PARTHENOGENESIS INVESTIGATION OF THE F1 GENERATION FROM A

CROSS BETWEEN *DAPHNIA PULEX* TEX 23

(MALES) AND TEX 85 (FEMALES)

by

EFE AMROVHE

Presented to the Faculty of the Honors College of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

HONORS BACHELOR OF SCIENCE IN BIOLOGY

THE UNIVERSITY OF TEXAS AT ARLINGTON

May 2023

ACKNOWLEDGMENTS

ABSTRACT

PARTHENOGENESIS INVESTIGATION OF THE F1 GENERATION FROM A

CROSS BETWEEN DAPHNIA PULEX TEX 23

(MALES) AND TEX 85 (FEMALES)

Efe Amrovhe, B.S. Biology

The University of Texas at Arlington, 2023

Faculty Mentor: Sen Xu

Daphnia pulex is a species of Daphnia that is capable of both cyclic and obligate parthenogenesis. In this experiment, two cyclically parthenogenetic (CP) isolates are crossed to access the frequency of obligately parthenogenetic (OP) offspring that will be produced. If OP animals are produced at a significant frequency from a CP-CP cross, the 'why' behind it must be examined. Prior research revealed that OP animals produced from CP isolates are genetically linked through introgression from a sister species, *Daphnia pulicaria*. This may be relevant to the results and serve as a focal point when researching the implications of them. The mating between Tex 23 (male) and Tex 85 (sexual female) isolates created new daphnia clones. To establish clonal lines, the newly hatched neonates were gathered and kept in different habitats. It was the ephippia produced by each clone that was dissected to determine the parthenogenesis of the clone. The results showed that

two out of eight crosses yielded an OP clone. There was no variation within clones. Results were conclusive so statistical analysis proved redundant. With this discovery, an investigation into the introgression with *D. pulicaria* can be explored. After that connection is confirmed, a gene map can be curated to examine the particular gene(s) responsible for this variation.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Daphnia

Daphnia possess various characteristics that are ideal for research (Lynch 1994). They are very accessible, have a short generation time, can mass produce offspring, and are quite sensitive to environmental changes. The *Daphnia pulex* Tex23 and Tex85 that were used for this experiment were obtained from Textile Road, Michigan. Daphnia, which plays a significant role in aquatic food webs to help balance the ecosystem, is a key genus found in ponds and lakes (Jurgens 1994; Colbourne et al., 2011). Thus, they are easily accessible and live in a habitat that can straightforwardly be replicated in the lab. This is because lakes and ponds are not nearly as biodiverse as coastal aquatic areas like oceans (Jurgens 1994). Their short generation time allows them to reach maturity in a short amount of time. Therefore, they can reproduce and establish large populations rapidly. If a population becomes contaminated, it will not affect the experiment much because a new population can be formed in a couple of weeks. Daphnia species can produce anywhere from 1-100 offspring per gestation period. This is another attribute that allows them to build large populations so quickly. Lastly, they are easily manipulated through changes to their environment (J. R. Shaw *et al*., 2008). Disruption of their photoperiod, food supply, and so on can induce male production or a switch to sexual reproduction.

Table 1.1: *Daphnia pulex* Isolates

Isolate	Sexuality	Male Production	Role
Tex 23	CP	MP	Paternal
Tex 85	CP	NMP	Maternal

1.2 Parthenogenesis

Daphnia implements cyclic parthenogenesis (CP) as well as obligate parthenogenesis (OP). Cyclically parthenogenetic animals refer to those that require fertilization of the egg to produce a diploid embryo. On the other hand, obligately parthenogenetic animals can produce diploid offspring without fertilization by a male (Britannica 2023). Within daphnia species, there is variation in that aspect. Furthermore, this experiment explores the variation of parthenogenesis, within the *D. pulex* species. The aim was to investigate the parthenogenetic variation within the f1 generation of a cross between 2 cyclically parthenogenetic *D. pulex* isolates, Tex 23 (males) and Tex 85 (females). The frequency with which OP offspring are produced from the f1 generation of this CP-CP cross sets a precedent for future research to be done.
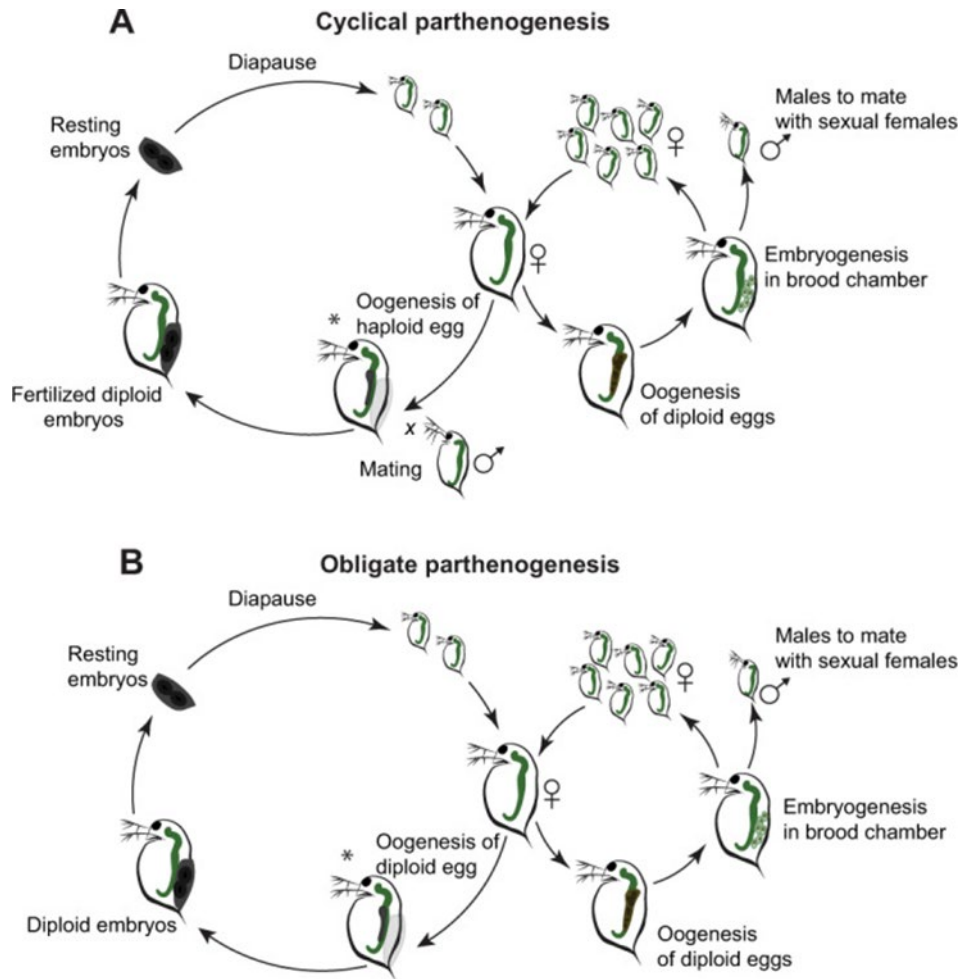
Figure 1.1: Types of Parthenogenesis. (A) Cyclical parthenogenesis. (B) Obligate parthenogenesis. It is important to note that some cyclically and obligately parthenogenetic daphnia lineages are non-male producing even though males are depicted in this figure (Xu et al., 2022).

1.3 Introgression

Introgression refers to the transfer of genetic material between species. This usually occurs due to backcrossing or hybridization. It serves as a source of variation in plants and animals alike (Nickel et al., 2021). The fact that freshwater ecosystems typically include various species of daphnia means that they are prone to this phenomenon. The results of this experiment serve the purpose of legitimizing a claim that OP offspring created from a *D. pulex* CP-CP cross is to be attributed to an introgression between *D. pulex* and *D. pulicaria*, a sister species. Essentially, if the OP offspring are produced at a significant rate, there is

a case for the introgression claim. Consequently, it will have to be examined to confirm the cause of the result.

CHAPTER 2

LITERATURE REVIEW

2.1 *Daphnia pulex*

Cladocera are common in inland aquatic areas all throughout the globe, but they are uncommon in coastal ecosystems. The about 150-species-strong cladocera genus daphnia has served as a fundamental model for research on ecological and evolutionary issues, including the effects of cyclical parthenogenesis. Daphnia is a crucial part of the zooplankton community. Although it has a short generation time (9–11 days at 20°C), it has a longer overall life span (over 60 days at 20°C) (Loxdale 2009).

When conditions are right, they reproduce via amictic parthenogenesis, giving birth to genetically identical offspring that grow a population of solely females. A clonal lineage can expand exponentially if this is sustained over numerous generations (Carvalho 1994). These clones' relative abundance reflects their respective performance in the environment (Decaestecker 2009). The creatures transition to sexual reproduction when unfavorable circumstances such a lack of food, crowding, the presence of predators, a change in day length, or a change in temperature occur. Females transition to producing sexual eggs as males are created parthenogenetically (Pijanowska and Stolpe 1996). A single female may initially lay two meiotic haploid eggs that must be fertilized before laying two diploid amictic eggs (De Meester et al. 2004). The fertilized haploid eggs are encased in an ephippium, a chitinous membrane secreted around the brood pouch of the female carapace (Schultz 1977). At the blastula stage, the development of the eggs is stopped, and

5

they enter a state of diapause. The ephippium is lost together with the old carapace during the following molt (Zaffagnini 1987). The encased latent eggs may survive harsh conditions (drying, freezing, digesting) and can be viable for up to 150 years (Brendonck and De Meester 2003).

One of the best-documented systems of contagious asexuality in animals, the *D. pulex* species complex (Crustacea, Anomopoda), contains numerous obligately parthenogenetic lineages descended from cyclically parthenogenetic ancestors (Lynch et al., 2008). It is a perfect system for investigating the genetic mechanisms underlying the transition to asexual reproduction.

2.2 Parthenogenesis

A small percentage of animal taxa transmit their genes asexually, while the majority reproduce sexually. A number of taxa have independently evolved a hybrid strategy throughout evolution that appears to combine the best aspects of both approaches: periods of asexual reproduction alternate with periods of sexual reproduction. This hybrid strategy is known as cyclical parthenogenesis (Davis 2012). The most well-known cyclical parthenogens include cladocerans, monogonont rotifers, and aphids, although protist, cnidarian, bryozoan, and plant life cycles that combine sexual and asexual reproduction are also widespread (Decaestecker 2009).

Evolutionary scientists have long been intrigued by the possibility of obligately parthenogenetic eukaryotic lineages that have completely abandoned sexual reproduction. In most multicellular species, the switch from sexual reproduction to obligate parthenogenesis happened independently, and this change is phylogenetically widespread (Snyman & Xu 2022). In literature, many cytogenetic expressions of obligate

parthenogenesis have been well-documented. However, little is known about the genes and molecular processes that underlie these cytogenetic alterations.

Animals can develop obligate parthenogenesis through a variety of methods, including infectious, hybrid, and spontaneous origins. The loss of sex in monogonont rotifers is one example of how spontaneous origin might result from changes in the genes responsible for sexual reproduction and meiosis (Serra & Snell 2009). When normal meiosis is interfered with by genomic divergences (such as chromosomal rearrangements) between hybridizing parental species, parthenogenesis may also take place (Kearney et al., 2009).

2.3 Introgression between *Daphnia pulex* and *Daphnia pulicaria*

Nuclear gene estimations suggest that *D. pulex* and *D. pulicaria* diverged about one million years ago (Omilian & Lynch 2009), yet morphological differences between these two species are minimal. But even though their geographic ranges overlap, these two species live in different ecosystems. While *D. pulicaria* inhabits stratified permanent lakes in North America, *D. pulex* only thrives in fishless ephemeral ponds with invertebrate predators. These two species most likely underwent local adaptation despite their recent divergence since they exhibit different life histories and physiological features (Moy et al., 2021).

By means of cyclical parthenogenesis, daphnia typically reproduce, with environmental cues determining the sex of the progeny. However, some females are no longer able to conceive males. Results show that a dominant allele at a single location is responsible for this loss of male procreation potential. A single 1.1-Mb nonrecombining area on chromosome I harbors 132 NMP-linked SNPs and 59 NMP-linked indels, which

allowed researchers to pinpoint the locus by comparing the complete genome sequences of 67 nonmale-producing (NMP) and 100 male-producing (MP) clones from five populations of *D. pulex*. One unannotated protein-coding gene (gene 8960) contains all seven of these markers, which are nonsynonymous mutations (Ye et al., 2019). The monophyletic cluster formed by all of the marker linked NMP haplotypes from various populations within this one gene suggests that the NMP phenotype originated by introgression from a sister species, *D. pulicaria*.

CHAPTER 3

METHODOLOGY

3.1 Sample Preparation

The *D. pulex* Tex23 and Tex85 were obtained from Textile Road, Michigan. Each isolate was cultivated in 250ml beakers filled (to 150ml) with lake water COMBO (Kilham et al., 1998) and fed with the green algae (*Scenedesmus obliquus*) at a concentration of 500,000 cells per ml every two days (Moy et al., 2021). Both isolates were spread across 8 beakers each and stored in an incubator that imitated their ideal photoperiod of 18:6 (light:dark).

When the Tex 23 animals matured (after about 10 days), about 20 of them were extracted and added to a new beaker to undergo a different treatment. This new beaker was treated with 30nM of methyl farnesoate (MF) to induce the production of male neonates (Lampert et al., 2012, Ye et al., 2019). The treatment was added on a Monday-Wednesday-Friday schedule and at the next week the males were collected, females disposed of, and a beaker of new mature animals was formed and ready to be treated. This was repeated every week to produce male isolates. On the other hand, when the Tex 85 animals matured, about 120 of them (60 per beaker) were collected and added to two beakers. To induce sexual reproduction, their photoperiod must be interrupted. Consequently, the two beakers were wrapped in foil and stowed away in a cabinet for two days. This stressed them enough to induce the desired switch.

3.2 Isolate Crossing

Animals from the maternal and paternal isolates were combined in another beaker that was stored at 18°C with a 0:24 hour (light: dark) photoperiod to conduct the crossing experiment. Low food and water availability caused the animals stress, encouraging the females to begin sexual reproduction. Asexually born offspring were eliminated on a regular basis to prevent genetic mixing between genders. Ephippia-contained sexually generated embryos were gathered and dissected. Subsequently, the embryos were kept in the dark for three weeks and then subjected to 410 nm UV radiation to promote hatching Luu et al., 2020). To establish clonal lines, the newly hatched neonates will be gathered and kept in different habitats with lake water COMBO and an 18:6-hour (light: dark) photoperiod.

3.3 Sexuality Test

As stated earlier, the research question for this experiment involves the realization of a statistically significant OP population in the f1 generation of a CP-CP *D. pulex* cross. Consequently, we must determine the number of CP and OP offspring produced during the experiment. This can be accomplished using the sexuality test for daphnia.

The sexuality test involved the inspection of the ephippia produced by adult female daphnia. Sexually isolated OP daphnia would produce ephippia containing one or two diploid embryos as they do not require male gametes to produce offspring. However, sexually isolated CP daphnia would produce empty ephippia because its eggs require sperm to form an embryo (Xu et al., 2013). Therefore, counting the number of empty ephippia and ephippia containing embryos revealed the ratio of CP to OP offspring produced.

However, OP offspring may also produce empty ephippia. So, a p-value from a t-test could verify if the findings depicted are significant.

These steps were repeated over 4 months to collate the results of this experiment. Methods that proved ineffective were addressed in the discussion section.

CHAPTER 4

RESULTS

4.1 Data

The number of ephippia, number of empty ephippia, and the number of embryos

counted during the sexuality test were the parameters used for the results (Table 4.1).

Table 4.1: Sexuality Test Results

Clones	Number of Ephippia	Number of Empty Ephippia	Number of Embryos
P1M5H1	10	10	0
P1M5H2	26	26	0
P1M5H3	39	29	26
P1M5H4	30	5	32
P1M5H5	23	23	0
P1M5H6	43	43	0
P1M5H7	5	5	0
P1M5H8	10	10	0

Clones P1M5H3 and P1M5H4 were the only two clones that produced embryos.

The rest of them produced no embryos at all. The P1M5H3 daphnia yielded an average of

2.60 embryos per ephippia while P1M5H4 yielded an average of 1.28 embryos per

ephippia.

CHAPTER 5

DISCUSSION

5.1 Result Analysis

This study builds on previous research done in this lab (Xu Lab) that have yielded OP progeny from a cross between two CP *D. pulex* isolates. The results are very clear cut as only 2 out of 8 (1:4) clones produced embryos. This means that they perform obligate parthenogenesis. The other clones produced no embryo at all, so they perform cyclic parthenogenesis (Innes & Herbert 1988). Because the results were very clear, statistical analysis proved redundant and unnecessary.

5.2 Drawbacks/Errors

This experiment was conducted for four months, so the sample size was relatively small. 25% of the clones produced were found to be OP. What if 100 clones were tested? Would that percentage remain the same? One could say that it compromises the credibility of the results, but each clone produced an ample number of ephippia for testing. Additionally, the distinct nature of the results testifies to the accuracy of them.

Inducing sexual reproduction in the Tex 85 isolates proved difficult. As stated earlier, stressing the isolates by disrupting their photoperiod causes them to switch to sexual reproduction. The first method used proved ineffective. The beakers of mature Tex 85 animals were placed on the bench in the lab to present them with a substandard photoperiod. However, lab work conducted at odd hours of the day seemed to affect the efficacy of the approach. Consequently, the beakers were stored in cabinets to facilitate

sexual induction. This also proved unsuccessful. Therefore, about three weeks (of the four months) were unproductive as the Tex 23 males lacked Tex 85 sexual females to mate with. Eventually, more animals were added per beaker to crowd them, and the beakers were wrapped in foil before (see Appendix A) storing them in the cabinets. Fortunately, this approach proved very effective, and the cross began.

5.3 Future Implications

The goal of this experiment was to establish if OP offspring would be produced from a CP-CP cross at a significant rate. Just as previous research showed, CP D. pulex produces OP progeny (Innes & Herbert 1988, Snyman & Xu 2022, Xu et al. 2013). The next question is why. One would not expect two CP animals to create OP offspring. Different theories have been cited as to the cause of this switch, most of which revolve around the hybridization and introgression between *D. pulex* and *D.pulicaria*. Those phenomena play an important role as they are pivotal to variation in species. (Nickel et al., 2021) However, conclusive research has yet to be conducted. The fact that the P1M5H3 and P1M5H4 clones produced from this experiment only produce OP offspring suggests that there may be potential in conducting further research using those clones. Furthermore, the goal is to compose a gene map and identify the gene responsible for the asexuality found in these animals.

APPENDIX A

TEX 23-TEX85 CROSSING BEAKERS CONTAINING DAPHNIA AND FLOATING

EPHIPPIA
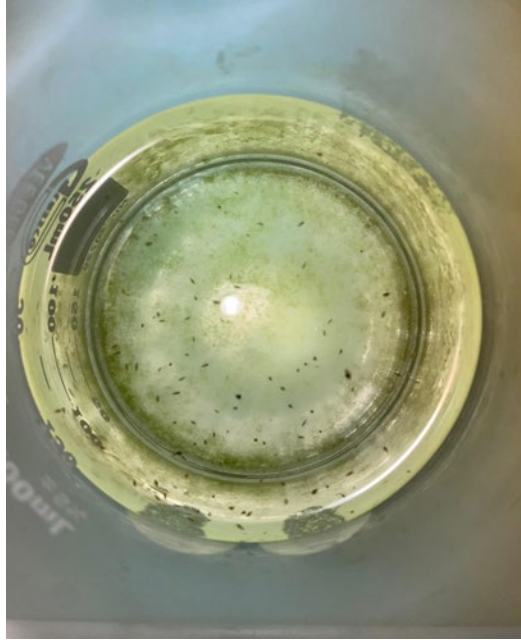
250ml beakers (filled to 150ml) of Tex 23 (male) and Tex 85 (female) *Daphnia pulex* and floating ephippia in lake water COMBO filled with green algae (*Scenedesmus obliquus*) at a concentration of 500,000 cells per ml.

APPENDIX B

FOIL COVERED BEAKERS

Beakers of Tex 85 (non-sexual females) covered in foil to keep out light and induce a switch to sexual reproduction.

APPENDIX C

PLATES CONTAINING INDIVIDUAL WELLS
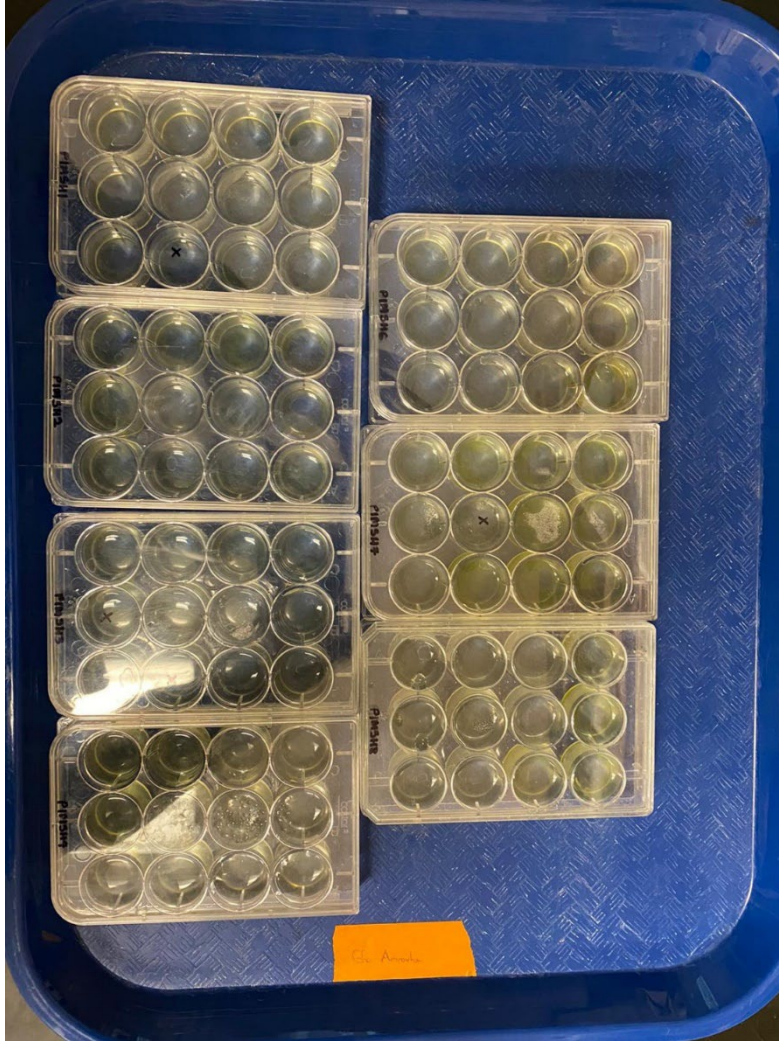
Plates containing each clone with one animal per well so that clonal lines could be established when ephippia was produced.

REFERENCES

Brendonck L, De Meester L. 2003. Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment. Hydrobiologia 491: 65–84

Britannica, T. Editors of Encyclopaedia. 2023. *parthenogenesis*. *Encyclopedia Britannica*.

Carvalho GR. 1994. Genetics of aquatic clonal organisms. In: Beaumont A (ed) Genetics and evolution of aquatic organisms. Chapman and Hall, London, pp. 291–323

Colbourne, J. K., Pfrender, M. E., Gilbert, D., Thomas, W. K., Tucker, A., Oakley, T. H., Tokishita, S., Aerts, A., Arnold, G. J., Basu, M. K., Bauer, D. J., Cáceres, C. E., Carmel, L., Casola, C., Choi, H., Detter, J. C., Dong, Q., Dusheyko, S., Eads, B. D., Boore, J. L. 2011. The Ecoresponsive Genome of Daphnia pulex. *Science.*

Davis Gregoryk. 2012. Cyclical parthenogenesis and viviparity in aphids as evolutionary novelties. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution 318:448–459.

Decaestecker E, De Meester L, Mergeay J. 2009. Cyclical parthenogenesis in daphnia: Sexual versus asexual reproduction. Lost Sex:295–316.

De Meester L, Gómez A, Simon JC. 2004. Evolutionary and ecological genetics of cyclical parthenogens. In Moya A, Font E (eds) Evolution from molecules to ecosystems. Oxford University Press, Oxford, pp. 122–134

Innes, D. J., & Hebert, P. D. N. 1988. THE ORIGIN AND GENETIC BASIS OF
OBLIGATE PARTHENOGENESIS IN DAPHNIA PULEX. *Evolution; international
journal of organic evolution*, *42*(5), 1024–1035.

J. R. Shaw *et al*. 2008. *Advances in Experimental Biology on Toxicogenomics,* C.
Hogstrand, P. Kille, Eds. pp. 165–219.

JURGENS, K. 1994. Impact of Daphnia on planktonic. *Marine Microbial Food
Webs*, *8*(1-2), 295-324.

Kearney M, Fujita MK, Ridenour J. 2009. Lost sex in the reptiles: constraints and
correlations. In: Schön I, Martens K, van Dijk P, editors. *Lost Sex.* Springer;
Dordrecht: pp. 447–474.

Kilham SS, Kreeger DA, Lynn SG, Goulden CE, & Herrera L. 1998. COMBO: a defined
freshwater culture medium for algae and zooplankton. *Hydrobiologia*, 377, 147–159.

Lampert W, Lampert KP, Larsson P. 2012. Induction of male production in clones of
Daphnia Pulex by the juvenoid hormone methyl farnesoate under short photoperiod.
Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology
156:130–133.

Loxdale HD. 2009. What's in a clone: The rapid evolution of aphid asexual lineages in
relation to geography, host plant adaptation and resistance to pesticides. Lost
Sex:535–557.

Luu DH, Vo HP, & Xu S. 2020. An efficient method for hatching diapausing embryos
of *Daphnia pulex* species complex (Crustacea, Anomopoda). *Journal of Experimental
Zoology Part A: Ecolical and Integrative Physiology*, 333, 111–117.

Lynch M, Seyfert A, Eads B, Williams E. 2008. Localization of the genetic determinants of meiosis suppression in *Daphnia pulex*. *Genetics.*180:317–327.

M. Lynch, K. Sptize. 1994. *Ecological Genetics,* L. Real, Ed, pp. 109–128.

Moy I, Green M, Pham TP, Luu DHK, Xu S. 2021. The life history fitness of F1 hybrids of the microcrustaceans *daphnia pulex* and *daphnia pulicaria* (Crustacea, Anomopoda). Invertebrate Biology 140.

Nickel, J., Schell, T., Holtzem, T., Thielsch, A., Dennis, S. R., C, B., Steiner, F. M., Möst, M., Pfenninger, M., Schwenk, K., & Cordellier, M. (2021). Hybridization Dynamics and Extensive Introgression in the Daphnia longispina Species Complex: New Insights from a High-Quality Daphnia galeata Reference Genome. *Genome Biology and Evolution*, *13*(12).

Omilian AR, & Lynch M. 2009. Patterns of intraspecific DNA variation in the *Daphnia* nuclear genome. *Genetics*, 182, 325–336.

Pijanowska J, Stolpe G .1996. Summer diapause in Daphnia as a reaction to the presence of fish. J Plankton Res 18: 1407–1412

Schultz TW (1977) Fine structure of the ephippium of Daphnia pulex (Crustacea: Cladocera). Trans Am Microsc Soc 96: 313–321

Serra M, Snell TW. 2009. Sex loss in monogonont rotifers. In: Schön I, Martens K, van Dijk P, editors. *Lost Sex.* Springer; Dordrecht: pp. 281–294.

Snyman M and Sen X. 2022. On the origin of obligate parthenogenesis in daphnia pulex. BioRxiv.

Toyota K, Miyakawa H, Hiruta C, Furuta K, Ogino Y, Shinoda T, Tatarazako N, Miyagawa S, Shaw JR, Iguchi T. 2015. Methyl farnesoate synthesis is necessary for the environmental sex determination in the Water Flea Daphnia Pulex. Journal of Insect Physiology 80:22–30.

Xu, S., Innes, D. J., Lynch, M., & Cristescu, M. E. 2013. The role of hybridization in the origin and spread of asexuality in Daphnia. Molecular ecology, 22(17), 4549.

Xu, S., Huynh, T. V., & Snyman, M. 2022. The transcriptomic signature of obligate parthenogenesis. *Heredity*, *128*(2), 132-138.

Xu, S., Huynh, T. V., & Snyman, M. 2022. The transcriptomic signature of obligate parthenogenesis. *Heredity*. 128(2): 132-138. Fig. 1, Life history of *Daphnia*.

Ye, Z., Molinier, C., Zhao, C., Haag, C. R., & Lynch, M. 2019. Genetic control of male production in Daphnia pulex. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(31), 15602-1560.

Zaffagnini F. 1987. Reproduction in Daphnia. In Peters RH, De Bernardi R (eds) Daphnia. Mem Inst Ital Idrobiol 45: 245–284

BIOGRAPHICAL INFORMATION

Efe Joseph Amrovhe is set to graduate from The University of Texas at Arlington (UTA) in Spring 2023 with an Honors Bachelor of Science in Biology. He is from Delta State, Nigeria. He joined the Honors College in his second semester at UTA to explore the scholarship and merit opportunities it possesses. On his quest for excellence in the Honors College, he contracted a genetics class for honors credit. This was when his interest in genetics was piqued, and he was inspired to join the Xu Lab.

During his time at UTA, Efe was an allrounder. He was heavily involved in the UTA soccer club, the African Student Organization, and other Black student organizations, while upholding a high grade point average. However, his passion for science always took priority as he aspires to become a Cardiothoracic Surgeon in the Future.

Ultimately, his goal is to apply the knowledge he will gain working in the medical field in the United States towards refining the medical field in Nigeria.