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A GENOTYPIC COMPARISION BETWEEN DAPHNIA PULEX HYBRID OFFSPRING AND ITS PARENTS

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A GENOTYPIC COMPARISION BETWEEN *DAPHNIA PULEX* HYBRID OFFSPRING AND ITS PARENTS

by

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ABSTRACT

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DAPHNIA PULEX HYBRID OFFSPRING

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The question of why most eukaryotic organisms engage in sexual reproduction has been studied extensively in evolutionary biology. A common approach to study why sex and recombination have been maintained and evolved is to understand asexual genomic evolution, which is hypothesized to impact genomes in various ways. A recent study in hybrids between species of *Drosophila* revealed a coevolution of *cis* and *trans* regulatory elements lead to gene misregulation in the F_1 offspring. Regulatory divergence can be detected by allele-specific gene expression assays. If the F_1 hybrid differs in gene expression to the same extent of alleles and parental species, it can be inferred to be *cis*-acting genetic differences. However, if it differs to a larger extent, it can be inferred to be *trans*-acting genetic differences. By performing this kind of gene regulatory divergence analysis in *Daphnia pulex*, we can have a better understanding of the consequence of how cis-trans compensatory evolution could lead to misexpression in hybrids of similar

species. The main objective of this study is to understand better a possible production of interspecific hybrid offspring from the crossing of EB1 (female) and STM2 (male) clones of *Daphnia pulex*. Our hypothesis is that the hybrid will be produced by the crossing of these two clones. The offspring produced by heterosexual parents is also predicted to have mixed genomic background of both parents with an equal ratio.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

CHAPTER 1

INTRODUCTION

1.1 Reproductive Strategy

In nature, there are two main types of reproduction: sexual and asexual. Sexual reproduction is a type of reproduction that happens due to the release of haploid gametes (sperm and egg cells) that are fused to produce a zygote with distinct genetic characteristics comprised from both dad and mom genomes. An asexual reproduction, also known as clonal reproduction, is a type of reproduction that produces two or more genetically identical offspring that does neither change the number of chromosomes nor involve the fusion of sex chromosomes. In animal science, the term parthenogenesis is frequently used as synonymous with asexual reproduction. However, it has a slightly different definition as it refers to the production of individuals without the need of male gametes from the parents. Parthenogenesis can be divided into four classes: arrhenotoky, deuterotoky, pseudo-arrhenotoky and thelytoky. Arrhenotoky is a production of males from unfertilized eggs, meaning they are haploid and appear to have a different set of genome as compared to their mother's. Thelytoky is a production of females from unfertilized eggs. Deuterotoky are similar to thelytoky except that some males are produced but have no role in reproductive process. Pseudo–arrhenotoky produces both male and female individuals from fertilized eggs, except that males subsequently become haploid (De Meeûs T et al 2007). When referring to parthenogenesis as asexual reproduction, it needs to be used carefully and must take into context since only thelytoky

and deuterotoky are characterized as asexual reproduction due to the fact they produce offspring that are genetically identical to their mother (De Meeûs T et al 2007).

Asexual reproduction has a significant advantage in natural fitness over sexual reproduction. One of the contributing factors is time and energy spent in finding mates. It could lead to more risks such as predator and parasite transmission (Roze, 2012). Another factor is “twofold cost of males”. Males only need to contribute their genomes to the next generation while sexual females oftentimes invest half of their resources into the production of males. This provides a two-fold advantage for asexual females (only producing female offspring) (Hurst et al 1996). Recombination offspring produced by sexual reproduction cannot keep the best combination gene together as they are broken down into the next generation. It can disrupt existing genomes created by natural selection, yielding to “recombination load”. (Kleiman et al 2015). Despite those listed reasons, a majority of higher eukaryotes engage in sexual reproduction. One known advantage of sexual reproduction is to increase the recombination of genes that could potentially randomly benefit. Muller’s ratchet might also play a role in providing advantage to sexual reproduction, which is a process of absence of recombination in asexual population results in accumulation of harmful mutation that could lead to extinction (Lynch and Gabriel 1990). Despite of multiple studies, there has not been a general agreement to explain this obvious contradiction.

It has been shown that asexuality plays important roles in the eukaryotic organisms medically and economically. In critical human diseases, such as malaria, it appears to have organisms that use asexual reproduction in at least one stage of their life cycle (De Meeûs T et al 2007). It is found that only asexual hybrid offspring have high

levels of heterozygosity while asexual offspring who is not hybrid origin are characterized by substantial homozygous genomes (Jaron et al 2019). By understanding the genetic rearrangement that happens during the recombination in species population, it plays an important role to locate genes that have important phenotypes.

1.1.1 Daphnia Pulex

Daphnia pulex, also known as water flea, is often used for freshwater ecosystem studies. *Daphnia* is widely used in genomic research due to its ability to switch between sexual and asexual reproduction in response to environmental conditions. Thus, it is good and accessible genomic models that allow researchers to study environmental influences on gene functions. It is shown that by studying traits and focusing on laboratory studies, it may represent phenotypic variation in natural ecosystems and partially explain why over 50% of many eukaryotic genomes are functional without being experimentally investigated (Pena-Castillo et al 2007).

1.1.1.1 Life Cycle and Development

Sex in *Daphnia* is characterized by different environment factors such as crowding, temperature and light period. The cladoceran crustaceans *Daphnia pulex*'s breeding system shows various types of reproduction, i.e. cyclical and obligate parthenogenesis. *Daphnia* typically reproduces by cyclical parthenogenesis (CP). Under favorable conditions, the females reproduce with a-meiotic eggs and produce genetically identical daughters. Under stressful environment (i.e., food shortage or crowding), the female *Daphnia* clonally produce males and produce haploid eggs through meiosis. The eggs, once fertilized, will be deposited in a protective case (ephippium), and become resting eggs. In this experiment, we use EB1 females, which theoretically only produce

females in the population. Some *Daphnia* reproduce by obligate pathogenesis (OP), meaning organisms only reproduce through asexual means. Under favorable conditions, it produces asexually; and under deteriorating environment, it also parthenogenetically produces ephippial resting eggs. We will be using STM2 species, which is known to reproduce by obligate pathogenesis. Most of the time STM2 cultures reproduce female; however, under harsh conditions (crowding, food shortage), they can reproduce males.

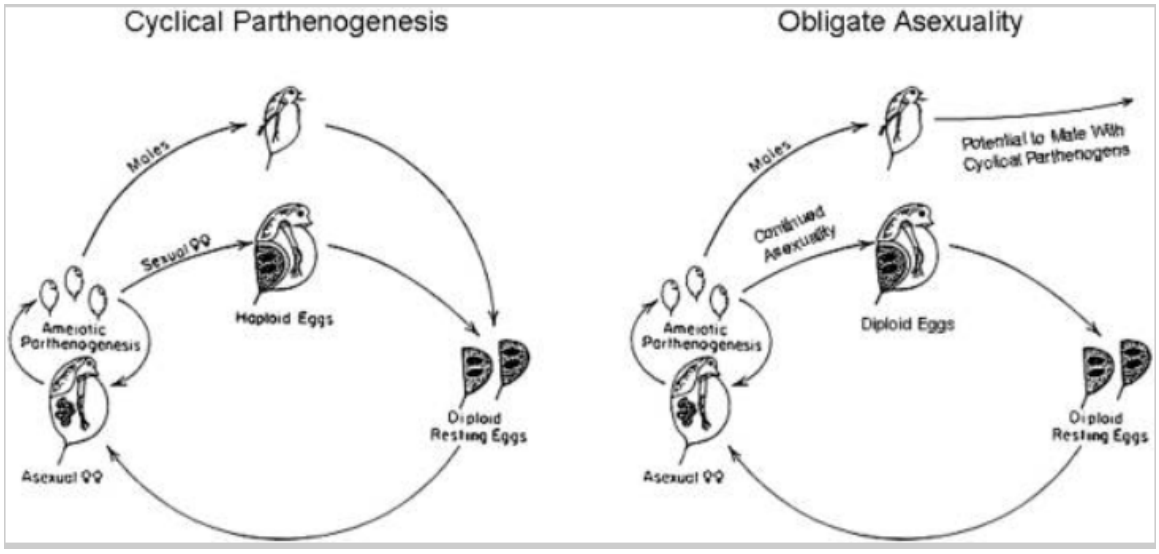

Figure 1.1: Schematic of *Daphnia pulex* breading system (1) cyclical parthenogenesis (CP) (2) obligate parthenogenesis (OP)

1.2 Statement of Research/Research Questions

A recent study has shown that a large amount of genes expression in F_1 hybrid offspring of *Drosophila* are found outside the range that found in the parental species (Comai et al. 2003; Wang et al. 2004). The conclusion drawn from the study is that a coevolution within species occurs so that cis-regulatory elements are compensated because natural selection acts to maintain optimum level of gene expression in *Drosophila* (Denver et al. 2005; Lemos et al. 2005). An allele-specific gene expression in

related species and hybrids can help determine the different types of regulatory divergence. When there is the same extent difference in allele and the parental species as in hybrid, it can be deduced that the genetic difference caused by cis-regulatory element (Yan et al. 2002; Wittkopp et al. 2004). When there is a larger difference in allele of parental species and the hybrids, it can be inferred that the genetic difference caused by trans-regulatory element. It also has been shown that intraspecific mating occurs frequently in the Cladocera, which leads to a possibility of an interspecific hybridization when *Daphnia* taxa crossing.

By analyzing gene regulatory divergence in *Daphnia pulex*, we can have a better understanding the consequence of how cis-trans compensatory evolution can lead to misexpression in hybrids of similar species. The main objective of this study is to understand better possible production of interspecific hybrid offspring from the crossing of EB1 (female) and SMT2 (male) subspecies of the *Daphnia pulex*. In this experiment, EB1 possesses a cyclically parthenogenetic while STM2 has obligate parthenogenesis. It has been shown that some of the OP descents can produce viable males (Innes and Hebert 1988). When *Daphnia pulex* OP male lineages mate with CP females, contagious asexuality occurs, meaning that asexual males can spread asexuality genomes to sexual females, leading to a conversion of sexual lineages into asexual ones. It has been predicted that these OP males can transmit those asexual element to about 50% progeny genome when mating with sexual CP females (Innes and Hebert 1988). Therefore, our hypothesis is that the offspring produced by heterosexual parents will have a mixture of genetics from both parents with an equal ratio, and some offspring are obligately parthenogenetic.

CHAPTER 2

LITERATURE REVIEW

2.1 Factors Affecting Types of Reproduction

Some OP *Daphnia pulex* over the course of evolution has lost the ability to participate in a sexual reproduction to give males. However, some still can produce males that are capable of haploid sperm production. It has been shown that in the cladoceran crustacean *Daphnia pulex*, the spread of asexuality is linked to sex-limited meiosis suppression. Meiosis-suppression effect is limited to the female species while the male in OP can still engage in meiosis. It is predicted that these underlies an important mechanism of the transmission of meiosis suppression genes to the new offspring by crossing between OP male with CP female (Innes and Hebert 1988). If sex-limited meiosis suppression mechanism is dominant, some OP hybrids will be produced from the crosses (Innes and Hebert 1988). It was hypothesized by Innes and Hebert that dominant sex-limited meiosis suppressors occur at a single locus in OP *Daphnia pulex* (Innes and Hebert 1998). Later on, it is discovered that it is in fact due to at least four unlinked loci in multiple genomic regions (Lynch et al 2008). Another study has shown that OP clones genome have a transposable element that is inserted into the genes while completely absent from the genome of CP clones (Eads et al 2012).

Approximately one out of ten thousand animal species obligate asexual (parthenogenetic reproduction) occurs (Lynch et al 2008). Yet it is not unusual occurrence that there are frequent males appearing in all female population

(Schön *et al*., 2009). Most of the rare males found in parthenogenetic species are functional; however, some of them appear to exhibit irregular spermatogenesis and problematic sterility (Lynch 1984). As these females are parthenogenetic, these rare males cannot fertilize with the females of its same species. Due to its low frequency of males in this population, they are often perceived as insignificant to the evolution. (Schön *et al*., 2009). If the population continues to produce these males consistently, these males might play a role in genetic exchange coexistence between parthenogenetic and sexual descent (Lynch 1984). It is predicted that when males produced by parthenogenetic females (OP) mate with sexual females (CP), the asexual elements may be transferred, which is known as contagious parthenogenesis (Innes and Hebert 1988). This process helps to introduce genetic diversity of asexual descent, increases chances of fitness in producing rare males and might contribute to evolutionary process of asexual lineages (Maccari et al 2013).

A similar species that have the variation in breeding system like *Daphnia pulex* is also found in parthenogenetic *Artemia*. It is shown that in most of the populations, there is a tendency for rare males to have an identical or closely related to haplotype as popular parthenogenetic females from their respective populations (Maccari et al 2013). The findings show that rare males from OP *A. parthenogenetica* populations are fully functional, go through normal meiosis, produce viable haploid sperm and have capability to fertilize eggs from three females of sexual Asian Artemia distant species (*A. urmiana*, *Artemia* sp. and *A. sinica*) (Maccari et al 2013). With the use of microsatellite marker, it shows the hybrid resulted from the cross shows a similar or even increased chances of fertile offspring viability from the control and showed a normal morphology. As these

Artemia rare males have potential reproductive role in the population, it is predicted that the hybrids between parthenogenetic males and sexual females could give a source of gene flow in different genotypes (MacDonald et al 1987).

Some studies in the past have indicated that the polyploid clones are dominant in *Daphnia pulex* complex from tundra regions (Beaton and Hebert 1988); however, only diploids are found in temperate zone of Canada. It is also noteworthy that polyploids are absent from Mexico and America indicating that the polyploid prefers low temperature making it restricted to the north (Hebert and Finston 2001). A similar tendency is found in polyploid lineages of a second cladoceran genus, *Bosmina*, which is also found only in the North area (Little et al, 1997).

Two related distant species of cladoceran crustacean, *Daphnia pulex* and *D. pulicaria*, are known to have a variation in their breeding system where some reproduce by cyclical parthenogenesis and some reproduce by obligate asexuality, which makes it is common to have them cross as hybrids (Hebert et al 1993). Studies have shown that these hybrids mostly reproduce by OP and could contribute to the lineages by their dispersal of asexual ephippial eggs. OP of *Daphnia pulex* populations dominate in polar region of Holarctic region (Weider et al 1987). However, CP of *Daphnia pulex* populations are found in temperate regions of Europe (Ward et al 1994). Based on a study conducted on Canadian population, it is predicted that OP and CP lineages and the region residence has a relationship with the differing distribution of glacial area (Hebert et al 1993). There has been evidence that OP population of *D.pulicaria* dominate the eastern margins and CP in west in North America (Hebert and Finston 2001). The variation in breading system in

different regions might provide the origin and effect of asexual transition from different areas.

Hatching embryos is critical in the process of producing hybrids when crossing between two distant related *Daphnia* species. Temperature factor is also exploited in the lab technique for hatching *Daphnia* purposes. In this experiment, a storage time at least 2 weeks (optimally 3 weeks) in the dark before exposing embryos to ultraviolet light was recommended to give higher chances of survival rate when hatching *Daphnia pulex* and *Daphnia pulicaria*. The embryos that are produced through OP, selfing and outcrossing from varying geographical *Daphnia* population can have a hatching rate 80% to 100% when putting under the ultraviolet light after the incubation period (Luu et al 2019).

CHAPTER 3

METHODOLOGY

The methodology is divided into three main parts: preparing the solutions, performing the crossing and hatching the embryos between the two related species of *Daphnia pulex* EB1 and STM2. One of the takeaways is to pay attention to the pipette or beaker in order to avoid contamination, which can result into inaccurate results.

3.1 Preparation of Chemicals

In order to feed the *Daphnia* with food (algae), a solution of concentrated algae and *Daphnia* combo are mixed together. Stock solutions need to be prepared before those Combos to be put together. These major stock solutions are common because they are used frequently in lab. If each bottle was filled with 1.0 L of ultrapure water, these chemicals can be added to make a stock solution: 36.76 g of Calcium Chloride, 36.97 g Magnesium Sulfate, 85.01 g Sodium Nitrate, 12.60 g Sodium Bicarbonate, 24.00 g Boric Acid, 8.71 g, 28.42 g Sodium Metasilicate and 7.46 g Potassium Chloride. If using only 500 mL of ultrapure water, then only half of these chemical are added. In order to make 1L Sodium Selenite (6.2×10^{-6}), 999mL of ultrapure water is mixed with 1 mL of Sodium Selenite (6.2×10^{-3}).

3.1.1. Preparation for Mini/Master Algae Trace Elements Stock Solutions

If a mini stock Algae Trace Element is needed, in 100 mL of UltraPure water, these chemicals are dissolved: 18.0 g $MnCl_2.4H_2O$, 0.1 g $CuSO_4.5H_2O$, 2.2 g $ZnSO_4$, 1.0 g $CoCl_2.6H_2O$, 2.2 g $Na_2MoO_4.2H_2O$, 0.16 g H_2SeO_3 and 0.18 g Na_3VO_4. If a larger

quantity major stock solution is needed, in a 1000 mL beaker, 4.36 g of $Na_2EDTA.2H_2O$ is dissolved in 1000 mL ultrapure water while gently heating the beaker. While heating the solution, pH is checked regularly. Then, 1.0 g of $FeCl_3.6H_2O$ is added into the solution and pH is checked. pH should be around 7.0 in order to dissolve $FeCl_3.6H_2O$ completely. If pH is too acidic, a small amount (approximately 4.2 mL) of KOH 5M would be needed to bring up the pH. When $FeCl_3.6H_2O$ is completely dissolved and cooled down, 1 mL of each chemicals listed above is added.

3.1.2. Preparation for Mini/Master Animal Trace Elements Stock Solutions

If a mini stock of Animal Trace Element is needed, in 100 mL of ultrapure water, these chemicals are dissolved: 31 g LiCl, 7 g RbCl, 15 g $SrCl_2.6H_2O$, 1.6 g NaBr and 0.33 g KI. If a larger quantity of major stock solution is needed, in a 1000 mL beaker, 1mL of each chemical above are combined with 995mL of ultrapure water.

3.1.3. Preparation for Master Vitamin Stock Solutions

In a 100mL bottle, 0.02g of Thiamine with 98.0mL of UltraPure water are dissolved. Then, 1mL of Biotin and 1mL of B_{12} are dissolved completely. For the Biotin stock solution, 10mg of Biotin is dissolved in 96.0mL of water. For the B_{12} stock solution, 10mg of B_{12} is dissolved in 89.0mL of water.

3.1.4. Preparation of Combo for Algae and Daphnia

A 20L Carboy was filled up with 19L of Nano-pure water. 3.4g of Tris base (THAM) and 5mL of hydrochloric acid (HCl) were added into the Carboy, shaken and set aside. In a cylinder, 650mL of Nano-pure water was filled up. Then, about 20mL of these chemical were added, respectively: Boric Acid, Sodium Bicarbonate, Magnesium Sulfate, Calcium Chloride, Sodium Metasilicate, Algae Trace Element and Sodium

Nitrate. After that, 10mL of VIM was added and mixed together. Nano-pure water was added to reach of 1000.mL. Before adding everything from the cylinder, 20mL of potassium phosphate was added into the carboy. A tape was put on the carboy with the date and time that the mixture was prepared. The solution was aerated for at least 24 hours prior use.

A 20.L Carboy was filled up with 19L of Nano-pure water. In a cylinder, 650mL of Nano-pure water was added to the mark and 20mL of these chemical were added: Boric Acid, Sodium Bicarbonate, Magnesium Sulfate, Calcium Chloride, Sodium Metasilicate, Animal Trace Element, Potassium Chloride and Sodium Selanite. After that, the cylinder was filled up with nano-pure water to reach to 1000mL. The solution from the cylinder was poured into the carboy and shaken before use for algae preparation.

3.1.5. Preparation for Methyl Farnesoate (MF6)

Ideally, we want to have a final concentration of 400nM methyl farnesoate, 0.003% EtOH in our standard *Daphnia* media. In order to do that, 30µL of EtOH is added into 1L of filtered lake water. This means that it requires 400nmol of MF per 30µL of EtOH in the MF stock. Because MF has a molecular weight of 250.38, and 400nmol of MF is approximately equal to 0.100152 mg. Therefore, 0.100152 mg MF is needed per 30µL EtOH in the stock. The following formula is used to determine the amount of ethanol needed to add to the MF vial to get the primary stock:

X mg MF in vial / 3.3384 = Y mL EtOH to be added.

3.2 Crossing Experiments

A small amount of animals EB1 and STM2 (3-5) are taken from the stock placed into multiple clean beakers. EB1 has a cyclical parthenogenetic cycle, so it invariably

reproduces female animals. On the other hand, STM2 has a tendency to produce both female and male animals. The crossing between EB1 and STM2 will produce a hybrid offspring. The goal is to grow it into a large quantity so we can perform multiple experiments. For approximately two to three weeks, it is fed continuously any other day.

3.2.1 Selecting Males from STM2

There are two ways we can collect STM2 males. One way is once a sufficient amount of STM2 is present, only big size female animals are isolated from the babies. Then, methyl farnesoate - a chemical dictating male production in *Daphnia*, will be applied so that male STM2 will be present in the environment for the crossing purposes. The other way is to find them manually. Most of the time, STM2 will produce female. However, under stress conditions (crowding or less food), some males will appear. After a couple weeks, when there is sufficient amount of STM2, we will use a pipette to collect the both male and female species and put under the microscope to individually collect the males. This technique is time consuming but it helps to avoid the hormonal change caused by the chemical, which is a confounding variable.

3.2.2 Crossing

Crossing technique is applied to produce a hybrid species. About 7-10 big females of EB1 and males of STM2 are placed into a plastic test tube with about 5mL of *Daphnia* combo and a small amount of algae. The mixture is placed under 25 or 37ºC incubators. Every other day, I will check back and remove the babies to avoid selfing and to dissect ephippia. The offspring produced will be an ephippia. When dissecting ephippia, we only collect the embryos (blackish eggs) and abandon the empty ones.

3.2.3 Hatching

The embryos are then placed into the Petri dish and fed with a small amount of *Daphnia* combo. It is then wrapped around aluminum foil and placed in the incubator. After three weeks under the dark, the petri dishes are placed under UV light condition. Once they are hatched, they will be fed with algae and move to the normal condition to continuously grow until they are mature. Theses F_1 hybrid will be fed to grow into high density and perform sexuality tests. If the offspring has no embryo, we expect it has the cyclically parthenogenetic cycle. If the offspring has embryo, we expect it has the obligately parthenogenetic cycle.

CHAPTER 4

RESULTS AND DISCUSSION

The experiment has produced many ephippia and about 70 embryos that were placed in the dark incubator. The embryos appeared to be black or yellow oval shape. So far, there have been 10 *Daphnia* after exposure to UV light. However, for some reasons, only about 4 of them were alive. One of the reasons was the petri dish continuously exposed the *Daphnia* under the UV light after it has showed some signs of liveliness (twitching, beating). It could have been too hot for the baby *Daphnia* to survive. Another reason could be due to not feeding the small *Daphnia* every day, which explains why over the weekend none of them was alive. Another main reason is the first batch was placed in the dark only for one week and then put under the UV light. This might have caused a disturbance in the development of the *Daphnia*, making all of the embryos did not hatch.

When performing crossing between EB1 and STM2, ephippia are frequently produced. If there are no embryos in there, it means that no hybrid occurs because only the females, but not the males present in the culture. The male STM2 cannot reproduce itself, so the ephippia produced most likely come from female EB1. However, if there are embryos in the ephippia, there is a high chance hybrids have been produced because it means that there must have been males STM2 (OP) and EB1 (CP) contribute to the mating process. Even though we did not have time to perform DNA extraction and sequencing, theoretically, these F_1 *Daphnia* are most likely hybrids produced. However,

whether or not these F_1 *Daphnia* will have CP or OP type of reproduction, it needs to be studied further with a larger quantity to provide reliable data. This could be done by sexuality test in which a large clone of F_1 hybrid are stressed and produced ephippia. The same concept is applied as if it is empty, we assume it is CP and if containing embryos, OP is recorded.

One improvement in the future is to place the embryos in the dark for three weeks and record the date putting in the incubator on sticky notes and in the lab notebook. From this experiment, it has shown that *Daphnia* tend to hatch easier when putting under the UV light after three weeks incubation. Another improvement is to conduct more tests crossing tube so that there is a higher possibility of STM2 and EB1 to mate. One of the errors I made was choosing a small EB1, which led to the production of multiple babies in the crossing tube. It consumes more time as I need to remove it every day but also do not yield a lot of ephippia. It could be it is still growing to its sexual stage and might take longer for it to actually fully mature. One of the recommendations is to choose the big EB1 or isolate the medium size EB1 in a beaker and watch it grow.

CHAPTER 5

CONCLUSION

The two clones that we are interested on are EB1 and STM2. EB1 reproduces by cyclical parthenogenesis while STM2 reproduce by obligate parthenogenesis pathway. Through this experiment, it has shown that there is about 95% certainties theoretically that STM2 and EB1 have reproduced viable F_1 hybrid. However, DNA extraction and sequencing are needed to be further conducted in order to give empirical evidence as well as comparisons in DNA genome in different ancestry lineages of the same or related species.

In further studies of this experiment, if the majority of the hybrid offspring show a cyclically parthenogenetic pathway, we can conclude that the gene expression most likely is due to the EB1. We can also question if there are any genetic consequences of meiosis abortion benefit leading to why it is preferred. If the majority of the hybrid offspring shows an obligately parthenogenetic pathway, STM2 plays a more dominant role in gene expression. Overall, it is questionable that which pathways play a more significant role in reproduction and why it is favored in the natural selection process. From there, we can be able to cross different clones of *Daphnia* to find a dominant trend as to why one reproduction and genetics are preferred versus another. Not only so, we could also look into the allele differences in those hybrid genes and parents to find out whether differences could be due to cis-trans regulatory elements like we have found in the previous paper for the *Drosophila*. The more knowledgeable we have on the *Daphnia*

reproduction, the more deduction we can gain on human reproduction evolution as well as molecular mechanisms in genetic consequence of asexuality.

In additional, this research enhances my knowledge in biological technique used in the lab and gives a better understanding in genomics. Not only so, it gives me a better idea of how to utilize my science knowledge particularly biology and chemistry courses into real world applications. Obtaining these skills give strong foundations in developing different projects as well as a wider scope of practical knowledges.

APPENDIX A

DAPHNIA PULEX STAGE OF DEVELOPMENT

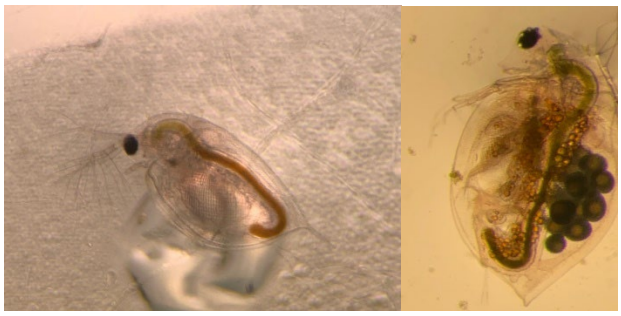

Figure A-1: (a) Male *Daphnia pulex* STM2 (b) female *Daphnia pulex* EB1

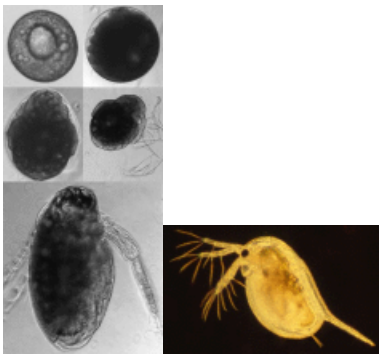

Figure A-2: (a) Development of *Daphnia* embryo (b) Newborn *Daphnia magna*

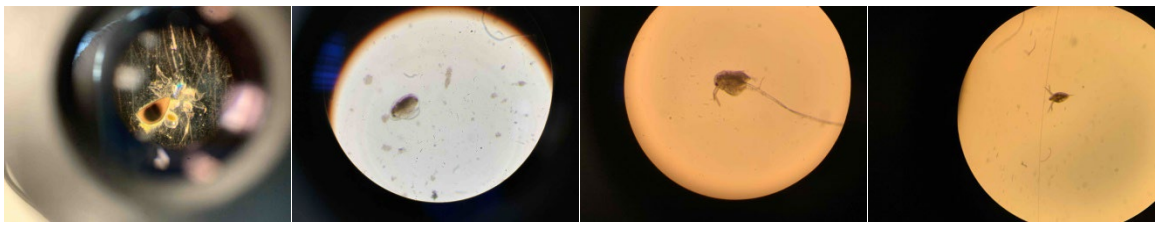

Figure A-3: (a) Epipphia collected in the crossing tube (b), (c), (d) embryos after exposure to UV light in the lab

REFERENCES

Beaton, M. J. Geographical Parthenogenesis and Polyploidy in Daphnia Pulex. *The American Naturalist* 1988, *132* (6), 837–845.

Comai, L.; Madlung, A.; Josefsson, C.; Tyagi, A. Do the different parental 'heteromes' cause genomic shock in newly formed allopolyploids? *Philosophical Transactions of the Royal Society of London.Series B: Biological Sciences* 2003*, 358*, 1149-1155.

Denver, D. R.; Morris, K.; Streelman, J. T.; Kim, S. K.; Lynch, M.; Thomas, W. K. The Transcriptional Consequences of Mutation and Natural Selection in Caenorhabditis Elegans. *Nature Genetics* 2005, *37* (5), 544–548.

Hebert, P. D. N.; Finston, T. L. Macrogeographic Patterns of Breeding System Diversity in the Daphnia Pulex Group from the United States and Mexico. *Heredity* 2001, *87* (2), 153–161.

Hebert, P. D. N.; Schwartz, S. S.; Ward, R. D.; Finston, T. L. Macrogeographic Patterns of Breeding System Diversity in the Daphnia Pulex Group. I. Breeding Systems of Canadian Populations. *Heredity* 1993, *70* (2), 148–161.

Hurst, L. D.; Peck, J. R. Recent Advances in Understanding of the Evolution and Maintenance of Sex. *Trends in Ecology & Evolution* 1996, *11* (2), 46–52.

Innes, D. J.; Hebert, P. D. N. The Origin and Genetic Basis of Obligate Parthenogenesis in Daphnia Pulex. *Evolution* 1988, *42* (5), 1024.

Jaron, K. S.; Bast, J.; Nowell, R. W.; Ranallo-Benavidez, T. R.; Robinson-Rechavi, M.; Schwander, T. Genomic Features of Asexual Animals. 2018.

Kleiman, M.; Hadany, L. The Evolution of Obligate Sex: the Roles of Sexual Selection and Recombination. *Ecology and Evolution* 2015, *5* (13), 2572–2583.

Lemos, B.; Meiklejohn, C. D.; Cáceres, M.; Hartl, D. L. Rates of divergence in gene expression profiles of primates, mice, and flies: stabilizing selection and variability among functional categories. *Evolution* 2005*, 59*, 126-137.

Little, T. J.; Demelo, R.; Taylor, D. J.; Hebert, P. D. N. Genetic Characterization of an Arctic Zooplankter : Insights into Geographic Polyploidy. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 1997, *264* (1386), 1363–1370.

Luu, D. H.-K.; Vo, H. P.; Xu, S. An Efficient Method for Hatching Diapausing Embryos of Daphnia Pulex Species Complex (Crustacea, Anomopoda). *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* 2019, *333* (2), 111–117.

Lynch, M. Destabilizing Hybridization, General-Purpose Genotypes and Geographic Parthenogenesis. *The Quarterly Review of Biology* 1984, *59* (3), 257–290.

Lynch, M.; Gabriel, W. Mutation Load and the Survival of Small Populations. *Evolution* 1990, *44* (7), 1725.

Lynch, M.; Seyfert, A.; Eads, B.; Williams, E. Localization of the Genetic Determinants of Meiosis Suppression InDaphnia Pulex. *Genetics* 2008, *180* (1), 317–327.

Maccari, M.; Gómez, A.; Hontoria, F.; Amat, F. Functional Rare Males in Diploid ParthenogeneticArtemia. *Journal of Evolutionary Biology* 2013, *26* (9), 1934–1948.

Macdonald, G. H.; Browne, R. A. Inheritance and Reproductive Role of Rare Males in a Parthenogenetic Population of the Brine Shrimp, Artemia Parthenogenetica. *Genetica* 1987, *75* (1), 47–53.

Meeûs, T. D.; Prugnolle, F.; Agnew, P. Asexual Reproduction: Genetics and Evolutionary Aspects. *Cellular and Molecular Life Sciences* 2007, *64* (11), 1355–1372.

Peña-Castillo, L.; Hughes, T. R. Why Are There Still Over 1000 Uncharacterized Yeast Genes? *Genetics* 2007, *176* (1), 7–14.

Roze, D. Disentangling the Benefits of Sex. *PLoS Biology* 2012, *10* (5).

Schön, I.; Marten, K.; Van Dijk, P. *Lost Sex: The Evolutionary Biology of Parthenogenesis* 2009, 99–131.

Wang, J.; Tian, L.; Madlung, A.; Lee, H.; Chen, M.; Lee, J. J.; Watson, B.; Kagochi, T.; Comai, L.; Chen, Z. J. Stochastic and epigenetic changes of gene expression in Arabidopsis polyploids. *Genetics* 2004*, 167*, 1961-1973.

Ward, R. D.; Bickerton, M. A.; Finston, T.; Hebert, P. D. N. Geographical Cline in Breeding Systems and Ploidy Levels in European Populations of Daphnia Pulex. *Heredity* 1994, *73* (5), 532–543.

Weider, L. J.; Beaton, M. J.; Hebert, P. D. N. Clonal Diversity in High-Arctic Populations of Daphnia Pulex, A Polyploid Apomictic Complex. *Evolution* 1987, *41* (6), 1335.

Wittkopp, P. J.; Haerum, B. K.; Clark, A. G. Evolutionary changes in cis and trans gene regulation. *Nature* 2004*, 430*, 85.

Yan, H.; Yuan, W.; Velculescu, V. E.; Vogelstein, B.; Kinzler, K. W. Allelic variation in human gene expression. *Science* 2002*, 297*, 1143.

BIOGRAPHICAL INFORMATION

Nguyen Nguyen was born in Saigon city, Vietnam. She came to the United States when she was fifteen years old and attended Juan Seguin High School. After graduating high school, she went to the University of Texas at Arlington with a desire to obtain an Honors Bachelor of Biochemistry, with a minor in biology. She loves to learn new things and plays tennis during free time. Recently, she has started learning guitar and piano in hoping to master those skills as well as proficiency in English. In the future, Nguyen wants to pursue career in healthcare professions.