. R. E. and Elgar, M. A. (2017). Anticipatory flexibility: larval population density in moths determines male investment in antennae, wings and testes. *Proc. R. Soc. B Biol. Sci.* **284**, 20172087.

Katsuki, M., Toquenaga, Y. and Miyatake, T. (2013). Larval competition causes the difference in male ejaculate expenditure in Callosobruchus maculatus. *Popul. Ecol.* **55**, 493–498.

Klepsatel, P., Procházka, E. and Gáliková, M. (2018). Crowding of Drosophila larvae affects lifespan and other life-history traits via reduced availability of dietary yeast. *Exp. Gerontol.* **110**, 298–308.

Kuznetsova, A., Brockhoff, P. B. and Christensen, R. H. B. (2017). lmerTest package: tests in linear mixed effects models. *J. Stat. Softw.* **82**,.

Lemaitre, J. F., Ramm, S. A., Hurst, J. L. and Stockley, P. (2011). Social cues of sperm competition influence accessory reproductive gland size in a promiscuous mammal. *Proc. R. Soc. B-Biological Sci.* **278**, 1171–1176.

Lenth, R. and Lenth, M. R. (2018). Package 'lsmeans.' *Am. Stat.* **34**, 216–221.

Lung, O., Kuo, L. and Wolfner, M. F. (2001). Drosophila males transfer antibacterial proteins from their accessory gland and ejaculatory duct to their mates. *J. Insect Physiol.* **47**, 617–622.

Lushchak, O. V, Karaman, H. S., Kozeretska, I. A., Koliada, A. K., Zabuga, O. G., Pisaruk, A. V, Koshel, N. M., Mechova, L. V, Inomistova, M. V, Khranovska, N. M., et al. (2019). Larval crowding results in hormesis-like effects on longevity in Drosophila: timing of eclosion as a model. *Biogerontology* **20**, 191–201.

McGraw, L. A., Fiumera, A. C., Ramakrishnan, M., Madhavarapu, S., Clark, A. G. and Wolfner, M. F. (2007). Larval rearing environment affects several post-copulatory traits in Drosophila melanogaster. *Biol. Lett.* **3**, 607–610.

Morimoto, J. and Pietras, Z. (2020). Natural history of model organisms: The secret (group) life of Drosophila melanogaster larvae and why it matters to developmental ecology. *Ecol. Evol.* **n/a**,.

Morimoto, J., Pizzari, T. and Wigby, S. (2016). Developmental environment effects on sexual selection in male and female Drosophila melanogaster. *PLoS One* **11**,.

Morimoto, J., Ponton, F., Tychsen, I., Cassar, J. and Wigby, S. (2017). Interactions between the developmental and adult social environments mediate group dynamics and offspring traits in Drosophila melanogaster. *Sci. Rep.* **7**,.

Parker, G. A. (2016). The evolution of expenditure on testes. *J. Zool.* **298**, 3–19.

Parker, G. A. and Pizzari, T. (2010). Sperm competition and ejaculate economics. *Biol. Rev.* **85**, 897–934.

Parker, G. A., Lessells, C. M. and Simmons, L. W. (2013). Sperm competition games: a general model for precopulatory male–male competition. *Evol. Int. J. Org. Evol.* **67**, 95–109.

Perry, J. C., Sirot, L. and Wigby, S. (2013). The seminal symphony: how to compose an ejaculate. *Trends Ecol. Evol.* **28**, 414–422.

Prasad, N. G., Shakarad, M., Anitha, D., Rajamani, M. and Joshi, A. (2001). Correlated responses to selection for faster development and early reproduction in Drosophila: the evolution of larval traits. *Evolution (N. Y).* **55**, 1363–1372.

Ramm, S. A., Edward, D. A., Claydon, A. J., Hammond, D. E., Brownridge, P., Hurst, J. L., Beynon, R. J. and Stockley, P. (2015). Sperm competition risk drives plasticity in seminal fluid composition. *BMC Biol.* **13**, 87.

Shenoi, V. N., Banerjee, S. M., Guruswamy, B., Sen, S., Ali, S. Z. and Prasad, N. G. (2016). Drosophila melanogaster males evolve increased courtship as a correlated response to larval crowding. *Anim. Behav.* **120**, 183–193.

Sirot, L. K., Wolfner, M. F. and Wigby, S. (2011). Protein-specific manipulation of ejaculate composition in response to female mating status in Drosophila melanogaster. *Proc. Natl. Acad. Sci.* **108**, 9922–9926.

Stockley, P. and Seal, N. J. (2001). Plasticity in reproductive effort of male dung flies (Scatophaga stercoraria) as a response to larval density. *Funct. Ecol.* 96–102.

Stockley, P., Gage, M. J. G., Parker, G. A. and Møller, A. P. (1997). Sperm Competition in Fishes: The Evolution of Testis Size and Ejaculate Characteristics. *Am. Nat.* **149**, 933–954.

Team, R. D. C. (2010). R: a language and environment for statistical computing. *R. Found.*

Stat. Comput.

Than, A. T., Ponton, F. and Morimoto, J. (2020). Integrative developmental ecology: a review of density-dependent effects on life-history traits and host-microbe interactions in non-social holometabolous insects. *Evol. Ecol.* **34**, 659–680.

Wickham, H. (2016). *ggplot2: elegant graphics for data analysis*. Springer.

Wigby, S., Perry, J. C., Kim, Y. and Sirot, L. K. (2015). Developmental environment mediates male seminal protein investment in Drosophila melanogaster. *Funct. Ecol.*

Figures

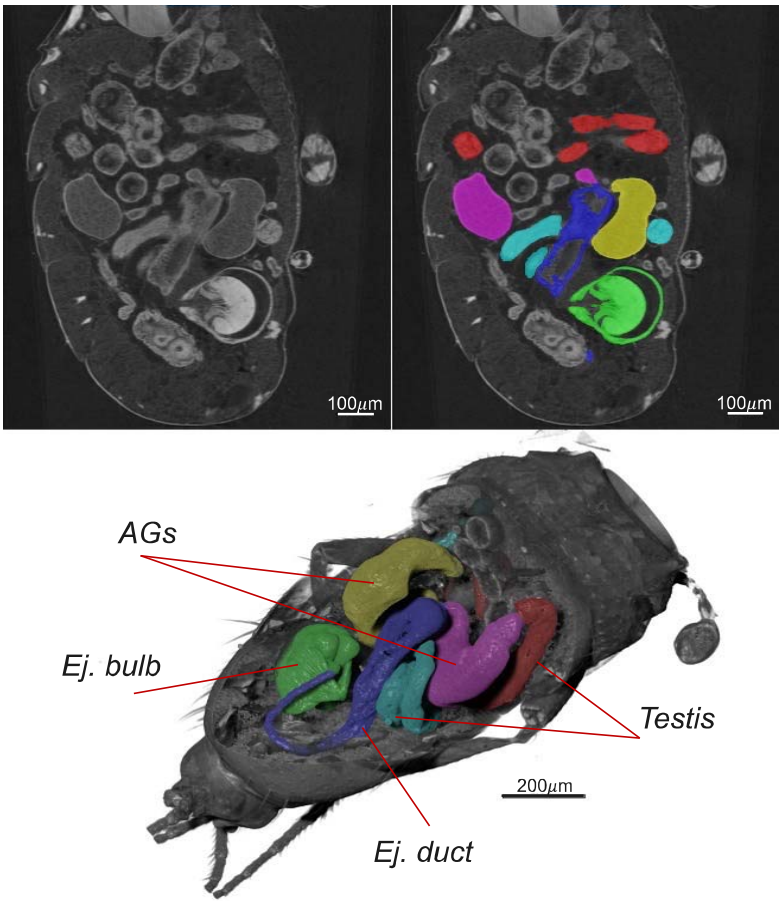


Fig 1. *μ*CT imaging of male reproductive system in *Drosophila*. (a) A slice generated after image reconstruction (b) the same slice as in *a* with segmented organs colored. (c) 3D image reconstruction genereted after segmentation.

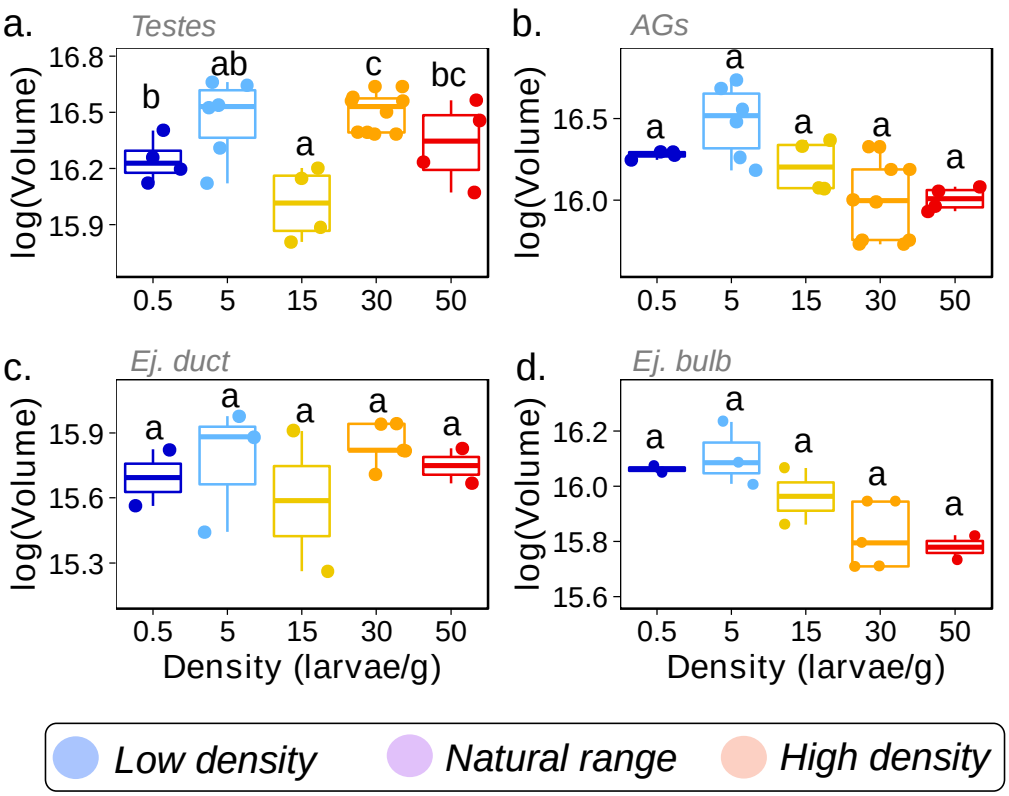
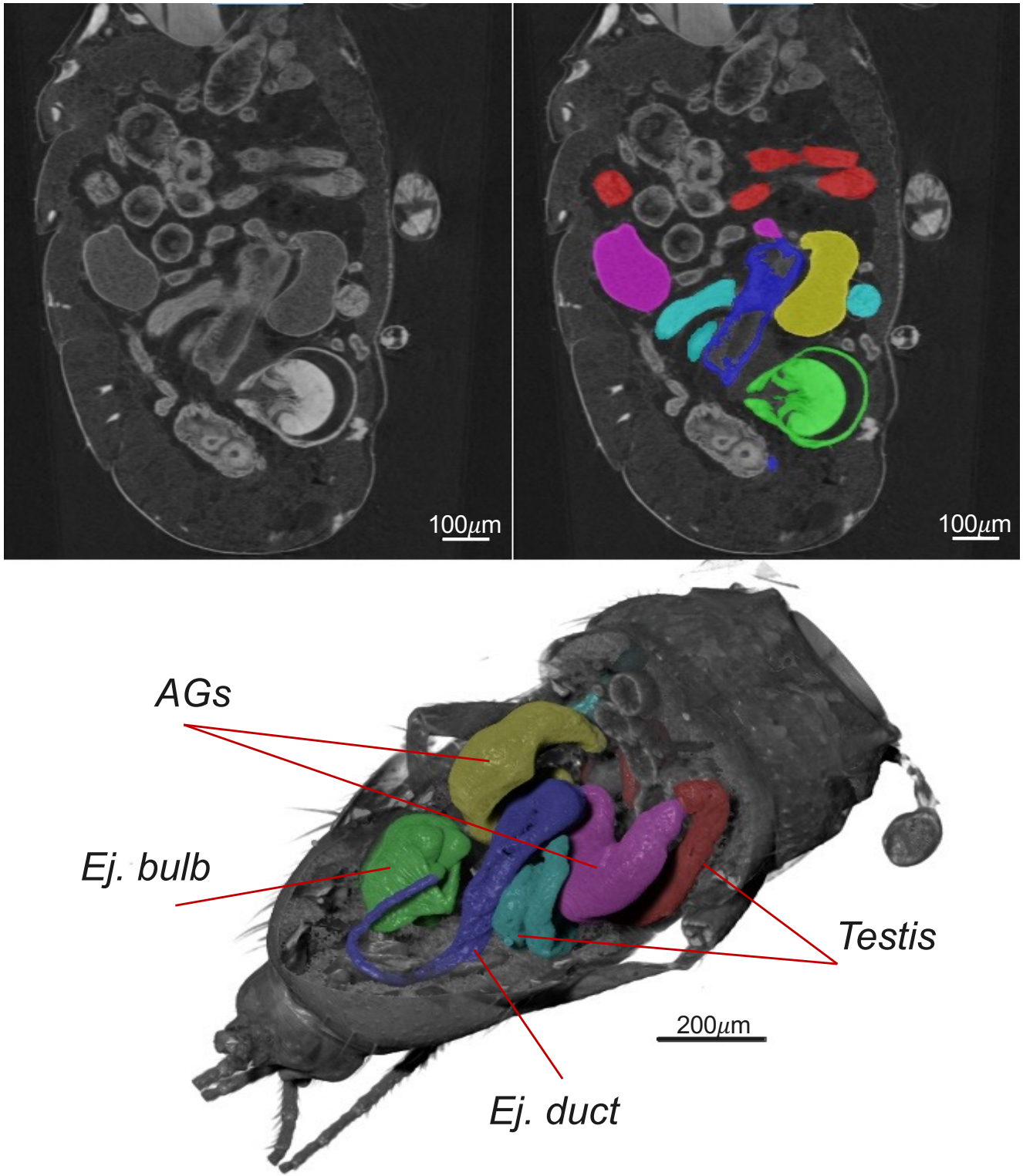
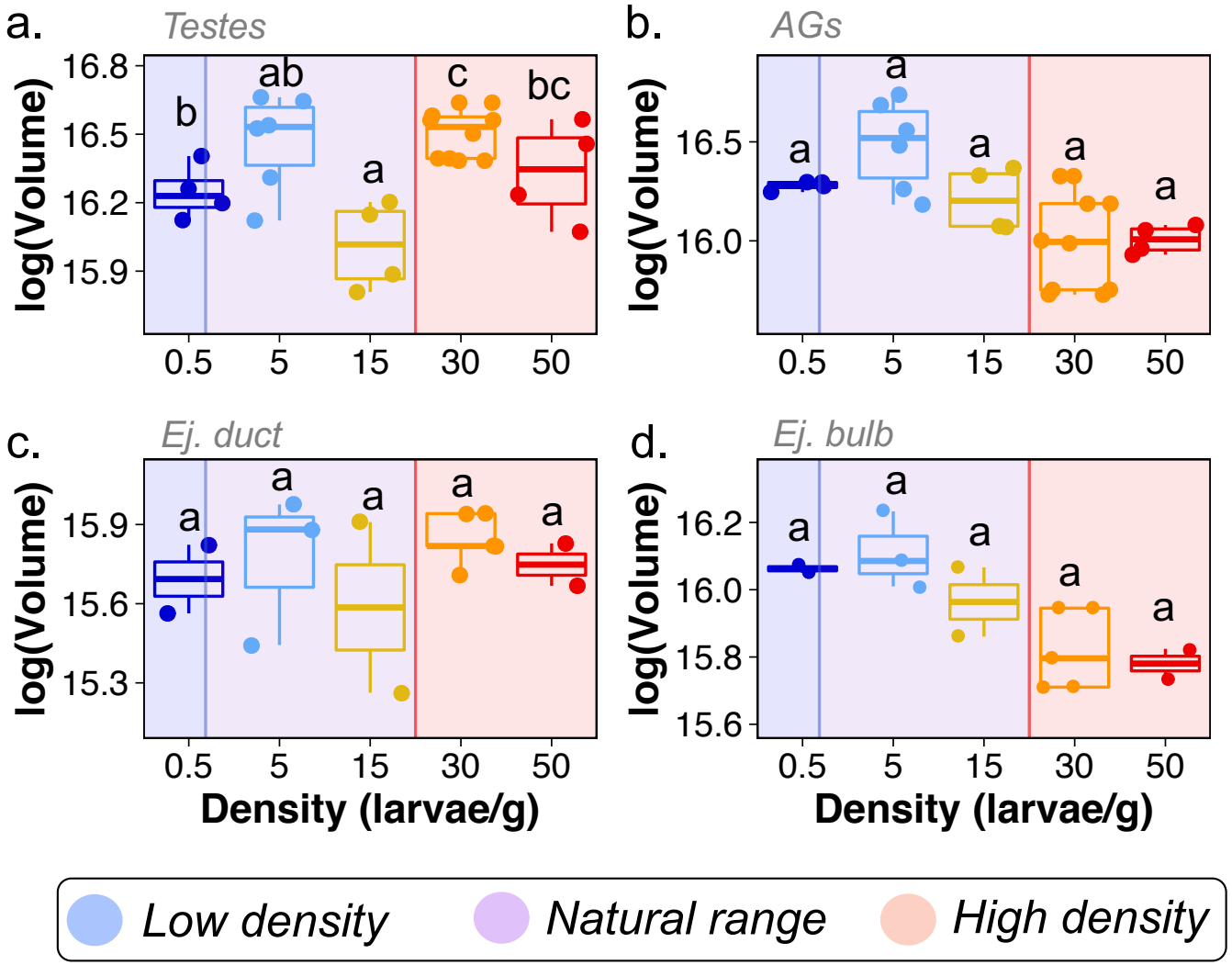


Fig 2. μCT reveals the effect of larval density on male reproductive system. Comparisons across larval density treatments (larvae/g) between the volumes of (a) testes (b) accessory gland (c) ejaculatory duct and (d) ejaculatory bulb. Volume was log-transformed for model fitting. Volume units are given in log(μm^3). Shaded region indicates the corresponding larval density observed in natural populations, whereby blue = low density, purple = natural range, and red = high density according to (Morimoto and Pietras, 2020).


100μm
100μm
AGs
Ej. bulb
Testis
200μm
Ej. duct


a.
Testes
b.
AGs
c.
Ej. duct
d.
Ej. bulb
log(Volume)
Density (larvae/g)
0.5
5
15
30
50
Low density
Natural range
High density